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Abdominal ascending interneurons in crickets: responses to sound at the 30-Hz calling-song frequency

Günter Kämper
Max-Planck-Institut für Verhaltensphysiologie, Abteilung Huber, D-8131 Seewiesen, Federal Republic of Germany

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Summary. 1. Ascending abdominal interneurons receiving inputs from the cerci were examined by stimulating the cerci with pulses of 30-Hz sound, a frequency that corresponds to the repetition rate of the syllables in the conspecific calling song.

2. 13 interneurons were morphologically identified and characterized physiologically.

3. Neurons showing little or no habituation during the sound pulse copy the stimulus pattern by discharging at a particular phase of each cycle of air motion (interneurons 10-2a, 10-3a; with qualifications also 8-1a, 9-1a and 9-3a).

4. Neurons in a second group act as frequency-dependent filters: three (9-1b, 10-1c and 11-1c) showed low-pass properties at 30 Hz, and one (8-1b) a band pass characteristic.

5. The direction of the stimulus source also affects the response; individual neurons have different best directions. In principle the animal could determine the direction of air-particle oscillation by comparing the response phases of two cells (10-2a and 10-3a) that are shown to discharge in synchrony or in alternation depending on the direction of the stimulus.

6. Changes of various parameters of the sound pulses during continuous stimulation of the cerci cause interneuron 11-1c and the newly described interneuron NN1 to give persistently increased responses.

Introduction

Female *Gryllus campestris* walk toward a singing male only if the song has a certain temporal structure (see, e.g., Thorson et al. 1982). The sound components above 2 kHz are detected by the tympanal organs in the forelegs and processed in the auditory pathway (for references see Huber 1983).

Kämper and Dambach (1981), by extracellular recordings of multi-unit potentials in the abdominal connectives, found that complete information as to the chirp and syllable rhythms is present in the discharge of abdominal ascending interneurons, both in the singing male itself and in females and non-singing males near the sound source. These interneurons, often called giant fibers, receive input from filiform-hair receptors on the cerci. The hairs are displaced by low-frequency sound and by wind. Air movements parallel to the long axis of the cercus are detected by L-hairs, and those transverse to the long axis are detected by T-hairs (Edwards and Palka 1974). Each of these populations is subdivided into two groups of hairs with opposite preferred directions (Tobias and Murphey 1979).

During stridulation, each inward (closure) movement of the wings produces a syllable, a pulse of 4- to 5-kHz sound. The same wing movement also generates periodic air-particle movements in the rhythm of syllable repetition, 25–30 Hz (Kämper 1981). Cercal filiform hairs detect these oscillations. (4- to 5-kHz sound is called 'low-frequency' in the cricket literature; in this paper the term 'low-frequency' refers to the 25- to 30-Hz component.) During normal singing the chirp rhythm (unlike the syllable rhythm) is relatively irregular, but when air puffs are applied to the cerci of a singing male at regular intervals in the chirp-repetition-interval range (ca. 3/s), the chirp rhythm of the male can be entrained; the air-puff input is superimposed upon the self-stimulation of the cercal receptors (Dambach et al. 1983). Therefore it appears that the cerci, in addition to sense organs on the wings (Elliot et al. 1982; Elliot and Koch 1983; Schäffner and Koch 1983) and in the region

Abbreviations: IN abdominal ascending interneuron; *I1-3* intensity 1-3

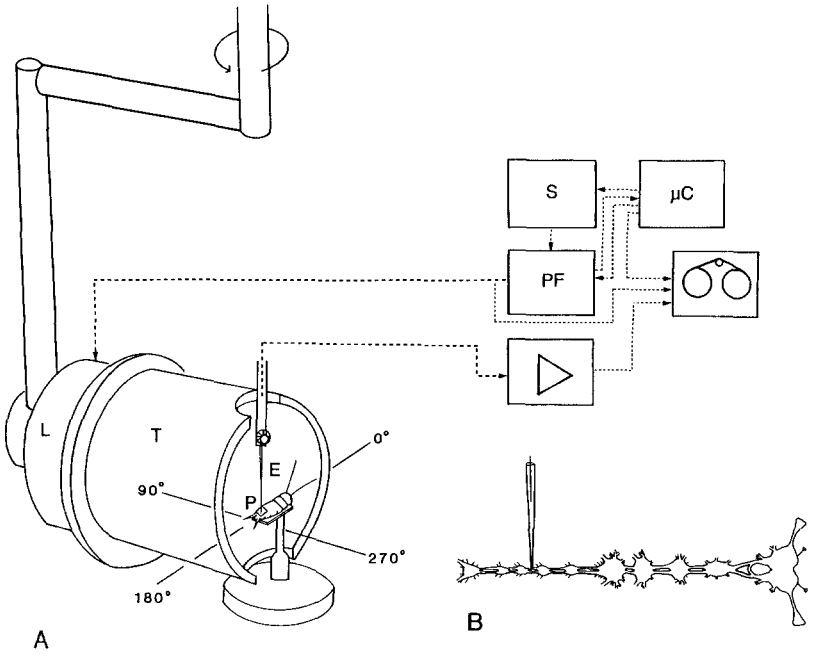

Fig. 1. A Apparatus for intracellular recording from cricket interneurons during stimulation of the cerci with low-frequency sound. Preparation *P* is mounted on a holder in the opening of a plexiglass tube *T*, the other end of which is closed by a loudspeaker *L*. This sound tube can be rotated about the preparation; in the coordinate system used here, the loudspeaker position illustrated corresponds to an angle of 90°. *E* Electrode, *S* sine-wave generator, *PF* pulse former, *μC* microcomputer. **B** position of recording electrode in the nervous system of the cricket

of the wing joint (Möss 1971), are involved in the proprioceptive feedback control of the stridulatory movements.

The responses of ascending interneurons to stimulation of the cerci with low-frequency sound have been studied in crickets by Edwards and Palka (1974), Counter (1976), Belosky and Delcomyn (1977), Palka and Olberg (1977), Matsumoto and Murphey (1977), Murphey et al. (1977), Levine and Murphey (1980a, b), Rozhkova (1980) and Morrissey and Edwards (1981). Because of the difficulty of producing low-frequency sound at well-defined air-particle velocities (cf. Tautz 1979), none of the above studies employed stimuli at frequencies below 50 Hz. Recently Kanou and Shimozawa (1984) have measured threshold curves at lower frequencies as well, for 7 identified and one non-identified ascending interneuron.

The present paper provides a survey of the various types of responses of identified abdominal interneurons. The main emphasis is on the processing of 30-Hz sound pulses: How are the physical parameters of the stimulus represented in the discharge of the interneurons? Does each neuron have a special function or is there functional overlap? How great are the differences in the responses of homonymous cells in different animals?

The cells are designated according to the nomenclature of Mendenhall and Murphey (1974) and Jacobs (1983). This numbering system assigns to each interneuron the number of the primitive abdominal segment and of the neuroblast cluster in which the soma lies.

Methods

Animals. The experiments were done with adult males and last-instar larvae of *Gryllus campestris* L. All the animals were caught in the field (in the southern Tyrol) and kept in individual containers at ca. 15–30 °C. Care was taken to select animals with externally intact cerci.

Auditory stimulation. Low-frequency sound pulses were generated with the 'sound tube' illustrated in Fig. 1. During the recording the animals were mounted on a holder in the opening of a plexiglass tube 12 cm in diameter and 13 cm in length. At the other end of the tube was an airtight attachment to a loudspeaker (Isophon BPSX 130). Oscillations of the loudspeaker membrane at low frequencies induced air oscillations that were homogeneous throughout a large region of the tube lumen; the layer near the wall in which amplitude was reduced was ca. 0.5 cm thick (Kämper 1981). At the position of the cricket the air particles oscillate in a direction parallel to the long axis of the tube. Direction, intensity and phase of the sound were calibrated with respect to the loudspeaker voltage by means of a microphone sensitive to air-particle velocity (Bennet-Clark 1984, slightly modified). The microphone itself was previously calibrated in a vibration box and for higher frequencies in a sound damped chamber. At 30 Hz the voltage waveform at the loudspeaker in the sound tube of Fig. 1 is nearly in phase with the air-particle displacement at the position of the animal (difference less than 18°, increasing at higher frequencies). The rising phase of the voltage signal corresponds to movement of the air particles toward the animal. The entire sound-producing apparatus could be rotated around the animal over the range 45–180°; angles are defined in Fig. 1. The cerci were at the center of rotation, so that they were always at a constant distance from the loudspeaker membrane. The sound tube was not in contact with the table bearing the animal, so that there was no discernible transmission of vibration from one to the other.

Sinusoidal voltage input to the loudspeaker was produced by a generator (Wavetek Model 114) and modulated by a pulse-generator system to provide pulses ca. 170 ms in duration with

gradual rising and falling flanks. The phase relationship of carrier and modulation envelope was constant within each experiment. Frequency, amplitude and number of pulses were controlled by a microcomputer (Rockwell Aim 65) with two digital-to-analog converters. The maximal departure of signal frequency from that selected was ca. −18% (only negative). Because of inaccuracy in the automatic intensity setting and the complicated calibration procedure, the intensity could vary by as much as ±25% from the nominal values (0.2 cm/s = I1, 2 cm/s = I2, 3.5 cm/s = I3 at 30 Hz, but also at 50 and 110 Hz; 0.06 cm/s = I1, 0.6 cm/s = I2, 0.7 cm/s = I3 at 500 Hz). Frequencies higher than 30 Hz were applied to detect frequency-filter effects at the 30 Hz test stimulus. All intensities are given as peak-to-peak air-particle velocities in order to facilitate comparison with published data where air puffs were used. I1 is an intensity near threshold for many neurons; I2 is well above threshold as a rule; I3 is the strongest stimulus the apparatus can produce. Note that all frequency-filter properties in this work are defined with respect to air-particle velocity, and that the stimulus intensities used at 500 Hz are considerably smaller than that at other frequencies.

Each stimulus pulse was repeated 15 times (ca. 2/s) at 4 loudspeaker positions: 45°, 90°, 135° and 180°. The stimulus 30 Hz/I2 corresponds approximately, in frequency and intensity, to the low-frequency sound pulse produced by the cricket during the calling song, as measured in the region of its own cerci (Kämper and Dambach, unpublished).

Electrophysiology. The animal was mounted on a holder, back upward, and the middle and hind legs and the wings were removed. The abdomen was opened and the gut, fat body and muscles resected together with part of the tracheal system, so as to expose the ventral cord. A small piece of wood was pushed under the cord to support the connectives between the 2nd and 3rd free abdominal ganglia. Application of ca. 5% collagenase solution (Sigma, Type 1A) in Ringer solution for 10 min facilitated later penetration of the connective sheaths. The Ringer solution had the following composition (in mMol/l): NaCl 140, KCl 10, $CaCl_2$ 7, $NaHCO_3$ 4, $MgCl_2$ 1, TES 5, trehalose 5 in distilled water. The preparation was positioned in the opening of the sound tube as shown in Fig. 1. During stimulation with 30-Hz sound pulses (I2, 90°) both connectives between the 2nd and 3rd free abdominal ganglia (Fig. 1) were explored with glass microelectrodes in search of axons responsive to the stimulus. All the data presented in this paper are from neurons on the left side of the body, with the loudspeaker at positions from 45° to 180°. Because the apparatus moves the air symmetrically back and forth over the animal, these positions also simulate positions in the range 225–0°. Therefore the directional diagrams (Figs. 4 and 11) are extended to the directions 225–0° by mirror-imaging of the points in the 45–180° range about the appropriate axis. That this procedure is justified was confirmed by the recordings obtained from the right side of the animal (with loudspeaker positions from 45° to 180°).

The electrodes were made of either thick-walled or thin-walled glass capillaries (Frederick Haer & Co.). Their resistances were in the range 30–150 MΩ. The tips of the electrodes were filled with a 5% solution of Lucifer Yellow CH (Sigma) for cell marking. A chlorided silver wire extending into the electrode tip was connected to a conventional amplifier apparatus; a second chlorided silver wire in the thorax served as reference electrode. The activity of the neurons was recorded on magnetic tape (Racal 4D recorder) together with the voltage applied to the loudspeaker and control pulses, and later evaluated by computer (PDP 11/40). The stimulus traces shown in the figures are the recorded loudspeaker voltages. The experiments were done at 20° to 23 °C.

After the stimulus program had been completed, Lucifer Yellow was injected into the cell by negative current (10 nA, ca. 10 min). Only if the electrode tip was intracellular during the injection and it was clear that the marked cell was the same cell that had produced the recorded responses were the data accepted (although one could often, during the search for a specific IN, identify physiologically nearly the complete set of IN's). Spreading of Lucifer Yellow was improved by keeping the preparation under Ringer solution in a humid chamber for 12–16 h at 8° C. The nervous system was subsequently dissected out, fixed (4% buffered formaldehyde, 2 h) and treated by the method of Steward (1978). The cells were photographed in wholemount in the fluorescence microscope at several horizontal levels and reconstructions were drawn from the photographs. In general the interneurons were stained from the terminal ganglion into the thorax, and in many cases the axons could be followed up into the cervical connectives. Some of the preparations were embedded and sectioned sagitally in 20-µm steps for better morphological resolution.

Results

Altogether 13 ascending interneurons (IN's) were identified on each side of the body, all of which responded to stimulation of the cerci with sound at 30 Hz. All of the IN's described in terms of morphology by Jacobs (1983) were found except for 7–1a. One IN has not been previously described, and was given the provisory name NN1. With the exception of NN1 ($n=2$) as well as 10–1c and 9–3a ($n=3$), each IN was identified at least 5 times.

All IN's continue to represent the stimulus repetition pattern (2 stimuli/s, as described in Methods) even after prolonged stimulation (20 min), although some show slight habituation in the first few seconds. Recordings were obtained from IN's 9–1a, 9–2a, and 8–1a for over 2 hours, and even in such periods there was no detectable change in the response characteristics. In no case did variation of the stimulus repetition rate within the range of the natural chirp rate (1.5–3/s) have a significant effect on the response.

The pattern of action potentials in the IN's discharge reflects the physical parameters of the sound – onset and duration, frequency, intensity, direction and phase of the air-particle oscillations. Despite the variability in the properties of homonymous IN's in different animals, there are criteria that make it possible to identify them according to the type of response. The terms 'phasic' and 'tonic' are not used in this classification, because a low-frequency sound pulse in general does not constitute a maintained stimulus; rather, the 'stimulus' is repeated at the frequency of the sound, with each oscillation of the air particles. Therefore we speak here of habituation during single sound pulses and over several consecutive pulses.

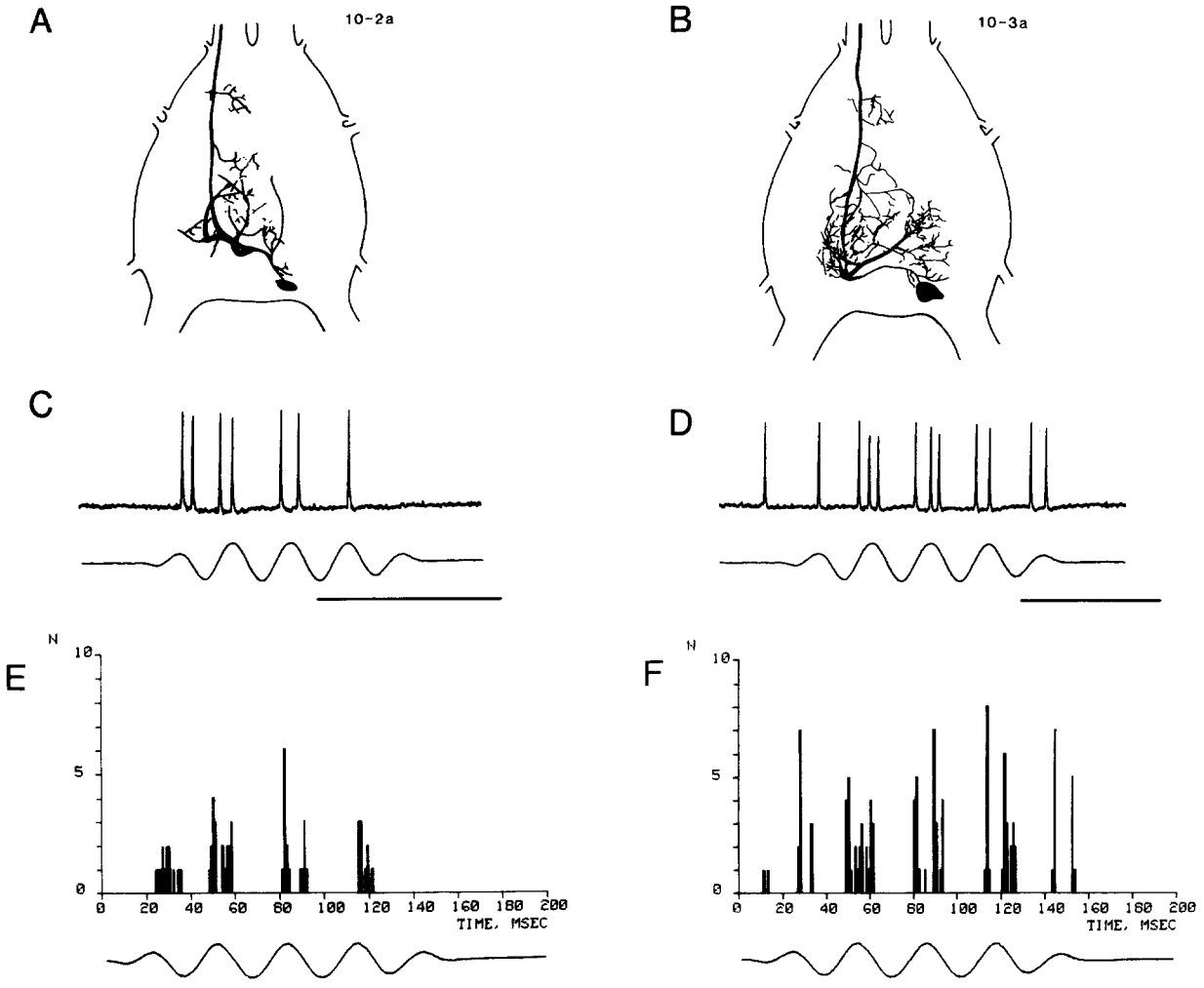

Fig. 2 A–F. Response characteristics of interneurons 10–2a (left) and 10–3a (right). **A, B** Morphology in the terminal ganglion. **C, D** Recordings of neuronal activity (upper traces) during a 30-Hz sound pulse (lower traces, intensity 2); scale, 100 ms. **E, F** Peristimulus histograms for 10 consecutive stimuli; N, number of spikes; stimulus traces below graphs (30 Hz, I2). Stimulus direction 90° in each case

Stimulus-cycle- and direction-resolving IN's

Interneurons 10–2a and 10–3a

The IN's 10–2a and 10–3a (Fig. 2 A and B) closely resemble one another in their responses to low-frequency sound stimuli. During stimulation at 90° both of them discharge from one to three spikes during the phase of the stimulus in which the air particles are moved to the animal (upper half of the stimulus trace, Fig. 2 C, D). The response does not habituate within the pulse, nor is there discernible habituation to repeated pulses. The action potentials are very precisely timed with respect to the stimulus period (Fig. 2 E, F). Both cells exhibit some activity (up to ca. 10 spikes/s) in the absence of stimulation in most, but not all animals. The intensity thresholds are below the lowest tested air-particle velocity of 0.2 cm/s. Increases in stimulus intensity produce only a relatively slight increase in number of spikes per stimulus.

Differences between the two neurons become apparent when the direction of the stimulus is varied. The nature of the cercal receptor system, with two groups each of T- and L-hairs on each cercus, makes it plausible that the directional information derives from the directional specificity of these hair populations. Given appropriate connections with the IN's, the directional information could be contained in the response phases of the IN's relative to one another. 10–2a and 10–3a are characterized by very constant phase relations – even in different individuals – between the discharge and the stimulus waveform. Therefore they are particularly well suited to convey directional information. Figure 3 summarizes, for all the recordings evaluated from these cells, the range of variation of the re-

Fig. 3. Phases of responses of three interneurons (bars) with respect to one stimulus cycle (30 Hz, I2) for 4 stimulus directions; bars give the complete range of spike timings observed in all recordings from these neurons. 8–1a has no clear phase relation to the stimulus at 90°. 10–2a and 10–3a respond nearly synchronously at 90° and in alternation at 180°. 10–2a has two activity phases at 45°, and 10–3a has two at 135°. The direction of the stimulus can be found by comparing the responses of 10–2a and 10–3a

sponses relative to a stimulus cycle at 30 Hz; similar data from another distinct type of IN (8–1a) are shown for comparison. Within an individual animal the scatter is still less (cf. Fig. 2E, F); here the precision of the time of occurrence of the spikes during repeated stimulation is ± 1 ms. With air movement transverse to the long axis of the animal (loudspeaker position 90°), the two IN's discharge approximately in synchrony (Fig. 3). After rotation of the loudspeaker to 180°, so that the air-particle motion is about parallel to the animal's long axis, 10–2a continues to respond in the same phase whereas 10–3a now responds in antiphase. The phase shift of 10–3a takes place within a relatively small sector between 120° and 150°. Within this sector spikes appear in both halves of the cycle (Fig. 3, 135°). The same effect is observed in 10–2a at loudspeaker positions near 45°.

Medial and lateral giant neurons

The IN's 8–1a (Medial Giant Interneuron, MGI) and 9–1a (Lateral Giant Interneuron, LGI), described in Fig. 4, can be said to convey frequency and directional information only with reservations. The response of 8–1a can vary greatly from one animal to another (Fig. 4C). Some examples copy the stimulus cycles very well and, others, very poorly. The response habituates during the stimulus. In neurons that copy well (Fig. 4C, upper trace), once the habituation phase is over the stimulus waveform is represented by phase-locking of the action potentials to it. With the loudspeaker position in the range ca. 90–135°, depending on the animal, there is a sector of ca. 10–15°, in which 1 spike is discharged per stimulus-cycle half – that is, the discharge has double the stimulus frequency (Fig. 4E). Like most other IN's, 8–1a is particularly sensitive to stimuli in particular directions. The resulting directional diagrams are reproducible in an individual animal, but can vary distinctly among individuals (Fig. 4G).

IN 9–1a habituates so strongly that stimuli in most directions elicit only one spike or a pair of spikes at the stimulus onset (Fig. 4D). However with air-particle oscillation in the direction of greatest sensitivity (about parallel to the long axis of the body, loudspeaker position 180°), after habituation the spikes copy the air-movement cycles relatively well (Fig. 4F). Beginning ca. 60–90 ms after stimulus onset the spikes are coupled 1:1 with the stimulus cycle. Considering a delay of some milliseconds by conduction- and other processes, the IN could respond to the air-particle acceleration. The directional diagram of 9–1a (Fig. 4H) reveals the sharpest directional characteristic of all IN's tested.

Increasing the stimulus intensity causes 8–1a to discharge more spikes per stimulus, whereas 9–1a begins to saturate at I2. Thresholds of both IN's are between I1 and I2.

Interneurons 9–2a and 9–3a

In most animals, 9–2a responds to 30-Hz stimuli relatively irregularly and habituates only little (Fig. 5C, D). At I2 the first spikes often do not appear until the second or third cycle of the stimulus. The phase of the spikes with respect to the stimulus waveform is constant in a given recording but varies among individual animals. The stimulus cycles are copied only at high intensities (I2 or greater, Fig. 5D). The directional diagrams for 9–2a were as variable as those (above) for 8–1a.

9–3a (Fig. 5) is even less sensitive for 30-Hz pulses than 9–2a. Only at I3 do 30-Hz signals regularly elicit single spikes (Fig. 5E). The responses of this cell are so weak, that no directional characteristic could be measured.

Band-pass and low-pass IN's

Owing to the mechanical properties of the receptor hairs (cf. Gnatzy and Tautz 1980), the cercal auditory system responds only at frequencies below ca. 1000 Hz; as a whole, then, it operates as a low-pass filter. Within this range the various IN's are matched to different frequency bands.

Low-pass characteristics are found in 9–1b, 10–1c (Fig. 6) and 11–1c (Fig. 8 and Discussion

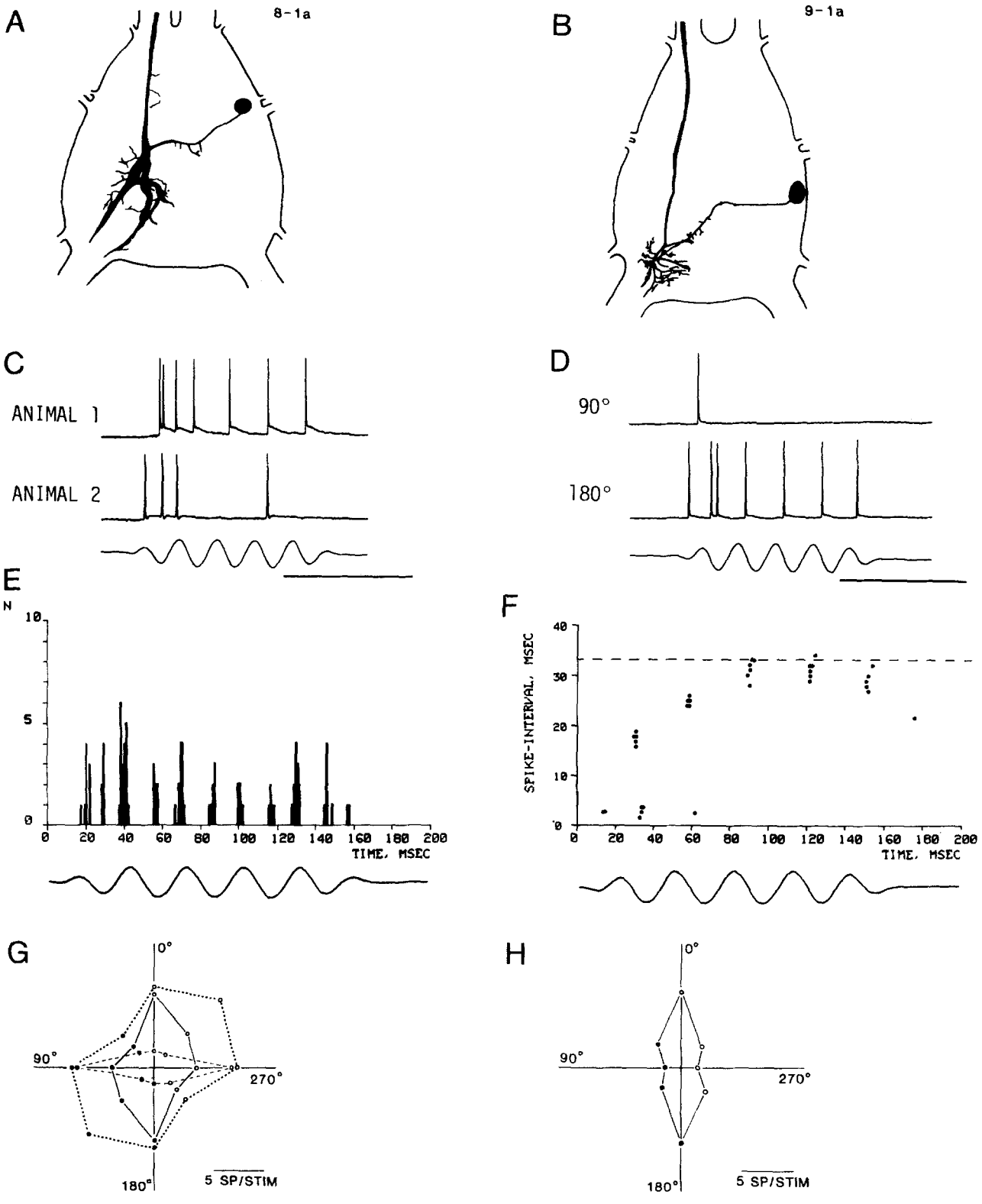

Fig. 4 A–H. Response characteristics of the medial and lateral giant interneurons 8–1a (MGI, left) and 9–1a (LGI, right). **A, B** Morphology in the terminal ganglion. **C** Recordings during a 30-Hz sound pulse (bottom trace) of the response of an IN 8–1a that resolves the frequency well (top trace) and another with poor resolution (middle trace); stimulus direction 90°. **D** Direction-dependent frequency resolution of a 30-Hz pulse (bottom trace) by a single 9–1a cell; response with sound at 90° (top trace) and at 180° (middle trace). Scale in **C** and **D**, 100 ms. **E** Peristimulus histogram for 8–1a at 135° (10 consecutive stimuli, 30 Hz; *N* number of spikes). With this stimulus direction there are two responses per stimulus cycle. **F** Progressive change in spike intervals of 9–1a during 30-Hz stimulation (10 consecutive stimuli, 180°); each dot represents the interval with respect to the preceding spike; dashed line, period of the stimulus. The stimulus waveforms are shown below graphs **E** and **F. G, H** Polar diagrams showing directional sensitivity; filled cycles, measured data; open cycles, inferred symmetrical responses (see Methods); for 8–1a (**G**) data from 3 animals are superimposed. Stimulus intensity 2 in **C–H**

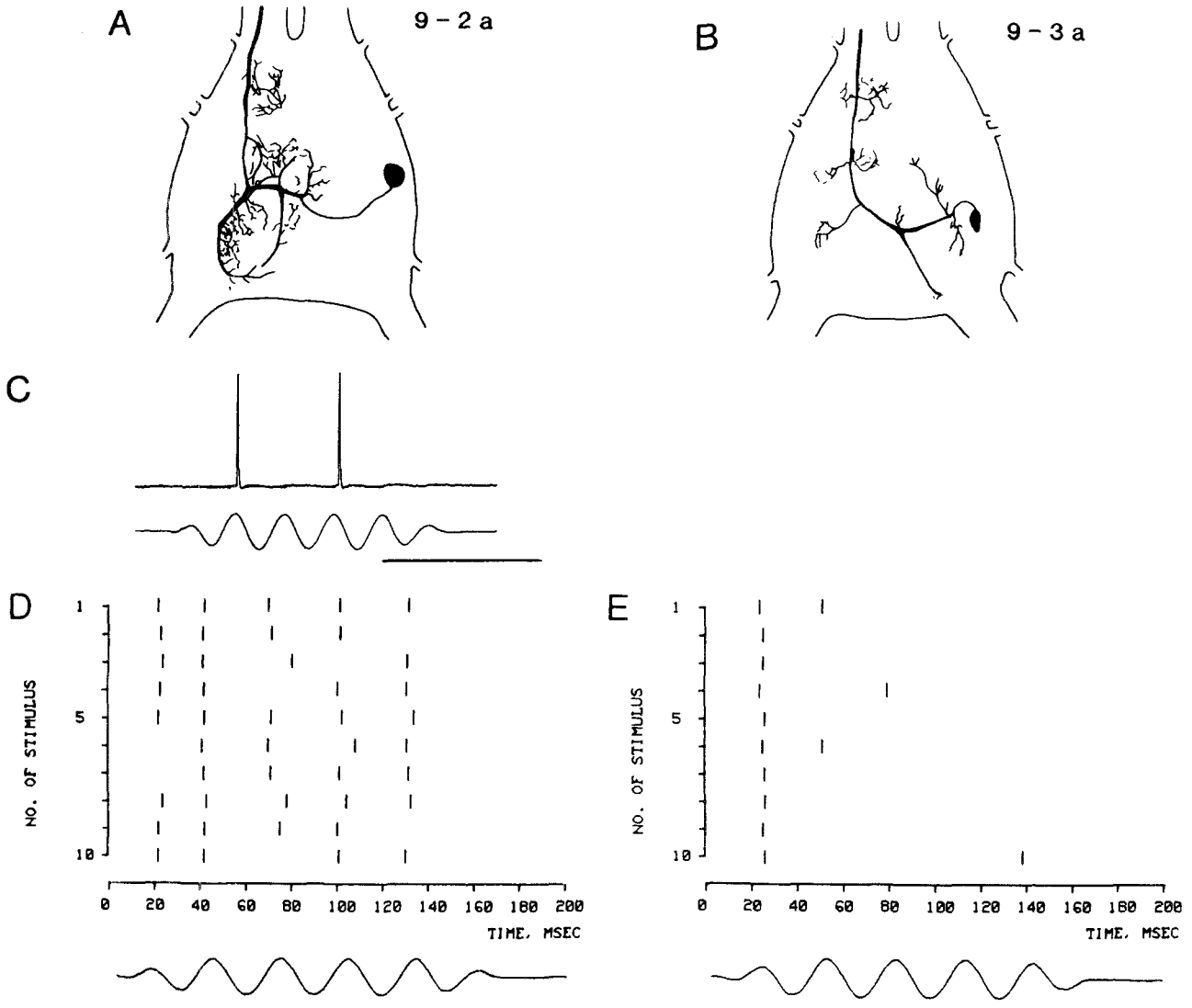

Fig. 5 A–E. Response characteristics of the interneurons 9–2a (left) and 9–3a (right). **A, B** Morphology in the terminal ganglion. **C** Typical recording from 9–2a (upper trace) during stimulation with 30-Hz pulses (lower trace; 90°; 12); scale, 100 ms. **D, E** Distribution of spikes during 10 successive 30-Hz stimuli at intensity 3 (direction 90°, stimulus waveform below graphs)

below). 9–1b habituates strongly during the 30-Hz pulses, and 10-1c, only slightly (Fig. 6C, D). In this frequency range both neurons have thresholds below 0.2 cm/s, and both exhibit a distinct aftereffect of each stimulus pulse (pause followed by activity during the interval between consecutive pulses, Fig. 6C, D); in the absence of sound stimuli both cells discharge irregularly at rates up to 20 spikes/s. The spikes are phase-locked to the 30-Hz waveform (for example, see 10–1c, Fig. 11). Regardless of the direction of the sound, the spike rate is twice the stimulus frequency (60 Hz for 30-Hz pulses), with spike occurrence at two preferred phases of the cycle rather than one. Both IN's also respond to 110- and 500-Hz stimuli. In this frequency range, 10–1c discharges fewer spikes per stimulus as the stimulus intensity is increased (Fig. 6E); at 30 Hz the reverse is true, and there is a transitional region (50 Hz) in which increasing the intensity does not significantly change the response. 9–1b responds less strongly at the highest intensity regardless of frequency (Fig. 6F).

IN 8–1b (Fig. 7) is particularly sensitive to stimuli at 50 Hz and 110 Hz (band-pass characteristic). During a 30-Hz pulse the response habituates rapidly and does not copy the stimulus cycles (Fig. 7B).

Disturbance-detecting IN's

Here a 'disturbance' is defined as any change in the physical nature of the stimulus during the periodic presentation of identical sound pulses – for example, a sudden change in the direction or frequency

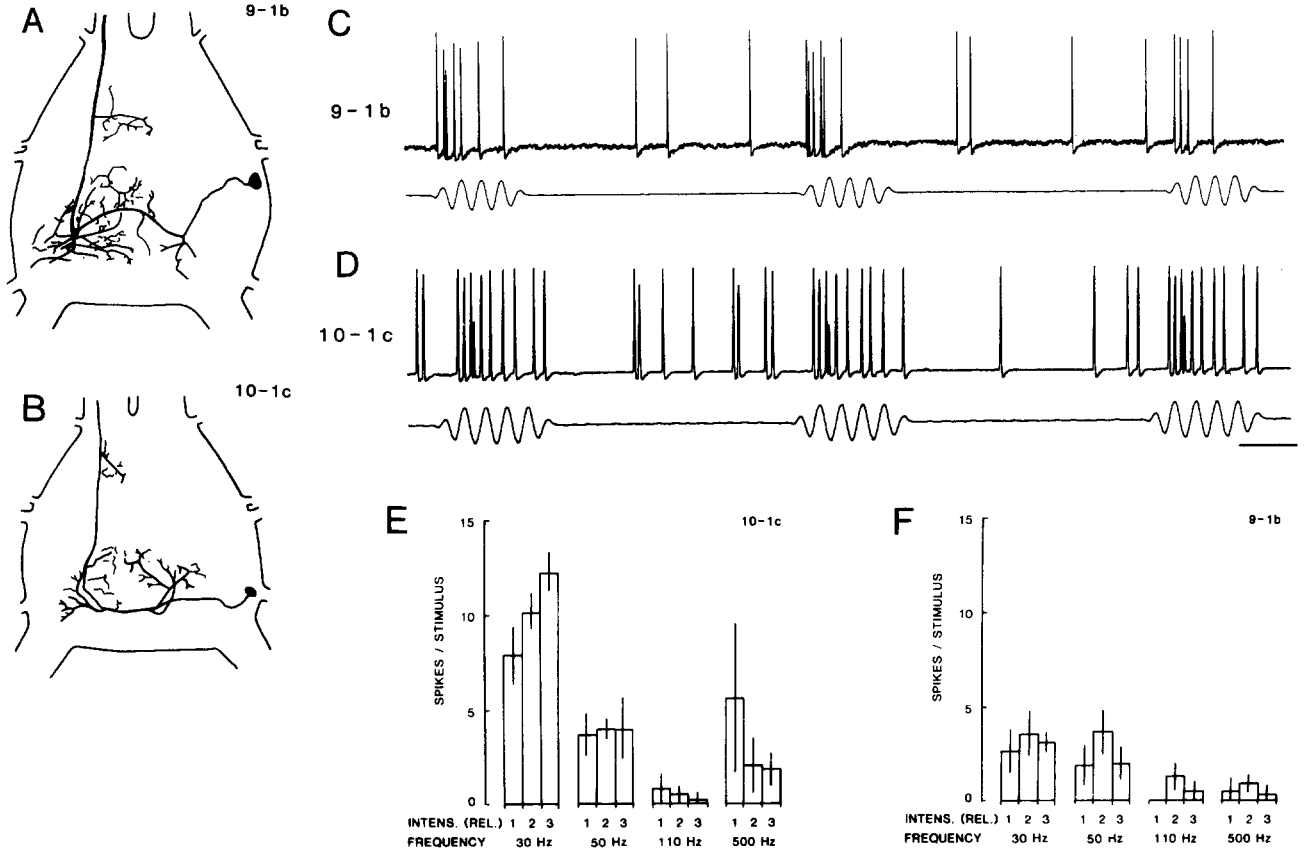

Fig. 6 A–F. Response characteristics of interneurons 9–1b and 10–1c. **A, B** Morphology in the terminal ganglion. **C, D** Recorded activity of 9–1b (**C**) and 10–1c (**D**) during repeated stimulation with 30-Hz sound pulses (lower traces, 90°). Scale, 100 ms. **E, F** Magnitude of response to signals at 4 frequencies and different relative intensities; mean and standard deviation ($n=10$)

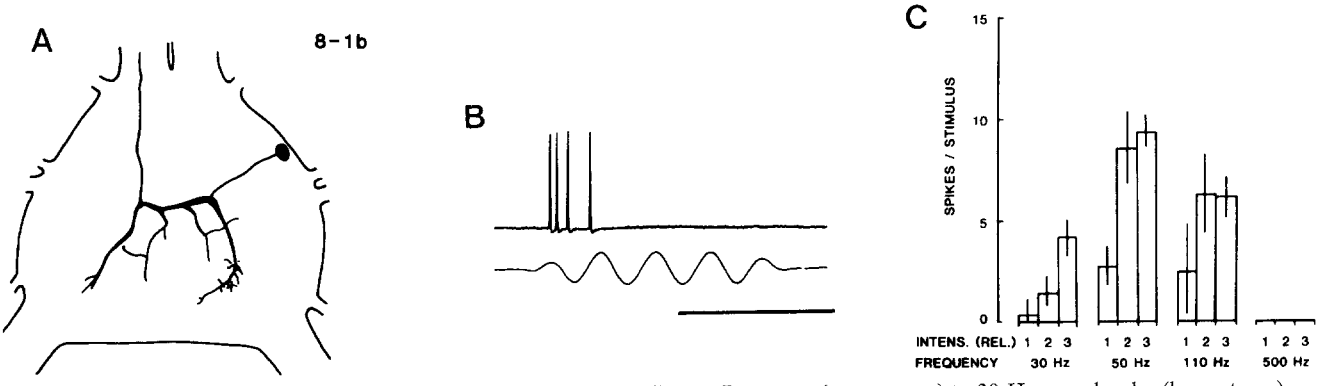

Fig. 7 A–C. Interneuron 8–1b. **A** Morphology in the terminal ganglion. **B** Response (upper trace) to 30-Hz sound pulse (lower trace) at intensity 2. Scale, 100 ms. **C** Magnitude of response to signals at 4 frequencies and different relative intensities; mean and standard deviation ($n=10$). The neuron does not respond to 500 Hz. Stimulus direction 90°

of the stimulus or an air puff presented in addition to an ongoing stimulus. Two cells of the tested IN's showed a specific response upon such stimulus changes.

IN 11–1c (Fig. 8) responds to continually repeated 30-Hz sound pulses with irregular activity, 2–10 spikes/stimulus, that habituates slightly during each stimulus (Fig. 8 B, C). A stepwise increase in intensity (e.g., from I1 to I2) causes an increase in the discharge rate that habituates during individual pulses but not over consecutive pulses (Fig. 8 C). But when a disturbing stimulus is presented (air puff or change in stimulus direction) the number of spikes per 30-Hz pulse rises sharply both during the following pulses and during the intervals between the pulses. Figure 8 D shows the responses of 11–1c during an experiment that lasted ca. 1.5 min. During this time disturbances were

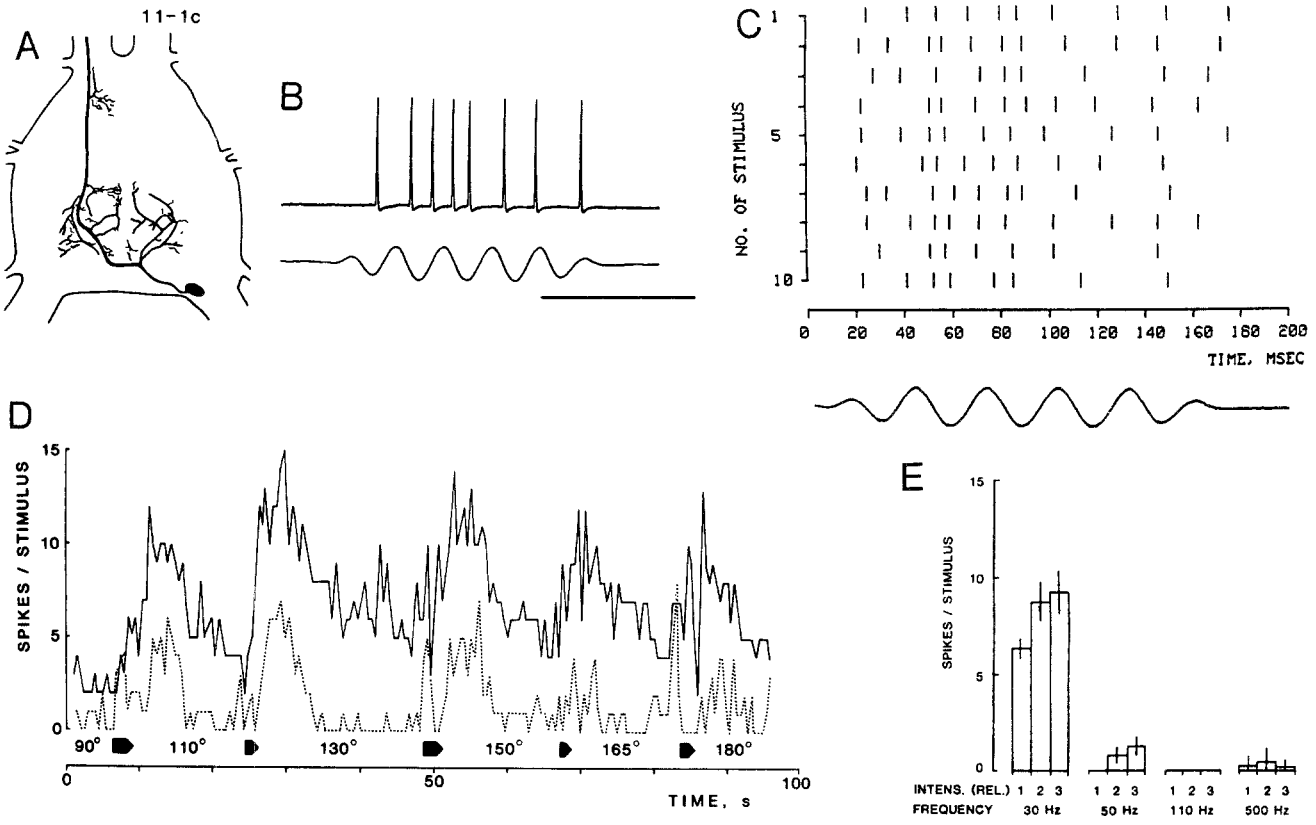

Fig. 8 A–E. Interneuron 11–1c. **A** Morphology in terminal ganglion. **B** Response (upper trace) to a 30-Hz sound pulse (lower trace; 90°, I2); scale, 100 ms. **C** Distribution of spikes during 10 consecutive stimuli (after stimulation with 30 Hz, I1; parameters as in **B**); stimulus waveform below graph. **D** Spike frequency during an experiment in which stimulus direction was changed 5 times; between black arrowheads direction is constant, and length of arrowheads indicates time required to move the loudspeaker; continuous line: spikes during stimuli; dotted line: spikes in intervals between stimuli. **E** Magnitude of response to signals at 4 frequencies and different relative intensities, 90°; mean and standard deviation ($n = 10$); the response has a marked low-pass characteristic

produced by turning the loudspeaker in 5 steps from 90° to 180°. The normal response of this cell consists of 2–5 spikes during each stimulus and 0–3 spikes in the intervals. During the turning the neuronal activity increased by both these measures, to 3–10 and 0–5 spikes/stimulus. When the loudspeaker was again stationary activity at first continued to increase to 9–15 and 3–8 spikes/stimulus, and then the response habituated over the next ca. 10 s. The magnitude and direction of the change in angle has no effect on the magnitude of the increase in activity. The cell responds predominantly to low-frequency sound (Fig. 8 E).

The interneuron NN1 (Fig. 9), studied in two animals, is sensitive to the frequency step from 500 Hz to 30 Hz, which occurs in the normal stimulus program. When previously stimulated with 500 Hz, the neuron gives considerably stronger responses to 30-Hz pulses (I2) than when previously stimulated with subthreshold 30-Hz pulses (I1; Fig. 9 B, C) – even when the 500-Hz pulses, as shown in this example, themselves are subthreshold. The increased response habituates over ca. 2–4 pulses, returning to the normal level (Fig. 9 D) for this stimulus. The effect of other frequency steps on this cell was not tested. When frequency and intensity are increased slowly, NN1 responds with only 0–2 spikes/stimulus over the entire frequency range tested. NN1 is the only IN stained that sends out laterally directed branches in the anterior part of the terminal ganglion (Fig. 9 A), as well as in the other ganglia. Because the axon of this cell runs very close to the midline, these branches cover the same area as do the medially directed branches of the other IN's.

IN's with weak sound-evoked responses: 7–2a and 8–2a

7–2a and 8–2a (Fig. 10 A, B) discharge about 10–20 spikes/s in the absence of stimulation, and also during the intervals between pulses. Sound pulses increase the discharge rate near stimulus onset (Fig. 10 C, D). In both cells there is evidence of weak coupling of the response to the stimulus cycle (Fig. 10 E, F) at a threshold between I1 and I2. The

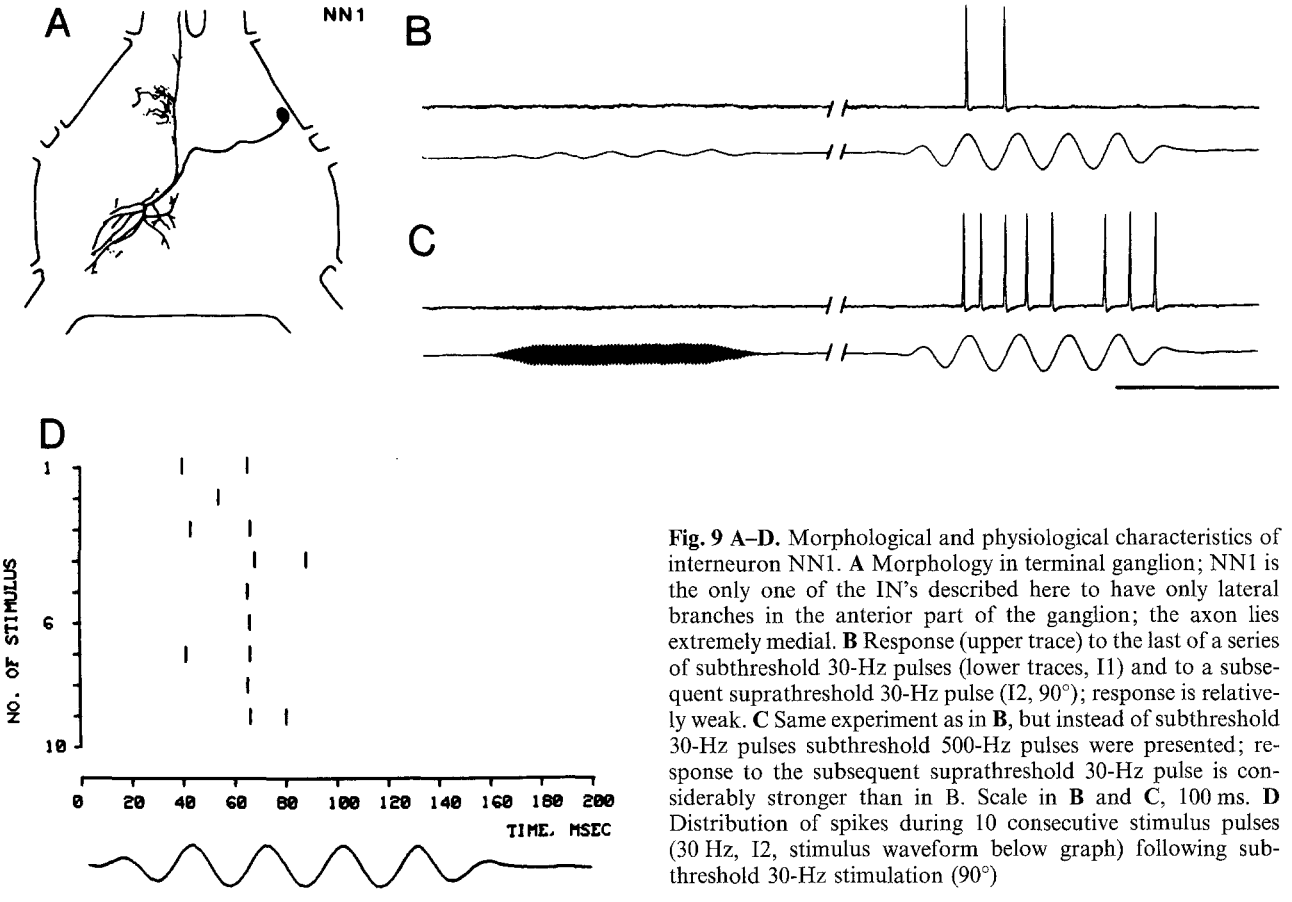

Fig. 9 A–D. Morphological and physiological characteristics of interneuron NN1. **A** Morphology in terminal ganglion; NN1 is the only one of the IN's described here to have only lateral branches in the anterior part of the ganglion; the axon lies extremely medial. **B** Response (upper trace) to the last of a series of subthreshold 30-Hz pulses (lower traces, I1) and to a subsequent suprathreshold 30-Hz pulse (I2, 90°); response is relatively weak. **C** Same experiment as in **B**, but instead of subthreshold 30-Hz pulses subthreshold 500-Hz pulses were presented; response to the subsequent suprathreshold 30-Hz pulse is considerably stronger than in B. Scale in **B** and **C**, 100 ms. **D** Distribution of spikes during 10 consecutive stimulus pulses (30 Hz, I2, stimulus waveform below graph) following subthreshold 30-Hz stimulation (90°)

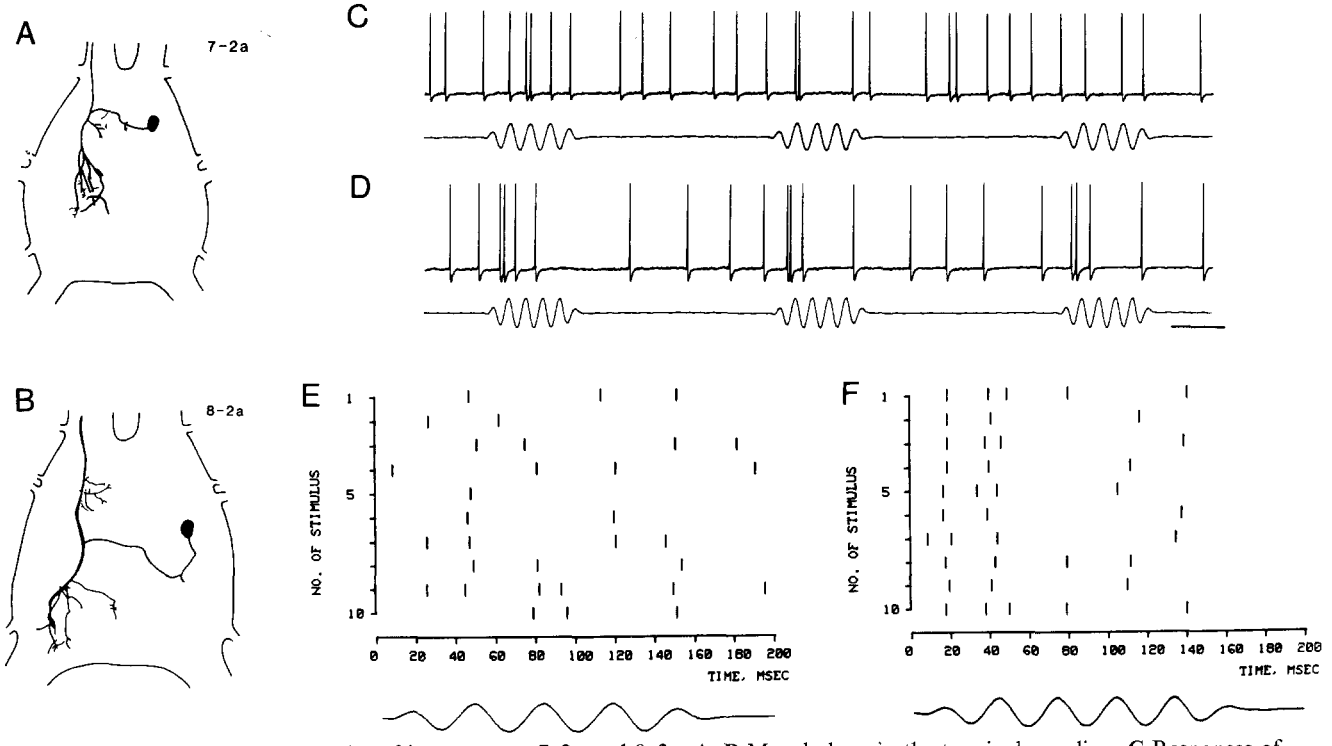

Fig. 10 A–F. Response characteristics of interneurons 7–2a and 8–2a. **A, B** Morphology in the terminal ganglion. **C** Responses of 7–2a, **D** of 8–2a (upper traces) to 30-Hz sound pulses (lower traces; I2, 90°); scale, 100 ms. **E, F** Distribution of spikes of 7–2a (**E**) and 8–2a (**F**) during 10 consecutive stimuli (30 Hz, I2, 90°; stimulus waveform below graphs)

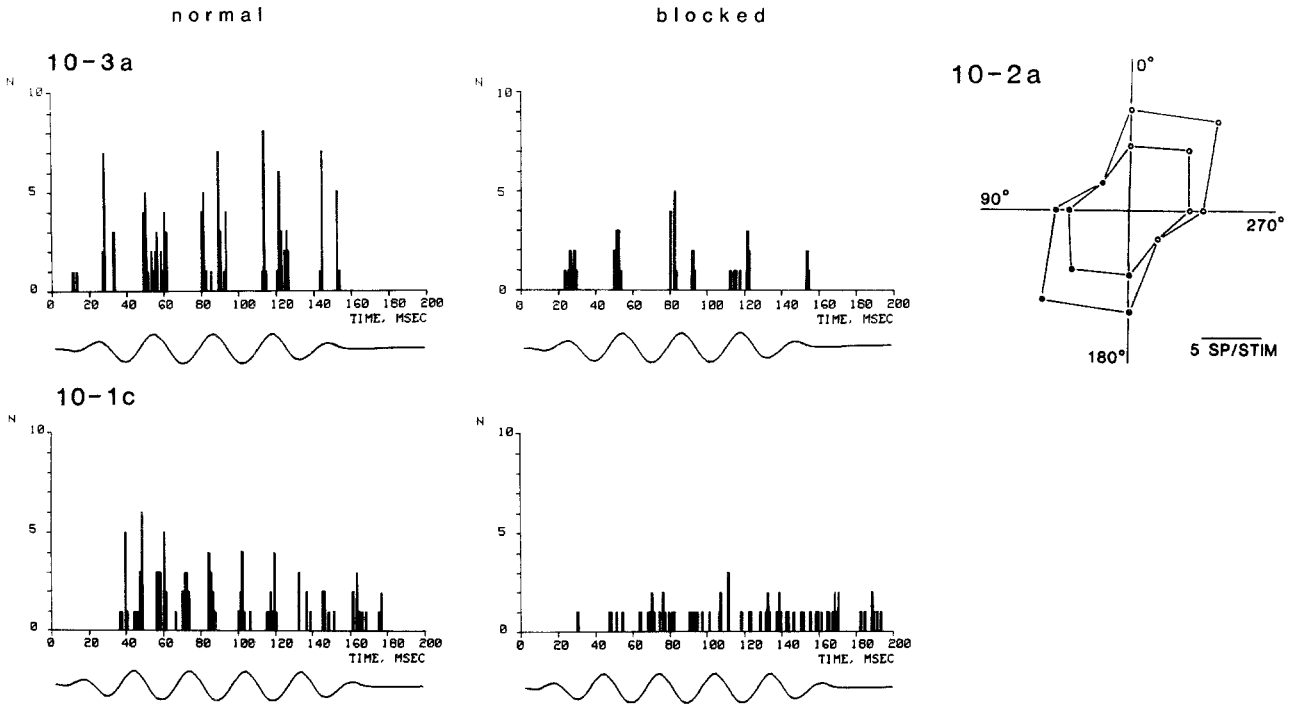

Fig. 11. Effect of blocking the contralateral cercus upon responses of interneurons 10–3a resp. 10–2a (upper row) and 10–1c (lower row). Left: peristimulus histograms with both cerci intact; stimulus waveform below graph; sound direction 90°. 10–1c gives two responses per stimulus cycle. Middle: right cercus blocked. Right: directional diagram of 10–2a with both cerci intact (outer curve) and with right cercus blocked (inner curve; filled circles, measured values; open circles, inferred symmetrical responses, see Methods)

spontaneous activity of both IN's is reduced for as long as ca. 100 ms following the sound pulse, perhaps because of post-stimulus inhibition.

Elimination of contralateral input

In some of the experiments, after the normal stimulus program was completed, the cercus contralateral to the recording site was covered with glue. With the contralateral input thus eliminated, a reduced program consisting of only 30-Hz stimuli (I2), in four directions, was tested once with each of six IN's: 9–1a, 9–2a, 10–1c, 10–2a, 10–3a and 9–1b. The effect on 10–3a and 10–2a (Fig. 11) is typical; the general form of the directional characteristic is retained, although the number of spikes per stimulus is reduced. The phase of the response with respect to the stimulus waveform also remains the same. The effects of blocking were similar in the cases of 9–1a and 9–2a. In 10–1c, although the directional characteristic was retained the phases of the spikes became much more variable after blocking, so that the previous rhythmicity was no longer discernible (Fig. 11). In the case of 9–1b the scatter in response phase, direction dependence and number of spikes per stimulus was so large both before and after blocking that no effect could be detected.

Discussion

All of the IN's described here respond to 30-Hz sound pulses. Apart from the weakly-responding 7–2a and 8–2a, each of them is distinct from all the others with respect to some features of the response at this frequency. The stimulus parameters frequency, time of occurrence, direction and intensity are represented in the discharge of the ascending IN's by variation of spike rate and number of spikes ('temporal coding'; cf. Schwab and Josephson 1977) as well as by the phase relations of the responses of the different IN's with respect to one another and the presence or absence of stimulus-related activity among the various IN's ('spacial coding').

Representation of stimulus frequency

The ganglia anterior to the terminal ganglion receive information about the carrier frequency within a sound pulse in two ways. First, neurons such as 10–2a and 10–3a discharge in the rhythm of the stimulus. Second, low pass neurons (9–1b, 10–1c, 11–1c) respond to low-frequency sound pulses without accurately representing the pulse waveform in their discharge. That is, they detect only the presence of a low-frequency stimulus.

Levine and Murphey (1980a) found that the IN 11–1c (which there is named 10–2) is inhibited at stimulus frequencies of 100–700 Hz, presumably based on the activity of local interneurons that have not yet been identified.

Directional characteristic

The IN responses offer two different ways of obtaining information about the possible position of the sound source.

1. Air-particle oscillations transverse to the long axis of the body are signalled by a synchronous burst pattern of the cells 10–2a and 10–3a (Fig. 3). On the other hand, oscillations parallel to the long axis cause the two to discharge bursts in alternation. Air movements in the two intervening sectors produce characteristic intermediate IN responses. Therefore analysis of the responses of these two cells on only one side of the body suffices for the discrimination of at least four directions of oscillation. The responses of the homonymous cells on the other side of the body generally provide no additional information with regard to localisation (see Fig. 11). In cockroaches, too, at least some weeks after elimination of one cercus, the remaining single cercus alone can correctly trigger the escape response (Vardi and Camhi 1982). It is unknown whether the cricket can resolve the ambiguity concerning the 180°-opposed sound-source positions that provide a given direction of periodic air movement, and also, what response pattern would be found with sound sources positioned above the animal.

2. The directional characteristics of most of the IN's are more or less constant from animal to animal. There is a striking interindividual variation in the directional characteristic of IN 8–1a (Fig. 4G). By contrast, 9–1a has a characteristic that is both extremely sharp and reproducible in all tested animals (Fig. 4H), a property that implies a special function of this neuron in direction-finding.

Changes in stimulus direction

11–1c responds to disturbance of a steady stimulus pattern with a prolonged increase in discharge (Fig. 8). Little is known about the neural mechanism that might underlie such a sustained change in response. Schildberger (1984) found local interneurons in the brain of *Acheta domesticus* that gave prolonged responses when the cerci were stimulated with air puffs. An interaction between the brain and the terminal ganglion in the form of excitatory influences of descending interneurons, given the long conduction times, could perhaps produce such an effect in 11–1c by positive feedback.

Stimulus intensity

With low frequency stimuli, all the IN's increased the number of spikes per stimulus as stimulus intensity was increased. In some cases saturation at I3 was observed (e.g., 9–1a). In general both these data and the thresholds found here agree with those published by Kanou and Shimozawa (1984) to the extend that the experiments overlap. With increasing stimulus intensity more IN's respond, for the IN thresholds vary.

The role of the abdominal IN system in behaviour

In cockroaches, the IN system that receives input from the cercal filiform hairs participates both in behaviour triggering and control mechanisms (Daley and Delcomyn 1980a; Ritzmann et al. 1980; Camhi and Nolen 1981; Ritzmann 1981; Ritzmann and Pollack 1981). The IN system of crickets can be expected to incorporate a similar separation of functions of the various IN's. The rapidly conducting giant IN's 8–1a and 9–1a could cause the cricket to adopt a more alert posture (abdomen flattened, antennal movement) when an external stimulus strikes the cerci. The other IN's apparently provide more detailed information about the stimulus parameters.

9–1a is especially sensitive to air movements approximately parallel to the long axis of the body (Fig. 4H) – that is, when predominantly L-hairs are stimulated. The defensive kicking response is most readily elicited by air puffs in the direction of the long axis of the cerci (Dumpert and Gnatzy 1977), which again stimulate the L-hairs and also campaniform sensilla beneath the sockets of the hairs. However, the threshold for triggering the kicking response is 200–230 cm/s (peak velocity of air puff), far above the threshold for discharge of 8–1a and 9–1a (ca. 0.2–2 cm/s peak-to-peak velocity at 30 Hz), which in turn is high compared with the thresholds of other IN's. 8–1a and 9–1a are suited for the rapid signalling of relatively strong (and hence threatening) stimuli. Their signals contain information about the direction and frequency of the stimulus. They do not trigger a kicking or escape response directly. The alarm reaction (see above) could also be released by the responses of the IN's 11–1c and NN1, which signal directional and frequency changes, respectively, in the stimulus pattern (Figs. 8, 9).

Various features of the abdominal IN's of crickets suggest that they may also function in the animal's communication system. The air oscillations generated by stridulating males have an intensity, at the animal's own cerci, of ca. 1–4 cm/s (peak-to-peak) at a frequency of 25–30 Hz (Kämper 1981). At a distance of only a few centimeters they are so weak that the only interneurons of other crickets (females or non-singing males) that could be expected to detect them are the highly sensitive IN's such as 9–1b, 10–1c, 10–2a, 10–3a and 11–1c. Of these, 9–1b, 10–1c and 11–1c are closely tuned to this frequency range (Figs. 6E, F and 8E). 9–1b signals the onset of the stimulus, 10–1c (with practically no habituation) signals its presence and duration, 10–2a and 10–3a make it possible to determine the air-oscillation direction (Fig. 3) as well as its exact frequency, and 11–1c detects changing in position of the sound source (Fig. 8).

When a stridulating male is stimulating its own filiform hairs, the activity in its IN system is more complex than when it is silently listening (Kämper and Dambach 1981). In addition to the highly sensitive IN's listed above, this stimulus could elicit responses of the IN's with low and intermediate sensitivity – 7–2a, 8–2a, 8–1b and 9–2a and perhaps 9–3a. The giant fibers 8–1a and 9–1a are likely to be subject to descending inhibition, as are the ventral giant fibers of the cockroach during locomotion (Ritzmann 1981). The information transmitted by the other IN's could be used by the animal to stabilize the song rhythm (Dambach et al. 1983). An additional possibility is that it might provide positive feedback into the song generating centers, to keep the song going. The parameters of the song of males with blocked cercal inputs are currently being examined in this regard.

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