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NEUROETHOLOGY OF THE KATYDID T-CELL

II. RESPONSES TO ACOUSTIC PLAYBACK OF CONSPECIFIC AND PREDATORY SIGNALS

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Summary

Although early work on the tettigoniid T large fiber suggested that it might mediate early-warning and escape behavior in katydids, the majority of research thereafter has focused on the ability of the T-cell to detect, localize and/or discriminate mate-calling song. Interestingly, T-cell responses to conspecific song are rarely examined for more than a few seconds, despite the fact that many katydids sing for minutes or hours at a time. In this paper, the second of a pair examining the physiology of the T-cell in *Neoconocephalus ensiger*, we recorded T-cell responses using longer-duration playbacks (3 min) of conspecific song (Katydid signal 30 ms syllables, 9–25 kHz bandwidth, 12–15 kHz peak frequency) and two types of bat-like ultrasound, a 10 ms, 80→30 kHz frequency-modulated sweep (Bat 10 signal) and a 30 ms, 80→30 kHz frequency-modulated sweep (Bat 30 signal). Spiking responses were distinctly biased towards the short-duration ultrasonic signal, with more spikes per pulse, at a shorter spike latency and at a higher instantaneous firing frequency to the Bat 10 signal than to the Katydid signal or, surprisingly, to the Bat 30 signal. The ability of the T-cell to encode the temporal pattern of the stimulus was particularly striking. Only for the predatory bat signals did T-cell spiking faithfully copy the stimulus; playbacks of conspecific song resulted in significantly weaker spiking responses, particularly in male katydids. The results demonstrate that responses from the T-cell alone may be sufficient for katydids to discriminate biologically relevant signals pertinent to the phonotactic behavior patterns involved in mate attraction and predator avoidance.

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

Introduction

As in vertebrates, hearing in the acoustic Orthoptera mediates not only the localization of singing conspecifics (e.g. mate attraction), but also serves for the detection of predators. For many insects in the family Tettigoniidae (katydids, bushcrickets or long-horn grasshoppers), the species-specific mate-calling song is broadband and contains both audio (<20 kHz) and ultrasonic (≥20 kHz) frequencies (e.g. Keuper et al., 1988; Belwood, 1990). Many katydids are active (e.g. sing, mate, disperse) and fly only at night and, like any night-flying insects, they risk predation by aerial-feeding echolocating bats, which also emit ultrasound for orientation and prey detection (e.g. Barclay, 1986). Moreover, even stationary singing katydids must detect and localize terrestrial sources of predatory ultrasound, either active signals or vocalizations (e.g. shrews, mice, substrate-gleaning bats; Sales and Pye, 1974; Belwood and Morris, 1987) or the incidental sounds produced by predators moving through the environment.

A basic task of the katydid auditory system is, therefore, to distinguish among classes of ultrasound so that the appropriate behavior patterns relating to mate attraction (positive phonotaxis) and predator avoidance (negative phonotaxis) are reliably performed, a fundamental question of perception and categorization. The use of ultrasound in two seemingly disparate behavioral contexts, intraspecific communication and predator detection, is not incongruous. Indeed, the calling songs of some katydids are purely ultrasonic (e.g. Mason et al., 1991; Morris et al., 1994), so distinguishing behaviorally relevant sources of ultrasound is probably routine for many species. Similarly, the neural mechanisms for categorizing ultrasound are probably also widespread within the Tettigoniidae.

The eastern sword-bearer conehead katydid *Neoconocephalus ensiger* Harris (Orthoptera: Tettigoniidae) is a common nocturnal singing insect found throughout the mid- and northeastern United States and southern Ontario, Canada (Gwynne, 1977; Shaw et al., 1982). Its calling song is broadband and contains both audible and ultrasonic frequencies (Faure and Hoy, 2000a). *Neoconocephalus ensiger* possess two types of acoustic startle responses: cessation of flight and cessation of song. When stimulated with pulses of bat-like ultrasound, flying *N. ensiger* alter their flight posture

and rapidly close all four wings, causing them to drop towards the ground (Libersat and Hoy, 1991), whereas stridulating males cease mate-calling or insert gaps (pauses) in their calling song (Faure and Hoy, 2000a). Both types of acoustic startle response confer survival by allowing katydids that perform these negative phonotactic behavioral responses to escape detection and thus evade acoustically orienting predators (for general reviews on insect acoustic startle responses, see Hoy, 1989, 1991, 1992, 1994).

Individual *N. ensiger* rarely perform an acoustic startle response when stimulated by pulses of conspecific song or a pure-tone mimic, but do so reliably when stimulated with pulsed ultrasound (Libersat and Hoy, 1991; Faure and Hoy, 2000a). *N. ensiger* is a good model system for studying the neural basis of sound categorization because, although there is overlap in the spectral and temporal features of conspecific song and predatory ultrasound, individuals reliably discriminate the two signal types. If, as previous authors have suggested (McKay, 1969, 1970), the pair of prothoracic T-cell interneurons (also known as the T large fibre or TN1) are involved in tettigoniid early-warning and escape behavior, then this still leaves a major question: can T-cell responses distinguish the same signal types? A similar question was raised by Nolen and Hoy (1984, 1986a,b) working on the interneuron-1 (Int-1)-mediated bat-avoidance response of flying field crickets (*Teleogryllus oceanicus*). Note that, for *N. ensiger*, behavioral context alone (i.e. flying *versus* standing or walking) is insufficient for gating T-cell responses to different neural networks controlling behavior (e.g. Ritzmann et al., 1980). This is because two types of acoustic startle response are present in *N. ensiger*, one that occurs in-flight, the other while singing on a substratum; thus, partitioning the world of sound into friendly and foe categories necessitates monitoring the concurrent activity of behaviorally specific neurons for the detection of conspecific song, such as ascending neuron 1 (AN1) (e.g. Schul, 1997).

Existing data on T-cell responses to conspecific song are equivocal. Specifically, contradictory evidence exists as to the ability of the T-cell to detect (respond) and follow (encode) the syllable pattern of mate-calling song. Published abilities range from little or no temporal coding (McKay, 1969), through some initial coding but with adapting responses (Kalmring et al., 1979; Zhantiev and Korsunovskaja, 1983), to a one-to-one correspondence with the pulsatory stridulatory sound (Suga and Katsuki, 1961; Suga, 1963) or the doublet syllable pulse (Schul, 1997). Unfortunately, T-cell responses in most of the above studies were monitored for only very brief periods (e.g. from a few to tens of seconds), yet in nature stridulating katydids sing for minutes or hours at a time.

In this paper, the second of a pair examining the physiology of the T-cell of *N. ensiger*, we use longer-duration (3 min) acoustic playback to investigate T-cell spiking when stimulating with syllables of conspecific song and pulses of bat-like frequency-modulated (FM) ultrasonic sweeps. The results demonstrate that the T-cell responses to short-duration bat-like frequency-modulated sweeps are distinctly superior to the responses evoked by the sounds of calling conspecifics. Although the T-cell in *N. ensiger* females more reliably encodes conspecific song than that in males, perhaps because of a difference in tuning in which females are more sensitive to the range of frequencies encompassing male calling song (Faure and Hoy, 2000c), nevertheless, in both sexes, the T-cell still responds best to stimulation with short-duration pulses of bat-like ultrasound. The ability of the T-cell to respond differentially, and thus to categorize conspecific and predatory signals, appears to be based on a combination of temporal and frequency tuning, as well as inhibition caused by stimulation with low-frequency audiosound.

Materials and methods

For details on the natural history and calling song of *Neoconocephalus ensiger* Harris, collecting katydids in the field, animal care, mounting and dissecting katydids for electrophysiology, the methods and apparatus used in extracellular recording, the history and identification of the prothoracic T-cell interneuron, and acoustic stimulation and calibration, see Faure and Hoy (2000a-c).

Acoustic playback stimuli

Experiments were conducted on 17 adult *N. ensiger* (13 males, four females); two katydids did not complete testing at all stimulus/amplitude combinations. Playback stimuli were recordings of conspecific song and digitally synthesized bat-like FM ultrasonic sweeps. All stimuli were controlled with a computer and array processor with A-to-D/D-to-A interface purchased from Tucker Davis Technologies (TDT: Apos II). The position of the loudspeaker was at 90 ° relative to the rostral–caudal body axis of the katydid.

The calling song of *N. ensiger* consists of a train of loud (93 dB sound pressure level, SPL, at 10 cm), short-duration (30 ms), broadband (–20 dB bandwidth approximately 15 kHz) syllables emitted at a rate of 5–15 Hz, depending on ambient temperature (Frings and Frings, 1957; Gwynne, 1977). The peak frequency of the calling song is 13.40±1.45 kHz (mean ± S.D., N=17), but appreciable energy extends into the ultrasonic spectrum (highest frequency 24.76±6.36 kHz; mean ± S.D., N=17). For a complete description of the mate-calling song of *N. ensiger*, see Faure and Hoy (2000a). The song from a single male with an excellent signal-to-noise ratio (Ne 173) was recorded with a Brüel & Kjær (B&K) type 4135 1/4 inch condenser microphone (flat ±3 dB from 20 Hz to 125 kHz), without protecting grid (diaphragm 0 ° incidence), coupled to a B&K type 2209 impulse precision sound level meter whose a.c. output was bandpass-filtered (1–60 kHz; Krohn-Hite, model 3550) prior to recording with a Racal Store 4DS instrumentation tape recorder operating at 19 cm s^{-1} (entire recording system flat ±3 dB from 300 Hz to 37.5 kHz). By feeding the output of the microphone into custom-built Schmitt trigger circuitry, the onset of individual syllables were recorded as TTL pulses. For acoustic playback, a 3 s segment of calling song was digitized (TDT: AD1, sampling rate

198 kHz), edited and stored in a buffer that was looped to provide a user-defined playback duration. Digitized song was converted to analog format (TDT: DA3-2) and low-pass-filtered prior to amplification (f_c=50 kHz; Krohn-Hite, model 3550).

Predatory bat sounds were synthesized using TDT hardware and custom-built software written by Timothy G. Forrest. The stimulus was a 10 ms, FM pulse that was swept downwards (linearly) from 80 to 30 kHz (rise/fall time 1 ms raised cosine). The duration and bandwidth were chosen to mimic typical search- and approach-phase biosonar pulses from North American species of aerial-hawking insectivorous bats (see Fenton and Bell, 1981; Simmons, 1987). The average syllable duration of the calling song of *N. ensiger* (henceforth referred to as the Katydid signal) is three times longer than the 10 ms, 80→30 kHz FM sweep (henceforth referred to as the Bat 10 signal), so we also generated a 30 ms, 80→30 kHz FM sweep (henceforth referred to as the Bat 30 signal) to control for the difference in pulse duration. Digitized FM pulses were converted to analog format (TDT: DA3-2) and band-pass-filtered (80–30 kHz; Krohn-Hite, model 3550) prior to amplification.

All playback signals were broadcast through a Panasonic EAS-10TH400B leaf tweeter, whose amplitude was adjusted using a programmable attenuator (TDT: PA4) and stereo amplifier (Nikko NA-790). Stimulus amplitudes, expressed in decibels sound pressure level (dB SPL rms re: 20 μPa), were measured with the type 2209 sound level meter (impulse mode, linear weighting network) fitted with either a type 4135 or type 4138 1/8 inch microphone (without protecting grid) and calibrated using a B&K type 4220 pistonphone (note that the rise time constant of the type 2209 sound level meter on impulse mode is 35 ms). Acoustic calibration was performed with the insect holder and any micromanipulators in place (for example calibration curves, see Faure and Hoy, 2000b). Each playback stimulus was presented at 50, 70 and 90 dB SPL.

To summarize, the stimuli used in the acoustic playback experiment were as follows: (i) Katydid signal, *N. ensiger* mate-calling song; (ii) Bat 10 signal, a 10 ms, 80→30 kHz FM sweep; and (iii) Bat 30 signal, a 30 ms, 80→30 kHz FM sweep.

In the previous paper (Faure and Hoy, 2000c), we showed that the ability of the T-cell to encode temporal patterns differs for pure-tone stimulus pulses mimicking the calling song peak frequency (15 kHz) of *N. ensiger* and a typical bat echolocation call peak frequency (40 kHz). That is, when the stimulus repetition rate increased from 1 to 100 Hz, T-cell temporal pattern-copying declined more rapidly for 15 kHz pulses than for 40 kHz pulses at both moderate and loud sound pressure levels (Faure and Hoy, 2000c). Hence, in the present experiments, the stimulus repetition rate was held constant at 14.25 Hz (period 70.18 ms), which is the natural syllable repetition rate for *N. ensiger* singing at 25 °C (Faure and Hoy, 2000a). For each stimulus type, the entire playback duration was 3 min, yielding a total of 2565 stimulus pulses per playback trial.

T-cell spike analysis

T-cell action potentials, recorded with tungsten hook and reference electrodes, were window-discriminated and analyzed with Igor Pro (Wavemetrics, Inc.) and custom-designed software written by Robert A. Wyttenbach (for details, see Faure and Hoy, 2000c). Variables extracted from T-cell recordings were the mean number of spikes per pulse, latency to the first spike (ms), instantaneous firing frequency (Hz) and the proportion of stimulus pulses encoded by one or more T-cell spikes during the 3 min of acoustic playback (a value of 1 indicates perfect temporal copying, a value of 0 indicates no temporal copying).

Statistical analyses

Individual T-cell responses within a playback trial are reported as the mean ± standard deviation (S.D.), whereas summary data for all katydids are reported as the mean ± standard error of the mean (S.E.M.). Spike variables were analyzed with a repeated-measures analysis of variance (Super ANOVA) using stimulus type (Bat 10, Bat 30 or Katydid signal), sex (male or female) and amplitude (50, 70 or 90 dB SPL) as factors. All statistical tests employ an experiment-wise error rate of $\alpha \leqslant 0.05$ (Zar, 1984; Rice, 1989).

Results

To illustrate how the T-cell of *N. ensiger* encodes each playback stimulus, we first present and discuss responses from a single representative male before summarizing the data for all the katydids used in the study. Example extracellular T-cell recordings in response to the Bat 10, Bat 30 and Katydid signals presented at 50, 70 and 90 dB SPL are shown in Fig. 1. At 90 dB SPL, the T-cell responds more vigorously to the Bat 10 signal than to either the Bat 30 or the Katydid signals. Note also that the temporal pattern of the Bat 10 stimulus is faithfully preserved by T-cell firing, whereas the response traces for the Bat 30 and Katydid signals have obvious gaps as a result of T-cell spike failures. At 70 dB SPL, T-cell spiking in response to the Bat 10 signal is still robust, with multiple spikes per stimulus pulse. Responses to the Bat 30 signal are rather poor and have declined relative to stimulation with the same signal broadcast at 90 dB (i.e. there are fewer consecutive spikes). Similarly, T-cell spiking in response to the Katydid signal presented at 70 dB SPL is also less frequent. However, close inspection of the Katydid signal response traces shows that there are neural units, other than the T-cell, that fastidiously copy the calling song pattern (note the small, regularly spaced spike bursts that stand out from the background activity at both 90 and 70 dB SPL). This demonstrates that the animal can hear both audio and ultrasonic frequencies, hence eliminating any possibility of a bad preparation. At 50 dB SPL, T-cell responses to the playback stimuli have declined in general; nevertheless, the Bat 10 signal is still fairly well encoded, especially in comparison with the Bat 30 and Katydid signals, in response to which T-cell spiking has all but disappeared.

Historaster displays of T-cell responses to the

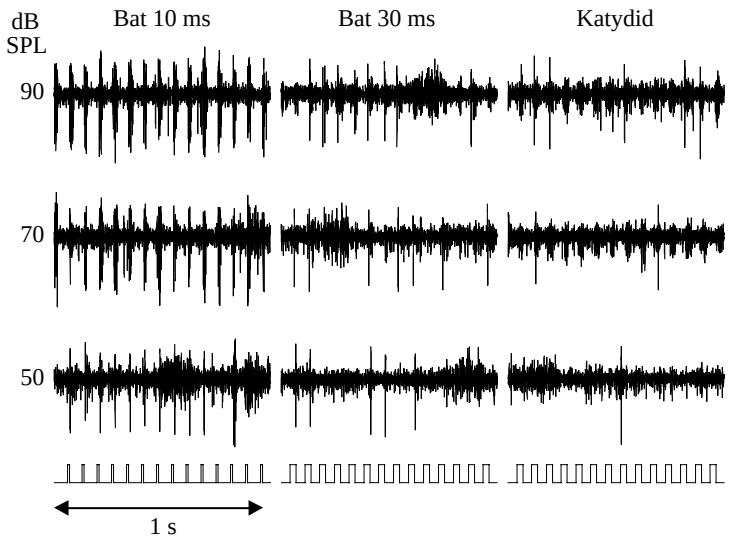

Fig. 1. Example extracellular response traces from the T-cell of an adult male *Neoconocephalus ensiger* (Ne 239). The T-cell spikes in response to ongoing stimulation with the three behaviorally relevant signals used in the long-term acoustic playback experiment presented at 50, 70 and 90 dB SPL (loudspeaker position 90 °) are shown. Note how the T-cell responds with more spikes per stimulus pulse to the 10 ms, 80→30 kHz FM sweep (Bat 10 signal) than to either the 30 ms, 80→30 kHz FM sweep (Bat 30 signal) or the conspecific song (Katydid signal). The stimulus presentation rate was 14.25 Hz, which is the natural calling rate of *N. ensiger* at 25 °C.

neuroethological stimuli broadcast at 50 dB SPL are shown in Fig. 2A. The top panel is a raster dot display, while the bottom panel is a post-stimulus time (PST) histogram for the 3 min of acoustic playback. The strong spiking behavior of the T-cell to the 10 ms, 80→30 kHz FM sweep can be seen in the left-hand column as a distinct band of dots extending over the entire playback duration. The T-cell responses to the 30 ms, 80→30 kHz FM sweep (middle column) are considerably weaker compared with responses to the Bat 10 signal; the number of spikes is lower and the relative timing of spikes is more variable (see PST display). Also note the increase in latency (by more than 10 ms) for the Bat 30 stimulus relative to the Bat 10 signal. At 50 dB SPL, T-cell spiking in response to conspecific song is rather weak. Although a faint band of spikes loosely correlated with the onset of the Katydid signal is present, the response is barely visible above background activity.

A more processed form of the 50 dB SPL data for Ne 239 is shown in Fig. 2B. Each column shows the number of T-cell spikes per stimulus pulse, the latency to the first T-cell spike relative to the onset of the stimulus, and the average instantaneous firing frequency (i.e. spike rate) elicited by the Bat 10, Bat 30 and Katydid signals. When looking at the number of T-cell spikes per pulse (Fig. 2B, top row), it is difficult to discern the difference in response strength between the stimuli. Nevertheless, the mean numbers of T-cell spikes are 0.72±0.71 for the Bat 10 signal, 0.36±0.68 for the Bat 30 signal and 0.16±0.51 for the Katydid signal (means ± S.D.). The difference in T-cell latency (Fig. 2B, middle row) for the three stimuli is, however, quite obvious. Note the absence of a distinct, time-locked band of spikes in response to the calling song stimulus. The mean latency was 17.31±4.71 ms for the Bat 10 signal, 29.28±10.63 ms for the Bat 30 signal and 32.66±13.89 ms for the Katydid signal (means ± S.D.). Because the stimulus presentation rate was fixed at 14.25 Hz, if the T-cell faithfully responded with at least one spike per stimulus pulse, then the expected average firing frequency would be 14.25 Hz. As Fig. 2B shows (bottom row), the Bat 10 stimulus has substantially more T-cell responses which fall above this expected firing frequency. The mean spike rate at 50 dB SPL was 27.90±88.71 Hz for the Bat 10 stimulus, 13.14±56.72 Hz for the Bat 30 stimulus and 8.76±54.17 Hz for the Katydid signal. Note that the standard deviations are larger than the means, which reflects the more varied nature of T-cell spiking at this amplitude. At 50 dB SPL, 39 % of the Bat 10 stimulus sweeps failed to elicit any T-cell spikes in this animal, with the percentage of spike failures worsening for the Bat 30 (72 %) and Katydid (88 %) signals.

Responses to the playback signals broadcast at 70 dB SPL are displayed in Fig. 3. When listening to the Bat 10 signal, the T-cell of Ne 239 responded with multiple spikes (1.6±0.84 spikes pulse^{-1}) at a shorter latency (14.70±1.67 ms) and at a higher instantaneous firing frequency (227.31±225.22 Hz) compared with stimulation with the same signal at 50 dB (compare with Fig. 2). The percentage of spike failures, i.e. when the stimulus failed to elicit at least one T-cell spike in response to the Bat 10 signal at 70 dB SPL, was extremely low (<1 %), whereas failures were obvious and common for both the Bat 30 (66 %) and Katydid (92 %) signals. Indeed, the difference in potency of the three signals is obvious (Fig. 3A). Moreover, there was little improvement (if any) in the responses of the T-cell at 70 dB SPL for the Bat 30 signal compared with responses evoked at 50 dB. The Bat 30 signal elicited relatively few spikes (0.40±0.64 spikes pulse^{-1}), occurring at a long latency (26.22±8.76 ms) and with a low spike rate (11.58±53.22 Hz). The latency to the Bat 30 signal is especially interesting in this animal because of its bimodal response nature: T-cell spikes are grouped either early or late after the onset of the stimulus, with an 8–10 ms refractory zone (Fig. 3A,B). T-cell spikes elicited by the Katydid signal at 70 dB also showed little improvement compared with stimulation at 50 dB SPL: there were few spikes (0.11±0.40 spikes pulse^{-1}), occurring at a relatively long latency (27.55±12.60 ms), and with an average spike rate that was lower than expected simply on the basis of the rate of stimulus presentation (5.29±41.70 Hz).

At 90 dB SPL, the T-cell of Ne 239 initially responded to the Bat 10 signal with a powerful burst of 9–12 spikes (Fig. 4). The

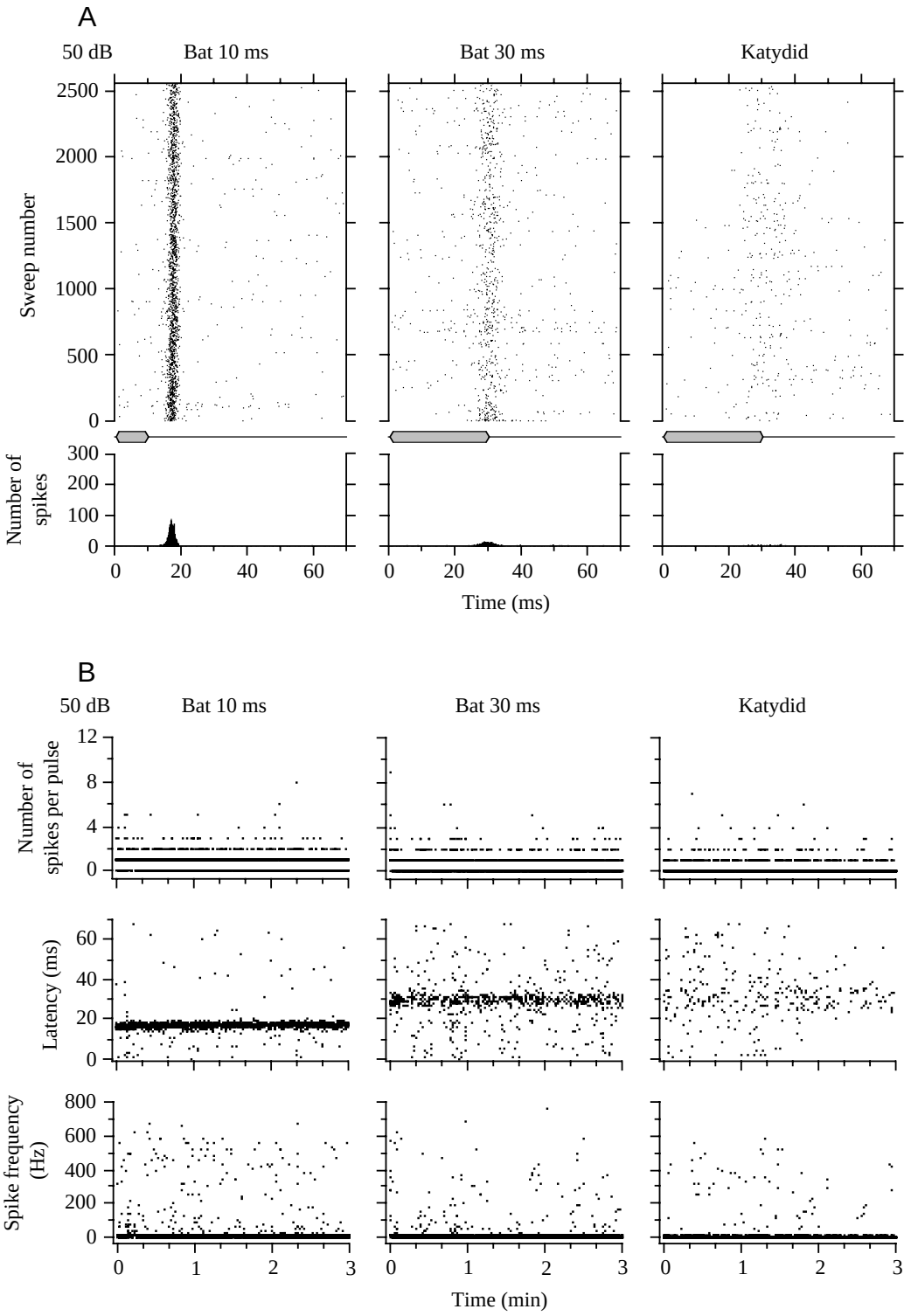

Fig. 2. T-cell spiking in response to conspecific and predatory signals at 50 dB SPL. (A) Historaster displays of T-cell responses to the three neuroethological stimuli used in the long-term acoustic playback experiment (Ne 239; an adult male *Neoconocephalus ensiger*). The upper panel represents the timing of individual T-cell spikes as dots relative to the onset of the stimulus, with successive stimulus presentations (i.e. sweeps) stacking on top of each other. Hence, time increases with each sweep number (playback duration 3 min, presentation rate 14.25 pulses s^{-1}, so the total number of stimulus pulses is 2565). The lower panel is a post-stimulus time (PST) histogram of the cumulative number of T-cell spikes over the entire playback duration (bin width 0.1 ms). Stimulus duration is illustrated schematically as a gray pulse (middle). (B) Rasterized T-cell response variables at 50 dB SPL (Ne 239; adult male). The number of T-cell spikes per pulse (top), the latency to the first T-cell spike (middle) and the average instantaneous firing frequency (bottom) over the 3 min of acoustic playback using the Bat 10 ms, Bat 30 ms and Katydid signals are shown.

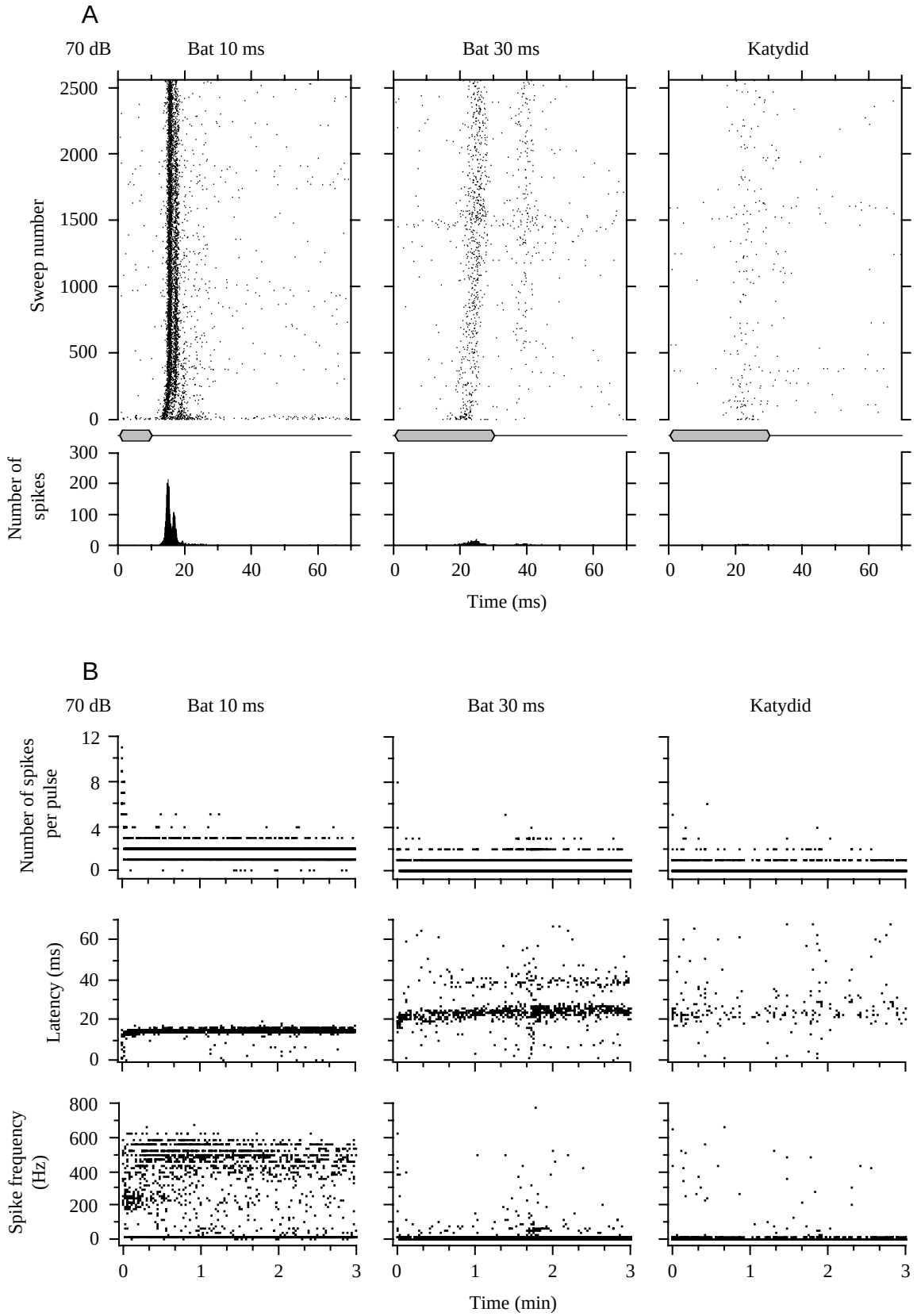

Fig. 3. T-cell spiking in response to conspecific and predatory signals at 70 dB SPL (Ne 239; an adult male *Neoconocephalus ensiger*). (A) Historaster displays of T-cell responses to the three neuroethological stimuli used in the long-term acoustic playback experiment. (B) Rasterized T-cell response variables. For details, refer to the legend of Fig. 2.

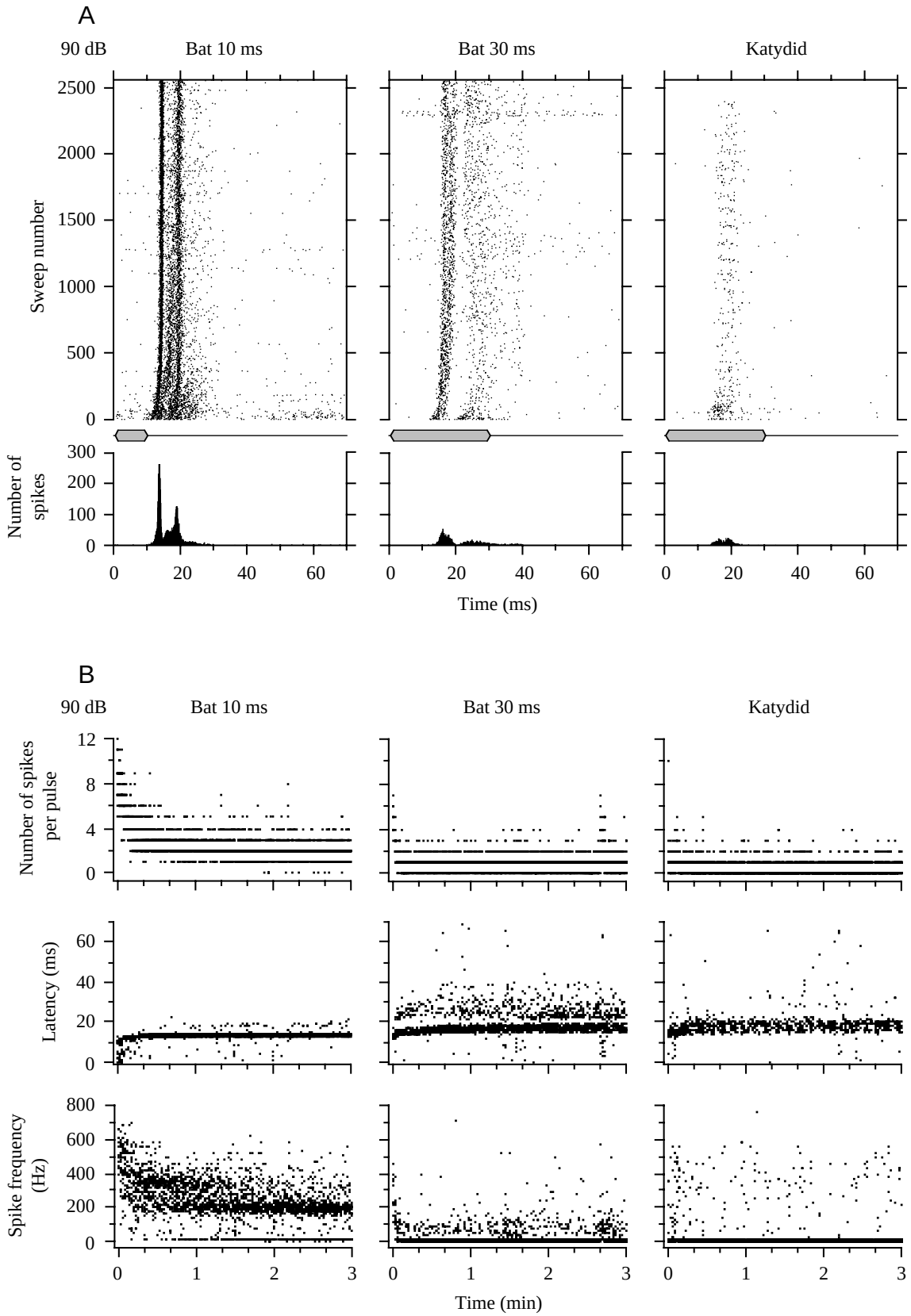

Fig. 4. T-cell spiking in response to conspecific and predatory signals at 90 dB SPL (Ne 239; an adult male *Neoconocephalus ensiger*). (A) Historaster displays of T-cell responses to the three neuroethological stimuli used in the long-term acoustic playback experiment. (B) Rasterized T-cell response variables. For details, refer to the legend of Fig. 2.

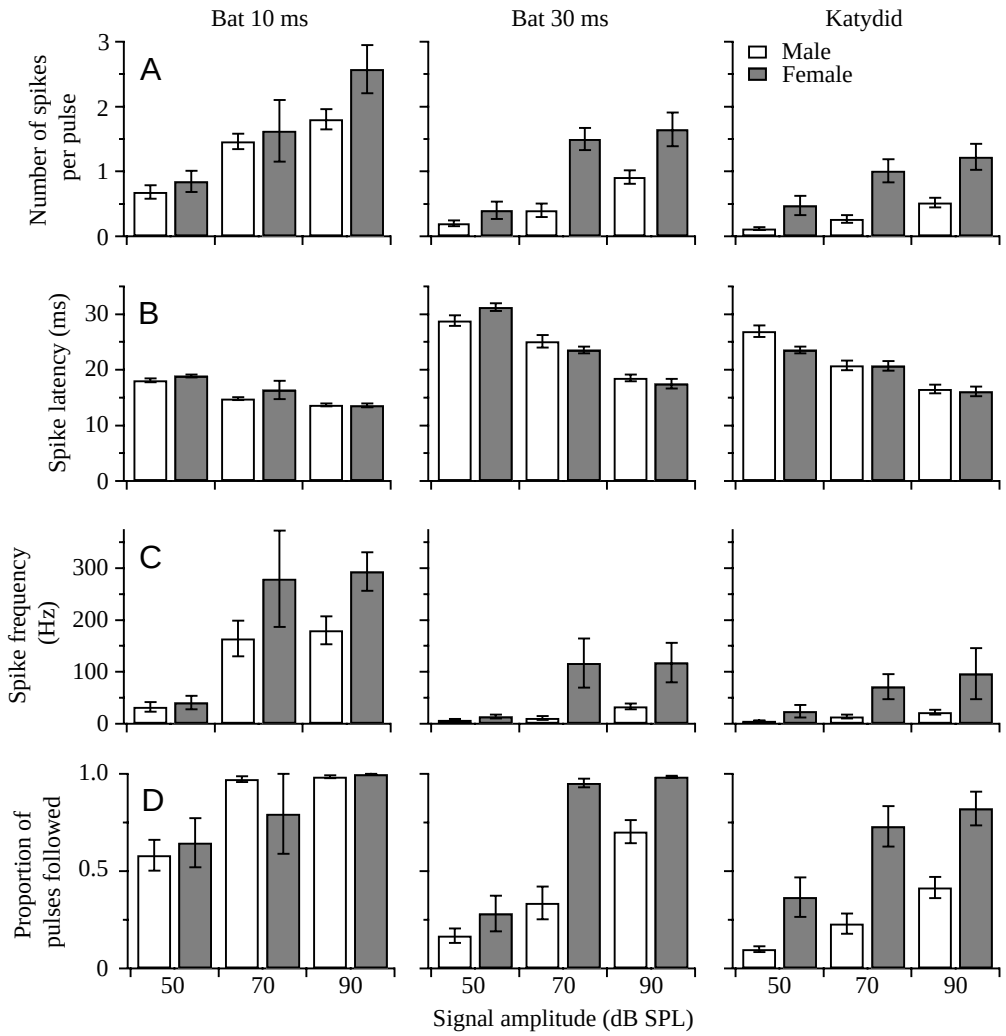

Fig. 5. Summary of T-cell response variables to the Bat 10, Bat 30 and Katydid signals for all animals used in the long-term acoustic playback experiment. The mean ± S.E.M. number of T-cell spikes per pulse (A), latency to the first T-cell spike (B), instantaneous spike frequency (C) and proportion of stimulus pulses encoded by one or more T-cell spikes (D) are presented as a function of sound pressure level for both male (N=11–13) and female (N=3–4) *Neoconocephalus ensiger*.

cell continued to respond vigorously for 10–20 s, and then slowly adapted to a lower but still robust response level. Despite adaptation, a trail of 2.60±1.40 spikes pulse^{-1} is evident over the entire playback duration (Fig. 4A). Relative to stimulation at 50 and 70 dB SPL, the latency of the T-cell decreased slightly for the Bat 10 signal at 90 dB (13.36±1.73 ms), while its average instantaneous firing frequency rose to 240.32±130.14 Hz (note that the spike rate standard deviation is now smaller than the mean). It is especially noteworthy that in this animal the Bat 10 signal did not fail to trigger a T-cell spike until almost 2 min into the experiment (Fig. 4B), with the total number of spike failures once again being extremely low (<1 %). T-cell responses to the Bat 30 signal broadcast at 90 dB were improved relative to lower sound pressure levels: the spike count rose to 0.98±0.82 spikes pulse^{-1}, the average latency shortened to 19.62±6.31 ms, while the spike rate increased to 27.93±56.82 Hz. Nevertheless, T-cell misfires begin to occur within 15 s of the onset of the Bat 30 stimulus, resulting in a spike failure rate of 26 %. Not surprisingly, augmented spiking was also observed in response to the Katydid signal presented at 90 dB SPL (response strength 0.45±0.72 spikes pulse^{-1}; spike latency 18.51±5.73 ms; instantaneous firing frequency 27.07±94.89 Hz); however, with a 64 % spike failure rate, T-cell responses to the Katydid signal were still weaker than those generated by the Bat 10 signal broadcast at 50 dB SPL (compare Figs 2 and 4).

Acoustic playback summary

Fig. 5 summarizes the results for all the katydids used in this study. The format is similar to that of panel B in the previous figures, with T-cell response variables arranged in rows, organized into columns by stimulus type. The only added complexity is that male and female responses have been separated, thereby allowing for a more detailed comparison. Note that the sample size for *N. ensiger* females (N=3–4) is considerably smaller than that for males (N=11–13). Also note that, within each column, T-cell spike variables are presented in order of increasing sound pressure level. In general, sound pressure level effects will not be discussed because it is understood that, as signal amplitude increases, so does the number of T-cell spikes, with a corresponding increase in spike rate and decrease in spike latency (Faure and Hoy, 2000c). Nevertheless, the format of Fig. 5 easily allows for the assessment of amplitude effects within and across stimulus types.

A repeated-measures ANOVA was conducted with stimulus

type (Bat 10, Bat 30 or Katydid), sound pressure level (50, 70 or 90 dB) and sex (male or female) as main factors and the number of spikes per pulse, spike latency, instantaneous firing frequency and the proportion of pulses encoded by one (or more) T-cell spikes as dependent variables. The effect of each factor was examined alone and in combination with other factors (i.e. interaction effects).

Beginning first with the number of T-cell spikes per stimulus pulse (Fig. 5A), there was a significant effect of stimulus type (F=133.553, d.f.=2, P=0.0001), with the Bat 10 signal evoking the strongest response at each amplitude (stimulus×SPL, F=13.977, d.f.=4, P=0.0001). In general, the female T-cell responded more vigorously than its male counterpart (F=22.363, d.f.=1, P=0.0006); however, the difference in response strength between the sexes did not change with stimulus type (stimulus×sex, F=0.48, d.f.=2, P=0.9534). Similarly, the stimulus×SPL×sex three-way interaction was also non-significant (F=2.207, d.f.=4, P=0.0837).

With regard to spike latency (Fig. 5B), there was a significant effect of stimulus type (F=47.443, d.f.=2, P=0.0001), with the Bat 10 signal evoking the shortest response latencies at all amplitudes (stimulus×SPL, F=15.232, d.f.=4, P=0.0001). There was no difference in spike latency between males and females (F=0.280, d.f.=1, P=0.6075). Similarly, the stimulus×sex (F=0.546, d.f.=2, P=0.5870), SPL×sex (F=0.247, d.f.=2, P=0.7835) and stimulus×SPL×sex interactions (F=2.249, d.f.=4, P=0.0790) were all non-significant (Fig. 5B).

The most dramatic physiological difference between the three stimulus types was in the instantaneous firing frequency of the T-cell (Fig. 5C), with the Bat 10 stimulus evoking the highest spike rates (F=75.480, d.f.=2, P=0.0001). Moreover, the difference in instantaneous firing frequency between the Bat 10, Bat 30 and Katydid signals increased with increasing sound pressure level (stimulus×SPL, F=18.771, d.f.=4, P=0.0001). T-cell spike rates were higher in females than in males (F=18.368, d.f.=1, P=0.0013), although the difference in instantaneous firing frequency between the sexes did not change with stimulus type (stimulus×sex, F=2.996, d.f.=2, P=0.0706) or with sound pressure level (stimulus×SPL×sex interaction, F=1.684, d.f.=4, P=0.1707).

Finally, the proportion of stimulus pulses encoded by one or more T-cell spikes differed significantly between the three neuroethological stimuli (F=66.807, d.f.=2, P=0.0001). In both sexes, the T-cell showed nearly perfect temporal copying of the Bat 10 signal broadcast at moderate (70 dB) and loud (90 dB) sound pressure levels (Fig. 5D), whereas this was not true when responding to syllables of conspecific song (stimulus×SPL, F=3.085, d.f.=4, P=0.0253). Temporal pattern-copying also differed between the sexes (F=16.081, d.f.=1, P=0.0021), with females exhibiting more faithful copying of all stimulus types (stimulus×sex, F=16.589, d.f.=2, P=0.0001). The difference between males and females is particularly obvious for the Bat 30 signal at 70 dB, but is also evident in the responses to the Katydid signal (Fig. 5D). Nevertheless, despite a sex difference in temporal pattern-copying of the Katydid signal, T-cell spiking in response to conspecific song was still significantly poorer than in response to the predatory Bat 10 and Bat 30 signals.

Discussion

One goal of neuroethology is to decipher the neural mechanisms underlying the production of natural behavior patterns, including the processing of behaviorally relevant sensory stimuli. Suggestions regarding the involvement of the T-cell in the early-warning and escape behavior of katydids date back more than three decades (McKay, 1969, 1970; Kalmring et al., 1979), yet almost no studies have examined the physiology of the T-cell specifically from a predator-detection perspective (but see Libersat and Hoy, 1991). Indeed, on the basis of experimental protocols as reported in the literature, the majority of T-cell studies simply assume or infer that its role is in the detection and localization of conspecifics (e.g. Suga and Katsuki, 1961; Suga, 1963; Rheinlaender et al., 1986; Rheinlaender and Römer, 1980, 1986; Schul, 1997). Moreover, the question of whether T-cell responses can distinguish conspecific song, which is broadband and contains both audio and ultrasonic frequencies, from predatory ultrasound has never been addressed.

Our results demonstrate that the T-cell of *N. ensiger* responds best to short-duration pulses of ultrasound that mimic the vocalizations of echolocating bats. The T-cell fired more spikes per pulse, at a shorter latency and at a higher instantaneous firing frequency in response to the 10 ms, 80→30 kHz FM sweeps than to pulses of conspecific song or, surprisingly, to longer-duration bat signals (i.e. 30 ms, 80→30 kHz FM sweeps). Furthermore, the temporal pattern of the stimulus, which remained constant at 14.25 Hz, was faithfully copied only for bat-like stimuli. T-cell encoding of conspecific song was significantly poorer, particularly in males, where fewer than 50 % of calling song syllables elicited one or more T-cell spikes (Fig. 5D). Given that the T-cell shows a fast throughput from auditory afferents (i.e. monosynaptic connection; Römer et al., 1988), combined with its large axonal diameter (i.e. giant fiber appearance; Kalmring et al., 1979; Rheinlaender and Römer, 1980, 1986; Römer et al., 1988; Schul, 1997), its broadband tuning, its high sensitivity to ultrasonic frequencies and its basic physiological response properties, which are distinctly ultrasound-biased (Faure and Hoy, 2000c), the data seem most consistent with the T-cell having a role in the processing of non-social acoustic stimuli (i.e. predator detection and escape).

An unexpected finding was the response of the T-cell to the Bat 30 stimulus. Both the number of spikes per pulse and the instantaneous spike rate were smaller, and the latency to the first spike was longer, when compared with responses to the Bat 10 signal (Figs 1–5). Rheinlaender et al. (1972) reported that T-cell responses in *Tettigonia viridissima* were strongest for 20 ms pure tones when durations from 1 to 100 ms were tested. In addition, while collecting data for a temporal summation and integration experiment, we also noticed reduced T-cell spiking in response to long-duration pure tones

(Faure and Hoy, 2000c). The present experiments were conducted with the loudspeaker positioned at 90 °, so a partial explanation for the reduction in response strength and more variable response latency to the Bat 30 signal (Figs 2–5) may be the increased inhibition originating from the contralateral ear because of the longer-duration bat stimulus (see Suga and Katsuki, 1961). Also, the Bat 30 signal, with its shallower rate of frequency modulation, may simply have taken longer to reach threshold, resulting in longer T-cell latencies. Regardless of the mechanism, the data indicate that, in addition to pulses of bat-like ultrasound, the T-cell responds best to short-duration or transient signals. T-cell responses are, therefore, shaped by a combination of frequency and temporal tuning, an idea consistent with detecting the incidental sounds produced by predators moving through the biotope and the rapid biosonar emissions of echolocating bats during the search, approach and terminal phases of hunting (Kalko and Schnitzler, 1989; Kalko, 1995).

The T-cell is thought to be part of a giant fiber system in katydids (Kalmring et al., 1979). In general, giant fibers increase action potential conduction velocities, thereby providing rapid, uninterrupted nervous transmission over relatively long distances. Giant fibers are also important sites of synaptic integration (Parnas and Dagan, 1971). Presumably the metabolic costs associated with maintaining large-diameter axons with increased conduction velocities are offset by the importance of these neurons in mediating arousal, early-warning and escape behaviors, acts crucial for the survival and (future) reproduction of any organism (for examples of giant-fiber-mediated evasive movements, see references in Friedel, 1999). Escape responses are extremely complex, requiring the activation and coordination of motor centers that have evolved to bring an animal into a state of alertness (see, for example, Eaton, 1984), including the inhibition (cessation) of motor activities such as flight or stridulation (Libersat and Hoy, 1991; Faure and Hoy, 2000a). Even if giant fibers themselves do not mediate specific behavioral acts, their rapid conduction velocities are nonetheless important for inhibiting motor programs prior to the arrival of motor commands directly responsible for escape behavior (Parnas and Dagan, 1971). With its strong spiking, short latency and high instantaneous firing frequency and temporal copying fidelity in response to short-duration pulses of ultrasound, the T-cell seems ideally suited to function in a predator-detection circuit.

Male *N. ensiger* sing continuously for periods of 30 min or longer, without interruption, whereas insect encounters with echolocating bats are typically short-lived events, lasting anywhere from hundreds of milliseconds to a few seconds (e.g. Kalko and Schnitzler, 1989). In our study, we used a playback duration of 3 min, which is a relatively brief period compared with bouts of stridulation but is, nonetheless, fairly lengthy for a predation event. That the T-cell responded vigorously and continuously to the Bat 10 stimulus, yet adapted to playbacks of male calling song, removes any possibility that the lack of responsiveness was due to overstimulation or nervous fatigue (e.g. ion or transmitter depletion). Conspecific song is simply not encoded well by the T-cell. Instead, male song is encoded by central auditory units other than the T-cell (e.g. ON, AN1, AN2; Rheinlaender and Römer, 1986; Römer and Bailey, 1986; Römer and Lewald, 1992; Schul, 1997), the existence of which is clearly manifest in the extracellular response traces of Fig. 1. Therefore, it would appear that some elements in the neural circuitry mediating positive and negative phonotactic behavior in katydids are fairly separate and distinct.

Initially, the T-cell responded to all the signals in the acoustic playback experiment; however, its responses to conspecific song rapidly adapted in comparison with bat-like ultrasound. Because the rise time of the Katydid signal was longer than the rise time of either Bat stimulus, this would result in reduced synchronous activity in the primary receptor array (Rössler et al., 1990), which could account for why the T-cell responded with fewer spikes per conspecific syllable (Fig. 5A). However, less synchronized afferent activity does not explain the adaptation of the T-cell to the Katydid signal. Adaptation was particularly evident in males, even at the highest sound pressure levels, whereas in females T-cell responses to conspecific song were improved. This suggests that a sex difference may exist in the physiology of the T-cell and, if true, then this would be interesting. In the previous paper (Faure and Hoy, 2000c), we showed that the tuning of the T-cell differs slightly between males and females, with females having increased sensitivity in the spectral band encompassing male song. With this in mind, it may not be surprising that the T-cell in *N. ensiger* females responded better to the Katydid signal than the T-cell in males. Perhaps females also use their T-cells at close range to assess and evaluate the ultrasonic components (quality?) of a male's song. Nevertheless, in both males and females, T-cell spiking responses are overwhelmingly biased in favor of bat-like echolocation signals (Fig. 5).

Suga and Katsuki (1961) were the first to demonstrate that the T-cell in *Gampsocleis buergeri* females responds to the syllable pattern of stridulating males. Their result, however, runs counter to the findings of McKay (1969), who reports that the T-cell of *Homorocoryphus* (=*Ruspolia*) sp. responds poorly, or not at all, to conspecific song. Kalmring et al. (1979) demonstrated neuronal adaptation to conspecific song in an auditory giant neuron of *Decticus verrucivorus* (probably the T-cell, but the soma did not stain), and a similar result was reported by Zhantiev and Korsunovskaja (1983) working on the T-cell of *Tettigonia cantans*. Indeed, close inspection of Fig. 5A in Suga and Katsuki (1961) reveals that spike adaptation is also manifest in the T-cell responses of *G. buergeri*. In crickets, a characteristic feature of TN1 is rapid adaptation to repetitive stimuli (Kühne et al., 1984). McKay (1969) pointed out that, if the T-cell does mediate escape and avoidance behaviors in katydids, then it is essential for its responses to be inhibited by conspecific song because the same motor apparatus is used for avoidance, orientation and singing and, in the absence of such inhibition, conflicting behavior patterns might be stimulated. The data from this and the previous paper (Faure and Hoy, 2000c) suggest that the mechanism of T-cell adaptation is *via* frequency-dependent inhibition from audio frequencies present in the male's song. If *N. ensiger* is like other orthopterans, with an over-representation of primary afferents

tuned to calling song frequencies (Kalmring et al., 1978; Esch et al., 1980), then this would provide a potential mechanism for low-frequency inhibition of T-cell responses when stimulated with conspecific song.

Previously, we showed that the T-cell of *N. ensiger* is capable of encoding the rapid changes in pulse rate that are typical of echolocating bats during the search, approach and terminal phases of hunting (Faure and Hoy, 2000c). The present results extend this work by demonstrating that responses from the T-cell alone are sufficient to discriminate between pulses of conspecific song and predatory ultrasound. Most researchers working on the acoustic Orthoptera conjecture that the putative signal analyzer must lie within the brain (e.g. Huber, 1978; Boyan, 1984). While our results do not refute this idea, they do, however, clearly demonstrate that a large degree of signal processing, sufficient for the discrimination and categorization of complex behaviorally relevant acoustic stimuli, occurs at the level of the prothoracic ganglion, which is only one or perhaps two synapses removed from the auditory periphery (Römer et al., 1988).

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