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# The function of auditory neurons in cricket phonotaxis

## I. Influence of hyperpolarization of identified neurons on sound localization

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**Summary.** In order to examine the role of particular identified auditory neurons of the cricket, *Gryllus bimaculatus*, in orientation to a sound source, a method has been developed by which intracellular recordings can be made while the animal walks on an air-suspended sphere, which is rotated by the leg movements (Fig. 1). The angular velocities of sphere rotation were found to depend on the direction of incident sound, on its intensity and frequency and on the temporal pattern of the sound stimulus (Figs. 2, 3).

While the cricket was walking, auditory neurons discharged extra action potentials, not correlated with the sound stimulus, and the neuronal response to the sound itself was reduced (Figs. 4, 5).

Suppressing the spike activity by hyperpolarization of a local neuron in the prothoracic ganglion (ON1) reduced in some animals the tendency to turn toward the sound source on the side of the ear that excites the ON1 (Figs. 6–8). Hyperpolarization of a neuron that ascends from the prothoracic ganglion into the brain (AN1), while sound was presented to the ear that excites this neuron, caused all animals to reverse direction; that is, they turned away from the sound source and from the side of the inactivated AN1 (Figs. 9, 10). Hyperpolarization of another ascending neuron (AN2) caused a reduction in turning velocity in half of the animals; but this effect occurred only with high sound pressure levels, and the direction of walking was not reversed (Figs. 11, 12).

From the influences on turning tendency observed in these experiments, it appears that the paired AN1s (and possibly the AN2s at high intensities) may provide inputs to a central comparator that dictates turning tendency in phonotaxis.

### Introduction

Analyses of the neuronal mechanisms underlying behavior are usually based on correlations between certain kinds of behavior and the properties of neurons and their connections. For causal analysis, however, the behavior should be modified by the direct manipulation of the physiological activity of identified neurons. Orthopteran insects have recently been used for such studies; the activity of identified neurons has been recorded during flight, walking and stridulation (Robertson and Pearson 1982; Nolen and Hoy 1984; Godden and Graham 1984; Kien 1983; Hedwig 1986). Here we extend this approach to the auditory system of crickets during phonotactic walking.

Female crickets give positive phonotactic responses to the calling song of conspecific males; that is, they track the song when free and show turning tendency when tethered. This behavior can be measured quantitatively to obtain information about sound localizing ability and its limits, and about the female cricket's criteria for recognition of the conspecific signal (Weber et al. 1981; Thorson et al. 1982; Schmitz et al. 1982; Stout et al. 1983; Huber and Thorson 1985).

Central processing of the conspecific song initially occurs in the prothoracic ganglion; several auditory neurons with cell bodies located here have been identified by physiological and morphological criteria (Casaday and Hoy 1977; Popov et al. 1978; Wohlers and Huber 1978, 1982; Moiseff and Hoy 1983; Stout et al. 1985).

All the auditory neurons that have been identified are present as mirror-image pairs with cell bodies in opposite halves of the ganglion, and each member of the pair is affected differently by the two ears. In the search for mechanisms of sound localization, interactions involving the members of

these pairs – either directly or by way of higher level comparisons – have been among the chief candidates. Because the results of the experiments presented here will be interpreted in terms of such interactions, a brief introduction to our terminology is in order. The omega neuron ON1 has a dendritic field on the same side as its cell body and an axon that crosses to the other side of the ganglion; it is excited by the ear on the same side as its cell body and dendrites and inhibited (indirectly) by the ear on the other side (Wohlers and Huber 1982). The ascending neurons AN1 and AN2, which have their cell bodies on one side of the ganglion and their dendritic fields and axons on the other, are excited by the ear on the same side as the dendrites; the ear on the opposite side has no effect on AN1 and inhibits (or, in some cases, weakly excites) AN2. Obviously, the 'laterality' of these neurons is ambiguous. In the original description (Wohlers and Huber 1982) the 'side' of a neuron was determined by the position of its cell body; the left neuron was the one with its cell body on the left. But because neurons of these three types are excited by the ear on the same side as their dendrites, regardless of the position of the cell body, for the present purposes it is more useful to adopt a different convention.

Here we regard a sound source as ipsilateral to a neuron if the neuron is excited by the ear on that side – that is, if the sound source is ipsilateral to the dendrites of the neuron. The directional characteristic of the ears is reflected in the activity of these central neurons and further enhanced by contralateral inhibition (Wiese and Eilts 1985; Kleindienst et al. 1981; Selverston et al. 1985). The omega neuron ON1 is thought to have the special function of binaural contrast enhancement.

In this paper we present a method that enables intracellular recording from these neurons while the animal is engaged in phonotactic walking (Fig. 1). Using it, we can observe whether the known response characteristics of the neurons are altered during the behavioral responses to sound. When recording from the integrating segment it is also possible to inactivate the cells reversibly by injecting negative current and to check for associated changes in walking. With such measures of the influence of a particular neuron on motor performance, one can determine the manner and degree of involvement of various neurons in the behavior.

## Materials and methods

Females of the gryllid species *G. bimaculatus*, raised in the laboratory, were acoustically isolated in the last instar and for 2–4 weeks after the imaginal moult, until they were taken for the experiment. The cricket is attached to a holder so that the head, thorax and abdomen are immobilized; the legs, which can move freely, are in contact with a hollow styrofoam sphere (diameter 12 cm, weight 5 g; see also Dahmen 1980) supported in an airstream. As the animal carries out walking movements, it turns the sphere in a direction opposite to the intended walking direction (Fig. 1).

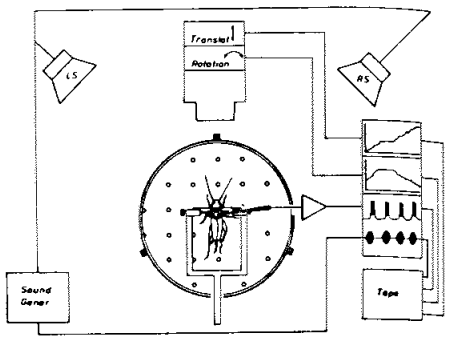


**Fig. 1.** Diagram of the experimental design for intracellular recording from neurons in the prothoracic ganglia during walking. The animal is fixed to a holder but can turn an air-suspended styrofoam sphere by its walking movements. The intended translational (Tra) and rotational (Rot) components are recorded separately. Two speakers are mounted 50° to the left (*LS*) and right (*RS*) of the long axis of the animal (not drawn to scale)

The rotation of the sphere is measured with a camera nearly identical to the one used in the walking compensator (see Kramer 1975; Weber et al. 1981). Reflecting dots (diameter 2 mm) are glued to the sphere at intervals smaller than the diameter of the camera's field of view (30 mm). The camera monitors the motion of the dot nearest to the lower left edge of this measurement area, and extracts the $x$ and $y$ components of the motion. Because the measurement area is situated on the equator of the sphere and in front of the animal, these data specify the translational and rotational components of the walk. When one point drifts out of the measurement field, the monitor switches to another; these discontinuities are eliminated automatically by the data processing system.

The measurement system operates with a temporal resolution of 10 ms, a spatial resolution of 0.3 mm, and a linear range of velocities from 0.3 to about 100 cm/s. To find the total path distance represented by the sphere's rotation, all movements in each 100 ms segment were added up separately for the two axes of rotation, and from these sums the total path length, the velocities and their distributions were calculated; parametric statistical tests ($t$-test, $F$-test) were applied to compare velocity distributions.

Two loudspeakers were situated 30 cm away from the animal, 50° to the right and left of the long axis of the body. The frequency characteristics of the speakers were equalized over the range of 3–20 kHz. The sound field was not homogeneous because of the presence of sound-reflecting objects, but the signals near 5 kHz could be equalized in the vicinity of the ears. Echos in this frequency range were more than 30 dB weaker than the signal. The sound stimulus was an artificial calling song (chirps of 4 syllables 20 ms in duration, separated by 20 ms pauses; chirp repetition interval 500 ms), at a carrier frequency that could be varied between 2 and 20 kHz and an intensity between 45 and 90 dB SPL (RMS).

The dorsal surface of the animal was opened and the sides of the thorax pulled slightly apart. The prothoracic ganglion was exposed by removal of the dorsal musculature and the gut; the lateral and ventral tracheae (including the acoustic trachea) remained intact, as did the nerves from the ganglion and the connectives.

The ganglion was stabilized mechanically by two spoons, one below serving as a platform and the other above with a hole for the electrode insertion. A glass microelectrode filled with 3% Lucifer Yellow was inserted into the ganglion from the dorsal surface and penetrated neurons in the auditory neuropil. Acoustic stimulation revealed the type of the neuron penetrated; e.g. receptor cells and interneurons were distinguished by their latency, threshold curves were used to discriminate between low-frequency (AN1, ON1) and high-frequency neurons (AN2), and the two neuron types AN1 and ON1 clearly copied the temporal pattern of the song more precisely than other neurons. For confirmation of the neuron type all cells were marked intracellularly with Lucifer Yellow; after the experiment the ganglia were processed histologically by the conventional procedures and photographed as a whole mount.

It was possible to record intracellularly for as long as one hour. An experiment was accepted for evaluation only if the recording was stable for at least 15 min, the animal exhibited an unambiguous phonotactic response during recording, and the injection of 1.5 to 3 nA of negative current caused a clear reduction in spike activity. For the latter reason the recording site was always the integrating segment where graded potentials and spikes are observable. The neuronal activity was recorded simultaneously with the sphere movement and evaluated with the aid of a computer.

## Results

### *Behavior*

Upon presentation of the calling song from one speaker the crickets, with the body immobilized, made leg movements that would have tended to turn them toward the side of the active speaker. Correspondingly, the free sphere counterrotated. When the song was switched to the other speaker, they reversed their turning tendency after 1–2 chirps. This reversal can occur with no pause in walking, especially if the sound is loud. In their phonotactic response these animals are as selective for the temporal pattern of the song as freely walking animals (see Thorson et al. 1982). Songs with syllable intervals equal to or less than 20 ms, or equal to or greater than 60 ms, elicited slower walking or no response at all (Fig. 2).

The mounting and dissection procedures can affect an animal's behavior; in some cases phonotaxis was eliminated altogether and in others the turning tendency was extremely asymmetrical. Such experiments have been excluded. In all the experiments described below, during the recording of interneuronal activity the cricket's walking behavior met the criteria for phonotaxis applied here: significant continuous turning tendency toward the active speaker for at least 2 min and change of turning tendency following change of speaker.



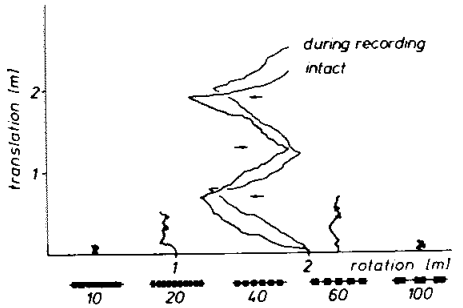


**Fig. 2.** Reconstruction of the path of a cricket walking in the presence of various sounds. The translational (tra) and rotational (rot) components are plotted additively on the ordinate and abscissa, respectively. At each starting point the sound is coming from the left speaker. Every 30 s thereafter it was switched from one speaker to the other (arrows). The temporal pattern of the sound stimulus is shown below the abscissa corresponding to the respective walking trace (syllable repetition intervals are 10, 20, 40, 60 and 100 ms). When the pattern is attractive (40 ms syllable repetition interval), the animal systematically attempts to turn toward the active speaker, changing its turning direction at each speaker switch. The two middle traces, showing the phonotactic behavior before preparation for recording and during recording from a neuron, do not differ significantly. Intensity (80 dB SPL) and timing of speaker switches were identical in all 6 tests

Most of the animals occasionally interrupted their phonotactic walking for a few seconds, although some walked continuously for over 20 min. The duration and frequency of the pauses were like those found for tracking behavior on the walking compensator (Weber et al. 1981; Schmitz et al. 1982). If the pauses lasted longer than 10 s, phonotaxis was considered to have stopped; after such a long pause the turning tendency no longer depended on speaker position and was not altered by a change in direction of the sound source. Crickets oriented less readily, the longer an experiment lasted. Even animals that were initially responsive stopped responding within an hour after preparation for the experiment was completed.

The translational velocities varied (both among animals and in a given animal) between 1 and 10 cm/s. The rotational velocities averaged 27 deg/s at 80 dB, but occasionally velocities as high as 100 deg/s were observed. When sound was presented on one side, the velocity of turning toward

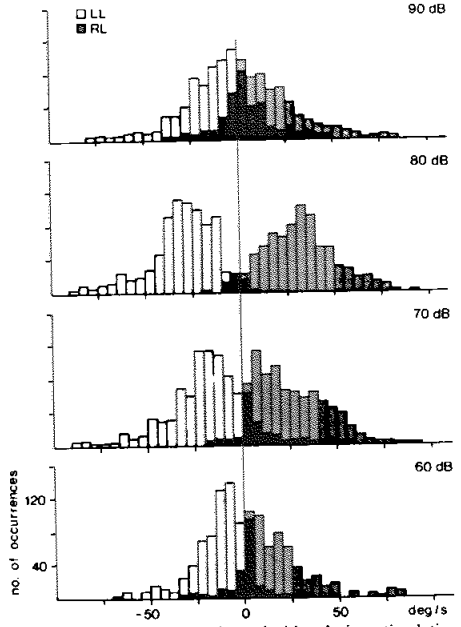


**Fig. 3.** Distribution of angular velocities during stimulation with calling song at different intensities from the right (*RS*) or left (*LS*) speaker. Positive values indicate turning to the right, negative to the left (as in all following figures). Under each stimulus condition 220 chirps were presented (=110 s). The angular velocities were measured while recording from the left ON1. The mean angular velocity increased between 60 and 80 dB and decreased again at 90 dB

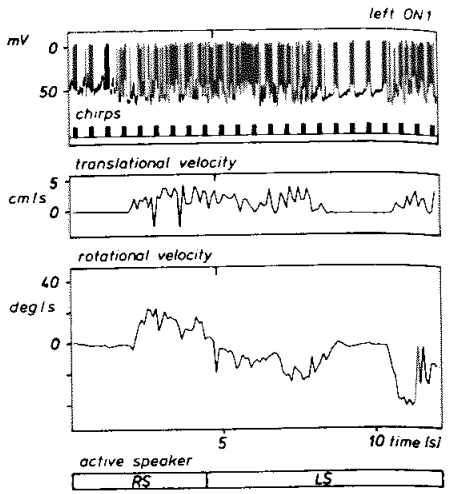


**Fig. 4.** Intracellular recording from the left ON1 during walking in the presence of calling song (70 dB). First trace (from top): intracellular recording of ON1 activity; 2nd trace: marks indicating the sound stimulus; 3rd trace: translational velocity, in which positive values indicate forward walking and negative values backward (as in all the following figures); 4th trace: angular velocity (positive to the right, negative to the left); letters at the bottom indicate the active speaker. Data in Figs. 4–6 come from the same preparation. During walking the auditory response of the neuron is no longer clearly apparent

that side varied greatly even though the sound intensity was constant; furthermore, even during clear phonotaxis, turns away from the speaker occurred (Fig. 3). In general, turning velocity depended on sound intensity. Sound intensities below 55 dB evoked no significant turning tendencies toward the side of the active speaker. In the range of 60–80 dB, the mean turning velocity of most animals rose to 25–35 deg/s. With higher intensities there was no further increase in angular velocity, and those of many animals decreased (Fig. 3).

### *Neuronal responses*

As long as the crickets were not walking, the responses of the auditory neurons in the prothoracic ganglion to conspecific calling song were no different from those recorded in a totally immobilized animal. For instance, the threshold of the omega neuron ON1 to tones at 5 kHz was about 40–45 dB for ipsilateral presentation and 10–15 dB higher for contralateral presentation. Auditory prothoracic neurons have little or no 'spontaneous' activity, and encode the song pattern in the manner characteristic of their type (see below). When the crickets were walking, two additional effects appeared. First, action potentials uncorrelated with the sound stimulus were discharged and, second, the responses to the calling song developed gaps that could become so large that the response disappeared altogether, especially for contralateral stimulation (Figs. 4, 5). However, if the intensity of the song was higher than 75 dB, there was always a clear response even during walking. The origin and possible significance of these effects are considered in a subsequent paper (Schildberger et al. 1988).

### *Inactivation of ON1*

The omega neuron ON1 is located in the prothoracic ganglion. It is tuned to the carrier frequency of the conspecific calling song and copies the temporal pattern of that song (Wohlers and Huber 1982). It receives excitatory input from the ear ipsilateral to the cell body and is inhibited by activity from the contralateral ear. This inhibition is mediated by its mirror-image partner, the ON1 on the opposite side (Selverston et al. 1985).

Because of these properties, the difference in level of excitation at the two ears is increased at the level of the ON1 pair. The question was whether the ON1 activity has a measurable influence on the behavior.

To test this, an electrode was inserted into an ON1 on one side and the animal was induced to walk (Figs. 6–8), tending to turn toward whichever of the speakers was broadcasting the calling song. The ON1 cell was distinctly more strongly excited by ipsilateral than by contralateral sound. During hyperpolarization, the number of action potentials per chirp was reduced, for sound from either side (Fig. 7, left). The average turning velocity in response to ipsilateral sound was also reduced because, although turning toward the speaker side continued, there was also a strong tendency to turn



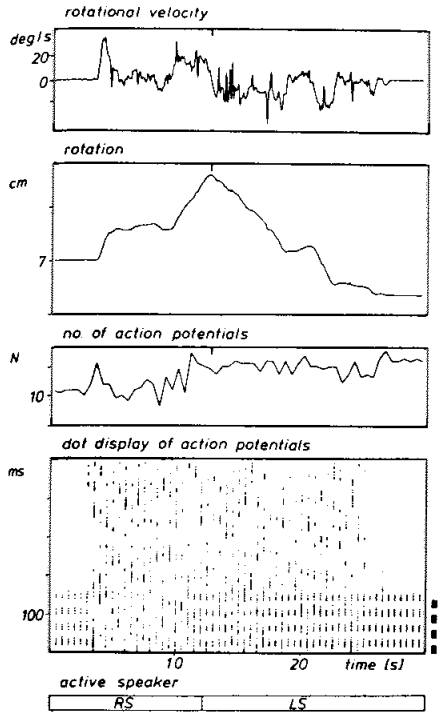


**Fig. 5.** Intracellular recording from the left ON1 during walking in the presence of calling song (80 dB). Top trace: angular velocity; 2nd trace: rotational component (integrated over time), with upward deflection indicating an intended right turn and downward a left turn (as in all following figures); 3rd trace: number of action potentials in the first 200 ms after the beginning of each chirp (as in all following figures); 4th trace: neuronal discharge, in which each dot represents a spike, timing of the spikes within a 500 ms cycle (chirp interval) on the ordinate, the successive chirps on the abscissa; the active speaker is indicated at the bottom. This 30 s sequence is part of the longer sequence shown in Fig. 6 (loudspeaker switch after the 4th min). With contralateral sound representation (*RS*), so that the ear that excites this neuron was less strongly stimulated than the other ear, the neuronal response to the sound during walking was more strongly affected than with ipsilateral presentation

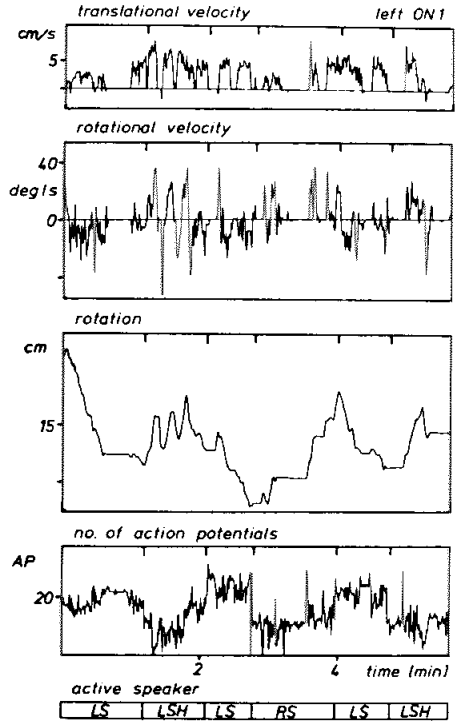


**Fig. 6.** Intracellular recording from the left ON1 during walking in the presence of calling song (80 dB). Top trace: translational velocity; 2nd trace: angular velocity; 3rd trace: rotational component, 4th trace: number of action potentials; the active speaker (*RS* or *LS*) and the injection of hyperpolarizing current (*H*) are indicated at the bottom. During injection of negative current the turning direction changed more often than when the cell was not hyperpolarized

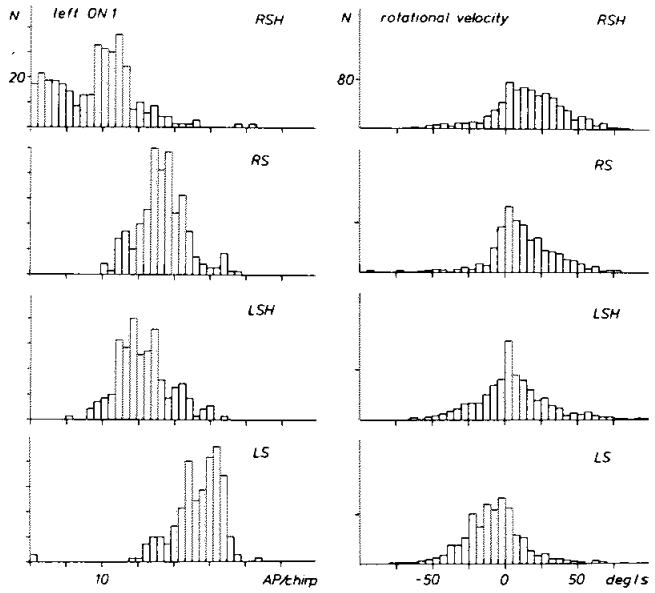


Fig. 7. Left: distribution of the number of action potentials per chirp discharged by the left ON1 in a walking animal; *RSH* right speaker active, neuron hyperpolarized; *RS* right speaker active; *LSH* left speaker active, neuron hyperpolarized, *LS* left speaker active; under each condition 240 chirps (5 kHz, 80 dB) were presented. Right: distributions of angular velocities measured at the same times as the spikes on the left. Hyperpolarization of ON1 with the sound ipsilateral (*LSH*) reduced the mean turning velocity and increased the scatter; hyperpolarization during contralateral sound presentation had no significant effect

away from it (Figs. 6, 7, right). These effects of hyperpolarization were reversible and reproducible. The turning velocity also depended on sound intensity, but the effect of hyperpolarization on turning velocity during ipsilateral sound presentation occurred at all the intensities tested. On the other hand, hyperpolarization of ON1 did not change the turning velocity during contralateral sound presentation, at any intensity (Fig. 8). The example in Fig. 8 demonstrates that these effects were observable even in animals that did not respond symmetrically to sound from the left and right. Without sound presentation, the mean turning tendency of this particular animal was 11 deg/s to the right. Thus, hyperpolarization during ipsilateral sound stimulation reduced the turning tendency to about the value of the no-sound condition. Not all animals exhibited significant effects of hyperpolarizing ON1 (Table 1).

### *Inactivation of AN1*

The cell body of the neuron AN1 is in the prothoracic ganglion; contralateral to it, the axon ascends to the brain (Wohlers and Huber 1982; Schildberger 1984). It has a low threshold to tones at the carrier frequency of the conspecific calling song, 35–40 dB, and copies the pattern of the song in its discharge. Like the other identified auditory interneurons, the AN1 has a mirror-image partner cell. These cells are excited by the ear ipsilateral to the ascending axon (as previously, a sound source is considered to be ipsilateral to an AN1 when it is on the same side as the ear that excites the neuron). The properties of the AN1 suggest that it sends to the brain information required for sound localization and pattern recognition.

Hyperpolarization of the ipsilateral AN1 during presentation of calling song caused the animal to reverse its turning tendency; having previously turned toward the side of the active speaker, during AN1 hyperpolarization the cricket turned toward the other side (compare LS and LSH in Fig. 9). This effect was reversible and reproducible. In the example of Fig. 10, turning velocity increased with sound intensity up to about 75 dB and then remained constant; between 55 and 75 dB hyperpolarization produced a reversal of direction. In this intensity range the turning velocity under conditions of ipsilateral sound presentation plus hyperpolarization was indistinguishable from that during contralateral sound presentation without hyperpolarization. At 80 dB the turning velocities during hyperpolarization were significantly different from those during sound presentation from either direction without current injection.

These effects on locomotion were closely correlated with the effects of hyperpolarization on the neuronal discharge. That is, sound stimuli between



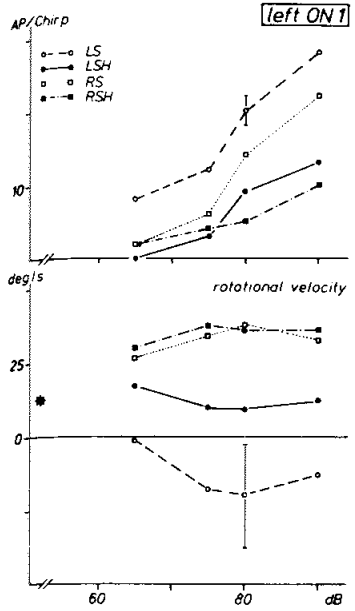


Fig. 8. Top: relation between discharge of the left ON1 and the intensity of the song, the sound direction, and the injection of hyperpolarizing current. Abbreviations as in Fig. 7. Under each condition 120 chirps were presented (= 60 s). Bottom: angular velocities measured at the same times as the spikes; at each intensity, the differences in angular velocity between LS and RS, LS and LSH and RS and LSH are significant (*t*-test, $P<0.001$); asterisk marks angular velocity when no sound is presented; vertical bars give standard deviation of the mean of the respective data points

Table 1. Effects of hyperpolarization of neurons on phonotaxis

| Neuron | ON1 | AN1 | AN2 | Other |
|---|---|---|---|---|
| No. recorded during walking | 25 | 5 | 20 | 8 |
| No. recorded during phonotaxis | 8 | 4 | 8 | 4 |
| No. in which hyperpol. decreases angular velocity | 3 | 1 | 4 | 0 |
| No. in which hyperpol. causes reversal of turning direction | 0 | 4 | 0 | 0 |

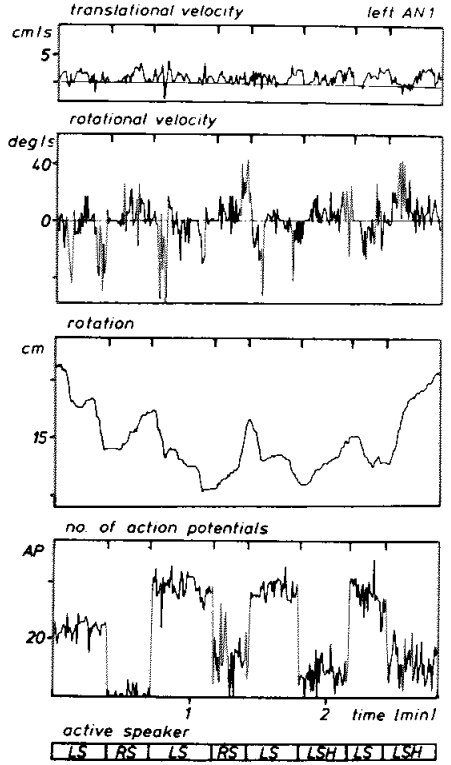


Fig. 9. Intracellular recording from the left AN1 during phonotactic response to calling song. Top trace: translational velocity; 2nd trace: angular velocity; 3rd trace: rotational component; 4th trace: number of action potentials; stimulus conditions indicated at bottom. Sound intensity 65 dB during the first LS/RS sequence, 75 dB during the others

55 and 75 dB elicited significantly less spike activity when presented ipsilaterally during current injection than when presented contralaterally without current injection (compare LSH and RS in the top graph of Fig. 10), but at 80 dB this difference disappeared. On the assumption that the members of the AN1 pair have mirror-image directional characteristics, it would follow that the animal always turns toward the side of the more strongly excited AN1. All the animals tested (see Table 1) reversed direction when one AN1 was hyperpolarized, as long as the neuronal response to ipsilateral sound during hyperpolarization was smaller than the response to contralateral sound. The other case

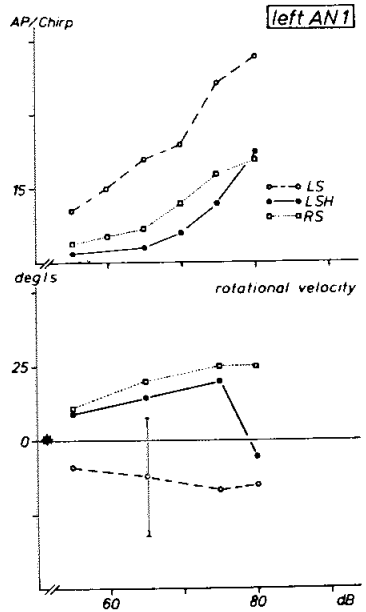


**Fig. 10.** Top: relation between the responses of the left AN1 in a walking animal and the sound intensity, sound direction, and current injection. Hyperpolarization of AN1 during ipsilateral sound presentation caused a reversal of turning direction; under each condition 120 chirps were presented; at each intensity differences between all the data for the different conditions are statistically significant ($t$-test, $P<0.01$) except for LSH and RS at 55 and 80 dB. Bottom: angular velocities measured at the same times as the neuronal responses; values for LS at each intensity are statistically different from those for the other conditions ($t$-test, $P<0.001$); except for the values at 80 dB the data for LSH and RS do not differ significantly; asterisk marks angular velocity when no sound is presented

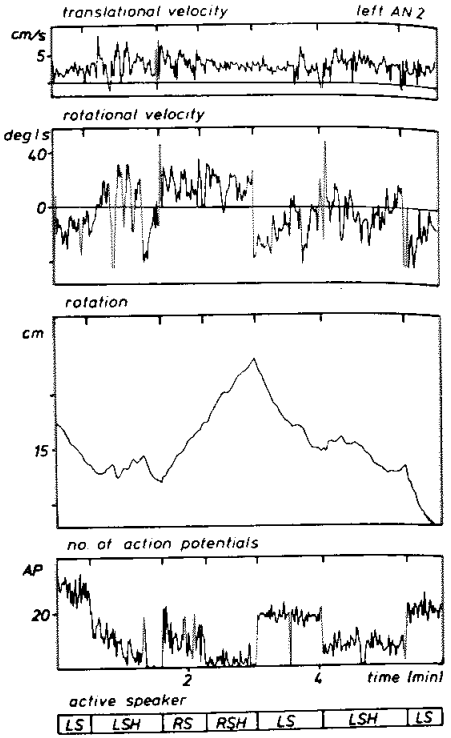


**Fig. 11.** Intracellular recording from the left AN2 during phonotactic responses to calling song. Top trace: translational velocity; 2nd trace: angular velocity; 3rd trace: rotational component; 4th trace: number of action potentials per chirp; stimulus conditions indicated at the bottom. Sound intensity 90 dB for the first LS sequence and 80 dB for the other sequences

noted as a decrease rather than a reversal reflects the response in Fig. 10 at 80 dB.

*Inactivation of AN2*

AN2 is a plurisegmental neuron similar to AN1. It originates in the prothoracic ganglion and terminates in the brain, but its tuning differs from that of AN1. AN2 responds to a broad band of frequencies above 10 kHz, as well as to the calling song at intensities of 50–60 dB or higher. It does not copy the song pattern as accurately as AN1. AN2 is excited by the ear ipsilateral to its axon and inhibited, or in some cases weakly excited, by the other ear (Wohlers and Huber 1982). The inhibition is mediated by the contralateral ON1 (Selverston et al. 1985). Although the main role of AN2 is thought to lie in the processing of high-frequency sound, a function in positive phonotaxis cannot be ruled out a priori.

Hyperpolarization of AN2 during ipsilateral sound presentation reduced the mean turning velocity (Fig. 11); as in the case of ON1 hyperpolarization, there was an increased tendency to turn away from the speaker. In the example of Fig. 12, when AN2 was not hyperpolarized the turning velocity rose with increasing intensity up to 80 dB and then fell off. The effect of hyperpolarization in this high-intensity range depended on the direction of the sound source. With ipsilateral presentation of sound at 80 dB or more, hyperpolarization of AN2 decreased the tendency to turn toward the



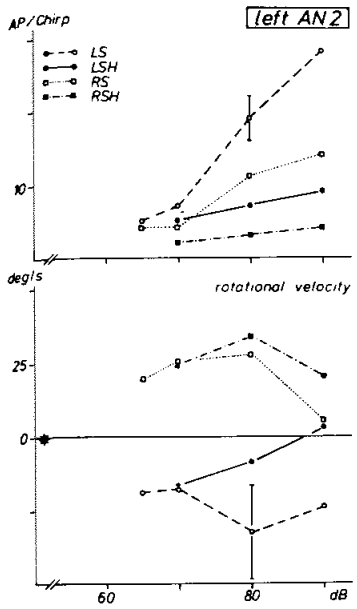


**Fig. 12.** Top: relation between the response of the left AN2 in a walking animal and the sound intensity, sound direction and current injection. Under each condition 120 chirps were presented; there are significant differences between all groups at 80 and 90 dB ($t$-test, $P<0.001$). Bottom: angular velocities measured at the same times as the neuronal activity; with ipsilateral presentation at over 70 dB hyperpolarization reduced turning velocities, and with contralateral presentation it raised turning velocity. Differences between LS and RS are significant at every intensity, as are those between LS and LSH at 80 and 90 dB and between RS and RSH at 90 dB ($t$-test, $P<0.001$)

speaker if the neuronal response was smaller than the response to contralateral sound without hyperpolarization. During contralateral presentation at high intensities, hyperpolarization increased the tendency to turn toward the speaker, by a significant amount at 90 dB. In either case, reduction of the spike rate of AN2 during ipsilateral stimulation below the rate during contralateral one affected turning tendency only at high sound intensities and never caused a complete reversal of direction such as was produced by inactivating AN1. Furthermore, hyperpolarization of AN2 affected the behavior in only 4 of 8 animals tested.

*Inactivation of other auditory neurons*

Auditory neurons other than the above have also been identified in the prothoracic ganglion – a second type of omega neuron (ON2), a descending neuron (DN1) and a neuron with ascending and descending axon (TN1). So far no change in turning tendency has been found to result from hyperpolarization of any of these cells (see Table 1).

## Discussion

In the experimental situation described here, crickets exhibit phonotactic behavior. As a measure of this phonotactic performance, we have recorded the direction and magnitude (in terms of angular velocity) of the turning tendency. The angular velocities we have observed are in the same range as those measured in the same cricket species with other methods (Stabel and Wendler 1986). Nevertheless, certain limitations of this method should be noted. For intracellular recording the cricket's body must be suspended from a rigid support, so that the forces acting on the legs are not necessarily the same as those that act when the animal is supporting its own weight. Correct loading of the joints is very important for properly coordinated walking (see Graham 1985). Therefore the system was carefully adjusted so that the positions of body and legs matched those of the freely walking animal as closely as possible. Under these conditions walking was initiated spontaneously, the normal tripod gait was used, walking was accompanied by the typical movements of antennae and palps, and the duration and frequency of the pauses in walking corresponded to those in free-moving animals.

The weight of the sphere was about 3/2 the average body weight of an adult female, so that the translational inertia corresponded approximately to that for a freely walking cricket (Dahmen 1980; Weber et al. 1981). The rotational moment of inertia, however, is considerably higher than in free walking, which can affect the animal's gait during attempted turns.

Asymmetric turning tendencies were also frequently observed, but were not due to the apparatus because different animals were asymmetric in different directions. Animals that did not clearly turn toward an active speaker were rejected.

Although the data base was limited for the reasons given above, the reproducibility and reversibility of the effects observed justify certain conclusions. Hyperpolarization of a neuron diminishes its response to sound. If there is a comparator that evaluates the difference in excitation of the left and right cells of a pair, when one cell is hyperpolarized the comparator will derive an erroneous estimate of sound-source position that could produce an

altered turning tendency. In the case of AN1 such an alteration was in fact observed; the direction of the turning tendency was reversed as long as the discharge of the hyperpolarized cell in response to ipsilateral sound was less than the response to contralateral sound without hyperpolarization. Given that the members of the AN1 pair have mirror-image directional characteristics, it follows that the animal will turn toward the side of the more strongly excited AN1. It remains unclear whether sound localization is possible when the AN1 neurons on both sides are inoperative. Therefore the demonstration of necessity of the AN1 pair for phonotaxis is not yet complete.

The turning tendency can also be influenced by inactivation of another ascending cell, AN2. But here the influence of hyperpolarization with ipsilateral sound does not suffice for a complete reversal of walking direction, as it does in AN1. In view of the higher threshold of AN2 to 5 kHz stimuli, one would expect an effect of hyperpolarization to become apparent only at higher sound intensities. Interindividual differences in threshold of the AN2 could also explain why hyperpolarization of the AN2 was not effective in all animals. An effect of hyperpolarization on turning velocity was observed chiefly in animals in which AN2 had a low threshold in the 5 kHz region.

In *Teleogryllus* an HF-neuron (INT-1, probably homologous to AN2 in *Gryllus*) has been shown to influence the flight behavior (Nolen and Hoy 1984). Hyperpolarization of INT-1 abolishes activity of contralateral abdominal flexion muscles, an action that is thought to diminish the negative phonotaxis of these animals in response to stimulation with ultrasound. By contrast, hyperpolarization of AN2 in walking crickets diminishes positive phonotaxis. These disparate findings are not necessarily contradictory. The sound frequencies used in the two experiments are different, as was the behavioral context. Furthermore, it has not been definitely established that the two neurons are indeed homologous and have identical functions.

Attempts have also been made to examine the influence of auditory neurons on phonotaxis in *Acheta* (Atkins et al. 1984; Stout et al. 1985). These authors report specific impairments of orientation after selective killing of individual auditory neurons. However, the results of these studies are not comparable with those described here. On one hand, it is unclear whether the physiological consequences of selective cell killing are comparable to those of reversible reduction of activity by hyperpolarization; on the other, the methods by which behavior is measured in the two cases are very different. In our experiments effects on behavior were assayed continuously in an open-loop situation at the same time that the activity of single neurons was monitored. The authors cited above analyzed runs in an arena. The effects on phonotaxis after cell killing that they reported were based on the description of a single run, in one particular stimulus configuration, as compared with another single run of the same animal before the cell had been killed.

The significance of the differences between the pre- and post-killing behavioral observations appears questionable for the following reasons: (i) Single runs in an arena are necessarily quite brief, because of the short distance between the starting point and the speaker (in this case, 56 cm). Given a mean velocity of 3 cm/s (Stout et al. 1976) the runs described in these papers would each have lasted about 20–30 s. (ii) The runs of intact animals, as well as those in some control experiments, are by no means always directed straight toward the speaker (see Atkins et al. 1984, Figs. 3, 4, 6, 7; Stout et al. 1983, Fig. 2). In relatively short single runs this fact can make the interpretation of orientation performance difficult or even questionable. (iii) The paths travelled by the same individual in successive test series, even when the animal is intact, vary greatly although the stimulus parameters are identical (see Stout et al. 1976, Fig. 3).

It is not clear why, in our experiments, hyperpolarization of AN2 or of ON1 in some cases does not produce a measurable change in behavior. One possibility is that individuals differ in the relative influence of these two neurons on the central comparator. Another explanation could lie in the restricted range of variation of the stimulus parameters. The auditory stimulus was always simulated calling song, and the sound was incident from only two directions. Therefore we cannot exclude the possibility that neurons other than AN1 might participate more actively in other stimulus configurations.

Inactivation of an AN1 did not abolish phonotaxis but induced only a directional error. The conspecific song pattern was recognized as well as before. The implication may be that there are other (as yet unidentified) neurons on the same side as the inactivated neuron that transmit the conspecific pattern to the brain. On the other hand, it might be sufficient for the recognition process if the patterned signal reaches the brain on only one side as transection experiments of cervical connectives indicate for crickets and grasshoppers (Weber pers. comm.; Regen 1926; Ronacher 1986). In any case, the neuron pair AN1 sends to the brain significant, if not absolutely necessary information for sound localization and pattern recognition. It is still entirely unclear whether or how these two aspects of the calling song are processed independently of one another in the brain, or how the brain triggers and controls phonotactic walking.

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