

## NEUROETHOLOGY OF THE KATYDID T-CELL

### I. TUNING AND RESPONSES TO PURE TONES

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#### Summary

The tuning and pure-tone physiology of the T-cell prothoracic auditory interneuron were investigated in the nocturnal katydid *Neoconocephalus ensiger*. The T-cell is extremely sensitive and broadly tuned, particularly to high-frequency ultrasound ( $\geq 20$  kHz). Adult thresholds were lowest and showed their least variability for frequencies ranging from 25 to 80 kHz. The average best threshold of the T-cell in *N. ensiger* ranged from 28 to 38 dB SPL and the best frequency from 20 to 27 kHz. In females, the T-cell is slightly more sensitive to the range of frequencies encompassing the spectrum of male song. Tuning of the T-cell in non-volant nymphs was comparable with that of adults, and this precocious ultrasound sensitivity supports the view that it has a role in the detection of terrestrial sources of predaceous ultrasound. In adults, T-cell tuning is narrower than that of the whole auditory (tympanic) organ, but only at audio frequencies. Superthreshold physiological experiments revealed that T-cell responses were ultrasound-biased, with intensity/response functions steeper and spike latencies shorter at 20, 30 and 40 kHz than at 5, 10 and 15 kHz. The same was also true for T-cell stimulation at 90° compared with stimulation at 0° within a frequency,

which supports early T-cell research showing that excitation of the contralateral ear inhibits ipsilateral T-cell responses. In a temporal summation experiment, the integration time of the T-cell at 40 kHz (integration time constant  $\tau=6.1$  ms) was less than half that measured at 15 kHz ( $\tau=15.0$  ms). Moreover, T-cell spiking in response to short-duration pure-tone trains mimicking calling conspecifics (15 kHz) and bat echolocation hunting sequences (40 kHz) revealed that temporal pattern-copying was superior for ultrasonic stimulation. Apparently, T-cell responses are reduced or inhibited by stimulation with audio frequencies, which leads to the prediction that the T-cell will encode conspecific song less well than bat-like frequency-modulated sweeps during acoustic playback. The fact that the T-cell is one of the most sensitive ultrasound neurons in tympanate insects is most consistent with it serving an alarm, warning or escape function in both volant and non-volant katydids (nymphs and adults).

Key words: auditory physiology, bioacoustics, bushcricket, hearing, insect, *Neoconocephalus ensiger*, neurophysiology, Orthoptera, Tettigoniidae, ultrasound.

#### Introduction

The functions of sound production and hearing in orthopteran insects (crickets, katydids, locusts and their allies) include territoriality and aggression, mate recognition, localization of conspecifics, courtship, defense and predator detection (see Alexander, 1962, 1967; Huber et al., 1989; Bailey and Rentz, 1990). Because of the tremendous diversity of sounds that are produced and the importance of these signals to their everyday lives (i.e. survival and reproduction), considerable research effort has been devoted to understanding peripheral and central auditory processing in the acoustic Orthoptera. In most orthopterans, it is the male that stridulates to produce the loud, long-range, species-specific advertisement signal (mate-calling song) to which sexually receptive females may be attracted (i.e. positive

phonotaxis). Given their small size, the problem of sound localization by insects is not trivial and, not surprisingly, this topic has been a central question in insect auditory research (e.g. Michelsen and Nocke, 1974; Lewis, 1992; Michelsen, 1992, 1998; Römer, 1992; Michelsen et al., 1994a,b; Schul et al., 1999). Indeed, the majority of research on the acoustic Orthoptera has focused on understanding the behavioral and/or neural mechanisms underlying sound localization during positive phonotaxis (e.g. von Helversen, 1984; Rheinlaender and Römer, 1980, 1986; Schildberger and Hörner, 1988; Schildberger et al., 1988; Stumpner, 1997; Schul, 1998, 1999; Römer and Krusch, 2000), with considerably fewer studies devoted to the detection and processing of other biologically relevant signals such as

sounds produced by predators (i.e. negative phonotaxis, but see reviews by Hoy et al., 1989; Hoy, 1992).

The purpose of this study is to identify neural correlates of behavioral responses to non-social acoustic stimuli in a nocturnal katydid, the eastern sword-bearer conehead *Neoconocephalus ensiger* Harris (Orthoptera: Tettigoniidae). When stimulated with pulsed ultrasound, tethered *N. ensiger* momentarily cease flying and alter their flight course, presumably to avoid predation by aerially hawking echolocating bats (Libersat and Hoy, 1991). Similar ultrasound-induced types of escape behavior, more generally referred to as acoustic startle responses, have been reported in a variety of nocturnal insects including moths (Roeder, 1962), crickets (Moiseff et al., 1978), lacewings (Miller and Olesen, 1979), locusts (Robert, 1989), mantids (Yager et al., 1990) and beetles (Forrest et al., 1995; Yager and Spangler, 1997). Recently, we have shown that *N. ensiger* possess a second, previously unknown, type of acoustic startle response: cessation of singing (Faure and Hoy, 2000a). When stimulated with pulsed ultrasound, stridulating males cease calling and may remain motionless (and cryptic), drop and/or jump to the ground, where they hide by burrowing into the vegetation, or take to the wing (evasive flight). As with cessation of flight, cessation of song (i.e. silencing oneself) presumably evolved as a way of avoiding predators, in this case acoustically orienting predators such as cats, mice, shrews and substrate-gleaning bats (Walker, 1964; Sales and Pye, 1974; Belwood and Morris, 1987).

Threshold tuning curves for the two acoustic startle responses are virtually identical, suggesting that a common neural mechanism underlies both forms of escape (Faure and Hoy, 2000a). For such a mechanism to function adequately, however, it must reliably distinguish conspecific song, which is broadband and contains both audio and ultrasonic frequencies, from predatory ultrasound. Despite considerable overlap in the spectral and temporal characteristics of these signal categories, *N. ensiger* rarely show startle responses when listening to conspecific song but do so reliably when stimulated with bat-like ultrasound (Libersat and Hoy, 1991; Faure and Hoy, 2000a). Thus, our goal was to discover neurophysiological correlates underlying behavioral perception and sound categorization (Wytenbach et al., 1996).

Within the acoustic Orthoptera, the presence of a large, prominent-spiking interneuron known as the T-cell has been recognized for nearly four decades. The T large fiber was first described in tettigoniids (katydids, bushcrickets or long-horned grasshoppers) by Suga and Katsuki (1961a,b) and later by McKay (1969, 1970). By simultaneously monitoring spike discharge patterns in the ascending and descending connectives of the prothoracic ganglion, it was demonstrated that T-cell spikes travel rostrally and caudally within the ventral nerve cord. Thus, auditory information entering the ganglion *via* the tympanic nerve bifurcates, or makes a T-junction, within the central nervous system. This characteristic physiological fingerprint was later linked to the anatomy of an auditory giant neuron (Kalmring et al., 1979) whose cell body

was later found to reside in the prothoracic ganglion (Fig. 1A; Oldfield and Hill, 1983; Zhantiev and Korsunovskaja, 1983; Rheinlaender and Römer, 1986; Römer et al., 1988). Aficionados of insect hearing will be interested to learn that the T-cell was originally named without reference to its T-shaped morphology: the T stands for tympanic organ; hence, the large unit excited by acoustic stimulation was named the T large fiber (N. Suga, personal communication). Technically, the T-cell is only one member of a class of T-shaped interneurons (e.g. crickets, Atkins and Pollack, 1987; mole crickets, Mason et al., 1998; haglids, Mason and Schildberger, 1993; katydids, Rheinlaender et al., 1972; Shen, 1993; Schul, 1997); however, to be consistent with the previous literature, we will refer to it as the T-cell.

Previous authors have suggested that katydid acoustic startle responses may be mediated by the pair of prothoracic T-cell auditory interneurons (e.g. McKay, 1969, 1970). Römer et al. (1988) found that the onset of synaptic activity in the T-cell was delayed by only 0.8–1.2 ms relative to primary receptors located micrometers away, implying a monosynaptic connection. Such fast throughput is consistent with the T-cell functioning in a predator-detection circuit, but other physiological data are inconclusive in assigning a role to the katydid T-cell. Various aspects of T-cell physiology have been investigated, including lesion and contralateral inhibition studies (Suga and Katsuki, 1961a; McKay, 1969, 1970; Rheinlaender et al., 1972), the effects of pharmacological agents (Suga and Katsuki, 1961b), encoding of directional acoustic stimuli (Suga, 1963; Rheinlaender et al., 1972, 1986; Rheinlaender and Römer, 1980), responses to conspecific and/or heterospecific katydid song (Suga and Katsuki, 1961a; Kalmring et al., 1979; Zhantiev and Korsunovskaja, 1983; Schul, 1997) and the use of the T-cell as a 'biological microphone' (Rheinlaender and Römer, 1986), but there have been few systematic studies of its basic response properties (but see Rheinlaender et al., 1972; Rheinlaender, 1975). This is surprising considering how easy it is to identify and record from the T-cell using simple extracellular techniques (e.g. Rheinlaender and Römer, 1986). Moreover, none of the above studies has examined T-cell responses specifically in relation to predator detection.

In this paper, the first of a pair dealing with the physiology of the T-cell in *N. ensiger*, we systematically investigate T-cell responses using pure-tone stimulation. We describe T-cell tuning in katydid nymphs and adults of both sexes, the tuning of the whole auditory organ in adults compared with that of the T-cell (i.e. peripheral *versus* central tuning), T-cell spike-amplitude and latency-amplitude intensity/response functions, temporal summation and integration, and the ability of the T-cell to encode the temporal pattern of short-duration stimulus trains mimicking calling conspecifics or the various stages of hunting by echolocating bats. Our companion paper (Faure and Hoy, 2000c) uses acoustic playback to compare T-cell responses in both sexes with syllables of conspecific song and predatory bat-like frequency-modulated (FM) sweeps.

## Materials and methods

### *Experimental animals*

*Neoconocephalus ensiger* Harris (Orthoptera: Tettigoniidae) were captured from fields near Ithaca, NY, USA (42°26'N, 76°28'W) between July and October 1993–1997. Adult males were localized by listening to their calling song. Female *N. ensiger* do not sing, so they were found by scanning the tops of grass seedheads with a flashlight. Katydid were housed in individual plastic cages containing apple, cat chow and water. Most animals were maintained in a cold room (12 °C) to slow their rate of senescence, but they were transferred to a rearing room (25 °C) at least 1 day prior to their use in electrophysiological experiments.

### *Neurophysiology*

Katydid were waxed (3:2, beeswax:rosin) ventral-side-up to the elevated platform of a custom-made insect holder with a magnetic base. The prothoracic legs were secured in a natural position by waxing the tip of each tarsus to a minuten pin on the holder; the meso- and metathoracic legs were waxed alongside the body of the animal. Because the tympanic organs are located on the proximal portion of the prothoracic tibia, care was taken not to damage the ears mechanically or thermally during waxing. The central nervous system was exposed for extracellular recording by removing the cuticular membranes overlying the ventral nerve cord. Katydid were minimally dissected, and care was taken not to cut or damage the internal acoustic trachea. Electrolytically sharpened tungsten electrodes (unipolar) hooked under the subesophageal–prothoracic and/or prothoracic–mesothoracic connectives were used for recording. The electrode tip was covered with a mixture of Vaseline and mineral oil to prevent tissue desiccation. A second sharpened tungsten electrode placed into the abdomen served as a reference. Action potentials were amplified with an A-M Systems (model 1700) differential a.c. amplifier (high pass 100 Hz, low pass 10 kHz, 60 Hz notch filter). T-cell spikes were measured on-line using a Hewlett Packard 54502A 400 MHz digitizing oscilloscope and/or recorded to VHS tape for off-line analysis (Vetter model 400 PCM recorder) or digitized directly using a Macintosh Centris 650 computer and GW Instruments MacAdiosII data-acquisition board (stimulus envelope and spike digitization rates 10 kHz). Spikes were window-discriminated and analyzed using Igor Pro (Wavemetrics, Inc.) and custom-designed software written by R. A. Wytenbach.

A more severe dissection was required for intracellular recording. In this case, the wings, the meso- and metathoracic legs, the gut and any muscles lying in close proximity to the prothoracic ganglion were removed before stabilizing the ganglion with a custom-shaped silver spoon, which also served as the reference electrode. Standard insect saline was applied to prevent desiccation. The anatomy of the T-cell was visualized by intracellular penetration with a borosilicate glass capillary electrode filled at the tip with Lucifer Yellow (2–4 % in 0.05 mol l<sup>-1</sup> LiCl, Sigma; electrode resistance >100 MΩ). The T-cell was stained ionophoretically by injecting –4 nA of

current for 8 min (A-M Systems model 1600 neuroprobe amplifier), after which the thoracic central nervous system was fixed *in situ* in 4 % paraformaldehyde in 0.1 mol l<sup>-1</sup> phosphate buffer and then removed from the animal and left in fixative overnight. The next day, the tissue was dehydrated in a standard ethanol series, cleared in methylsalicylate, and photographed with a Leitz Dialux 20 fluorescence microscope.

### *Acoustic stimulation*

Electrophysiological recordings were made inside a 1.1 m × 0.65 m × 0.65 m (length × width × height) chamber lined with sound-attenuating foam (Sonex). Mean chamber temperature was 24.1 ± 0.8 °C. The entire chamber was mounted on a TMC Micro-g vibration table. Pure-tone pulses (2–100 kHz) with 1 ms linear or raised cosine rise/fall times were produced either by a custom-made sine-wave generator and pulse-shaper circuitry (analog) controlled by an AMPI Master-8 pulse generator or a by computer with an array processor and A–D/D–A interface purchased from Tucker Davis Technologies (TDT: Apos II).

All sounds were broadcast through an ESS or Panasonic EAS-10TH400B leaf tweeter located approximately 33 cm from the insect preparation. Sound levels, measured with a Brüel & Kjær (B&K) type 2209 impulse precision sound level meter and type 4135 1/4 inch condenser microphone (flat ±3 dB from 20 Hz to 125 kHz without protecting grid; diaphragm 0° incidence) and calibrated with a B&K type 4220 pistonphone, are expressed in decibels sound pressure level (dB SPL rms re: 20 μPa) equivalent to the peak amplitude of continuous tones of the same carrier frequency (Stapells et al., 1982). Amplitudes were adjusted using a programmable attenuator (TDT: PA4) and stereo amplifier (Nikko NA-790). The magnetic base of our insect holder allowed visual positioning of katydid at 90° or 0°. The position of the loudspeaker is given by its angle relative to the mid-sagittal body axis of the katydid: anterior 0°, lateral 90°. At 90°, ipsilateral refers to the ear and prothoracic auditory spiracle closest to the loudspeaker, whereas at 0° both ears are approximately the same distance from the loudspeaker so there is no ipsilateral side, only a recording side. Calibration curves were constructed for each position with the insect holder and micromanipulator in place (see Fig. 1 in Faure and Hoy, 2000b).

### *T-cell tuning in adult males and females*

T-cell spikes are large and conspicuous in extracellular recordings and are amenable to quantitative analyses, so all physiological data were collected using this technique. Threshold determination was on-line (resolution 0.5 dB) and was defined as the minimum dB SPL required to elicit one T-cell spike in four of five consecutive stimulus presentations using 10 ms pure tones presented once per second (note that, when stimulating at 90°, the contralateral T-cell is below the threshold of the ipsilateral T-cell; see also Rheinlaender and Römer, 1980). Variables extracted from T-cell tuning curves were best frequency (BF), best threshold (BT) and tuning

quality at 10 dB ( $Q_{10\text{dB}}$ ). For definitions of these variables, see Faure and Hoy (2000b).

#### *T-cell tuning in nymphs and adults*

We compared the tuning and sensitivity of the T-cell in late-stage immature katydids with that of adult katydids. Katydid nymphs do not sing, so they were located in the same manner as adult females, by scanning the top of the vegetation at night with a flashlight. Extracellular T-cell recordings in nymphs were conducted in the same manner as for adults.

#### *Leg nerve versus T-cell tuning*

Using a paired design, we compared the tuning of the T-cell with extracellular multi-unit responses from the whole auditory (leg) nerve in the same animal ( $N=10$  katydids: nine males, one female). The prothoracic leg nerve (nerve V) was exposed for recording by removing the cuticle overlying the proximal portion of the coxa. Sound-evoked potentials were recorded using tungsten hook and reference electrodes; evoked responses appear as a synchronous wave of summed action potentials from the auditory afferents (see Fig. 1a in Boyd and Lewis, 1982). The stimulus was a 10 ms pure tone (1 ms raised cosine rise/fall time) varying from 2 to 70 kHz, presented at 0.5 Hz (leg nerve recordings) or 1 Hz (T-cell recordings), with the loudspeaker positioned at 90°. Summed action potentials were averaged for 10 sweeps and displayed on the HP digitizing oscilloscope, and leg nerve thresholds were determined on-line by decreasing the stimulus in 3 dB steps (TDT: PA4) until summed action potentials were no longer discernible from background activity. For comparison, T-cell thresholds were measured from the subesophageal–prothoracic connective on the same side of the body as the nerve V recording. T-cell thresholds, determined as previously described, were measured semi-blind, with the sequence of data collection reversed in some preparations (i.e. nerve V thresholds were hidden from view before determining T-cell thresholds, and *vice versa*).

#### *Intensity/response functions*

Single, 10 ms pure tones presented over a broad range of amplitudes (20–100 dB SPL) and frequencies (5, 10, 15, 20, 30 and 40 kHz) were used to investigate non-adapting responses of quiescent T-cells (presentation rate  $\leq 1$  Hz). Experiments were conducted with the loudspeaker positioned at 90° and 0°. T-cell spikes occurring in a 65 ms post-stimulus response window were captured on-line using the HP digitizing oscilloscope. We counted the number of spikes per pulse and measured the latency to the first T-cell spike using the measurement cursors of the oscilloscope. Stimuli were repeated at least five times for each intensity/frequency combination to obtain an average response, although for some combinations it was not possible to obtain five responses (e.g. at lower amplitudes and frequencies).

#### *Temporal summation/integration*

In 11 katydids (seven males and four females), we determined T-cell thresholds using pure-tone pulses of variable

duration (0.1–500 ms; 50  $\mu\text{s}$  linear rise/fall time) presented at 1 Hz (loudspeaker position 90°). Katydid nymphs were tested at two behaviorally relevant frequencies: 15 kHz and 40 kHz. The 15 kHz stimulus was chosen to mimic the peak spectral frequency of the calling song of *N. ensiger* (Faure and Hoy, 2000a), whereas 40 kHz is a typical peak spectral frequency of a biosonar pulse emitted by a North American species of insectivorous bat (Fenton and Bell, 1981). The thresholds of the T-cell were normalized relative to the lowest sound pressure level for each katydid, independent of carrier frequency, before averaging across individuals. The integration time constant ( $\tau$ ) of the T-cell was estimated using a least-squares fit to the data according to a leaky integrator energy detector model with an exponential decay (Plomp and Bouman, 1959). The predicted threshold ( $T_P$ ) change at each duration ( $d$ ) is described by the equation:

$$T_P = -10 \log(1 - e^{-d/\tau}).$$

#### *Short-term temporal pattern coding*

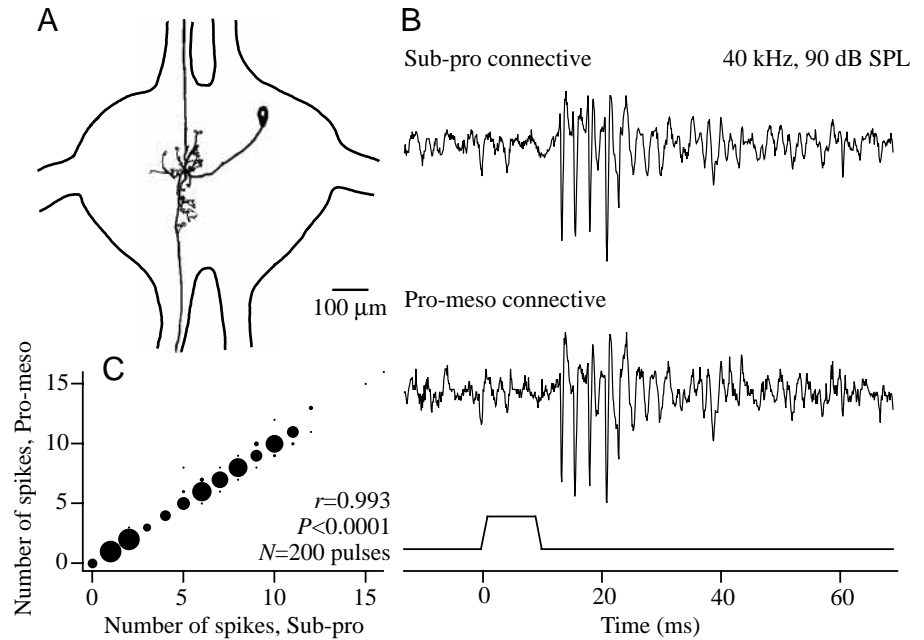
The ability of the T-cell to encode short-duration temporal pattern trains was measured using 10 ms pulses presented at repetition rates varying from 1 to 100 Hz. These rates encompass the range of syllable periods exhibited by *N. ensiger* singing in the field (Frings and Frings, 1957) and the vocalization rates of echolocating bats during the search, approach (tracking) and terminal phases of hunting (Simmons et al., 1979; Kick and Simmons, 1984). The train duration was 25 pulses, so constant energy was presented at each rate, and all stimuli were broadcast at 50, 70 and 90 dB SPL. The sequence of testing was randomized within trials, and T-cells were given at least 30 s to recover between presentations.

The temporal pattern-copying ability of the T-cell was quantified by computing a pulse synchronization index (SI), which is simply the proportion of stimulus pulses encoded by one or more T-cell spikes during a user-defined post-stimulus window. The pulse synchronization index can vary from 0 to 1: a value of 1 indicates perfect temporal copying, whereas a value of 0 indicates no copying. The pulse repetition rate corresponding to a synchronization index value of 0.5 was defined as the cut-off rate ( $R_c$ ), equivalent to the  $-3$  dB point on a decibel scale, and  $R_c$  values at 15 kHz and 40 kHz, interpolated from individual synchronization index functions, were compared using a paired *t*-test or a Wilcoxon signed-rank test. A three-way analysis of variance (ANOVA) was used to assess the effects of the factors carrier frequency, sound pressure level and loudspeaker position on T-cell temporal copying with  $R_c$  as the dependent variable.

#### *Statistical analyses*

All data are reported as the mean  $\pm$  standard deviation (S.D.), except when reporting the mean of averages, in which case the mean  $\pm$  standard error of the mean (S.E.M.) is given. Data were first tested for normality (Shapiro and Wilk, 1965) and/or equality of variances (homoscedasticity); non-normal or heteroscedastic data were analyzed with an equivalent non-

Fig. 1. T-cell identification. (A) Anatomical reconstruction of a T-cell stained with Lucifer Yellow in the prothoracic ganglion (ventral view) of an adult male *Neoconocephalus ensiger* (Ne 405). The composite drawing was made by tracing slides projected at various focal planes. Scale bar, 100  $\mu$ m. (B) Simultaneous extracellular recordings from another katydid (Ne 120) confirm that time-locked (stimulus evoked) T-cell spikes travel in both the subesophageal–prothoracic (Sub-pro) connective (upper trace) and in the prothoracic–mesothoracic (Pro-meso) connective (middle trace). Lower trace: stimulus envelope. (C) Bubble plot correlating the number of T-cell spikes in the subesophageal–prothoracic and prothoracic–mesothoracic connectives ( $N=2$  preparations). The size of the bubble is proportional to the sample size at each point, e.g. bubble (0,0)  $N=9$ ; bubble (15,15)  $N=1$ .



parametric test (Statview, Super ANOVA). All statistical tests employ a rejection criterion of  $P \leq 0.05$  (Zar, 1984).

## Results

### T-cell identification

Three independent methods confirmed that the large spikes observed in our extracellular recordings were from the same (homologous) T-cell as that described by Suga and Katsuki (1961a,b), McKay (1969, 1970) and Rheinlaender and Römer (1980).

### Intracellular dye injection

In one preparation, we made intracellular recordings from and stained a cell whose physiology was similar to that of the T-cell in extracellular recordings. The morphology of this T-cell (Fig. 1A) is very similar to that described for other katydids (e.g. Zhantiev and Korsunovskaja, 1983; Rheinlaender and Römer, 1986; Rheinlaender et al., 1986; Römer et al., 1988; Schul, 1997; Stumpner, 1999).

### Paired extracellular recordings

In two preparations, we recorded simultaneously from the ascending and descending connectives of the prothoracic

ganglion during acoustic stimulation using various amplitudes and frequencies; large phasic spikes evoked by the stimulus were recorded in each connective (Fig. 1B). Analysis of both

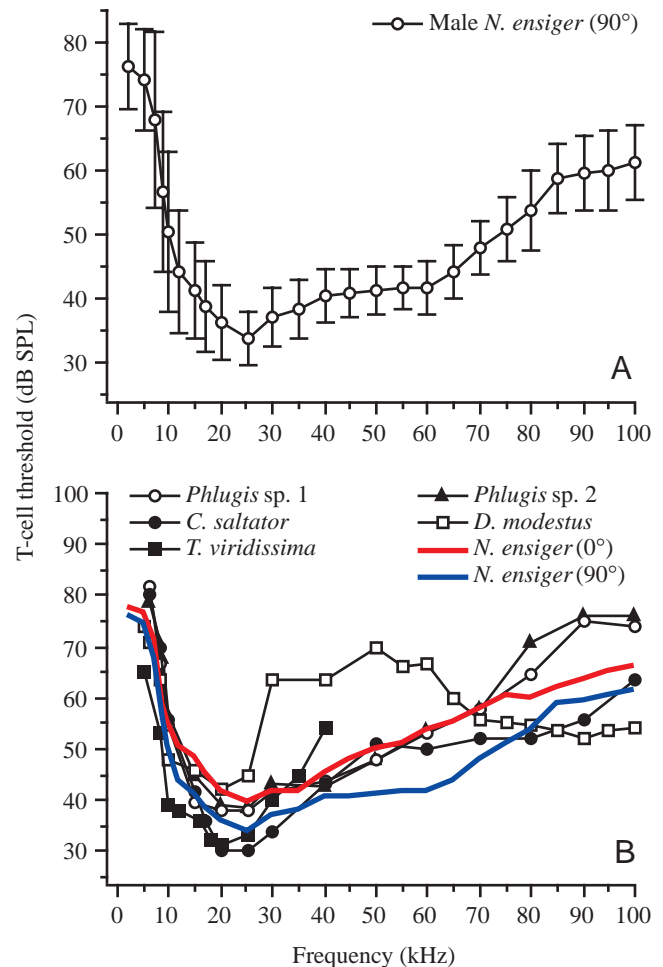


Fig. 2. T-cell tuning and cross-species tuning comparison. (A) Mean  $\pm$  S.D. excitatory threshold tuning curve for the T-cell in *Neoconocephalus ensiger* with the loudspeaker positioned at 90°. Note the higher degree of variability in audiosound (7–17 kHz) thresholds. (B) Average T-cell tuning curves from European and neotropical katydids compared with that of the T-cell from *N. ensiger* with the loudspeaker positioned at both 90° (blue) and at 0° (red). Note the overall similarity in neural thresholds. References: *Phlugis* spp., *Conocephalus saltator* and *Drepanoxiphus modestus* (Suga, 1966); *Tettigonia viridissima* (Rheinlaender and Römer, 1980).

the number of spikes (Fig. 1C) and the spike latency (data not shown) confirmed that the impulses traveling in each connective were from a single cell.

#### Tuning comparison

Fig. 2A shows the average threshold tuning curve for the T-cell in a male katydid with the loudspeaker positioned at 90° (i.e. when the ipsilateral ear and prothoracic auditory spiracle are directly facing the loudspeaker). The T-cell is very sensitive and broadly tuned, having a sensitivity bandwidth at +10 dB re: best threshold ranging from 14.0±5.5 kHz to 49.3±13.8 kHz, yielding a  $Q_{10\text{dB}}$  of less than 1.0. Note that T-cell thresholds appear to show greater variation in the lower skirt of the tuning curve at audiosound frequencies (7–17 kHz), whereas thresholds at ultrasonic frequencies (20–100 kHz) are more consistent. When T-cell thresholds in *N. ensiger* are compared with T-cell thresholds from other katydids reported in the literature, except for the W-shaped audiogram of *Drepanoxiphus modestus* (Suga, 1966), the tuning curves are remarkably similar (Fig. 2B). This is despite the fact that auditory thresholds were measured (i) by different workers, (ii) in different species of katydid, (iii) from various locations around the world and (iv) over a span of more than 30 years.

#### T-cell tuning in adult males and females

An examination of average threshold tuning curves in males and females reveals that the T-cell is very sensitive in both sexes. Note, however, that in females the T-cell is more sensitive near its best frequency than in males (Fig. 3A). The largest sensitivity difference (6–12 dB) occurs for frequencies between 5 and 25 kHz, and this is true for stimulation at both 90° (Fig. 3A, left) and 0° (Fig. 3A, right), whereas at frequencies of 30 kHz or above, the tuning curves and threshold difference functions ( $\Delta\text{dB}$ ) converge. A slightly different picture emerges when individual thresholds are averaged across audiosound (2–17 kHz) and ultrasound (20–100 kHz) hearing categories (Fig. 3B). At 90°, males are significantly less sensitive than females for audiosound (90° audiosound,  $t=-3.942$ ,  $P=0.0002$ ) but not for ultrasound (90°

ultrasound, Wilcoxon signed-rank test  $z=-0.678$ ,  $P=0.4979$ ) frequencies, whereas at 0° males are slightly, but significantly, less sensitive than females in both the audiosound and ultrasound ranges (0° audiosound,  $z=-2.758$ ,  $P=0.0058$ ; 0° ultrasound  $t=-4.352$ ,  $P=0.0001$ ).

Table 1 summarizes mean best frequency, best threshold and  $Q_{10\text{dB}}$  values of the T-cell for male and female *N. ensiger* stimulated with the loudspeaker positioned at 90° and 0°. In both sexes, the mean best frequency of the T-cell was usually  $\geq 20$  kHz and its best threshold was normally  $< 40$  dB SPL. At both 90° and 0°, best frequencies and best thresholds were significantly higher in males than in females; however, no differences between the sexes were found in the  $Q_{10\text{dB}}$  values. So, while the T-cell in females is slightly more sensitive to audio frequencies than in males, the overall shape and breadth of the tuning curves do not differ between the sexes.

#### T-cell tuning in nymphs and adults

Not enough replicates were available to produce separate tuning curves at each developmental stage, so T-cell thresholds from nymphal instars -3, -2 and -1 were pooled in this comparison (i.e. the third-last, second-last and penultimate instars before the final adult molt, respectively). As in adults, the T-cell in *N. ensiger* nymphs is quite sensitive and broadly tuned. The mean best frequency, best threshold and  $Q_{10\text{dB}}$  for juvenile males are 22 kHz at 34 dB SPL with a  $Q_{10\text{dB}}$  of 0.97; values for juvenile females are 25 kHz at 24 dB SPL with a  $Q_{10\text{dB}}$  of 1.36. These values are similar to those measured in adults (see Table 1). Fig. 4 compares T-cell tuning curves in nymphal and adult katydids (loudspeaker position 90°). Although the overall shape of the tuning curve of nymphs is similar to that of adults, some small differences are apparent. Adult thresholds are, on average, lower than juvenile thresholds across most frequencies. The average juvenile minus adult T-cell threshold difference ( $\Delta\text{dB}$ ) in males is 5.4±4.7 dB SPL and that in females is 3.0±4.5 dB SPL. The difference in sensitivity is particularly obvious in males from 40 to 80 kHz (Fig. 4A), whereas in females the difference is rather slight (Fig. 4B).

Table 1. Summary data of the best frequency (BF), best threshold (BT) and  $Q_{10\text{dB}}$  index of the T-cell for male and female *Neonoconocephalus ensiger* stimulated at two loudspeaker positions (90° and 0°)

| Tuning variable   | Speaker position (degrees) | Male        |    | Female      |    | P-value* |
|-------------------|----------------------------|-------------|----|-------------|----|----------|
|                   |                            | Mean        | N  | Mean        | N  |          |
| BF (kHz)          | 90                         | 26.9±9.4    | 64 | 20.2±3.5    | 18 | 0.0001   |
| BT (dB)           | 90                         | 32.4±3.7    | 64 | 28.4±10.6   | 18 | 0.0183   |
| $Q_{10\text{dB}}$ | 90                         | 0.873±0.467 | 63 | 1.030±0.481 | 18 | 0.1322   |
| BF (kHz)          | 0                          | 24.0±6.8    | 27 | 19.7±3.7    | 9  | 0.0326   |
| BT (dB)           | 0                          | 38.2±3.6    | 27 | 33.1±2.1    | 9  | 0.0003   |
| $Q_{10\text{dB}}$ | 0                          | 0.802±0.302 | 27 | 0.773±0.317 | 9  | 0.4761   |

N, number of adult katydids tested.

\*Two sample *t*-test or Mann-Whitney *U*-test comparing males and females.

Values are means ± s.d.

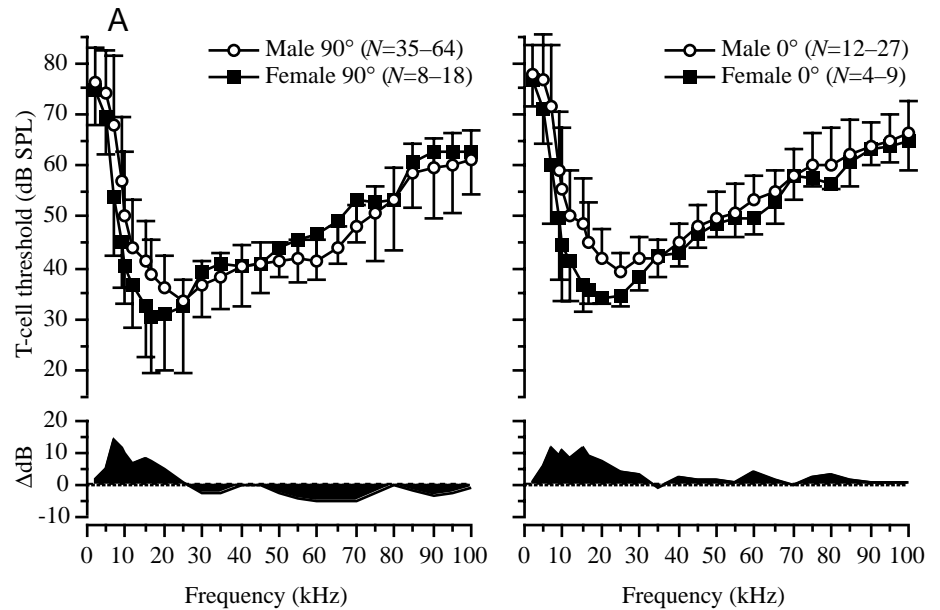
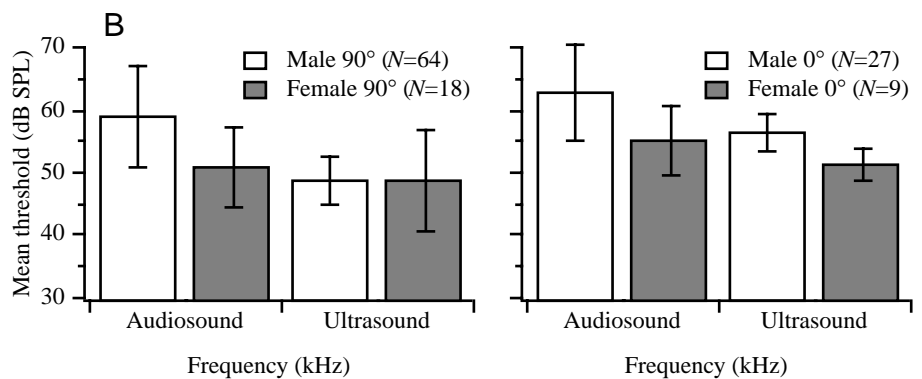


Fig. 3. T-cell tuning in adult male and female *Neoconocephalus ensiger*. (A) Mean  $\pm$  s.d. T-cell threshold tuning curves in male and female katydids for loudspeaker positions of  $90^\circ$  (left) and  $0^\circ$  (right). The shaded area (below) is the threshold difference function ( $\Delta\text{dB}$ =male threshold minus female threshold). (B) Mean  $\pm$  s.d. pooled audiosound (2–17 kHz) and ultrasound (20–100 kHz) T-cell thresholds at  $90^\circ$  (left) and  $0^\circ$  (right).



#### Leg nerve versus T-cell tuning

Average threshold tuning curves for the whole auditory organ and for the T-cell recorded on the same side of the body are shown in Fig. 5A (the abscissa is plotted on a logarithmic scale to emphasize audio frequencies). In general, the overall shapes of the tuning curves are quite similar: the mean T-cell minus leg nerve threshold difference ( $\Delta\text{dB}$ ) from 2 to 70 kHz is  $1.2 \pm 5.5$  dB, and the distribution of  $\Delta\text{dB}$  is not significantly different from a distribution with a mean  $\Delta\text{dB}$  of zero (one-way  $t$ -test,  $t = -0.942$ ,  $P = 0.3585$ ), indicating that the tuning curves are not significantly different from each other (see Faure and Hoy, 2000b). When the analysis is partitioned into audiosound and ultrasound frequency ranges, the tympanic organ is, on average, 6 dB more sensitive than the T-cell at audio frequencies, with the largest sensitivity difference occurring at 7 kHz ( $\Delta\text{dB} \approx 15$  dB), but with differences of 3 dB or more being common between 5 and 12 kHz (Fig. 5B). At ultrasonic frequencies, the average threshold difference is negligible ( $\Delta\text{dB} \approx 1$  dB). Quantitatively, the magnitude of the T-cell minus leg nerve  $\Delta\text{dB}$  is significantly larger at audio than at ultrasonic frequencies ( $z = -2.803$ ,  $P = 0.0051$ ). Also note that there is less variability in  $\Delta\text{dB}$  at ultrasonic frequencies (Fig. 5B).

#### Intensity/response functions

Intensity/response functions (i.e. input/output curves) for the T-cell of *N. ensiger* show the classic sigmoidal shape: as stimulus amplitude increases, the number of action potentials per pulse also increases, slowly at first, but rising rapidly with further increases in sound pressure level and eventually saturating at the highest levels (Fig. 6A,B). Interestingly, the magnitude of the intensity/response function of the T-cell varies with stimulus frequency. When stimulating with single 10 ms pulses of audiosound (5, 10 or 15 kHz), the quiescent T-cell responds with fewer spikes per pulse than when pulses of ultrasound (20, 30 or 40 kHz) are broadcast. For ultrasound stimulation at  $90^\circ$ , the T-cell shows an almost monotonic increase in spike number between 40 and 80 dB SPL, whereas this is not true for audiosound stimulation (Fig. 6A). At 5 kHz, it is difficult to evoke T-cell spiking, even at the highest sound pressure levels. At 10 kHz, the response strength begins to increase, but it is still poor relative to ultrasound stimulation. At 15 kHz, which is only slightly above the peak spectral frequency of the mate-calling song of *N. ensiger* (peak spectral frequency  $13.4 \pm 1.5$  kHz; Faure and Hoy, 2000a), T-cell responses are greater than at 10 kHz. However, for amplitudes  $\geq 60$  dB SPL, the intensity/response

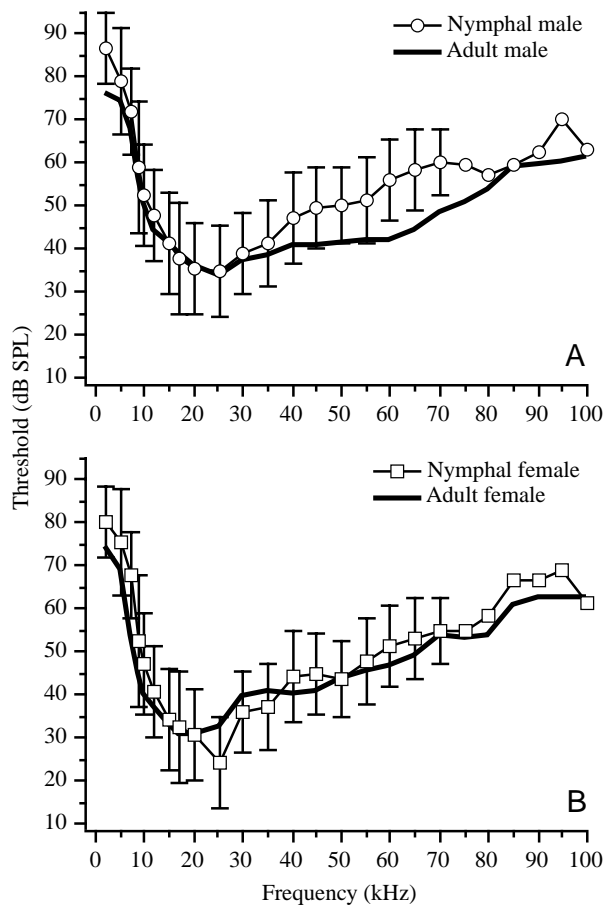


Fig. 4. T-cell tuning in late-instar nymphs and adult *Neoconocephalus ensiger* (loudspeaker position  $90^\circ$ ). (A) Mean  $\pm$  s.d. T-cell threshold tuning curves in juvenile ( $N=6$ , except at 2 kHz, where  $N=5$ , and from 75 to 100 kHz, where  $N=1$ ) and adult ( $N=35-64$ ) male katydids. (B) Mean  $\pm$  s.d. T-cell threshold tuning curves in juvenile ( $N=4$ , except at 2 kHz, where  $N=3$ , and from 75 to 100 kHz, where  $N=1$ ) and adult ( $N=8-18$ ) females. For clarity, adult thresholds (from Fig. 3) are shown without their associated error bars.

curve plateaus, and in some preparations a decline in response strength was readily apparent (i.e. at 15 kHz, T-cell responses were reduced or inhibited at moderate-to-loud sound pressure levels). This general pattern held true regardless of whether intensity/response curves were measured with the loudspeaker positioned at  $90^\circ$  (Fig. 6A) or at  $0^\circ$  (Fig. 6B), although the difference in response strength between audiosound and ultrasound frequencies is most pronounced for lateral ( $90^\circ$ ) stimulation.

The latency of the T-cell also varies as a function of sound pressure level and frequency. As is typical of most sensory systems, spike latencies decrease in a non-linear fashion as the amplitude of the stimulus increases from 20 to 100 dB SPL (Fig. 6C,D). Within a given sound pressure level, latencies at ultrasound frequencies are shorter than at audio frequencies, with the effect being most apparent for single-pulse stimulation with the loudspeaker positioned at  $0^\circ$  (Fig. 6D). As mentioned above, reliable T-cell spiking is difficult to invoke when

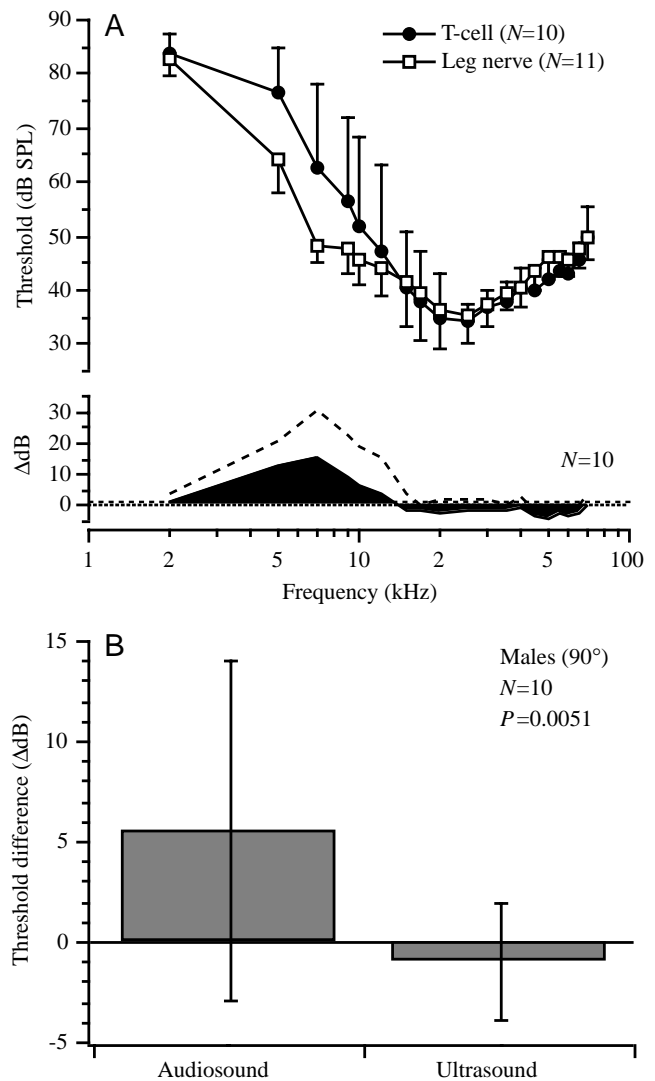


Fig. 5. Peripheral versus central tuning in adult *Neoconocephalus ensiger*. (A) Mean  $\pm$  s.d. T-cell thresholds compared with the tuning of the whole auditory organ (tympanal leg nerve) in the same animals (loudspeaker position  $90^\circ$ ). The shaded region and dashed line represent the mean  $+1$  s.d. threshold difference ( $\Delta$ dB) function, respectively. (B) Mean  $\pm$  s.d. T-cell minus leg nerve threshold difference ( $\Delta$ dB) for pooled thresholds from the audiosound and ultrasound spectral bands ( $N=10$ , paired design).

stimulating with pulses of 5 kHz, and this can be seen by the displaced intensity/response curves in Fig. 6C,D. While the latency curves at 10 kHz and 15 kHz are more like the curves for ultrasonic stimulation, close inspection reveals that T-cell latencies are consistently shorter at 20, 30 and 40 kHz over the majority of sound pressure levels tested. Finally, spike latencies measured at  $90^\circ$  are slightly shorter than those measured at  $0^\circ$ , especially at low-to-moderate sound pressure levels (compare Fig. 6C and 6D).

#### Temporal summation/integration

When plotted on a logarithmic scale, T-cell thresholds decrease more-or-less linearly with increasing pulse duration,

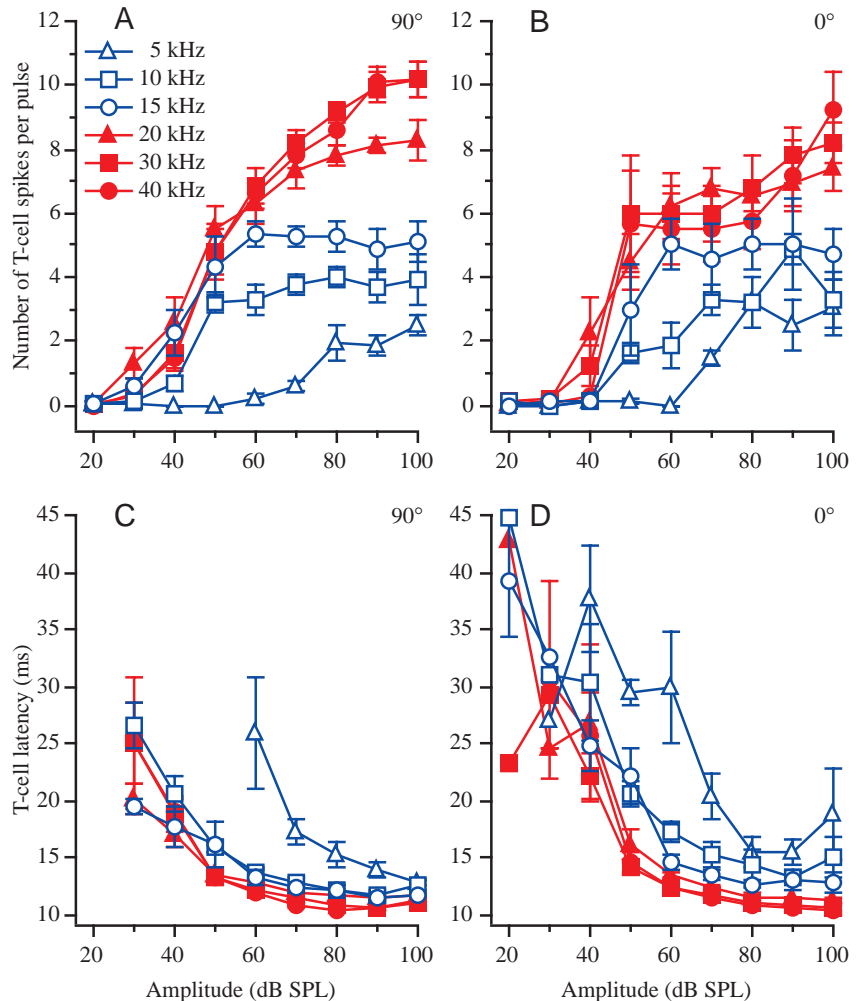


Fig. 6. Mean  $\pm$  S.E.M. T-cell intensity/response functions. Number of T-cell spikes per stimulus pulse as a function of stimulus amplitude (A) with the loudspeaker positioned at  $90^\circ$  ( $N=4-13$ ) and (B) with the loudspeaker positioned at  $0^\circ$  ( $N=4$ ). Latency to the first T-cell spike as a function of stimulus amplitude (C) with the loudspeaker positioned at  $90^\circ$  ( $N=2-13$ ) and (D) with the loudspeaker positioned at  $0^\circ$  ( $N=1-4$ ). Audiosound frequencies (5, 10 and 15 kHz) are shown with open symbols and blue lines; ultrasound frequencies (20, 30 and 40 kHz) are shown with filled symbols and red lines.

but only for durations from 0.1 to 10 ms; beyond this, further increases in stimulus duration result in little or no change in T-cell threshold (Fig. 7). For pulse durations of less than 10 ms, T-cell thresholds measured at 15 kHz are consistently higher than thresholds measured at 40 kHz (note that all thresholds were normalized relative to the lowest dB value for each animal, independent of carrier frequency). The rate of threshold decay also depends on carrier frequency. At 15 kHz, T-cell thresholds decline by  $-2.7$  dB per doubling of duration ( $-9.1$  dB per decade), which is very close to the theoretical rate of decay of  $-3.0$  dB per doubling of duration ( $-10.0$  dB per decade) predicted by the Plomp and Bouman (1959) equation (see Materials and methods). At 40 kHz, threshold changes are shallower, declining by only  $-2.0$  dB per doubling of duration ( $-6.5$  dB per decade), which results in a poorer fit to the model. Nevertheless, using a least-squares fit to the data, the integration time constant ( $\tau$ ) of the T-cell calculated at 15 kHz ( $\tau=15$  ms) is more than twice that at 40 kHz ( $\tau=6$  ms).

#### Short-term temporal pattern coding

Example spike volleys from the T-cell of a male katydid elicited in response to 15 kHz and 40 kHz pulse trains presented

at 5, 20 and 30 Hz are shown in Fig. 8 (amplitude  $90$  dB SPL; loudspeaker position  $90^\circ$ ; 25 pulses per presentation). As observed in the intensity/response plots (Fig. 6), T-cell spiking at 40 kHz is stronger (i.e. more spikes per pulse) than at 15 kHz. Temporal pattern-copying is also stronger at 40 kHz. At low pulse repetition rates (e.g. 5 Hz), T-cell pulse synchronization indices (SI) at 15 kHz and 40 kHz are identical (SI=1.0). However, when the repetition rate increases to 20 Hz, the T-cell is able to follow only approximately half of the 15 kHz stimulus pulses (SI=0.52), whereas at 40 kHz the entire pulse train is faithfully copied (SI=1.0). Increasing the repetition rate to 30 Hz results in even larger temporal pattern-copying differences: at 15 kHz, T-cell responses adapt and only the first few pulses in the stimulus train are therefore encoded (SI=0.16), whereas at 40 kHz spike adaptation is not manifest (SI=1.0).

The results from all the katydids used in the short-term temporal pattern-copying experiment (13 males, three females) are summarized in Fig. 9. Each panel shows the mean SI at 15 kHz and 40 kHz as a function of stimulus repetition rate and sound pressure level with the loudspeaker positioned at  $90^\circ$ . The SI functions at both carrier frequencies have a distinct low-pass characteristic, declining with increasing pulse repetition

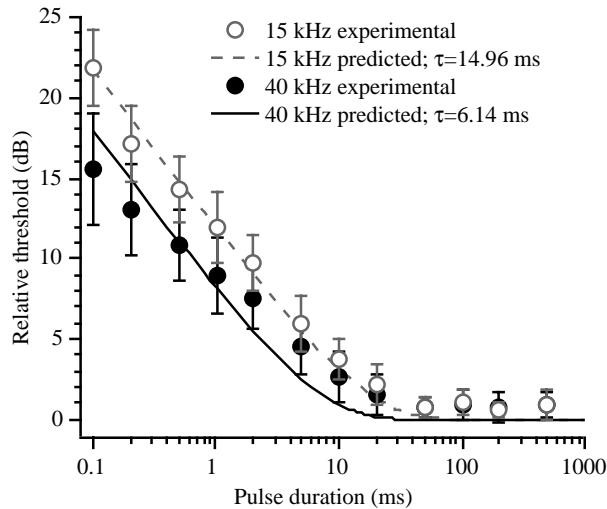


Fig. 7. T-cell temporal integration/summation. The mean  $\pm$  S.D. relative T-cell thresholds to pure-tone pulses of variable duration (50  $\mu$ s linear rise/fall time) are shown at two behaviorally relevant carrier frequencies: 15 kHz (open circles) and 40 kHz (filled circles). The inset shows the calculated (least-squares fit) integration time constant ( $\tau$ ) of the T-cell at 15 kHz and 40 kHz using the Plomp and Bouman (1959) leaky integrator energy detector model with an exponential decay (see Materials and methods). The lines show the theoretical threshold changes predicted by the model using the calculated  $\tau$  at 15 kHz (dashed line) and 40 kHz (solid line).

rate. Note that the temporal resolution of the T-cell is markedly better at 40 kHz than at 15 kHz, particularly at moderate-to-loud sound pressure levels (Fig. 9). This is despite the fact that T-cell thresholds at 40 kHz were slightly, but significantly, higher than at 15 kHz for the katydids used in this experiment (threshold at 15 kHz,  $36.8 \pm 6.8$  dB SPL; threshold at 40 kHz,  $40.5 \pm 4.8$  dB SPL; means  $\pm$  S.D.,  $t = -3.620$ , d.f. = 14,  $P = 0.0335$ ). Stimulating the T-cell with 40 kHz pulses at 90 dB SPL results in strong temporal pattern-copying at all but the fastest modulation rates. By comparison, temporal copying of 15 kHz pulses at 90 dB is significantly worse. At 70 dB SPL, the temporal copying response of the T-cell at 40 kHz drops slightly relative to its performance at 90 dB, but it is still superior to temporal coding measured at 15 kHz at either 70 dB or 90 dB SPL. At 50 dB SPL, the SI functions at 15 kHz and 40 kHz converge.

Fig. 10 shows SI functions obtained when the temporal pattern-copying experiment was repeated with the loudspeaker positioned at  $0^\circ$  (12 males, two females; three of the animals were also tested at  $90^\circ$ ). The overall low-pass pattern is similar to the results presented in Fig. 9, although SI functions realized at  $0^\circ$  are shifted leftwards (i.e. to lower modulation rates) relative to the same functions measured at  $90^\circ$  (compare Figs 9 and 10). Nevertheless, temporal copying at 40 kHz is still superior to that at 15 kHz. Moreover, T-cell thresholds measured with 40 kHz pulses at  $0^\circ$  were not significantly different from thresholds measured at 15 kHz in the same katydids (threshold at 15 kHz,

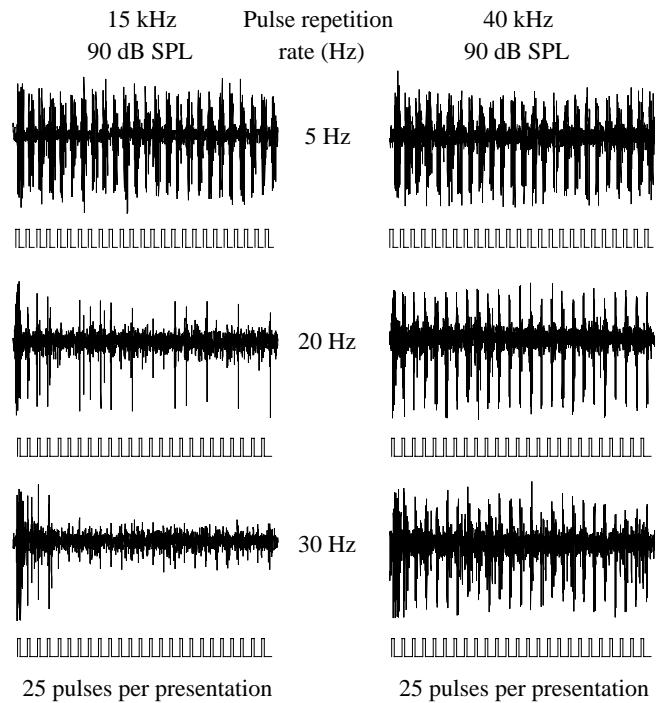


Fig. 8. Example extracellular response traces from the T-cell of *Neoconocephalus ensiger* 174 (an adult male) in the short-term temporal pattern-copying experiment (pulse duration 10 ms; amplitude 90 dB SPL; loudspeaker position  $90^\circ$ ). The T-cell reliably encodes the temporal pattern of the stimulus at each repetition rate when stimulated with pulses of 40 kHz bat-like ultrasound, but not when stimulated with a 15 kHz conspecific song peak frequency mimic. Note that time is not to scale: each trace shows the spiking response of the T-cell to 25 stimulus presentations.

$47.6 \pm 8.3$  dB SPL; threshold at 40 kHz,  $44.7 \pm 3.5$  dB SPL; means  $\pm$  S.D.  $z = -1.049$ ,  $P = 0.2942$ ).

We quantitatively compared T-cell SI cut-off rates ( $R_c$ ) measured at  $90^\circ$  and at  $0^\circ$  in katydids tested at both 15 kHz and 40 kHz, and found that, irrespective of loudspeaker position,  $R_c$  values measured at 40 kHz were consistently higher than, often more than double,  $R_c$  values measured at 15 kHz, with the difference being statistically significant at moderate (70 dB) and loud (90 dB) sound pressure levels (Table 2). Note also that SI  $R_c$  values measured at  $90^\circ$  are larger than those at  $0^\circ$ . This was confirmed in a three-way ANOVA with  $R_c$  as the dependent variable and carrier frequency (15 or 40 kHz), loudspeaker position ( $90^\circ$  or  $0^\circ$ ) and sound pressure level (50, 70 or 90 dB SPL) as factors. When each factor was examined alone, SI  $R_c$  values were, on average, larger for stimulation with 40 kHz pulses ( $F = 65.892$ , d.f. = 1,  $P = 0.0001$ ), for stimulation with the loudspeaker positioned at  $90^\circ$  ( $F = 25.623$ , d.f. = 1,  $P = 0.0001$ ) and for higher sound pressure levels ( $F = 36.703$ , d.f. = 2,  $P = 0.0001$ ). There was also a significant frequency  $\times$  sound pressure level two-way interaction ( $F = 18.844$ , d.f. = 2,  $P = 0.0001$ ), with the difference in  $R_c$  values between 15 kHz and 40 kHz being greatest at 90 dB SPL (Figs 9, 10). However, none of the remaining interaction effects, frequency  $\times$  loudspeaker position

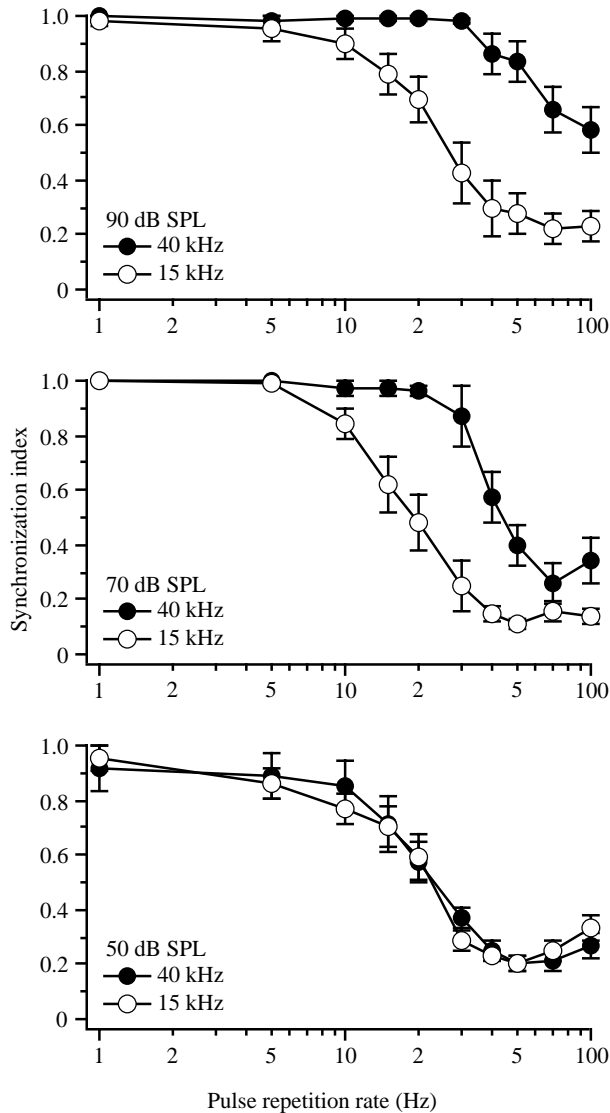


Fig. 9. Summary data for the short-term temporal pattern-following experiment with the loudspeaker positioned at  $90^\circ$ . The mean  $\pm$  S.E.M. pulse synchronization indices (SI) are shown as a function of pulse repetition rate and sound pressure level ( $N=11$  at 50, 70 and 90 dB SPL). The SI is the proportion of stimulus pulses encoded by one or more T-cell spikes.

( $F=0.593$ , d.f.=1,  $P=0.4427$ ), loudspeaker position  $\times$  sound pressure level ( $F=0.708$ , d.f.=2,  $P=0.4946$ ) and frequency  $\times$  loudspeaker position  $\times$  sound pressure level ( $F=1.069$ , d.f.=2,  $P=0.3469$ ), was significant.

### Discussion

The katydid T-cell (T large fiber or TN1) was discovered almost 40 years ago yet, despite its longevity and prominence in the literature on insect hearing, a surprising number of basic questions remain unanswered. Indeed, until recently, we still lacked an understanding of T-cell terminal projections in the brain and lower ganglia (for a complete morphological

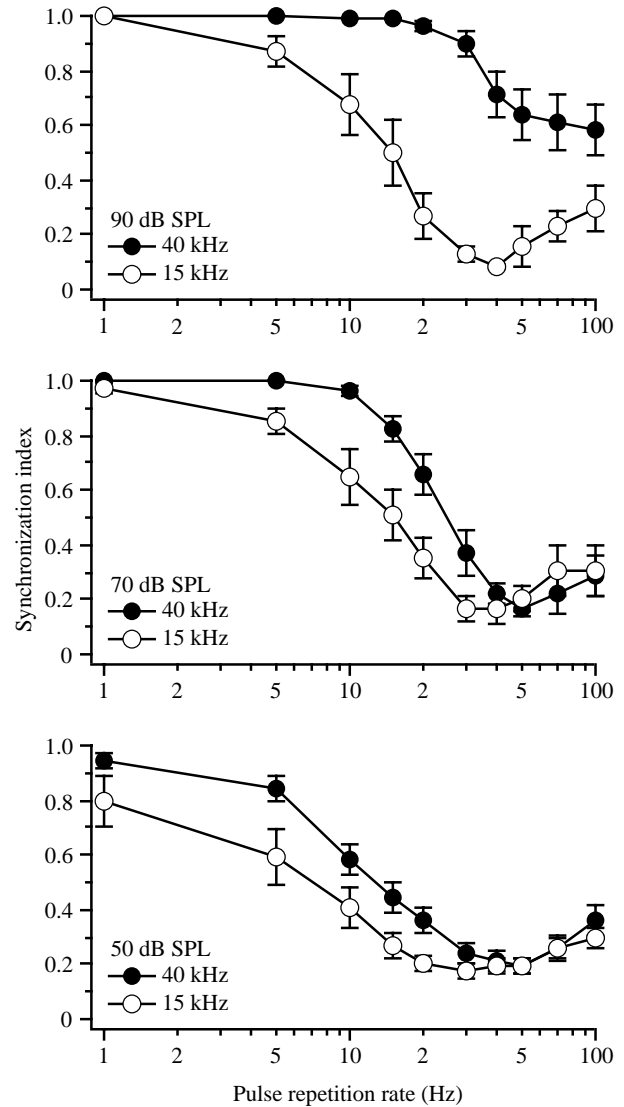


Fig. 10. Summary data for the short-term temporal pattern-following experiment with the loudspeaker positioned at  $0^\circ$ . The mean  $\pm$  S.E.M. pulse synchronization indices (SI) are shown as a function of pulse repetition rate and sound pressure level ( $N=14$  at 50 dB SPL;  $N=11$  at 70 and 90 dB SPL). The SI is the proportion of stimulus pulses encoded by one or more T-cell spikes. Note how SI functions measured at  $0^\circ$  are shifted to the left compared with the same functions measured at  $90^\circ$  (see Fig. 9).

staining, see Stumpner, 1999). We know that auditory activity in the ipsilateral tympanal organ has a strong inhibitory influence on contralateral T-cell spiking (Suga and Katsuki, 1961a,b; McKay, 1969; Rheinlaender et al., 1972) and that the T-cell is directionally sensitive (Suga, 1963; Rheinlaender and Römer, 1980; Rheinlaender et al., 1986), presumably as a result of side-to-side inhibitory interactions mediated by local (intraganglionic) interneurons (e.g.  $\Omega$ -neuron or ONs; Selverston et al., 1985; Schul, 1997). These findings have led some researchers to interpret their experimental results and/or employ stimulus paradigms that assume or infer that the

Table 2. Mean synchronization index (SI) cut-off rates ( $R_c$ ) at 15 kHz and 40 kHz in the temporal pattern-copying experiment

| Amplitude (dB SPL) | Speaker position (degrees) | N  | SI $R_c$ (Hz) |        | P-value* |
|--------------------|----------------------------|----|---------------|--------|----------|
|                    |                            |    | 15 kHz        | 40 kHz |          |
| 90                 | 90                         | 8  | 29±17         | 71±24  | 0.0027   |
| 70                 | 90                         | 10 | 20±10         | 42±12  | 0.0002   |
| 50                 | 90                         | 10 | 22±08         | 24±08  | 0.5513   |
| 90                 | 0                          | 7  | 12±07         | 55±26  | 0.0180   |
| 70                 | 0                          | 11 | 17±12         | 26±07  | 0.0368   |
| 50                 | 0                          | 9  | 10±04         | 14±04  | 0.0937   |

N, number of adult katydids tested.  
\*Paired *t*-test or Wilcoxon signed rank test comparing 15 and 40 kHz.  
Values are means ± s.d.

primary function of the katydid T-cell is in the detection and localization (lateralization) of conspecifics (e.g. Suga and Katsuki, 1961a; Suga, 1963; Rheinlaender et al., 1986; Rheinlaender and Römer, 1980, 1986; Schul, 1997).

However, evidence presented in this paper and Faure and Hoy (2000c) suggests that this assumption may be unwarranted because the physiology of the T-cell in *Neoconocephalus ensiger* is distinctly biased towards ultrasonic frequencies. Tuning curves in adults and non-volant nymphs are comparable in overall sensitivity (Fig. 4); in adults, the T-cell is more narrowly tuned than the whole auditory (tympanic) organ, a difference due to higher T-cell thresholds at audio frequencies (Fig. 5); the T-cell shows stronger spiking responses, a wider dynamic range and responds with shorter latencies to ultrasonic frequencies (Fig. 6); and, in addition, the integration time of the T-cell is shorter (Fig. 7) and its temporal resolution is sharper when stimulated with pulsed ultrasound (Figs 8–10). Combined with the large axonal diameter of the T-cell (i.e. giant fiber appearance, Kalmring et al., 1979; Rheinlaender and Römer, 1980, 1986; Römer et al., 1988; Schul, 1997), its sensitivity to ultrasound and equivocal spiking when stimulated with mate-calling song (Suga and Katsuki, 1961a; McKay, 1969; Zhantiev and Korsunovskaja, 1983; Faure and Hoy, 2000c), the data seem most consistent with a role in detecting and processing non-social acoustic signals (i.e. predator detection and escape).

#### T-cell tuning in adult males and females

The ears of katydids function to detect both the sounds of conspecifics (e.g. mate-calling song) and the sounds produced by predators (e.g. echolocating bats). The calling song of *N. ensiger* consists of a train of short-duration (30 ms), loud (>90 dB SPL at 10 cm), broadband (9–25 kHz, peak frequency approximately 14 kHz) syllables that are emitted continuously each evening (Faure and Hoy, 2000a). Our results show that the T-cell of *N. ensiger* is sensitive to a broad range of frequencies encompassing both conspecific song and predatory

ultrasound. Suga and Katsuki (1961a) found that directional coding by the T-cell in *Gampsocleis buergeri* was best for sounds centered near the peak spectral frequency of the calling song (approximately 17 kHz), which coincided with the best frequency range of the T-cell, and concluded that the T-cell was well adapted for detecting species-specific stridulatory sounds. But coincidences between the peak spectral frequency of conspecific song and the best frequency of the T-cell are less common in other studies. Indeed, Suga (1966) later found that only one of the four neotropical katydid species he studied had such a match. Libersat and Hoy (1991) reported that the best frequency of the T-cell in male *N. ensiger* was close to the peak spectral frequency of the calling song, but our more recent data show that this match is close only for females. Bailey and Römer (1991) observed a large mismatch between the peak spectral frequency of the calling song (50 kHz) and the best frequency of the T-cell (35 kHz in both males and females) in the Australian zaprochiline tettigoniid *Kawanaphila nartee*.

Although no difference was found in the breadth of tuning between male and female *N. ensiger*, on average, the best threshold of the T-cell was significantly lower in females by approximately 5 dB and the best frequency was significantly lower by approximately 5 kHz (Table 1; Fig. 3; Faure and Hoy, 2000b). This suggests that the T-cell in females will respond better to playbacks of conspecific song than in males. This hypothesis was tested in an acoustic playback experiment, the results of which are reported by Faure and Hoy (2000c). Only one previous study has reported a sex difference in T-cell tuning, but to be fair it seems never to have been rigorously investigated. Bailey and Römer (1991) observed that T-fiber thresholds in male *K. nartee* were 10 dB higher than in females, but reported no differences in superthreshold response physiology. Male *K. nartee* lack auditory bulla and have smaller spiracular openings (Bailey and Römer, 1991), so anatomical differences in the acoustic tracheal apparatus probably account for the lower sensitivity of the male. Suga (1966) reported no difference in T-cell sensitivity between males and females in a study of four species of neotropical katydid, but furnished no data backing this claim. In a related study, Counter and Henke (1977) found no difference in the tuning of the tympanal organ between male and female *N. ensiger*, but again no data were provided.

As to the origin of the sex difference in T-cell tuning, we can only speculate. Male and female *N. ensiger* are dimorphic: in addition to possessing a long and conspicuous ovipositor (length 31.6±1.1 mm,  $N=23$ ; see also the cover of Dethier, 1992), females have longer wings and a significantly greater mass (female mass 0.747±0.165 g,  $N=30$ ; male mass 0.451±0.058 g,  $N=363$ ; means ± s.d.,  $z=-8.833$ ,  $P<0.0001$ ). Although we did not measure the cross-sectional area and length of the acoustic spiracles and auditory tracheae of *N. ensiger*, it would not be surprising to learn that both were larger in females (e.g. Heinrich et al., 1993). If true, then this should cause an increase in sensitivity at some frequencies (Bailey and Römer, 1991; Stumpner and Heller, 1992). In addition, because females are the phonotactic sex, their tympanal organs may

contain a higher proportion of auditory afferents tuned to male song. Alternatively, peripheral tuning may be similar in both sexes, but with females having more afferents synapsing on the T-cell that are tuned to male song. It is also possible that the number of afferents synapsing on the T-cell is the same, but with the synaptic efficacy differing between the sexes. Regardless of the mechanism, examination of the T-cell  $\Delta$ dB function of males and females shows that the maximum sensitivity difference occurs between 5 and 25 kHz (Fig. 3A), which coincides precisely with the range of frequencies dominating the spectrum of the song of *N. ensiger* (Gwynne, 1977; Faure and Hoy, 2000a).

#### *T-cell tuning in nymphs and adults*

Despite the small number of katydid nymphs tested, T-cell best frequencies, best thresholds and  $Q_{10dB}$  indices in juveniles were remarkably similar to values in adults. This is somewhat surprising because in katydid nymphs neither the prothoracic auditory spiracles, the major sound guide to the rear of the tympanum (Michelsen et al., 1994a), nor the tympanal slits had reached their final (adult) dimensions. However, it should be noted that, even though the auditory spiracles and tympanal slits were not yet fully developed, openings to these structures were present and unoccluded in all nymphs tested. In a related study, McKay (1969) reports that the ears of *Homorocoryphus* sp. (= *Ruspolia*, the closest relative of the genus *Neoconocephalus*) are functional from the fourth instar onwards. Unfortunately, data on the activity and ultrasound sensitivity of the T-cell in *Homorocoryphus* nymphs were not provided.

Because adult katydids are not present and singing during most of nymphal development, from a conspecific song processing point-of-view the development of acute ultrasonic hearing in *N. ensiger* nymphs is puzzling since there should be little selection pressure for the song recognition and phonotactic neural pathways to be functional until the adult molt (e.g. Feaver, 1983). In crickets, the neural circuits involved in song production are actively suppressed until nymphs complete their final molt, and the same is essentially true for the circuitry involved in flight, with fine tuning of the oscillatory network and flight pattern generators continuing into adulthood (Bentley and Hoy, 1970). Precocious ultrasound sensitivity in katydid nymphs is, however, consistent with the hypothesis that the T-cell functions in early-warning and escape behavior. Katydid nymphs remain flightless until after the adult molt, and thus are not subject to predation by aerially feeding bats, but terrestrial sources of predatory ultrasound abound and include the incidental sounds produced by approaching predators, the echolocation calls of substrate-gleaning bats and the active signals and/or vocalizations of non-volant mammalian predators such as rodents and shrews (e.g. Walker, 1964; Sales and Pye, 1974; Belwood, 1988). Furthermore, there are even carnivorous katydids with specialized diets and predatory lifestyles (e.g. Togashi, 1980; Kaltenbach, 1990; O'Donnell, 1993), while most other tettigoniids are opportunistic and will take insect prey when available (D. Gwynne, personal communication).

There is reason to speculate that audition in the Ensifera may have evolved before intraspecific sound-signaling systems were predominant, in which case the primitive auditory system from which modern hearing evolved may have originally served a role in predator detection (Bailey, 1990). A similar hypothesis has been advanced to explain the maintenance of hearing and the evolution of intraspecific communication *via* sexual selection in some diurnal moths (e.g. Surlykke and Fullard, 1989). If true, this could account for the presence of acute ultrasound hearing in katydid nymphs.

#### *Leg nerve versus T-cell tuning*

Using a paired design, we found that the tuning of the T-cell was narrower than that of the tympanic organ, but only for audio frequencies; at stimulus frequencies of 15 kHz and above, there was no difference in auditory thresholds (Fig. 5). This supports previous observations by Suga and Katsuki (1961a), who reported that the response range of the T large fiber in *Gampsocleis buergeri* was narrower than that of the whole auditory organ, although in their case the stricture in tuning was not specific to audio frequencies. Counter and Henke (1977) measured the sensitivity of the tympanic organ in male ( $N=6$ ) and female ( $N=4$ ) *N. ensiger* and found that its best frequency was between 8 and 13 kHz, which is considerably lower than our measurements (Fig. 5). A possible explanation for this discrepancy is that we used signal averaging to determine leg nerve summed action potential thresholds, which undoubtedly yielded a better resolution than was possible in the study of Counter and Henke (1977).

The data suggest four possible hypotheses (non-exclusive) to account for the observed results. (i) The greater sensitivity of the leg nerve to audiosound frequencies may result from significant input by low-frequency receptors present in the subgenual and intermediate organs (Kalmring et al., 1978, 1990; Lakes and Schikorski, 1990). (ii) Primary receptors may not synapse on T-cell dendrites in direct proportion to their occurrence in the tympanic organ (i.e. the distribution of receptor inputs to the T-cell from the low- and high-frequency afferent populations is not 1:1, but is biased towards higher frequencies). (iii) Alternatively, receptor inputs may be in direct proportion to their occurrence in the tympanic organ, but with low-frequency afferents providing less synaptic drive or efficacy (i.e. smaller postsynaptic potentials). (iv) T-cell responses may be inhibited by low frequencies. As yet, we have no evidence to support the three former hypotheses, but results from this and our companion paper (Faure and Hoy, 2000c) are suggestive of the latter (e.g. input/output curves, temporal summation and temporal pattern-copying experiments, acoustic playback with conspecific and predatory signals). Also, Zhantiev and Korsunovskaja (1983) report that the T-cell in *Tettigonia cantans* is inhibited by frequencies below 10 kHz.

#### *Intensity/response functions*

The spike-amplitude and latency-amplitude input/output functions of the T-cell show ultrasound biases. Stimulation with audiosound produced substantially weaker, and in some cases

reduced (inhibited?), T-cell spiking, whereas responses to ultrasound were robust, resulting in steep increases in spike number and shorter latencies with higher sound pressure levels (Fig. 6). Suga and Katsuki (1961a) reported an almost monotonic increase in T-cell spiking in *G. buergeri* with increasing sound pressure level at 17 kHz; unfortunately, no other frequencies were tested. Libersat and Hoy (1991) report that, in *N. ensiger*, the number of T-cell spikes in response to 30 kHz pulses increased by more than twofold compared with stimulation with 15 kHz tones (both at 85 dB SPL). An increase in spike number and decrease in spike latency with increasing stimulus amplitude is typical for any sensory system, and in the case of insect hearing this phenomenon has been known for some time (e.g. Roeder, 1966; Mörchen et al., 1978; Rheinlaender and Mörchen, 1979; Mörchen, 1980). The input/output curves in Fig. 6 were specifically plotted as isointensity rather than isothreshold (i.e. dB above threshold) because this experiment was designed to examine non-adapting T-cell responses to sounds presented at biologically realistic amplitudes. Nevertheless, when plotted as isothreshold, T-cell responses to ultrasound stimuli are still superior to responses evoked by audio frequencies. A similar result was reported for the  $\Omega$ -neuron in field crickets (Pollack, 1994).

There are at least three mechanisms (non-exclusive) that could account for the input/output functions of Fig. 6: (i) the T-cell may receive centrally mediated inhibition from neurons primarily responsive to audio frequencies (e.g. ascending neuron 1 or AN1; Schul, 1997); (ii) the T-cell may receive a higher proportion of auditory afferents tuned to ultrasound than audiosound frequencies; and (iii) the synaptic efficacy of auditory afferents tuned to ultrasound frequencies may be more potent than that of audiosound afferents (see Pollack, 1994). In determining which of these hypotheses is correct, future researchers may wish to explore the physiology of the T-cell using two-tone stimulation. If the T-cell functions in a manner similar to the bat-detecting interneuron-1 (Int-1=AN2) of flying field crickets, then such experiments may find that ultrasound-induced excitation is suppressed (inhibited) during the simultaneous presentation of a low-frequency tone (e.g. Nolen and Hoy, 1986). Low-frequency two-tone suppression was observed in the T-neuron of the Australian katydid *Caedicia simplex* (Oldfield and Hill, 1983); unfortunately, ultrasonic stimuli were rarely employed as the excitatory stimulus.

Realizing that intensity coding in central auditory neurons is more complex than in primary afferents, and assuming that intensity information is processed separately for different frequencies, Rheinlaender (1975) discussed three classes of intensity/response coding for interneurons in the ventral nerve cord of the katydid *Decticus verrucivorus*: (i) monotonically increasing input/output functions covering a wide dynamic range, (ii) shallow input/output functions covering a small dynamic range and with responses that saturate at relatively low sound pressure levels, and (iii) bell-shaped input/output curves with band-pass characteristics for reporting preferred stimulus sound pressure levels. Our data on the T-cell of *N. ensiger* show that a single neuron can have multiple intensity

coding characteristics, depending on stimulus frequency, in which case the utility of distinguishing input/output response classes becomes less clear.

#### *Temporal summation/integration*

It is well known that, for short-duration acoustic stimuli, auditory thresholds decrease as the duration of the stimulus is lengthened, but only until reaching a certain duration beyond which further increases in pulse length have little or no effect on auditory thresholds. This duration is known as the integration time ( $\tau$ ; Plomp and Bouman, 1959). For stimulus durations much shorter than  $\tau$ , the product of duration and intensity at threshold remains nearly constant (i.e. intensity/duration trading), indicating that auditory thresholds are of constant energy (Tougaard, 1996).

Unlike vertebrate audition, in which studies of temporal integration are common, particularly at higher levels in the central auditory pathway, there have been fewer studies of temporal integration in insects. Using laser vibrometry and three different stimulus paradigms (pure tones, noise and broadband clicks), Schiolten et al. (1981) measured the mechanical time constant ( $\tau$ ) of the tympanum in moths as 60  $\mu$ s and that in locusts as 90  $\mu$ s. Using variable-duration pure tones and employing an Urkowitz energy detector model (Au, 1988), Surlykke et al. (1988) estimated the integration time ( $\tau$ ) of the noctuid moth A1 auditory receptor to be 25 ms. Later Tougaard (1996), also working on the moth A1 receptor, employed a double-click paradigm and estimated a value of  $\tau=4$  ms by fitting the data to the leaky integrator model of Plomp and Bouman (1959) with an exponential decay [note that Tougaard also used this model to re-estimate the integration time of Surlykke et al. (1988) as  $\tau=9.8$  ms]. In all the insect studies discussed above,  $\tau$  has been measured only at the auditory periphery. This is not inappropriate because, as Tougaard (1996) clearly points out, the basic limitations for any sensory system in stimulus encoding and temporal feature extraction will originate at the receptor level, thus establishing a clear need for measuring temporal integration and resolution in primary afferents. However, given the importance of temporal information to the survival (e.g. Fullard et al., 1994) and reproduction (e.g. Schildberger, 1984) of tympanate insects, as well as most other hearing organisms, it seems equally important to measure temporal integration at deeper levels in the central auditory pathway to determine how information coding varies with different categories of biological signals between the sensory afferents and motor efferents.

In the case of the T-cell of *N. ensiger*, the time constant measured at 40 kHz ( $\tau=6$  ms) was less than half that measured with 15 kHz pulses ( $\tau=15$  ms), which also demonstrates that the physiology of the T-cell interneuron is ultrasound-biased (Fig. 7). At present, it is difficult to speculate how or where in the auditory system the difference in  $\tau$  arises because similar experiments were not conducted at the auditory periphery using whole-leg nerve summed action potential thresholds. Indeed, it would be interesting to see whether  $\tau$  continues to increase when the T-cell is stimulated with even lower carrier frequencies (e.g. 5 kHz and 10 kHz). Also, because the T-cell is most sensitive to

short-duration (transient) signals and does not respond well to stimulation with long tones (Rheinlaender et al., 1972; P. A. Faure and R. R. Hoy, unpublished observations), it would be worthwhile to re-estimate the integration time of the T-cell using a double-click paradigm to avoid confounding influences associated with sensory adaptation and/or probability integration (Tougaard, 1996, 1998).

#### Short-term temporal pattern coding

T-cell SI functions revealed strong temporal copying over a wide range of repetition rates when the neuron was stimulated with 40 kHz pulses, a typical bat echolocation frequency, but not with 15 kHz pulses, our mimic of the peak spectral frequency of the calling song of *N. ensiger* (Figs 8–10). In this respect, the katydid T-cell is similar to the bat-detecting Int-1 of field crickets (*Teleogryllus oceanicus*), which does not reliably encode the temporal pattern of calling song stimuli presented at fast repetition rates (e.g. Schildberger, 1985; Hennig, 1988). Unfortunately, the comparison between the T-cell and Int-1 is incomplete because these authors did not test temporal coding in Int-1 with pulsed ultrasound; however, it is known that Int-1 responds well to ultrasonic pulse trains (R. R. Hoy, unpublished observations).

Synchronization index (SI) cut-off rates at 40 kHz were significantly greater than at 15 kHz at all but the lowest sound pressure levels tested (Table 2). These findings are consistent with the results from our intensity/response (Fig. 6) and temporal summation experiments (Fig. 7) and reinforce the idea that the physiology of the T-cell is ultrasound-biased. In many respects, our SI functions resemble the temporal modulation transfer functions (TMTFs) extensively used in the study of mammalian hearing (e.g. Viemeister, 1979). An important difference between the two is the nature of the stimulus: TMTFs routinely employ sinusoidally amplitude-modulated (SAM) stimuli, whereas T-cell SI functions were measured with trapezoidally amplitude-modulated (TAM) signals (Lesser et al., 1990). We found TAM signals necessary for measuring temporal pattern-copying in *N. ensiger* because preliminary results with SAM stimuli (100% modulation depth) resulted in poor T-cell spiking, except at the fastest modulation rates. This suggests that, in addition to pulses of short duration (Rheinlaender et al., 1972), the T-cell also prefers signals with rapid onsets and offsets.

Reduced temporal synchronization in response to sound pulses centered near the peak spectral frequency of the calling song of *N. ensiger* might be expected if the T-cell was simply less sensitive (with respect to threshold) at 15 kHz than at 40 kHz. To address this possibility, we measured T-cell thresholds at 90° at both carrier frequencies and indeed found a significant difference, but in the direction opposite to that predicted by our results: T-cell thresholds at 40 kHz were significantly higher than thresholds at 15 kHz; thus, sensitivity differences cannot explain the relatively poor performance of the T-cell in encoding 15 kHz *versus* 40 kHz pulse trains (Fig. 9).

Another possible explanation for the difference in temporal pattern synchronization is based on differential levels of

peripherally generated central inhibition originating from the contralateral ear because of the decreased diffraction of sound at 15 kHz relative to 40 kHz. The temporal pattern-copying experiment was originally conducted with the loudspeaker positioned at 90°, so the contralateral ear and auditory spiracle were sound-shadowed by the body of the katydid. Because diffraction increases at frequencies with wavelengths shorter than the size of the diffracting body, diffraction effects are most prominent for ultrasonic frequencies (Michelsen and Nocke, 1974; Michelsen, 1992). The wavelength of 15 kHz pulses in air (22.93 mm) is 2.67 times greater than that of 40 kHz pulses (8.6 mm), and the diameter of the body of *N. ensiger* is 5–7 mm. Therefore, stimulating the ipsilateral T-cell with 40 kHz pulses at 90° should result in reduced auditory input at the contralateral ear relative to stimulation at 15 kHz. Because auditory input at the ipsilateral ear has a strong inhibitory influence on contralateral T-cell spiking (Suga and Katsuki, 1961a,b), poorer temporal copying at 15 kHz relative to 40 kHz may not be surprising if differential levels of contralateral inhibition were present simply on the basis of physical acoustic effects alone.

However, unequal diffraction and dissimilar levels of peripherally generated contralateral inhibition cannot explain the difference in temporal copying at 15 kHz *versus* 40 kHz in the short-term temporal pattern-copying experiment at 90°. This is because, when we repeated the experiment with the loudspeaker positioned at 0°, now with both tympanal organs and T-cells receiving more-or-less equal sound pressure levels (see Fig. 1 in Faure and Hoy, 2000b), temporal copying differences at 15 kHz and 40 kHz were maintained (Fig. 10). Moreover, T-cell thresholds measured at 0° for 15 kHz and 40 kHz pulses were not significantly different, so once again sensitivity differences cannot explain the observed effect. This is not to say that increased levels of binaural inhibition at 15 kHz relative to 40 kHz did not influence T-cell responses at 90° *per se*, merely that such effects cannot account fully for the pattern of variation observed. This conclusion is supported by our ANOVA results: carrier frequency, sound pressure level and loudspeaker position were all significant main factors, but none of the interaction effects with loudspeaker position was statistically significant. That is, even though SI  $R_c$  values were larger (i) at 90° than at 0°, (ii) at 40 kHz than at 15 kHz, and (iii) for higher sound pressure levels, with the difference in  $R_c$  between 15 kHz and 40 kHz being greatest at 90 dB SPL, no evidence exists to support the conclusion that the difference in  $R_c$  between 15 kHz and 40 kHz changed with loudspeaker position. So, although  $R_c$  decreased when the loudspeaker moved from 90° to 0° (compare the leftward-shifted SI functions in Fig. 10 with Fig. 9), probably as a result of increased levels of binaural inhibition at 0°, the pattern of change was similar for both frequencies.

Echolocating bats increase their pulse repetition rate from 5 to 20 Hz during the search phase, from 20 to 50 Hz during the approach phase, and from 50 to more than 100 Hz during the terminal phase of an attack sequence (Kalko, 1995). Because bats also decrease their call intensity during an

approach (Kick and Simmons, 1984), pulse amplitude becomes an ambiguous cue as to the proximity of an approaching bat. The pulse repetition rate is, however, a reliable indicator of time to attack (Fullard, 1984; Fullard et al., 1994). Thus, the high sensitivity, short latency and strong spiking responses of the T-cell to pulses of ultrasound make it ideally suited for encoding information relevant to predator detection and escape; however, the exact nature of the involvement of the T-cell in such behaviors is still unknown. It is possible that T-cell spikes arouse higher control centers in the brain while simultaneously priming motoneurons in the meso- and metathoracic ganglia that initiate and coordinate the powerful flight and jumping muscles used in rapid escape. In the following paper (Faure and Hoy, 2000c), we use playbacks of conspecific song and pulses of bat-like ultrasound (i.e. frequency-modulated sweeps) to probe further the neuroethology and physiological response properties of the katydid T-cell.

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