

HEARING IN MOLE CRICKETS (ORTHOPTERA: GRYLLOTALPIDAE) AT SONIC AND ULTRASONIC FREQUENCIES

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Summary

We have studied auditory responses in two species of mole cricket (*Scapteriscus borellii* and *S. abbreviatus*) to determine (1) whether they show sensitivity to ultrasound, (2) whether their hearing (at both low and high frequencies) is based on the same neural circuitry as that of true crickets, and (3) whether ultrasound sensitivity in different mole cricket species varies with their ability to fly.

S. borellii are sensitive to ultrasonic frequencies. There is evidence of a segregation of frequency bands in prothoracic auditory neurons. There are two pairs of omega neurons (ONs) with similar morphology to ON1 of true crickets. The two pairs of ONs differ in tuning. One pair has two sensitivity peaks: at the frequency of the calling song of this species (3 kHz), and in the ultrasonic range (25 kHz). The other pair lacks the high-frequency sensitivity and responds exclusively to frequencies in the range of the species song. These two types are not morphologically distinguishable. In *S. abbreviatus*, only one class of ON was found. *S. abbreviatus* ONs are narrowly tuned to the frequency of the species' calls. A T-neuron had

the best ultrasonic frequency sensitivity in *S. borellii*. This cell showed a broad tuning to ultrasonic frequencies and was inhibited by low-frequency stimuli. A morphologically similar neuron was also recorded in *S. abbreviatus*, but lacked the high-frequency sensitivity peak of that in *S. borellii*.

We also assessed the responses of flying *S. borellii* to ultrasound using field playbacks to free-flying animals. The attractiveness of broadcast calling song was diminished by the addition of an ultrasound signal, indicating that *S. borellii* avoid high-frequency sound.

The results indicate that mole crickets process low-frequency auditory stimuli using mechanisms similar to those of true crickets. They show a negative behavioural response to high-frequency stimuli, as do true crickets, but the organization of ultrasound-sensitive auditory circuitry in mole crickets differs from that of true crickets.

Key words: Orthoptera, mole cricket, *Scapteriscus borellii*, *Scapteriscus abbreviatus*, hearing, ultrasound, evolution.

Introduction

Auditory processing in ensiferan insects has been extensively studied from the perspective of both intraspecific acoustic communication (Kalmring *et al.* 1997) and the detection of predators (Hoy, 1992; Libersat and Hoy, 1991). The majority of these studies have concentrated on two common families of Ensifera, the true crickets (Gryllidae) and the katydids (Tettigoniidae). Comparative studies of other families in this suborder of the Orthoptera have been fewer (Ball and Field, 1981; Cokl *et al.* 1995; Field *et al.* 1980; Jeram *et al.* 1995; Mason, 1991; Mason and Schildberger, 1993) and have been confined to the tettigoniid clade (Gwynne, 1995).

This work has established that, despite considerable variation between the Gryllidae and the Tettigoniidae in peripheral auditory anatomy (Bailey, 1993; Ball *et al.* 1989) and signal characteristics (Bennet-Clark, 1989; Kalmring *et al.* 1997; Morris *et al.* 1988, 1994), these distantly related ensiferan families (Gwynne, 1995) show a remarkable similarity in the central neural circuitry devoted to processing

intraspecific acoustic signals (Mason and Schildberger, 1993; Wohlers and Huber, 1982). The true crickets and katydids appear to be less similar, however, in the organization of their ultrasound-sensitive auditory circuitry. Specifically, ultrasound-elicited acoustic startle responses (ASRs), which have been associated with the avoidance of echolocating bat predators in both these families, are mediated by different central auditory neurons in true crickets and katydids (Libersat and Hoy, 1991; Nolen and Hoy, 1984).

The mole crickets (Gryllotalpidae) are the ensiferan family most closely related to the true crickets (Gwynne, 1995) but, although mole cricket acoustic behaviour has been studied (Bennet-Clark, 1987; Daws *et al.* 1996; Forrest, 1980, 1983, 1986, 1991; Forrest and Green, 1991; Ulagaraj and Walker, 1973; Walker and Forrest, 1989), less is known about their hearing. Suga (1968) found that the hearing of several mole cricket species was most sensitive to ultrasound frequencies and speculated that this was related to the high-frequency

content in their songs. Subsequent studies of mole cricket sound production have found their songs to contain exclusively low frequencies (Bennet-Clark, 1989; Forrest, 1983), so that this interpretation is not supported. Many species of mole cricket are frequent nighttime fliers, however, and are likely to be subject to predation by bats. Because mole crickets, unlike true crickets, have no social signals that contain high-frequency components (cf. true cricket courtship calls; Bennet-Clark, 1989; Libersat *et al.* 1994), the most plausible context in which ultrasound hearing could have evolved in this group is for the detection of non-social (predator-related) cues. We have therefore undertaken a study of auditory sensitivity and anatomy in mole crickets with the following aims. (1) To determine the relative sensitivity of mole crickets to high- and low-frequency sound, and to characterize central auditory neurons on the basis of their possible roles in processing intraspecific songs and ultrasound. (2) Another goal was therefore to compare the central auditory anatomy of mole crickets and true crickets, in particular to establish whether high-frequency hearing in the two groups is based on similar neural elements. (3) To determine whether there are behavioural correlates or interspecific differences between flying and flightless mole cricket species that are consistent with the use of high-frequency hearing for the detection of bats. This would be expected if high-frequency hearing in this group evolved solely in the context of bat predation and independently of its origin in true crickets.

Materials and methods

Animals

We used adult *Scapteriscus borellii* (Giglio-Tos) and *S. abbreviatus* (Scudder) of either sex in all experiments. *S. borellii* were collected from the field. As adults, members of this species make dispersal flights each night during their breeding seasons. *S. borellii* were collected by attracting them to traps broadcasting their species' calling song during their nightly dispersal flight (see below). We obtained specimens of *S. abbreviatus*, a flightless species, from a laboratory colony at the University of Florida.

Hearing

Acoustic stimuli

Acoustic stimuli were tone pulses of 20 ms duration with 1 ms rise/fall times. Typically, single pulses were delivered at a rate of 2 s^{-1} , but in some cases we created trains of 10 pulses with varying repetition rates and presented these at a rate of 1 s^{-1} . Stimuli were generated using either of two systems. (1) We used a microcomputer and D/A board (IBM PC-compatible, Data Translation DT2821) to set a voltage-controlled oscillator (Tektronix FG501), the output of which was connected to a custom-built pulse shaper that was modulated by a square-pulse generator (Tucker Davis TG6). (2) Stimulus pulses were synthesized with a DSP board (Tucker Davis APOS II) and output through a D/A interface (Tucker Davis DA3-2). With both systems, the tone pulses

were attenuated (Tucker Davis PA4), amplified (Harman Kardon HK660) and broadcast *via* loudspeakers (Realistic piezo tweeter or 4 inch woofer) placed 50 cm from the position of the preparation. The frequency range of both systems was 1–70 kHz. Stimulus levels at the position of the animal were calibrated with continuous tones, using a microphone (B&K type 4135 or 4138) and sound level meter (B&K type 2209) or measuring amplifier (B&K type 2606). Unless otherwise indicated, sound intensities are given in dB SPL (re $20\text{ }\mu\text{Pa}$).

Neurophysiology

Auditory neurons were located using a search stimulus consisting of two temporally offset tone pulses of different frequencies (3 and 25 kHz). We recorded responses to acoustic stimuli from interneurons in the prothoracic ganglion. After removing the wings, we mounted the animals ventral side up on a metal platform using low-melting-point wax. We removed the ventral cuticle of the prothorax, lifted the ganglion onto a chlorided silver spoon that served as reference electrode, and softened the ganglionic sheath using an enzyme (Sigma pronase) to facilitate electrode penetration. Recording electrodes were electrolyte-filled (0.1 mol l^{-1} LiCl), thin-walled (1.0 mm o.d.) glass micropipettes the tips of which were filled with 2.5 % Lucifer Yellow (Sigma). Electrode resistance varied from 50 to $100\text{ M}\Omega$. Neural responses were amplified (Axoclamp 2A), digitized (Data Translation DT2821 or Tucker Davis AD3 and APOS II) and stored on disk. Following recording, we stained cells with Lucifer Yellow by injection of 0.5–2.5 nA of hyperpolarizing current. Ganglia in which cells had been stained were removed, fixed in 4 % paraformaldehyde for 12–24 h, dehydrated in an alcohol series, and cleared in methyl salicylate. Filled neurons were photographed as whole mounts using a Leitz Dialux 20 or captured as digital images using a BioRad MRC-600 confocal microscope.

Recordings of the summed activity in the neck connectives were made from some animals using silver wire hook electrodes.

Behaviour

We tested the behavioural significance of ultrasound sensitivity in *S. borellii* in a field study. We used two sound traps (Walker, 1982) to test whether ultrasound influenced the phonotactic behaviour of flying *S. borellii*. In this species, individuals of both sexes are attracted to conspecific calling song during evening dispersal flights.

The two traps were placed 10 m apart at the University of Florida's Green Acres Farm (Alachua Co., FL, USA). Each trap consisted of a 1.5 m diameter sheet metal funnel. Centred in the opening of each funnel was a Motorola piezoelectric horn tweeter that broadcast simulated calling songs of male *S. borellii*. Flying crickets attracted to the songs and landing near the speaker fell into the funnel and were collected in a 19 l plastic bucket beneath each funnel (Fig. 1). The Motorola speakers broadcast the same synthetic calling song: a 2.7 kHz carrier having a 50 % duty cycle modulated at 50 Hz with 20 % raised-cosine ramps on the onset and offset of each pulse. However, the two broadcasts of calling

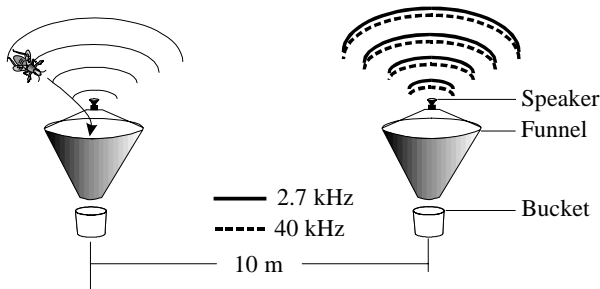


Fig. 1. Schematic diagram of the sound-trapping apparatus used to measure the effect of ultrasound on flying *Scapteriscus borellii* in the field. Both speakers broadcast synthetic calling song (2.7 kHz), one 6 dB louder than the other. The louder speaker also broadcast ultrasound (40 kHz) with a temporal pattern similar to that of the synthetic calling song. Flying *S. borellii* attracted to the calling song from one of the speakers landed in the funnel and were captured in the bucket. With no effect of ultrasound, the louder source of calling song was a more attractive stimulus, such that one would predict that only 17 % of mole crickets collected should be caught in the quieter trap (Forrest, 1980; Forrest and Raspet, 1994). We measured the responses of *S. borellii* to ultrasound by recording the proportion of mole crickets actually captured in the quieter trap as a function of the energy of ultrasound added to the louder trap.

song differed in level by 6 dB (106 and 100 dB SPL at 15 cm, adjusted at each speaker). The proportion of crickets attracted to the low-intensity trap is predicted to be approximately 17 % (Forrest and Raspet, 1994). Empirical results support this prediction (16 %, $N=2979$; Forrest, 1980). Along with the high-intensity calling song, we simultaneously broadcast a 5 ms duration ultrasound stimulus (40 kHz carrier with 1 ms raised cosine ramps) from a Panasonic piezoelectric ultrasound transducer (P-9934). The level of the ultrasound was the independent variable in the experiment, and we measured the attraction of mole crickets to the two calling songs as a function of the energy of ultrasound relative to the calling song. If the phonotactic behaviour of flying mole crickets is negatively influenced by ultrasound (bat predation), we predicted that the relative number of crickets collected at the trap broadcasting low-intensity calling song would increase significantly as we increased the level of the ultrasound in the other trap. All stimuli were computer-generated using custom-built software and played back through 16-bit D/A converters (TDT, Quikki D/A converter) at a sampling frequency of 100 kHz. Anti-aliasing filters with a roll-off of more than 90 dB per octave were used to remove aliased frequencies from the broadcast. The sound pressure levels of the calling song and ultrasound were calibrated prior to each broadcast using a Larsen-Davis model 2520 1/4 inch microphone located 15 cm above each speaker. The total harmonic distortion of the broadcast system was below -40 dB relative to the broadcast signals.

On each of seven nights (28 April to 4 May 1996), broadcasts from the two traps began at about sunset and continued throughout the nightly flying period (Forrest, 1983). Each night, the broadcast of ultrasound and high-intensity calling song was

randomly assigned to one of the traps. To control for the effects of trap positions, the speakers broadcasting the high-intensity calling song and ultrasound were switched to the other trap and the experiment continued until approximately equal numbers of crickets were caught while ultrasound was broadcast from each trapping location. Results were analyzed using logistic regression (SAS Institute, 1985).

Results

Characterized neurons

Fig. 2 shows the anatomy of auditory interneuron types that were recorded and stained in both *S. borellii* and *S. abbreviatus*. By analogy with *Gryllus* spp., we will designate neurons in *Scapteriscus* using 'canonical' names. Omega neurons (ONs; Fig. 2A) and T-neurons (TNs; Fig. 2B) in *S. borellii* (but not *S. abbreviatus*) responded to high-frequency stimuli. Detailed data for these will be presented below.

All descending neurons were narrowly tuned to the species' call frequencies and had similar responses in the two species. All have bilateral projections in the auditory neuropil and soma-contralateral axons. The cell shown in Fig. 2C is identifiable as DN1, which has previously been described in true crickets (Wohlers and Huber, 1982) and haglids (Mason and Schildberger, 1993). DN1 has its main dendritic projection in the soma-contralateral auditory neuropil. A secondary branch arises from the axon and crosses the midline posterior to the dendritic region to project to the soma-ipsilateral neuropil. The neuron shown in Fig. 2D (mDN) has a medially located soma and similar projection areas to DN1, but differs primarily in the origin of the secondary branch, which in mDN arises from the primary neurite anterior to the main dendritic projection. These two neurons are also distinguished by the nature of their responses to acoustic stimuli. Auditory neurons similar to mDN have been recorded in haglids (A. C. Mason, unpublished data). DN1 shows tonic responses with bursts of spikes for tone pulses and accurate copying of pulse trains, whereas the responses of mDN are phasic, with only a single spike per sound pulse and poor temporal following. The third descending neuron type (pDN, Fig. 2E) lies entirely in the posterior half of the ganglion. The soma of pDN lies near the base of the posterior connective. The neurite gives rise to a large soma-ipsilateral dendritic branch and a mid-line crossing segment which branches contralaterally into the descending axon and a smaller projection which is nearly symmetrical with the main dendritic branch. Despite apparently less overlap with auditory neuropil, pDN receives strong auditory input, responding to tone pulses with bursts of spikes and accurately copying temporal patterns.

Response properties of omega and T-neurons

Scapteriscus borellii

Both sexes of *S. borellii* engage in nightly dispersal flights during their breeding seasons (Forrest, 1980). The auditory responses of *S. borellii* indicated strong ultrasound sensitivity in this species. While some prothoracic interneurons were tuned only to frequencies in the range of the species' calling

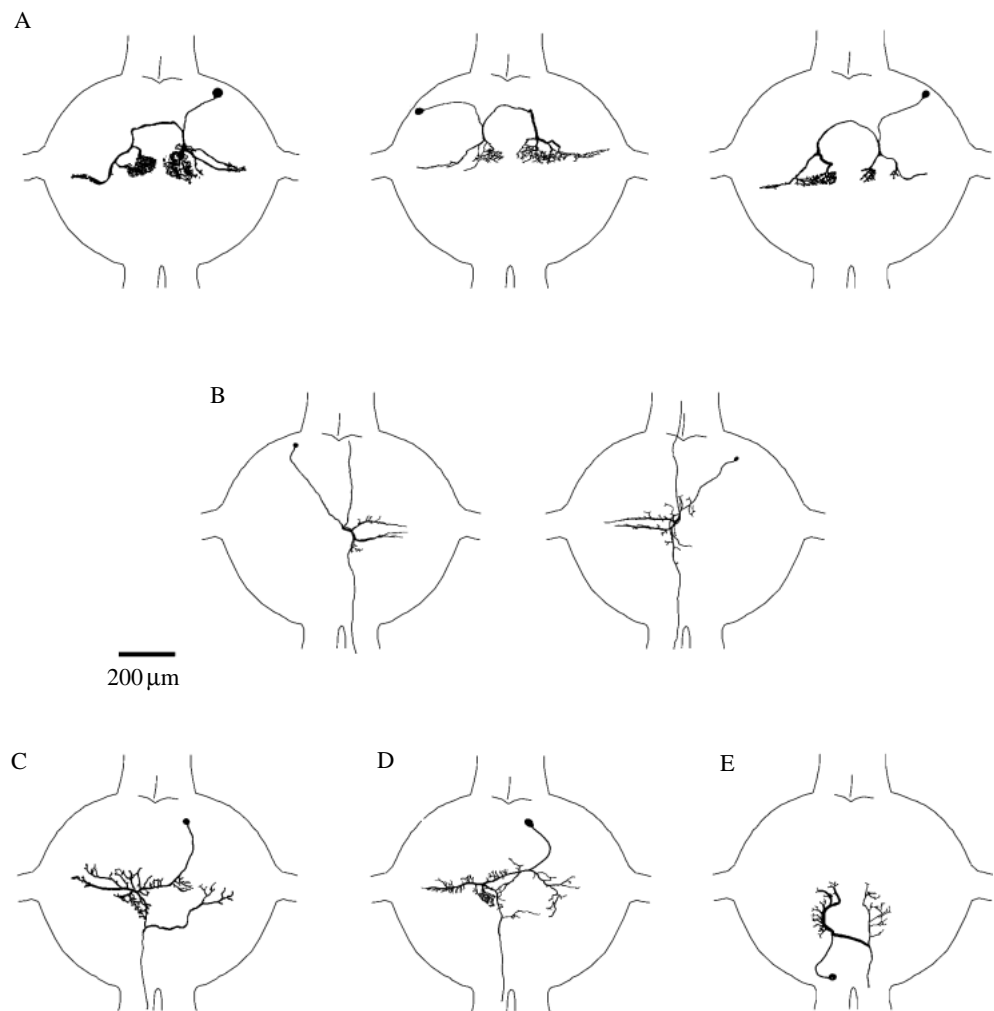


Fig. 2. Anatomy of prothoracic auditory interneurons recorded in both *Scapteriscus borellii* and *S. abbreviatus*. (A) Omega neurons (ONs). The left-hand (LON) and middle (HON) examples are from *S. borellii*, the right-hand ON is from *S. abbreviatus*. (B) T-neurons (hfTs) with high-frequency sensitivity in *S. borellii* (left) and only weak auditory input in *S. abbreviatus* (right). (C–E) Descending neurons: DN1 (C), mDN (D) and pDN (E).

song, others exhibited tuning curves with two sensitivity peaks: one corresponding to the species' call and the other in the ultrasonic range (20–30 kHz).

Omega neurons. Two classes of omega neurons were discovered in the prothoracic ganglion of *S. borellii*, as in true crickets, which also possess two bilateral pairs of omega neurons, ON1 and ON2 (Wohlers and Huber, 1982). Unlike true crickets, there was no consistent morphological difference between the two pairs of omega cells in *S. borellii*. However, double stains of both cells in a single preparation confirmed that these were distinct cell types. The omega neurons of *S. borellii* were anatomically similar to ON1 of true crickets. Their axons connect the auditory neuropils of the two hemiganglia, and both dendritic and axonal arborizations extend laterally from this region towards the leg nerves (Fig. 2A).

In *S. borellii*, the two omega cell types differed in their frequency tuning (Fig. 3A). One type showed two sensitivity peaks, at calling song frequencies and at ultrasonic frequencies. Absolute sensitivity at low frequencies was similar in both omega cell classes, with thresholds at the species' song frequency (3 kHz) of approximately 50 dB SPL. For the low-

frequency-tuned omega cells (LONs, $N=12$), thresholds increased continuously for higher frequencies and were above 80 dB SPL in the ultrasonic range. High-frequency-sensitive omega cells (HONs, $N=13$) had W-shaped threshold curves, with absolute thresholds of approximately 60 dB SPL for frequencies of 20–30 kHz.

Both ON types responded to trills with phasic bursts of spikes that copied the temporal pattern of the stimulus at repetition rates up to 50 pulses s^{-1} (Fig. 3C) and responded tonically to long-duration tones at song frequencies (data not shown). But while LONs showed weak excitation for some ultrasound frequencies (up to 25–30 kHz), they did not copy trills or long tones at these frequencies (Fig. 3C). HONs copied temporal patterns equally well at audio and ultrasonic frequencies (Fig. 3C). Omega neurons of both types responded to variations in stimulus amplitude with a wide dynamic range (over 50 dB at low frequencies) with responses varying in both spike number and latency (Fig. 3B,D). HONs had dynamic ranges of approximately 30 dB for 25 kHz stimuli (Fig. 3B) and responded with fewer spikes per stimulus than for low frequencies but with a similar latency shift (Fig. 3E).

In some ON recordings, inhibitory potentials were visible. In HONs, inhibition was not frequency-dependent and was present over a wide range of intensities. The depth of hyperpolarization was greater for contralateral than for ipsilateral stimuli at all intensities and had a wider dynamic range. Contralaterally evoked inhibitory postsynaptic potentials (IPSPs) were also larger when stimuli eliciting equal numbers of spikes were compared (Fig. 4).

In LONs, inhibitory potentials in response to low frequencies were more easily observed for contralateral stimulus presentation

at low intensities. In contrast, high-frequency stimuli evoked inhibitory potentials in some preparations in the absence of any excitatory response, for both ipsi- and contralateral presentation and over a wide range of intensities (Fig. 5).

High-frequency-tuned T-neurons. One type of auditory unit with a single sensitivity peak in the ultrasound range was recorded in *S. borellii*. This was a T-neuron (Fig. 2B left), characterised by two large soma-contralateral dendritic branches and soma-contralateral axons in the medial connectives. The two dendritic branches are displaced from

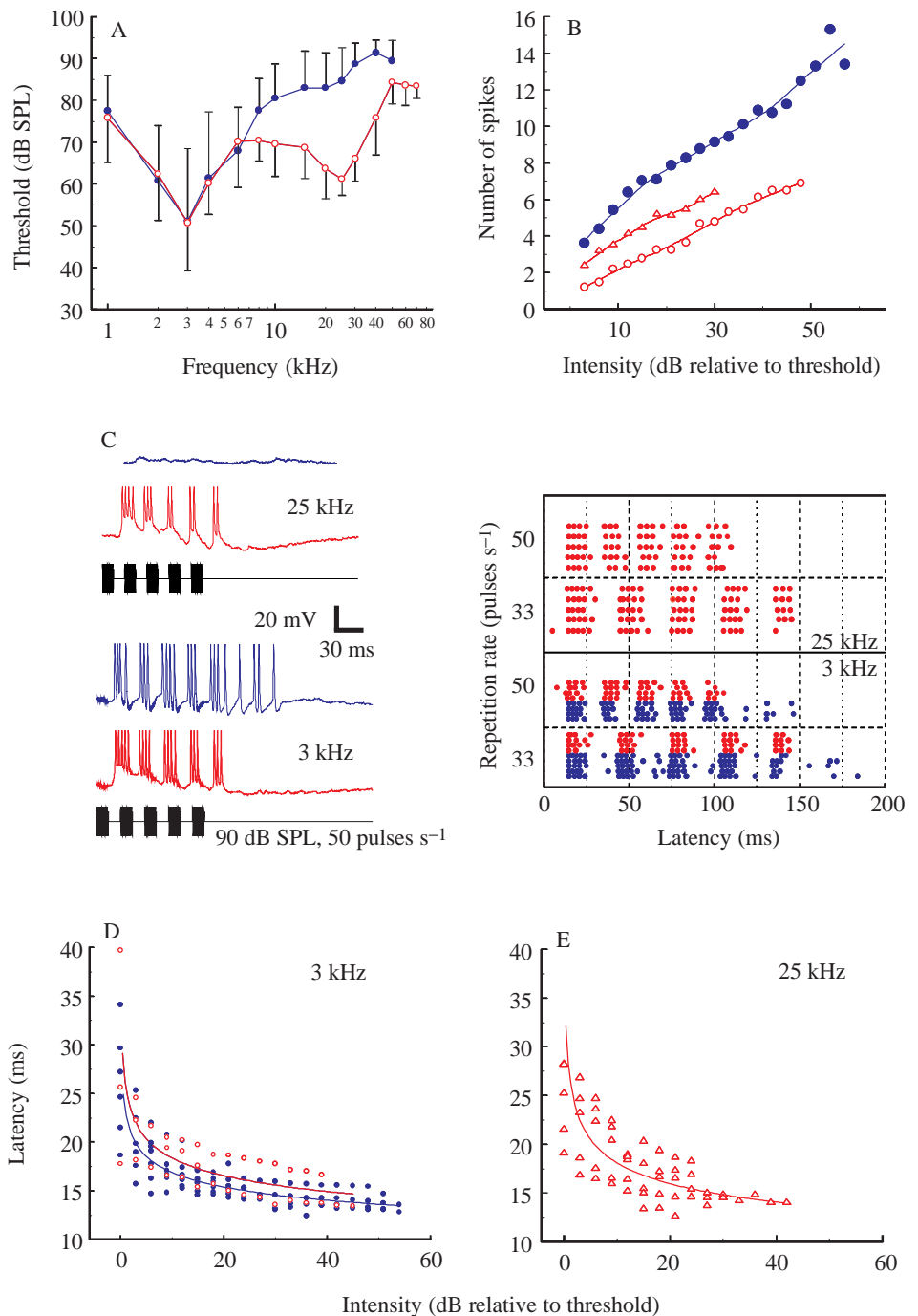


Fig. 3. Response characteristics of omega neurons in *Scapteriscus borellii*. In all panels, data shown in blue are for low-frequency-tuned omega neurons (LONs) and data shown in red are for high-frequency-tuned omega neurons (HONs). (A) Mean frequency-tuning curves (\pm s.d.) for the two types of omega cell. (B) Intensity-response functions for 3 kHz (circles) and 25 kHz (triangles; HONs only). Data points are global means for each cell type ($N=12$ LONs, $N=13$ HONs, five repetitions of each stimulus value averaged within individual cells). Lines were generated by LOWESS curve-fitting with the stiffness parameter set to 0.6 (Wilkinson *et al.* 1992). (C) Temporal pattern copying by the two ON types. On the left are sample traces (spikes clipped) showing responses to trains at 50 pulses s⁻¹ of 3 and 25 kHz tone bursts (upper, LON; lower, HON). On the right is a raster plot showing spike times for responses to two pulse repetition rates (33 and 50 pulses s⁻¹). Responses of both HONs and LONs are shown for 3 kHz stimuli and for HONs only for 25 kHz stimuli. (D,E) Response latency plotted against stimulus intensity for 3 kHz (D) (circles) and 25 kHz (E) (triangles; HONs only). Data points are means of five repetitions, and data from multiple preparations are combined. Lines were fitted with a power function.

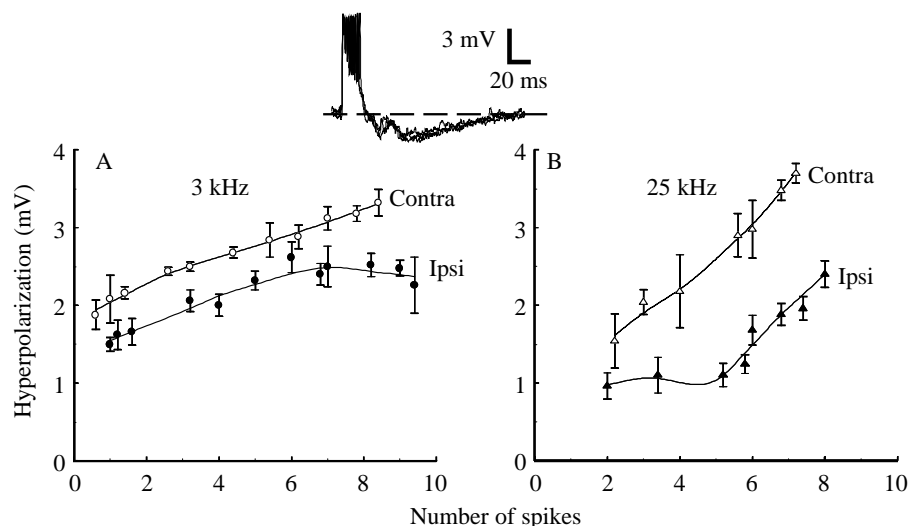


Fig. 4. Hyperpolarization following excitation in high-frequency-tuned omega neurons. Ipsi, ipsilateral stimulation; Contra, contralateral stimulation. (A) 3 kHz stimuli; (B) 25 kHz stimuli. Data points are the mean maximum deviation from resting potential (five repetitions) at each stimulus intensity. Lines are LOWESS curve fits (see Fig. 3). Inset: sample response traces (spikes clipped) for 3 kHz stimulus; five traces superimposed.

one another both antero-posteriorly and dorso-ventrally, such that the more anterior branch lies more dorsally in the ganglion while the posterior dendrite is more ventral.

High-frequency T-neurons (hfTs, $N=8$) had a distinctive pattern of frequency tuning. Thresholds were lowest for frequencies of 20–30 kHz (Fig. 6A). They responded most strongly and with the widest dynamic range to ultrasonic frequencies (Fig. 6B), some preparations showing strong responses up to at least 70 kHz. Low-frequency stimuli elicited a combination of excitation and inhibition (Fig. 6C). The time courses of hfT responses to low-frequency stimuli indicate that excitatory and inhibitory components had similar latencies but that the initial response was usually depolarizing. This initial excitation was usually limited to one or a few spikes before further activity was suppressed by inhibitory input. In some preparations, low-frequency stimuli failed to elicit spikes, although the same pattern of combined IPSPs and excitatory postsynaptic potentials (EPSPs) was apparent in these cases. Inhibitory inputs declined with increasing frequency, such that responses to ultrasonic stimuli were predominantly excitatory (Fig. 6C).

This combination of excitation and inhibition resulted in variable threshold measurements for low-frequency stimuli in hfT neurons and limited dynamic range. Nevertheless, in most preparations, hfT neurons fired at least a single spike over a 20 dB range of stimulus intensities at 3 kHz. Response latencies were approximately 15 ms and showed no significant change over this range of intensities (Fig. 6D). In contrast, latencies for ultrasonic stimuli changed by approximately 15 ms (from 30 ms near threshold to 15 ms at high intensities) over a similar dynamic range (Fig. 6E).

Temporal pattern copying by hfT neurons was poor at low carrier frequencies. Pattern copying was much more accurate with more robust responses at high frequencies (Fig. 7). Responses to combined stimuli of 3 and 25 kHz were reduced relative to those of 25 kHz alone.

Ultrasound responses in cervical connectives. To determine whether there might be other auditory neurons, in addition to

hfT, carrying ultrasound responses to the brain, we recorded responses in the neck connectives. Without signal averaging, no single units responding with short latency to acoustic

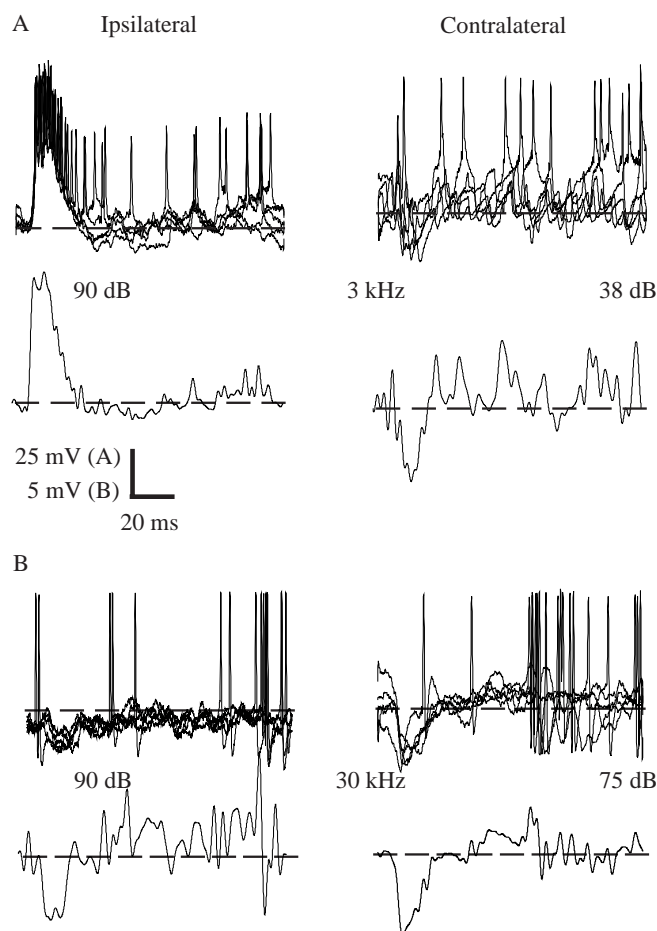


Fig. 5. Hyperpolarization of low-frequency-tuned omega neurons in response to 3 kHz (A) and 30 kHz (B) stimulus frequencies. Upper traces in each panel show five responses superimposed; lower traces show the average of the same five responses low-pass-filtered (60 Hz cut-off) to remove spikes.

stimuli were detectable in the summed activity of the ascending connectives. Signal averaging revealed short-latency (15–20 ms) responses to both audio and ultrasonic stimuli (Fig. 8). Averaged responses to audio stimuli had greater amplitude and duration than high-frequency responses (Fig. 8A). High-frequency responses consisted of a single or double peak in the averaged trace. These results suggest the presence of multiple units carrying ascending low-frequency responses, but only one or a few cells carrying ascending ultrasound responses (Suga, 1968).

In single sweeps, bursts of large-amplitude spikes with longer latency (35–40 ms) could be detected, but only in response to high-frequency stimuli (Fig. 8B). These responses

habituated rapidly and therefore did not appear in averaged responses. This may represent descending activity associated with ultrasound startle responses (Hoy *et al.* 1989).

Behavioural responses to ultrasound. Free-flying *S. borellii* of both sexes were attracted to sound traps broadcasting their species' calling song. A total of 203 crickets were collected in the traps over the seven nights of the experiment. There was a significant relationship between the intensity of ultrasound added to the louder song playback and the relative attractiveness of the quieter song source. With no ultrasound added to the broadcast, the proportions attracted to the two traps were as predicted by their relative broadcast power (Forrest, 1980; Forrest and Raspet, 1994). As the intensity of

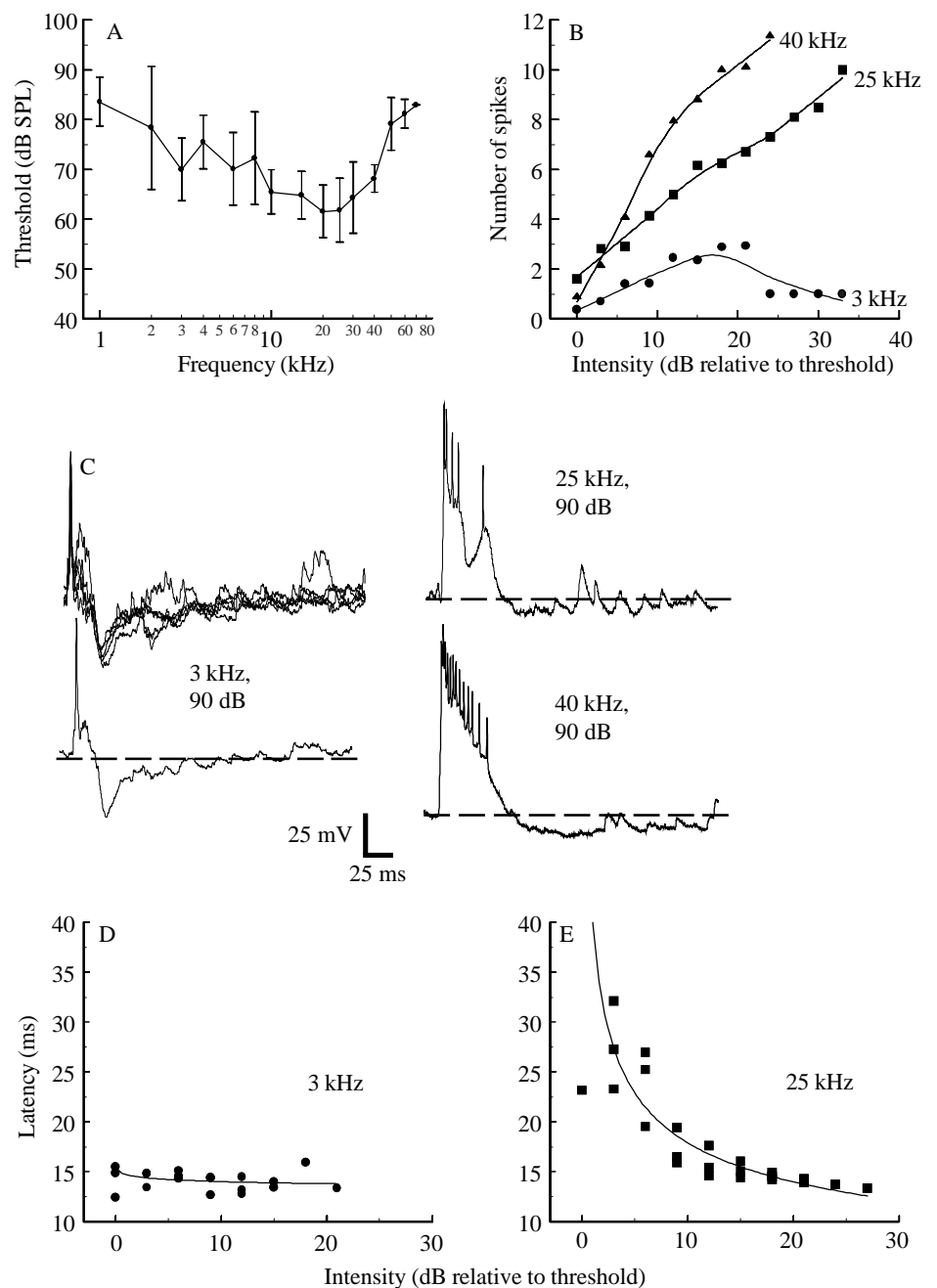


Fig. 6. Response characteristics of high-frequency-sensitive T-neurons (hfTs) in *Scapteriscus borellii*. (A) Mean tuning curve (\pm S.D.) for hfT cells. (B) Intensity-response functions for 3 kHz (circles), 25 kHz (squares) and 40 kHz (triangles). Data points are global means ($N=8$ cells, five repetitions of each stimulus value averaged within individual cells). Lines are LOWESS curve fits (see Fig. 3). (C) Sample response traces for 3, 25 and 40 kHz. For 3 kHz, both five single traces superimposed (upper) and their average (lower) are shown. Only single traces are shown for 25 and 40 kHz. Responses to 3 kHz show brief excitation followed by a pronounced IPSP. High-frequency responses are only excitatory. (D,E) Response latency plotted against stimulus intensity for 3 kHz (D) and 25 kHz (E). Data points are means of five repetitions. Lines were fitted with a power function.

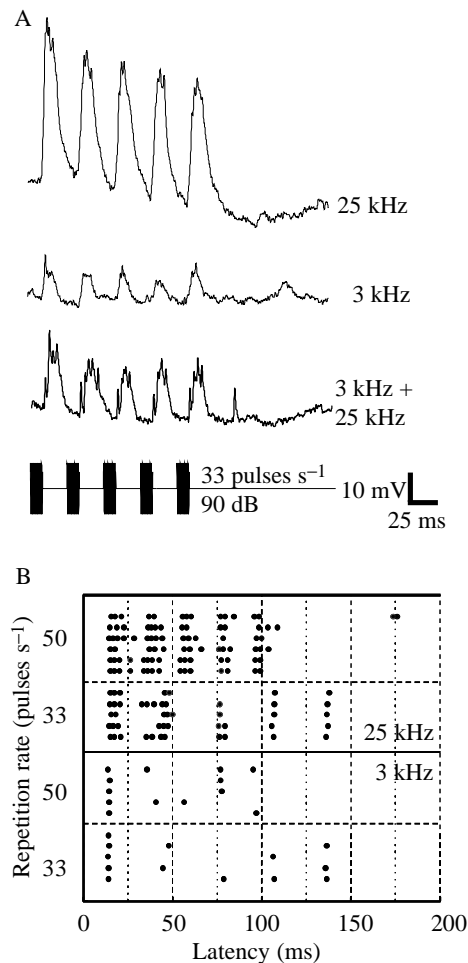


Fig. 7. Temporal pattern copying by hfT neurons in *Scapteriscus borellii* at 3 and 25 kHz stimulus frequencies. (A) Sample response traces. Strong responses with bursts of spikes and large-amplitude excitatory potentials are elicited by 25 kHz stimuli (upper trace). Responses are much weaker, with only intermittent spiking, for 3 kHz stimuli (middle trace). Simultaneous presentation of 3 and 25 kHz stimuli results in intermediate activity (bottom trace). (B) Raster plot showing spike times for two pulse rates (33 and 50 pulses s⁻¹) and two stimulus frequencies.

ultrasound added to the louder song source was increased, however, the relative attractiveness of this trap diminished such that larger proportions of captured mole crickets were attracted to the quieter song source (Fig. 9).

Scapteriscus abbreviatus

S. abbreviatus is a flightless species. The frequency sensitivities of all auditory interneurons recorded in this species were similar, and no evidence of ultrasound sensitivity was found. We did, however, identify anatomical homologues in *S. abbreviatus* of each of the auditory cell types we have described for *S. borellii*. These are described below.

Omega neurons. All omega neurons recorded in *S. abbreviatus* (ONs, $N=7$) had similar tuning and response properties to those of LON in *S. borellii* and all were

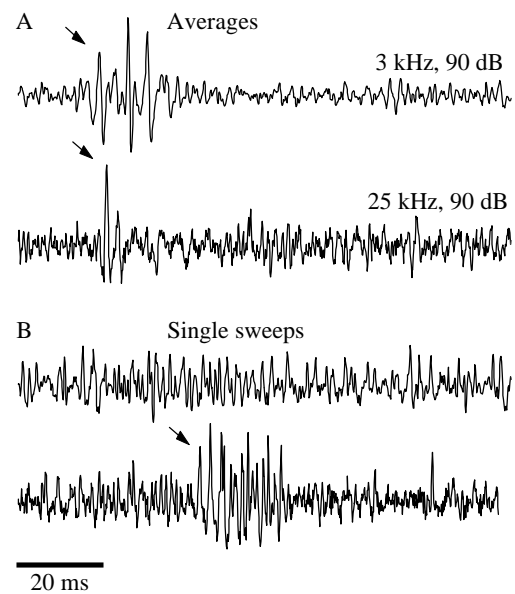


Fig. 8. Summed activity recorded in the neck connectives of *Scapteriscus borellii* for 3 kHz (upper traces) and 25 kHz (lower traces) stimuli. (A) Averages of multiple stimulus presentations (3 kHz, $N=500$; 25 kHz, $N=250$). (B) Single responses. Arrows indicate activity correlated with acoustic stimuli.

anatomically similar to *S. borellii* omega neurons (Fig. 2A). Omega neurons recorded from *S. abbreviatus* were narrowly tuned to frequencies from 2 to 4 kHz, with thresholds of approximately 50 dB SPL and a dynamic range of approximately 50 dB SPL (Fig. 10A,B). Thresholds were approximately 90 dB SPL or higher for frequencies above 8 kHz. ONs showed tonic responses and accurate copying of temporal patterns (Fig. 10C). Latencies decreased by approximately 15 ms with increased stimulus intensity (Fig. 10D).

'High-frequency' T-neurons. A single example of a T-neuron, which had a similar anatomy to hfT neurons of *S. borellii*, was recorded and stained in *S. abbreviatus*. This neuron responded to acoustic stimulation but with thresholds greater than 80 dB SPL at all frequencies. However, details of the responses of this neuron, in addition to its anatomical similarity, indicate that it is a homologue of the hfT neuron. In response to low-frequency stimuli, this neuron showed a combination of excitatory and inhibitory inputs (Fig. 11A). Latencies were shorter for excitation than for inhibition, with the result that, at high intensities (above 85 dB SPL), responses consisted of an initial single spike with a latency of approximately 15 ms, followed by a pronounced IPSP with a latency of 20–25 ms. Below 85 dB SPL, spiking did not occur. At higher frequencies and intensities (above 85 dB SPL), this neuron fired one or several spikes. Inhibition was not observed for high-frequency stimuli (Fig. 11B).

Discussion

The two mole cricket species examined in this study, *S.*

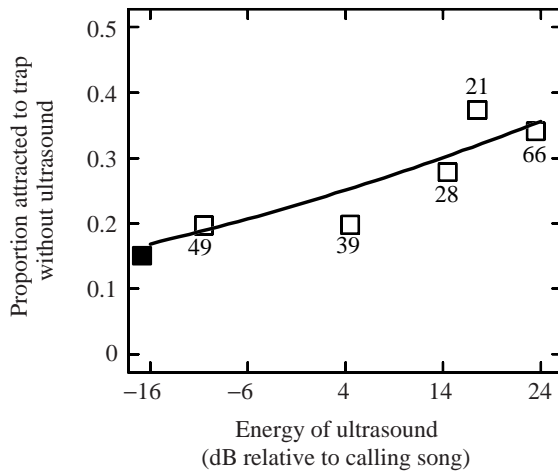


Fig. 9. Effect of ultrasound on flying *Scapteriscus borellii* in the field. The plot shows the proportion of individuals attracted to the quieter of two sources broadcasting *S. borellii* calling song as a function of the energy of ultrasound added to the louder source. See text for further details. Numbers beside symbols give the total number of individuals attracted in each experimental treatment. The filled symbol represents data from previous experiments (Forrest, 1980; $N=2979$) which were not included in the regression analysis for this experiment. The equation for the logistic regression was $y = [\text{Exp}(0.025x - 3.844)] / [1 + \text{Exp}(0.025x - 3.844)]$, $P < 0.05$.

borellii and *S. abbreviatus*, are similar in terms of their low-frequency hearing. *S. abbreviatus* are completely flightless, whereas *S. borellii* are frequent nighttime flyers and have an additional range of auditory sensitivity to ultrasonic frequencies. Low-frequency hearing is consistent with the nature of the intraspecific acoustic signals of both species. High-frequency ultrasound hearing in *S. borellii* suggests a role for hearing in the avoidance of predation by bats in this species, as has been demonstrated in several other insect taxa (Forrest *et al.* 1995; Libersat and Hoy, 1991; Miller, 1975; Roeder, 1967; Yager and Hoy, 1986; Yager and Spangler, 1997). The behaviour of *S. borellii* in response to broadcast ultrasound supports this interpretation.

Auditory anatomy and processing

The overall anatomical and cellular organisation of the prothoracic auditory system is similar to that of true crickets, particularly in anatomy and in the response properties of auditory units that are specialized for intraspecific communication. Omega neurons (ONs) are able to encode the temporal patterns and intensity of acoustic signals through variations in spike number, latency and timing. Categorical frequency coding (Wytenbach *et al.* 1996) is available in the differential tuning of the two ONs in *S. borellii*. Tonic responses that copy the temporal pattern of song-like acoustic signals are typical of ensiferan ONs (Pollack, 1986, 1988; Rheinlaender and Roemer, 1986; Schul, 1997; Wohlers and Huber, 1982). True cricket ONs are characterised by a combination of excitatory and inhibitory responses to acoustic stimuli. Mutual contralateral inhibition by pairs of ONs and

contralateral inhibition of other auditory interneurons by ONs are the basis of sound localization in true crickets (Horseman and Huber, 1994a,b; Schildberger and Hörner, 1988; Schildberger *et al.* 1988). In addition, biphasic responses in ONs (i.e. excitatory responses followed by a longer-latency hyperpolarization) have been identified as a mechanism for 'selective attention' of auditory processing in true crickets (Pollack, 1988) and katydids (Römer, 1992). This refers to the ability of the cricket auditory system accurately to encode one signal among many on the basis of intensity differences. The pattern of inhibitory responses we recorded in *S. borellii* ONs suggests that similar mechanisms operate in mole crickets.

Two types of ON were recorded in *S. borellii*. In *S. abbreviatus*, only one type was recorded. The distinguishing characteristic of the two ON classes in *S. borellii* is their sensitivity to high frequencies. In contrast to true crickets, in which ONs show both physiological and anatomical differences (Wohlers and Huber, 1982), no anatomical differences between the two ON types were observed. Since *S. abbreviatus* apparently lack high-frequency auditory input, it is likely that there are also two pairs of omega neurons in this species with similar anatomy and response properties.

Ultrasound sensitivity in *S. borellii* appears to be mediated primarily by one of the ON pairs (HON) and one type of T-neuron (hfT). This is similar, in general terms, to the organization of prothoracic auditory circuitry devoted to ultrasound hearing in other acoustic Ensifera. In both true crickets (Popov *et al.* 1978; Wohlers and Huber, 1982) and katydids (Römer *et al.* 1988), ON tuning is as broad as that of the peripheral auditory system. Insects in both of these families are also known to show ultrasound-induced acoustic startle responses (ASRs) (Libersat and Hoy, 1991; Moiseff *et al.* 1978). In true crickets, to which mole crickets are most closely related (Gwynne, 1995), ultrasound-avoidance behaviour depends on the activity of the ascending auditory neuron Int-1 (Nolen and Hoy, 1984, 1986a,b). Despite this anatomical difference, the response properties of hfT and Int-1 are strikingly similar. Both show short-latency inhibitory inputs in response to low frequencies, leading to variable responses to frequencies in the range of intraspecific signals; both show maximal sensitivity with high spike rates and tonic responses to ultrasonic frequencies; and in both Int-1 and hfT, ultrasound responses are suppressed by the simultaneous presentation of low-frequency stimuli (Nolen and Hoy, 1987).

Our behavioural data indicate that flying *S. borellii* avoid sources of ultrasound. While the results of these sound-trapping experiments are not directly comparable with measurements of thresholds for negative phonotaxis (Nolen and Hoy, 1986a,b), they clearly demonstrate an analogous ultrasound-avoidance response in *S. borellii*. In the absence of other acoustic stimuli, calling song sources will attract conspecifics in proportion to their relative broadcast power (Forrest and Raspet, 1994). The significant reduction in attractiveness of the calling song when combined with ultrasound cannot be attributed to a degradation of the temporal pattern of this signal, since the ultrasonic component had a

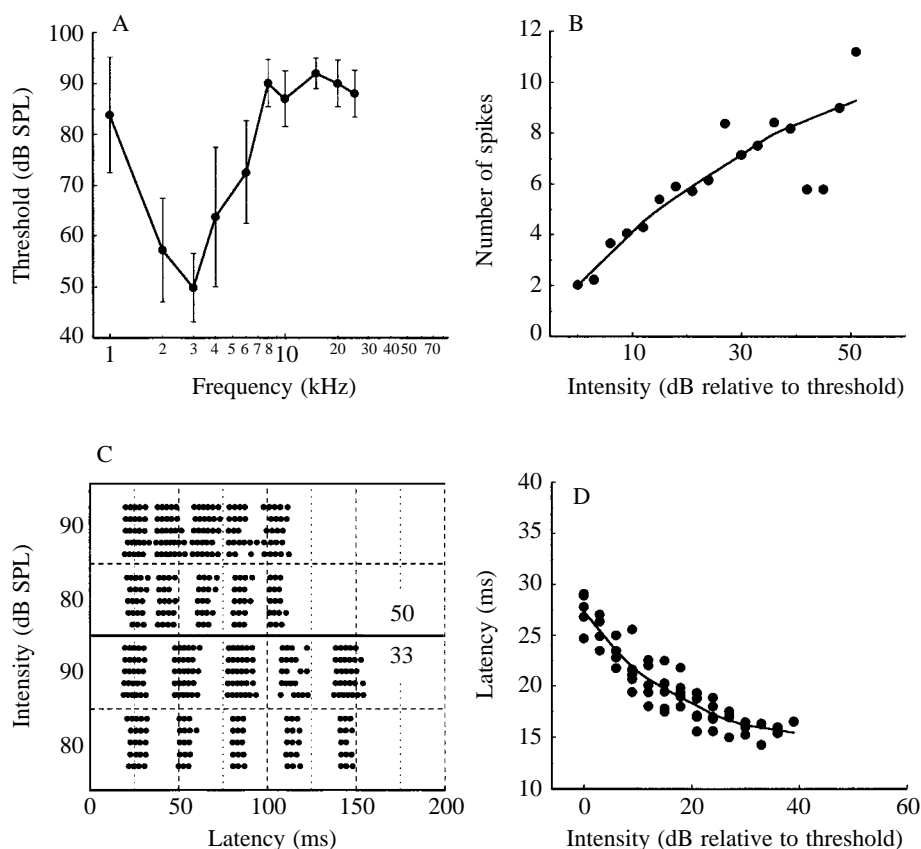


Fig. 10. Response characteristics of omega neurons (ONs) in *Scapteriscus abbreviatus*. (A) Mean tuning curve (\pm S.D.) for ONs. (B) Mean intensity-response function for 3 kHz ($N=7$ cells, five repetitions averaged within cells). (C) Raster plot showing temporal pattern copying for 3 kHz stimuli at two intensities (80 and 90 dB) and pulse rates (50 and 33 pulses s^{-1}). (D) Latency shift for variation in intensity of 3 kHz stimuli. Data points are means ($N=5$), and the line was fitted with a power function.

similar temporal pattern. Therefore, only the frequency content of the broadcast signal was altered. The attraction to the source of the song of proportionally fewer mole crickets as a function of the power of the ultrasonic component of the signal indicates an aversive response competing with the attraction of the calling song.

Our data do not directly demonstrate a link between this ultrasound-avoidance behaviour and the responses of hfT, such as has been shown for Int-1 in true crickets (Nolen and Hoy, 1984). However, the similarity between the responses of hfT

and those of Int-1 and the similar behavioural responses of mole crickets and true crickets suggest such a function for hfT. Ultrasound hearing has been demonstrated in other mole cricket species that are nocturnal flyers. Suga (1968) measured auditory responses in the nerve cord of South American mole crickets. These responses were most sensitive to ultrasonic frequencies (see Fig. 1 in Suga, 1968) and very similar to our data for hfT tuning (Fig. 6A). Suga further suggested that there are few ascending auditory units in the neck connectives, and only one in the prothoracic-mesothoracic connective. Our neck

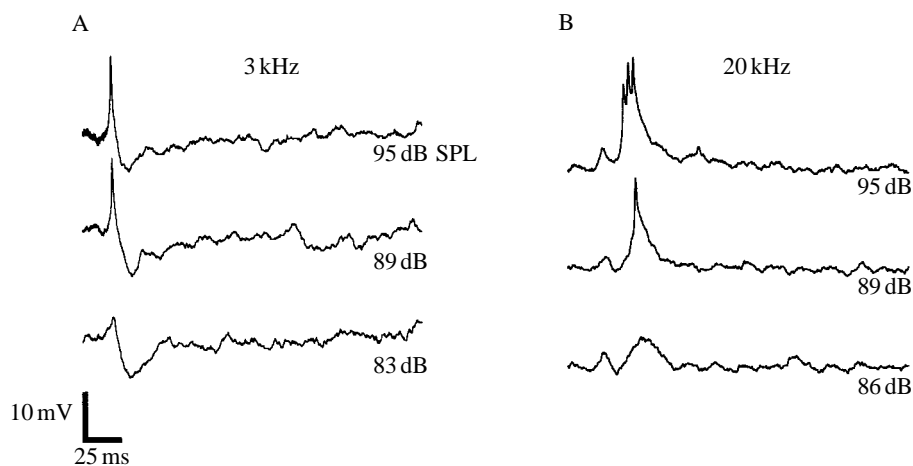


Fig. 11. Responses of the hfT cell in *Scapteriscus abbreviatus* to 3 kHz (A) and 20 kHz (B) stimulus frequency. Auditory responses are insensitive but show a similar time course to those of *S. borellii*. Low-frequency responses show a brief excitation followed by a pronounced IPSP. High-frequency responses are only excitatory.

connective recordings corroborate these results and suggest that hT is the only source of ascending ultrasound activity. Also, the facts that single pulses of ultrasound, but not audio sound, can elicit a delayed burst of activity in the neck connective and that this activity habituates are consistent with an ultrasound-mediated ASR in *S. borellii* (Brodfuehrer and Hoy, 1989). In contrast, in a non-flying species, *S. abbreviatus*, high-frequency hearing is absent and hT neurons show very weak auditory input, while auditory responses to low frequencies are identical to those of *S. borellii*.

Evolution

On the basis of comparative morphology, it is probable that mole cricket omega neurons are homologous with those of previously studied Ensifera (Mason and Schildberger, 1993; Römer *et al.* 1988; Wohlers and Huber, 1982). Two pairs of ONs are found in true crickets (Gryllidae; Wohlers and Huber, 1982) and haglids (Haglidae; Mason and Schildberger, 1993). Auditory responses have been studied in two other ensiferan taxa, katydids (Tettigoniidae) and weta (Stenopelmatidae). Omega neurons are known from tettigoniids, but only a single pair of ONs per individual has been described (Römer *et al.* 1988; Schul, 1997). Peripheral auditory responses have been recorded in weta (Field *et al.* 1980), but their central auditory anatomy is unknown. True cricket ON1 responses are better suited than those of ON2 for a role in processing intraspecific acoustic signals, and the function of ON2 is unclear (Horseman and Huber, 1994a,b; Schildberger and Hörner, 1988; Schildberger *et al.* 1988; Wohlers and Huber, 1982). In the case of *S. borellii*, both ON types respond similarly to song-like signals, and they differ only in their responses to ultrasound. In *S. abbreviatus*, no functional differentiation of ONs was observed. Thus, we could not determine a clear correspondence between the two ON subtypes of mole crickets and true crickets. In some species of true crickets, however, ON1 shows a secondary sensitivity peak at ultrasonic frequencies which is similar to that of HON (Atkins and Pollack, 1986).

It is more difficult to infer homology between neurons mediating ultrasound-avoidance behaviour in different ensiferan families, since these show more diversity than song-processing elements. It is possible that hT is homologous to Int-1 of true crickets. In addition to similar acoustic responses, these two neurons show several anatomical similarities: soma position, the location of the midline-crossing segment, the position of dendritic branches and the location of the ascending axon in the anterior connective (Casaday and Hoy, 1977).

Regardless of the specific homologies, the diversity of ultrasound-processing neurons in different ensiferan families raises interesting evolutionary questions. Hearing and acoustic communication in Ensifera existed prior to the evolution of echolocating bats (Novacek, 1985; Otte, 1992; Sharov, 1971). Ultrasound hearing in relation to bat detection is, therefore, a secondary adaptation of the ensiferan auditory system (Hoy, 1992). Differences in the processing mechanisms associated with ultrasound avoidance in different ensiferan taxa may

reflect their independent evolution in already divergent groups. Gwynne (1995) argues that hearing and acoustic communication evolved independently in the two main ensiferan clades: the Tettigoniodea, which includes the families Tettigoniidae (katydids), Haglidae and Stenopelmatidae (weta), and the Grylloidea, which includes Gryllidae (true crickets) and Gryllotalpidae (mole crickets). This obviously implies an independent origin for ultrasound hearing in grylloids and tettigonioids, but within the grylloids the origins of ultrasound hearing in different families are unknown. More detailed study of the functional differences in the organization of the ultrasound auditory pathway in true crickets and mole crickets could address these questions. Identification of the destination and function of the descending axon of hT in mole crickets would allow comparisons with both true crickets and katydids. In addition, we have demonstrated significant differences in ultrasound hearing between flying and flightless mole cricket species even within the same genus. These results highlight the lability of specialized sensory systems under varying ecological conditions (Fullard *et al.* 1997). The variation among mole cricket species we have described suggests that even more direct tests of the function of identified neurons in conspecific communication compared with predator avoidance are possible. These data, combined with phylogenetic analyses, could help distinguish historical effects from functional specializations in the evolution of these sensory systems.

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