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The sounds of silence: cessation of singing and song pausing are ultrasound-induced acoustic startle behaviors in the katydid *Neoconocephalus ensiger* (Orthoptera; Tettigoniidae)

Abstract Previous studies of acoustic startle in insects have dealt with behavioral and/or neural mechanisms employed in evading aerially hawking, echolocating bats; however, insects also face terrestrial predators. Here we describe an acoustic startle response of the nocturnal katydid, *Neoconocephalus ensiger*. Stridulating males disturbed in the field perform obvious anti-predatory behaviors – cessation of singing, freezing, jumping, and evasive flight. Under controlled laboratory conditions we found that cessation of singing and song pausing are ultrasound-specific behaviors: when stimulated with pulsed ultrasound (20–100 kHz), but not audio-sound (<20 kHz), males cease mate calling or insert pauses in their song. A second factor influencing acoustic startle is the phase of stimulation: an acoustic startle response occurs only when the pulse of ultrasound arrives during the window of silence between stridulatory syllables. The average startle threshold and response latency was 70 ± 5 dB SPL and 34.2 ± 6.0 ms, respectively. *N. ensiger* is particularly useful for examining acoustic startle responses of non-flying insects because (1) its calling song is broadband and contains ultrasound, thus the possibility exists of confusion over the biological meaning of ultrasound, and (2) this species shows the classic bat-avoidance response while flying, so a direct comparison between two types of acoustic startle is possible within the same species.

Introduction

Hearing is well developed in only two major groups of terrestrial organisms – vertebrates and insects – and both groups use their ears for two primary purposes: the detection and identification of sounds, and the localization of sound sources (see chapters in Webster et al. 1992 for recent reviews). Both functions are important, and auditory systems have evolved to be most sensitive to the features of sound waves that impart significance toward an organism's survival and reproduction. Within this context, the detection of sounds produced by predators, whether they be active signals, vocalizations, or incidental noises, must have been an important driving force in the evolution of audition.

Probably the best-documented example of how predatory selection pressures can drive the evolution of prey audition is the interaction between moths and insectivorous bats (Roeder and Treat 1961; Roeder 1967a). Many moths have evolved tympanal hearing organs whose sole function (with few exceptions) is the detection of the high-frequency echolocation calls uttered by sympatrically foraging, aerially hawking bats (Fenton and Fullard 1981; Fullard 1987). When stimulated by bat ultrasound, moths initiate defensive flight behaviors that result in the insect flying away from the sound source (i.e., negative phonotaxis), or if the bat is in close proximity, a series of last-ditch evasive maneuvers designed to avoid predation (e.g., cessation of flight and dropping to the ground; Roeder 1965, 1967a).

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Interestingly, the notion that moths may use their ears to detect "the shrill squeaking of bats" was suggested even before the discovery of echolocation itself (White 1877). Since Roeder and Treat's pioneering work, we now know that many species of nocturnal insects (e.g., lacewings, locusts, crickets, katydids, beetles, and mantids) have evolved ultrasound-sensitive tympanal hearing organs and ultrasound-induced acoustic startle responses (ASRs). Because all night-flying insects are potentially vulnerable to bat predation, the ASR serves as a countermeasure by warning or priming the insect for escape (for recent reviews see Hoy 1992; Hoy et al. 1989).

To date, almost all studies of insect acoustic startle have focused on the behavior and underlying neural mechanisms for insects in flight (free flying or tethered). Assuming ASRs evolved for dealing solely with the predatory threat imposed by aerially hawking bats, this approach is justified. However, aerial-hawking bats are not the only source of predaceous ultrasound in the environment; other sources include the rustling of vegetation (see Fullard 1988) caused by an approaching predator, the echolocation calls of substrate-gleaning bats, and the exploratory squeaks and vocalizations of non-volant mammalian predators such as rodents and/or shrews (Sales and Pye 1974). Furthermore, there are even groups of ultrasound-emitting insects, such as the Tettigoniidae (katydids), that include members with specialized predatory lifestyles and carnivorous diets (e.g., Togashi 1980; O'Donnell 1993), while most other tettigoniids are opportunistic and will take insect prey when available (D. Gwynne, personal communication).

In spite of the diversity of potentially dangerous sources of terrestrial ultrasound, ASRs of non-flying insects have rarely been investigated. The best work is that of Spangler (1984) who found that male lesser wax moths and creosote bush katydids cease mate calling during the overhead passage of free-flying bats. This suggests insects silence themselves to avoid attracting predators. A similar self-silencing behavior was noted for stridulating *Conocephalus* spp. by Sales and Pye (1974) while tape-recording bats in Africa. Werner (1981) found that non-flying tympanate moths respond to intense pulses of ultrasound by ceasing movement, tucking their antennae close to their body, and pulling their body and wings close to the substrate, and concluded that this behavior should render moths less conspicuous to substrate-gleaning bats. Unfortunately, the artificial signals used in Werner's experiment were inappropriate mimics of gleaners (Faure et al. 1990), and subsequent work has shown that the echolocation calls of gleaning bats appear to be acoustically mismatched, and thus less detectable, to the ears of moths (Faure et al. 1993; Acharya and McNeil 1998). Riede (1993) studied the response of sitting locusts to intense pulses of broadband sound as a tool for assessing sensory thresholds. This startle reaction does not involve any displacement of the insect, so its function (still an open question) is perhaps to prime the locust for evasive jumping.

The purpose of this study was to investigate the ASRs of eastern sword-bearing conehead katydids (*Neoconocephalus ensiger*) during bouts of stridulation. In nature, calling males reliably startle to disturbing sounds by performing obvious anti-predator behaviors, such as cessation of singing, hiding, evasive jumping and flight. This species is particularly interesting for studying the ASR of a non-flying insect because: (1) the song of *N. ensiger* is broadband and contains both sonic (<20 kHz) and ultrasonic frequencies (≥20 kHz); thus within a species there exits the possibility of confusion over the biological meaning of ultrasound (i.e., predator versus conspecific), and a detailed examination of the ASR may shed light on solutions to this problem and the neural mechanisms underlying the behavior; and (2) *N. ensiger* has previously been shown to possess the classic bat-avoidance ASR while flying (Libersat and Hoy 1991); consequently, a direct comparison between two forms of acoustic startle – one elicited in flight and the other while singing when perched on a substrate – is possible within the same species.

Materials and methods

Natural history

Singing male *N. ensiger* Harris (Orthoptera; Tettigoniidae) were collected at night from the meadows and fields in the vicinity of Ithaca, New York, USA (42°26′N, 76°28′W) between July and October 1993–1996. In midsummer males begin calling at sunset and continue singing well into the night, uninterrupted, for periods of 30 min or longer (Scudder 1874; Frings and Frings 1957; Gwynne 1977). In early fall when the evenings are cool the activity of *N. ensiger* becomes more diurnal and stridulation begins in late afternoon. However, should the weather warm, thereby making nocturnal activity favorable, males will resume singing at night (Allard 1911; Cantrall 1943; P.A. Faure, personal observations). Singing males were located by listening to their song and capturing them by hand. Katydids were housed in individual plastic cups with ad libitum access to food (cat chow) and water. Most animals were kept in a cold room (12 °C) to slow their rate of senescence, but were transferred to a rearing room (25 °C) at least 1 day before experimentation.

Bioacoustics

The calling song of 17 male *N. ensiger* was recorded inside a darkened 1.1 m × 0.65 m × 0.65 m (length × width × height) chamber lined with sound-attenuating foam (Sonex), except for one side covered with black corduroy (average chamber temperature 24.1 ± 0.8 °C). The entire chamber was mounted atop a TMC Micro-g vibration table. Within the chamber, katydids were placed inside a 9 cm × 6 cm × 6 cm aluminum mesh cage (mesh diameter 6.35 mm) mounted on a 9-cm magnetic post, also covered with acoustic foam, to ensure that individuals sang on-axis with respect to a Brüel & Kjær (B&K) Type 4135 1/4″ condenser microphone (flat ±3 dB, 20 Hz to 125 kHz) suspended 10 cm above (microphone diaphragm at 0° incidence). The microphone, without protecting grid, was coupled to a B&K Type 2209 Impulse Precision Sound Level Meter (fast mode, linear weighting network) whose a.c. output was connected to a single channel of a Racal Store 4DS instrumentation tape recorder operating at 19 cm s⁻¹ or 38 cm s⁻¹ (entire recording system flat ±3 dB from 300 Hz to 37.5 kHz or 75 kHz). Song amplitudes were measured directly with the sound-level meter. The entire system was recalibrated daily using a B&K

Type 4220 piston-phone. Sound amplitudes are expressed in decibels sound pressure level (dB SPL re: 20 μPa) equivalent to the peak amplitude of a continuous tone of the same carrier frequency (Stapells et al. 1982).

Calling songs were analyzed using the Canary bioacoustics software (Charif et al. 1995). We measured the pulse (syllable) duration (d), pulse period (T; where T = the time from the onset of one syllable to the onset of the next syllable), and pulse duty cycle (duty cycle = $d/T \times 100$) for 30 syllables from each animal (minimum time resolution = 0.01 ms). Song spectral features were measured by calculating the power spectrum of 30 consecutive syllables using a Fast-Fourier Transform (FFT) with a Hamming analysis window (FFT size = 2048 points, 98.44% overlap, filter bandwidth = 380.62 Hz, minimum frequency resolution = 46.88 Hz). The resulting power spectrum was normalized relative to its peak (maximum spectral) frequency (PF amplitude = 0 dB). From this spectrum we measured the lowest (LF) and highest spectral frequencies (HF), defined as the −20-dB points on either side of the PF; the song bandwidth was defined as the HF–LF. The normalized power spectrum from each katydid was averaged across individuals to produce a population spectrum.

Acoustic startle experiments

Experiments were conducted using the anechoic chamber and mesh cage previously described. Pure tones, generated with a BK 3011 Dynascan 2-MHz Precision Function Generator, were gated with a Grass S44 Stimulator, and shaped into 10-ms pulses (1-ms linear rise/fall) using custom-built shaper circuitry. Sound amplitudes, calibrated with the B&K Type 2209 meter fitted with a Type 4135 microphone, were adjusted using a Harman Kardon PM640 Vxi Stereo Amplifier and Hewlett Packard 350D Attenuator Set. During calibration, the microphone (without protecting grid) was positioned inside the mesh cage so that its diaphragm faced (0° incidence) the ESS tweeter located 32 cm away. During acoustic startle experiments, the Type 4135 microphone was suspended 10 cm above the center of the mesh cage; this allowed us to record singing males on one channel of the Racal tape-recorder while a second channel recorded the stimulus onset time from the Grass stimulator. All data have been corrected for the time delay (1.46 ms) between the Grass stimulator and the arrival of sound at the katydid.

Two types of acoustic startle experiments were conducted:

Behavioral audiograms

Short-duration pulse trains were used to measure startle thresholds of 20 male katydids for carrier frequencies of 5–100 kHz. Starting at the lowest amplitude for a given frequency, a 1-s stimulus consisting of ten 10-ms pulses presented at a rate of 10 Hz, was delivered to a singing katydid. Three types of behaviors were observed (Fig. 1): (1) Cessation of singing; the katydid stops singing for ≥1 s. (2) Song pausing; the katydid inserts a gap into the temporal pattern of its song. The duration of song pausing was always < 1 s, otherwise it was defined as cessation of singing. (3) No response. If cessation of singing occurred, the stimulus was decreased by 5 dB and the animal was re-tested once singing resumed. Threshold was defined as the lowest amplitude that reliably elicited cessation of singing. Some animals, especially upon repeated stimulation, tended to exhibit only song pausing rather than cessation of singing, and in these cases threshold was defined as the lowest amplitude that reliably elicited song pausing. If no response occurred, the stimulus was repeated (after a few seconds of silence) to verify the lack of response. If still no response, the stimulus amplitude was increased by 10 dB and the katydid was re-tested. For purposes of analysis, animals which could not be induced to startle at the maximum output amplitude of the stimulus delivery system (120 dB SPL) for a given frequency were arbitrarily assigned a minimum startle threshold of 125 dB SPL (note: this only happened for frequencies < 20 kHz). By repeating this process, ASR thresholds were measured to within ±5 dB at each frequency.
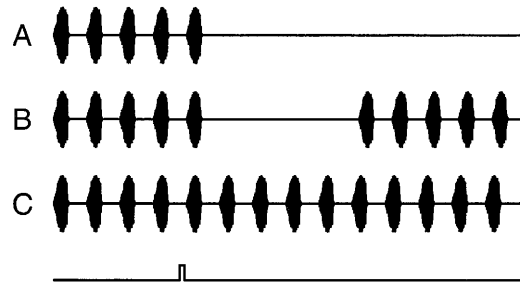

Fig. 1A–C Schematic oscillogram illustrating the acoustic startle response (ASR) of singing *Neoconocephalus ensiger*. In the figure, the onset time of the stimulus (t_s) occurs during the 4th syllable period. By definition, the period of stimulation = period P. **A** Cessation of singing: a katydid stops singing for ≥1 s. **B** Song pausing: a katydid momentarily stops singing by inserting a pause (< 1 s) into its calling song. In the figure, the response (pause) occurs in period $P + 1$. **C** No response: no significant increase or decrease in any of the ten pulse periods post-stimulation (i.e., periods P through $P + 9$). *Bottom trace* = stimulus

Timing of the ASR

Fourteen male katydids were used in a total of 567 stimulus presentations (trials) to determine the temporal features associated with acoustic startle (e.g., phase of stimulation, response latency, duration of pausing). Calling males were presented with a single, 10-ms pure-tone pulse, and their behavior noted (Fig. 1). If cessation of singing occurred, a new stimulus amplitude and/or frequency was tested when singing resumed. If song pausing occurred, the stimulus was re-delivered (two to five times) to try and induce cessation of singing before testing with a new stimulus. If no response occurred, the stimulus was re-tested (two to five times) to verify the lack of response before testing with a new stimulus. The minimum inter-trial interval was 5 s, and the stimulus was non-sequentially varied in SPL and frequency in order to prevent animals from habituating. Pulses of ultrasound were presented most often (86% of 567 trials), with 30 kHz being the modal frequency (48%). Stimuli were normally broadcast at or above 60 dB SPL (88%), with 90 dB SPL being the modal amplitude (59%).

Because the timing of the stimulus was random with respect to *N. ensiger's* period, each stimulus was considered as an independent trial. Designating P as the period of stimulation, then $P + n$ (where n is an integer > 0) identifies all periods subsequent to P. For each trial we measured: (1) mean ± standard deviation of the pre-stimulus period ($T_{pre} \pm$ SD), calculated from the ten periods preceding period P; (2) mean post-stimulus period (T_{post}), calculated from periods P through $P + 9$; (3) onset time of the stimulus (t_s); (4) onset time of the syllable beginning period P (t_p); and (5) onset times of the 1st, 2nd, 3rd,..., and 10th syllables subsequent to t_p, designated as $t_{p+1}, t_{p+2}, t_{p+3},..., t_{p+10}$, respectively. Stimulus phase (ϕ) was defined as:

$$\phi = [(t_s - t_p)/T_{pre}] \times 360° \qquad (1)$$

Cessation of singing trials were scored as a "response" (Fig. 1A). Unambiguously distinguishing a "response" from a "no response" was more difficult in trials with song pausing (Fig. 1B), particularly when the duration of the pause was ≤1.5 × T_{pre}. Therefore, a conservative working definition was constructed: for each trial a "response" was scored if the duration of any of the ten post-stimulus periods was greater (or less) than $T_{pre} \pm$ 4SDs; otherwise "no response" was scored (Fig. 1C). Using this definition, the probability of a type I error – that is, incorrectly classifying a response in any one of the ten post-stimulus periods – is $P < 0.0001$.

Data analysis

Unless stated otherwise, all data are reported as the mean ± SD, except where data conform to a circular distribution (e.g., stimulus

phase), in which case the mean vector angle ($\phi°$) $\pm$ mean angular deviation (s), and the mean vector length (r) are reported (Batschelet 1981). All statistical tests are two-tailed and employ a rejection criterion of $P \leq 0.05$ (Zar 1984).

Results

Bioacoustics

The mate-calling song of *N. ensiger* (Fig. 2A) is perhaps the most conspicuous nocturnal sound a human listener is likely to encounter from mid-July through October in the northeastern regions of the United States and in the southeasterly portions of Canada. The song consists of a train of loud, short duration, raspy or lisping syllables emitted at a rate of 5–15 pulses s^{-1}, depending on the ambient temperature (Frings and Frings 1957). Each syllable is composed of two parts (Fig. 2B): the first part is low in amplitude and represents the opening of the forewings (elytra), whereas the second part contains most of the sound energy and is produced during the closing stridulatory movements (Walker 1975; Gwynne 1977). Individual syllables are composed of a series of clicks, with each click representing the passage of the scraper (plectrum), located on the dorsal surface of the right forewing, over a single tooth of the file (pars stridens), located on the ventral surface of the left forewing. The sound pulses are broadband with no ordered frequency-time structure (Fig. 2C). The peak sound energy falls between 10 kHz and 20 kHz, but appreciable energy extends well into the ultrasonic spectrum. Comparing power spectra across katydids reveals little variation in the LF or PF, whereas the HF, and thus the relative amount of ultrasound within the song, is much more variable (Fig. 2D). Quantitatively this is shown in Table 1, which summarizes the acoustical characteristics of *N. ensiger*'s song, but can also be seen by examining the magnitude of the SD function in the population spectrum, which reaches a minimum between 8 kHz and 15 kHz (Fig. 2E).

Acoustic startle response – field observations

Singing katydids are exquisitely sensitive to sounds and vibrations in their environment and are capable of performing rapid escape when disturbed (e.g., Arak and Eiriksson 1992). Most tettigoniids are cryptically colored leafy green or brown, which makes locating them by sight extremely difficult by day, and virtually impossible at night were it not for their nocturnal activities (Nickle and Castner 1995). This is particularly true for coneheads, such as *N. ensiger*, whose body coloration and morphology give it the appearance of a blade of grass. When disturbed, stridulating *N. ensiger* may perform the following behaviors.

Freezing/hiding

In this behavior, males cease singing and remain motionless. If perched on a stem or blade of grass, often the insect will quickly reduce its body profile before becoming motionless. When approached, a katydid may slowly reposition its body so as to keep the substrate (e.g., the stem) between itself and the predator, thus dynamically concealing itself.

Rapid escape

In this behavior, katydids cease singing and move off the singing perch, often away from the disturbance. The
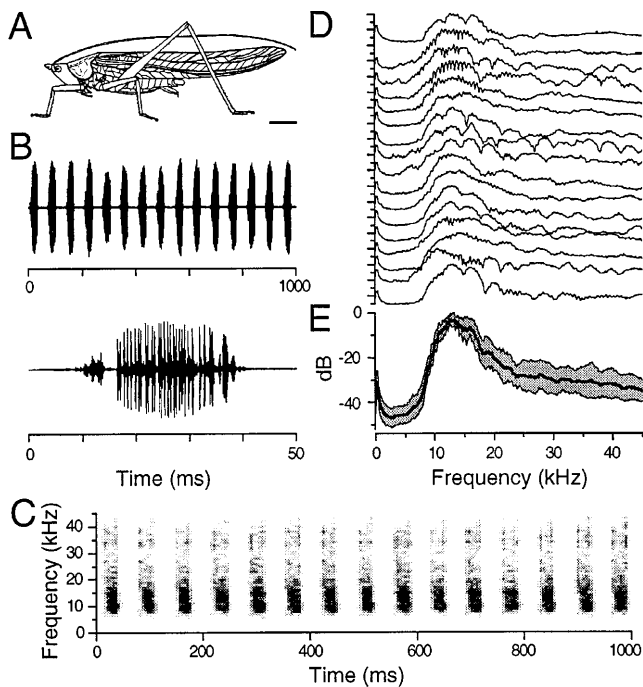

Fig. 2A–E Bioacoustics of the eastern sword-bearing conehead katydid. **A** Male *N. ensiger* (drawing by Martha B. Lackey in Alexander et al. 1972). Scale bar = 5 mm. **B** *Upper trace*: time-domain display of a train of calling song syllables. *Lower trace*: time-expanded view of one syllable. **C** Spectrogram of a train of calling-song syllables. **D** Frequency-domain displays of the average power spectrum of 30 syllables from 17 individual males. **E** Mean $\pm$ SD power spectrum of the 17 males in **D**

Table 1 Temporal and spectral characteristics of the calling song of male *Neoconocephalus ensiger* (n = number of individual katydids measured)

Calling song variable	n	Mean	SD	Min	Max
Syllable duration (ms)	17	30.76	5.27	14.27	37.65
Pulse period (ms)	17	72.08	3.11	67.39	78.21
Duty cycle (%)	17	42.68	6.93	18.86	50.16
Lowest frequency (kHz)	17	9.35	0.46	7.88	9.82
Peak frequency (kHz)	17	13.40	1.45	10.50	16.32
Highest frequency (kHz)	17	24.76	6.36	17.88	37.44
Bandwidth at −20 dB (kHz)	17	15.41	6.51	8.06	27.72
Amplitude at 10 cm (dB SPL)	17	93.1	5.8	80.5	99.6

most common means of escape are by active jumping or passive dropping, after which the highly cryptic insect may remain motionless or sneak away under the protective cover of the vegetation. The latter strategy is effective even in cool weather when animals are lethargic. Another form of rapid escape is active flight. This strategy is effective against terrestrial predators, but has the disadvantage that it can only be used when the thoracic musculature is elevated to a temperature suitable for flight – a condition that is satisfied during warm summer evenings or after an individual has been singing (males only) or shivering for some minutes (e.g., Heath and Josephson 1970).

Acoustic startle response – laboratory experiments

Behavioral audiograms

Of 20 *N. ensiger* tested, 16 (80%) consistently responded to pulsed sounds by cessation of singing and/or song pausing. Figure 3 shows the average threshold tuning curve for the ASR and the proportion of katydids that responded at each frequency. Startle thresholds decline sharply for stimuli between 5 kHz and 30 kHz, but remain essentially constant at higher frequencies. Note also that threshold variability decreases with increasing stimulus frequency. Males were reticent to startle when presented with pulsed audio-sound, but reliably startled to pulses of ultrasound. The lack of responsiveness to audio-sound is especially evident at 5 kHz and 10 kHz, where most animals did not startle even to sounds presented at the maximum amplitude of our stimulus delivery system. In these instances, katydids were assigned
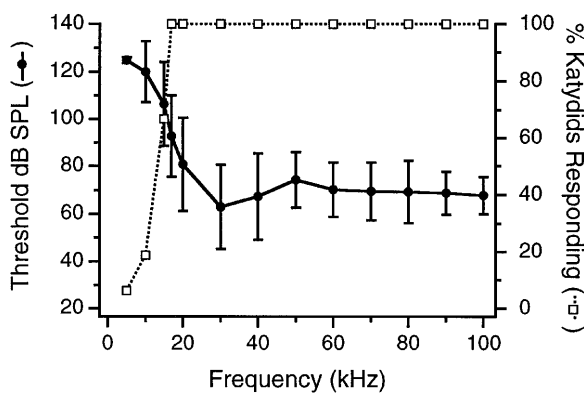

Fig. 3 Behavioral audiogram of cessation of singing and song pausing in *N. ensiger*. Mean ± SD threshold-tuning curve (*filled circles*) for the ASR (n = 16 katydids, except at 15 kHz where n = 15). The stimulus was a 1-s train of ten, 10-ms pulses (1-ms linear rise/fall) presented at 10 pulses s^{-1}. Note how threshold variability decreases at higher ultrasonic frequencies. Also shown is the proportion of katydids that responded at each frequency (*open squares*). Animals which could not be induced to startle upon reaching the maximum output amplitude of the stimulus delivery system were assigned minimum startle thresholds of 125 dB SPL for that frequency

minimum startle thresholds of 125 dB SPL (which accounts for the lack of variation at 5 kHz).

Temporal features of the ASR

The syllable period of *N. ensiger* during uninterrupted solo singing is highly stereotyped (coefficient of variation = 2.4 ± 1.6%), hence syllable period deviations are usually easy to detect. Of 567 single pulse presentations, behavioral responses occurred in 219 trials (38.6%). Cessation of singing occurred in 59 of 219 responses (26.9%), while the remaining 160 responses (73.1%) were instances of song pausing. This experiment was not designed to statistically test for stimulus amplitude and frequency effects; however, trends in the data were apparent. The majority of the 219 responses occurred to pulses of ultrasound (93%) that were ≥60 dB SPL (89%), even though ultrasonic stimuli ≥60 dB comprised only 74% of all trials. This corroborates the results obtained from the behavioral audiogram experiment (Fig. 3).

Figure 4A plots T_{pre} against T_{post} for all 567 trials. The data are also coded for trials where katydids responded or showed no response according to our mean ± 4SD criterion (see Timing of the ASR in Materials and methods). All the no-response trials fall along the 1:1 line, whereas the response trials fall into two groups: one group is intermingled with the no response data, while the other group spreads upward along the ordinate and falls above the 1:1 line. Figure 4B compares the pre- and post-stimulus pulse period variance, again coded by trials with responses and no responses. The post-stimulus variance is two to three orders of magnitude larger than the pre-stimulus variance and is bimodal, which causes the data to group into two clusters yielding both small and large responses. Note how the small responses fall along the upper margin of the lower cluster, while the large responses are separate and distinct.

The majority of responses, including stops, were pulse period increases (195, 89.0%), although pulse period decreases (11, 5.0%) and mixed responses (13, 5.9%) occurred, albeit rarely. Figure 4C not only demonstrates this but also shows that most responses occurred within the first two (72.8%) or three (81.4%) post-stimulus periods. That is, remembering that *P* equals the period of stimulation, then the majority of responses were period increases that occurred for periods *P*, *P* + 1, and *P* + 2 (Fig. 4C). This is to be expected for a time-correlated response. That the stimulus had no lasting effects on the intrinsic period of *N. ensiger*'s song was confirmed by comparing T_{pre} with the mean syllable period for periods *P* + 6 through *P* + 9 in trials with song pausing (paired $t = 1.493$, $df = 159$, $P = 0.1374$).

Knowing that pulses of ultrasound reliably cause *N. ensiger* to startle (Fig. 3), we next wanted to determine what other stimulus parameters affected singing katydids. Is it possible to predict which post-stimulus period will be lengthened (i.e., period *P*, *P* + 1, or
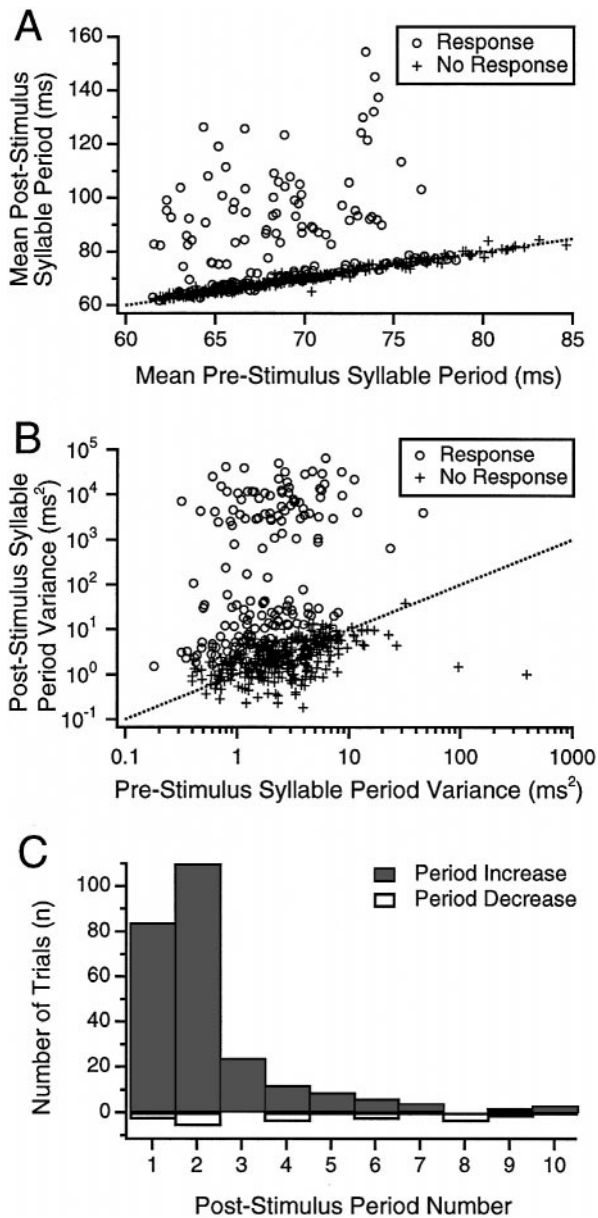
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Fig. 4A–C Identification and time-course of the ASR. **A** Average pre- and post-stimulus syllable period duration, and **B** pre- and post-stimulus syllable period variance ($n = 567$ trials). The *dashed line* = 1:1 line. In panel **B** note the difference in scale between the ordinate and the abscissa. See Timing of the ASR in Materials and methods for the definition of a response. **C** Histogram showing the relative distribution of responses (i.e., syllable period increases or decreases) in each of the ten post-stimulus periods. In the figure, post-stimulus period No. 1 = period of stimulation = period P

Fig. 5A, B Temporal features of acoustic startle in *N. ensiger*. **A** Distribution of stimulus phase angles (ϕ) for the response ($n = 219$) and no response ($n = 348$) trials (response = song pausing or cessation of singing). Both distributions are significantly non-uniform and are significantly different from each other. Note the lack of responses during the production of the stridulatory syllable (i.e., when ϕ is between 0° and 140°). **B** Distribution of ϕ relative to the onset of the final stridulatory syllable for the 59 cessation of singing trials. Acoustic startle occurs only when the stimulus does not overlap in time with the emission of a syllable. The positive phase angle data were used to estimate the range and average minimum ASR latency

response and no-response trials was highly non-uniform (response: $\chi^2 = 97.36$, $df = 17$, $P < 0.001$; no-response: $\chi^2 = 54.52$, $df = 17$, $P < 0.001$). Response trial ϕs were clustered between 140 and 340° – that is, during the quiet portion of period P – whereas no response trials had ϕs clustered in time with the production of the stridulatory syllable. The mean vector angle ± angular dispersion and mean vector length for response trials was 232.54 ± 62.54° and $r = 0.404$, compared to 49.75 ± 70.78° and $r = 0.237$ for no-response trials (Fig. 5A); the difference between response and no response trials in the distribution of ϕ was highly significant ($\chi^2 = 137.23$, $df = 17$, $P < 0.001$).

The distribution of ϕs for the 59 trials that evoked cessation of singing is shown in Fig. 5B. The presentation differs from Fig. 5A to show the timing of the stimulus relative to the katydid's final stridulatory syllable. In 30 of 59 trials the startle stimulus arrived during the penultimate calling period – that is, although the stimulus was delivered in period P, a subsequent syllable and calling period ($P + 1$) were produced. These data are displayed with negative phase angles ($-\phi$) to indicate

$P + 2$), and if so, by how much? Much of the variation in predicting whether or not a katydid will startle can be accounted for by examining the phase angle (ϕ) of the stimulus relative to the onset time of the syllable defining period P (t_p). Figure 5A shows the distribution of ϕs for all 567 trials, split by whether katydids responded (top) or not (bottom). Although stimuli were uniformly delivered across all ϕs ($\chi^2 = 13.73$, $df = 17$, $P > 0.5$; mean vector length $r = 0.013$), the distribution of ϕ for
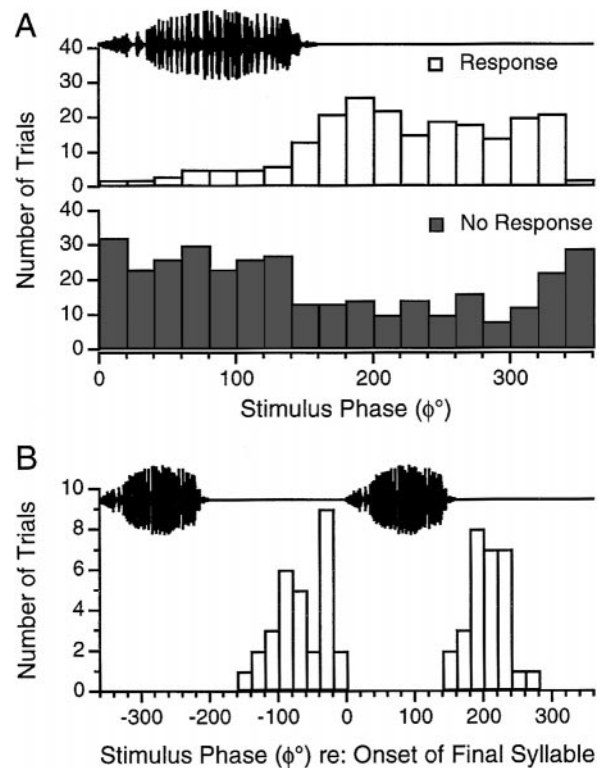
that the stimulus arrived before the final syllable. In the remaining 29 cessation of singing trials both the stimulus and the response occurred in period P, and these data are displayed with positive angles ($+\phi$). Given that stimulus pulses were uniformly delivered across all ϕs (Fig. 5A), Figure 5B clearly demonstrates that cessation of singing occurs only when the stimulus does not overlap in time with the production of a stridulatory syllable. Because *N. ensiger*'s pulse period is extremely stereotyped (CV = 2.4 $\pm$ 1.6%) and highly predictable at a given temperature (Frings and Frings 1957), it is possible to estimate a minimum startle latency (reaction time) for cessation of singing by converting the positive phase angle data in Fig. 5B to units of time (re: T_{pre}): this gives a range of 20.30–49.68 ms, with a mean latency of 34.2 $\pm$ 6.0 ms.

Because cessation of singing and song pausing typically result in a lengthening of post-stimulus periods P and/or $P + 1$, the rest of our analysis concentrates only on the 127 trials in which responses occurred during these periods. A phase response curve for song pausing in *N. ensiger* is shown in Fig. 6. This plot shows the normalized duration of the post-stimulus pause as a function of ϕ for periods P and $P + 1$ (cessation of singing trials not included), and was calculated as

$$(t_{p+1} - t_p - T_{pre})/T_{pre} \qquad (2)$$

if the response occurred during period P, or as

$$(t_{p+2} - t_{p+1} - T_{pre})/T_{pre} \qquad (3)$$

if the response occurred during period $P + 1$. Note the large degree of variation in response magnitude. Pause durations appear greatest when ϕ is between 160° and 340°. The data are plotted on a $\log_{10}$ scale to emphasize that the normalized increase in syllable period length

(i.e., the increase in syllable period length over and above T_{pre} – where a syllable normally would have occurred) is bimodally distributed, forming two horizontal clusters across all ϕs: a lower cluster corresponding to small period increases from 3% to $<100\%$ of T_{pre}, and an upper cluster corresponding to large period increases from 100% to $>1000\%$ of T_{pre}. Also note that the transition phase between responses in period P versus those in $P + 1$ occurs between $\phi \approx 180$–220°. In other words, in trials where ϕ was ca. $\leq 200°$, katydids responded by lengthening period P, whereas in trials where ϕ was ca. $>200°$, katydids responded by lengthening period $P + 1$. The mean ϕ angle $\pm$ angular dispersion and mean vector length for period P responses is 159.19 $\pm$ 41.85° and $r = 0.733$, compared to 271.43 $\pm$ 44.30° and $r = 0.701$ for period $P + 1$ responses (Fig. 6), and the distribution of ϕs between period P and period $P + 1$ responses is significantly different (Watson-Williams $F = 144.8$, $n = 119$, $P \ll 0.0005$). Although the eight dual-response trials were omitted from the above statistical analysis, the same pattern held true: in seven of eight dual-response trials the period that increased by the largest amount was the same as when only one period increased, and was predicted by ϕ.

One obvious question is: when katydids perform song pausing, do they resume singing in phase with where they left off? That is, does the neural central pattern generator (CPG) responsible for song production continue to oscillate in multiples of T_{pre} during acoustic startle, in a manner similar to the flash skipping behavior of unstimulated fireflies (Buck et al. 1981a), perhaps being reset by the stimulus but continuing to run nonetheless, or does the ASR stop the pacemaker altogether? Designating the onset time of the stimulus as t_s, and the onset time of the first post-response syllable as t_r ($t_r = t_{p+1}$ or t_{p+2}, depending on whether the response occurred in period P or $P + 1$, respectively), if the neural CPG continues to oscillate in multiples of T_{pre} during acoustic startle (hypothesis 1), then

$$t_r - t_p \approx (k \times T_{pre}) + \varepsilon, \qquad (4)$$

where k is a positive integer ≥ 2 and ε is an error term to account for intrinsic variation within the free-running neural oscillator. If the stimulus resets the neural CPG (hypothesis 2), then

$$t_r - t_s \approx (k \times T_{pre}) + C + \varepsilon, \qquad (5)$$

where element C accounts for the fact that (1) the CPG may be inhibited for the duration of the stimulus, and/or (2) inhibitory resetting is non-instantaneous (i.e., there are neural delays). It may also be true that the delay introduced in resetting the neural oscillator will vary with ϕ (hypothesis 3), in which case $C \approx f(\phi)$. Finally, if the ASR causes the CPG to stop altogether, then katydids should resume singing randomly with respect to t_p, t_s, and ϕ (hypothesis 4, the null relationship), giving k a random, non-integer value.

Hypotheses 1–3 were tested by dividing Eqs. 4 and 5 by T_{pre} and examining the modulus or remainder. (Note:
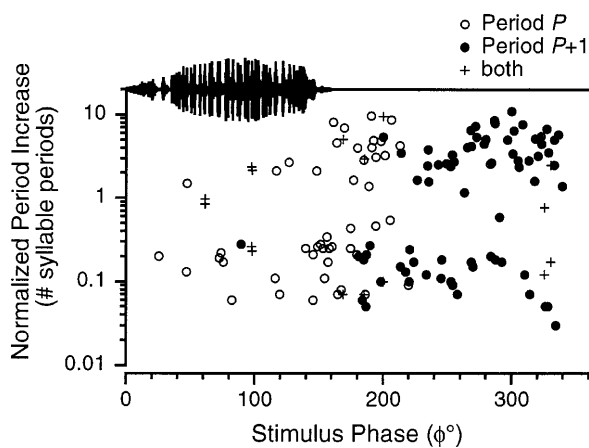

Fig. 6 The duration of song pausing. Shown is the phase response curve for the 127 trials with syllable period increases in period P (*open circles*), period $P + 1$ (*filled circles*), or both periods (*crosses*). The logarithmic ordinate shows the duration of song pausing over and above T_{pre} (i.e., where the next syllable normally would have occurred), plotted as function of ϕ. See text for calculations. Note how ϕ reliably predicts the post-stimulus response period, with the transition phase falling between 180° and 220°

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because *N. ensiger*'s pulse period is highly stereotyped, ε will be small, and is the same for each hypothesis; thus its influence has been ignored.)

If hypothesis 1 is true, then

$$\mathrm{mod}[(t_r - t_p)/T_{pre}] \approx 0 \text{ or } 1. \quad (6)$$

If hypothesis 2 is true, then

$$\mathrm{mod}[(t_r - t_s)/T_{pre}] \approx C/T_{pre}. \quad (7)$$

If hypothesis 3 is true, then

$$\mathrm{mod}[(t_r - t_p)/T_{pre}] \approx f(\phi), \quad (8)$$

and a plot of φ versus $\mathrm{mod}[(t_r-t_p)/T_{pre}]$ should reveal a clear function or relationship. Finally, if hypothesis 4 – the null relationship – is true, then the remainders obtained by evaluating Eqs. 6 and 7 should be uniformly distributed between 0 and 1, and Eq. 8 should show no relationship with φ.

Figure 7 shows the data that test each hypothesis. The dotted line in panels A–F is the expected value for a uniform (random) distribution. The distribution in Panel A was obtained by evaluating

$$\mathrm{mod}[(t_{p+10} - t_p)/T_{pre}] \quad (9)$$

over the ten post-stimulus periods for the 348 no response trials, and reinforces how precisely T_{pre} is maintained. That the distribution is significantly non-uniform is not surprising, given that (1) all the remainders cluster around 0 or 1, and (2) none of the ten post-stimulus

Fig. 7A–I Timing and control of the neural central pattern generator (CPG) during song pausing. *Column 1*: no response trials (*n* = 348); *column 2*: all response trials (*n* = 119); *column 3*: response trials with normalized pause durations > 0.5 × T_{pre} (*n* = 63). **A–C** Hypothesis 1: if the neural CPG continues to oscillate during acoustic startle, then the onset of the first post-response syllable (t_r) will occur at an integer multiple of the mean pre-stimulus period (T_{pre}) beginning from the onset of the final pre-stimulus syllable (t_p). **D–F** Hypothesis 2: if the CPG is reset by the stimulus but continues to oscillate thereafter, then t_r will occur at an integer multiple of T_{pre} beginning from the onset of the stimulus (t_s). **G–I** Hypothesis 3: similar to hypothesis 2 except that the CPG is reset by an amount that varies as a $f(\phi)$. Hypothesis 4: if acoustic startle stops the CPG altogether, then katydids will resume singing (i.e., t_r) randomly with respect to t_p, t_s, and φ. Note: response trials (columns 2 and 3) are compared against the no response trials (column 1) as well as the expected value for a random (uniform) distribution (*dotted line*). See text for additional details
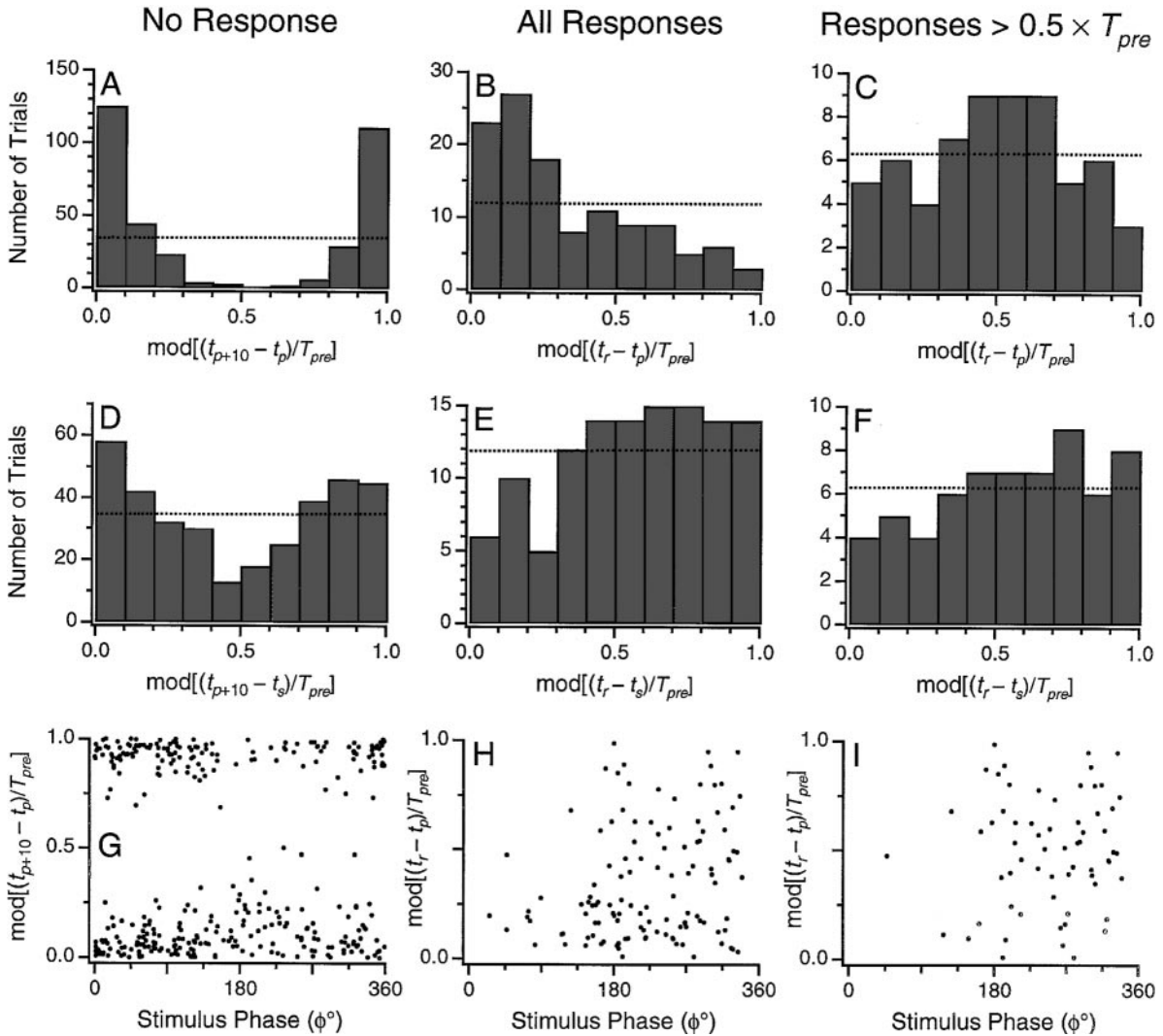
periods in these trials were, by definition, significantly different from T_{pre}. Panel A's usefulness, however, comes in providing an expected distribution against which hypotheses 1 and 2 can be tested. The significantly non-uniform distribution in panel B, obtained by evaluating Eq. 6 for the 119 trials with responses in periods P or $P + 1$ only, results from the numerous small responses with normalized pause durations $\leq 0.5 \times T_{pre}$. When these small pauses are excluded, leaving only responses with normalized pause durations $> 0.5 \times T_{pre}$, this yields panel C which contains 63 trials where katydids skipped at least half a period before singing resumed. The distribution in panel C is significantly different from the expected distribution of panel A, but is not significantly different from a uniform distribution (hypothesis 4). This result shows that when katydids pause for more than half a cycle during an ASR, they do not resume singing in phase with where they left off, thereby falsifying hypothesis 1. Panels D–F show the distribution of remainders assuming that the stimulus resets the neural CPG (hypothesis 2). Note how correcting for ϕ in the no-response trials (panel D) degrades the tight clustering of remainders previously observed in panel A; Panel D is significantly different from both panel A and from a uniform distribution. Panel E shows the distribution of remainders for all response trials, whereas panel F shows the data for only those trials with normalized pause durations $> 0.5 \times T_{pre}$. Although both distributions have been corrected for ϕ, neither is significantly different from a uniform distribution, thus falsifying hypothesis 2. Panels G–I are scatter plots of ϕ against the remainders obtained from Eq. 8, which says that the delay introduced in resetting the neural CPG varies as a $f(\phi)$. Not surprisingly, the data from the no response trials (panel G) shows no relationship with ϕ even though a clear pattern exists: all of the remainders cluster around 0 or 1 (modulus phase = 0° or 360°). When one examines the scatter plots for the data from all of the response trials (panel H), or for only those trials with normalized period increases $> 0.5 \times T_{pre}$ (panel I), neither a relationship nor a pattern is observed, thereby falsifying hypothesis 3. Therefore, the null relationship (hypothesis 4) cannot be rejected because there is no evidence to conclude that N. ensiger's CPG continues to oscillate – with or without a stimulus resetting effect – during song pausing.

Discussion

Startle responses of singing Neoconocephalus spp. have previously been described, but surprisingly, auditory stimuli were never credited with eliciting the behaviors. Cantrall (1943) observed that male N. ensiger "invariably cease singing when the beam of the headlight falls upon them," and that "a strong light usually causes them first to dodge behind the stem on which they rest, and then drop to the ground, where they hide by burrowing in the vegetation." Regarding the escape

behavior of N. robustus, Counter (1977) wrote "the slightest mechanical disturbance may cause cessation of singing" and later suggested that the abdominal cerci probably mediate the escape responses observed in the field. Visual and vibrational cues are important releasers of startle responses in all orthopteran insects (e.g., Pearson and O'Shea 1984; Kühne et al. 1985; Dambach 1989), and while our results do not address the initiation of startle via sensory modalities other than hearing, nevertheless they clearly demonstrate that acoustic cues alone are important and sufficient for the initiation of startle in non-flying insects.

Katydids are a staple food for many animals, particularly vertebrates (e.g., mice, shrews, primates, lizards, snakes, toads, frogs, bats, and birds) and arthropod predators (e.g., mantids, spiders, scorpions, and carnivorous katydids). Thus, it is not surprising that they have evolved numerous and elaborate predator avoidance strategies (for reviews see Belwood 1990; Nickle and Castner 1995). Such strategies seem particularly important for males because they are the sex (usually) that produce the loud, long-range advertisement signals that serve as conspicuous beacons to sexually receptive females and acoustically orienting predators (e.g., Walker 1964; Belwood and Morris 1987).

Although cessation of singing seems like an obvious candidate for a defensive behavior, it is worth mentioning that it fulfills most of the established criteria for acoustic startle, including those of the mammalian ASR (see chapters in Eaton 1984). Startle responses are fast, stereotypic motor acts that occur with short latency in response to unexpected or alarming stimuli with a rapid onset. In invertebrates these behaviors are usually, but not always, expressed with an all-or-none characteristic when the stimulus rises above a certain intensity (Hoy 1989). Cessation of singing is a highly stereotyped behavior that occurs with a short latency to brief pulses of ultrasound. It tends to have an all-or-none expression, with full expression occurring most reliably at moderate-to-loud ultrasonic frequencies, and can be elicited by even single pulses of ultrasound, although short-duration pulse trains are also effective. It can also habituate with repeated stimulation. Cessation of singing is a nondirectional ASR that does not result in a translocation of the animal away from a threatening stimulus; however, as Bullock (1984) reminds us, not all startle responses automatically lead to escape (e.g., freezing). Cessation of singing is only one member of a suite of ASRs that constitute a katydid's defenses. Finally, there can be little doubt that silencing is an effective strategy for concealing oneself from acoustically orienting predators.

The function of cessation of singing and song pausing is obvious: in response to disturbing sounds, katydids insert gaps in their calling song so that a window of silence is available for monitoring the environment and listening for predators. If a startle stimulus arrived early in the pulse period and before the transition phase of

ca. 180–220°, *N. ensiger* lengthened its concurrent pulse period, whereas if the stimulus fell late into the calling cycle the subsequent period was increased. The L response of snowy tree crickets stimulated with playback sounds (Walker 1969) and the delay response of singing ground crickets to substrate vibrations (Dambach 1989) both work in a similar fashion. Indeed, Walker (1969) reported a similar transition phase (220°) for acoustic synchrony in tree crickets. A mechanistic explanation for this behavior is analogous to the photically induced phase shifting (resetting) of the simple relaxation oscillator proposed to control rhythmic flashing in fireflies (Buck et al. 1981b): if a stimulus arrives after the transition phase of the calling cycle, the neural pacemaker (CPG) responsible for song production can no longer be reset, hence the animal is committed to the production of another syllable before lengthening the subsequent period. That no strong relationship exists between ϕ and the duration of the post-stimulus pause (Fig. 6), combined with the lack of evidence supporting various models (hypotheses) concerning the timing and control of the CPG with or without a stimulus resetting effect (Fig. 7), leads us to conclude that acoustic startle halts the song CPG altogether. This predicts that the CPG – and not just its output motor neurons – is the site of neural inhibition within the CNS during acoustic startle.

In many stridulating Orthoptera the song CPG is both inhibited and excited by the chirps of conspecifics (Jones 1966a, b, 1974; Shaw 1968; Heiligenberg 1969). Calling males advance or retard the onset of their next syllable after hearing a neighboring male's song, and such reciprocal interactions can generate rather precise relationships in the form of phase synchrony or alternation (Sismondo 1990) between rival males "singing to lead" to attract sexually receptive females (Greenfield and Roizen 1993). Cessation of singing and song pausing, as reported in this paper, differ from the inhibitory resetting mechanisms proposed for the control of song in other rhythmically signaling insects. The bimodal distribution in the variance of T_{post} (Fig. 4B) and in the duration of the post-stimulus pause (Fig. 6) both indicate that there are at least two ways of introducing pulse

period variation. The first mechanism produces small period increases with relative magnitudes (from 3% to < 100% of T_{pre}) comparable to that of other orthopterans during male-male interactions (Shaw 1968; Sismondo 1990; Greenfield and Roizen 1993). That *N. ensiger*'s phase-response curve (Fig. 6) and modulus-response function (Fig. 7) did not show an obvious relationship with ϕ for the small (or large) responses may not be surprising given that the maintenance of precise phase relationships between stridulating males has not been demonstrated for insects calling at rates > 5 Hz (see Greenfield 1994) – which is the case for *N. ensiger* (14 Hz; Table 1).

The second mechanism produces large period increases (from 100% to > 1000% of T_{pre}) that should not be confused with the period adjustments made by interacting males, although the underlying mechanism(s) may incorporate neural elements common to both behaviors. We suggest that these large period increases are part of the ASR proper. First, the magnitude of the pause duration provides a reasonable time window to listen for predators (Fig. 6), is longer than that required for male-male interactions, and is unrelated to T_{pre} and the onset or phase of the stimulus (Fig. 7). Note that the upper boundary for large period increases is unlimited; in this study cessation of singing was defined as any pause ≥1 s, but clearly cessation of singing and song pausing fall along the same continuum. Second, large period increases occur in response to single pulses of ultrasound and do not require pulse entrainment or pre-pulse conditioning, unlike male-male interactions. Third, mutual period adjustments made by singing crickets and katydids occur in response to pulses (or mimics) of *conspecific song*; however, *N. ensiger* responded poorly or not at all when stimulated with sounds centered near the peak frequency of its calling song, but startled consistently and with lower thresholds when stimulated with ultrasound (Fig. 3). Fourth, the short response latency for cessation of singing is typical of insect ASRs (see Table 2). Finally, the overwhelming majority of responses were syllable period increases. Rarely did *N. ensiger* advance the onset of its next syllable, and when it did the decrease was usually less

Table 2 Comparison of the behavioral context, frequency, threshold, and response latency for all of the insect orders known to have acoustic startle responses

Insect (context)	Frequency (kHz)	Threshold (dB SPL)	Latency (ms)	Reference
Moth (walking)	40	50–87	?	Werner (1981)
Moth (flying[a])	40	40	45–60	Roeder (1967b)
Lacewing (flying[a])	30–70	55–77	40–106	Miller (1975)
Beetle (tethered)	20–70	60–70	30–60	Forrest et al. (1995)
Beetle (flying[a])	30–60	75–80	90–150	Yager and Spangler (1997)
Cricket (flying[a])	30	55	25–66	Nolen and Hoy (1986)
Locust (flying[a])	15–35	44–50	40–88	Robert (1989)
Locust (sitting)	2–40[b]	77–82	5–60	Riede (1993)
Mantis (flying)	40	64	125–230	Yager et al. (1990)
Katydid (flying[a])	30–60	78	30–70	Libersat and Hoy (1991)
Katydid (singing)	30–100	63–74	20–50	Present study

[a] Stationary flight
[b] Band-limited white noise

than 10% of T_{pre} and was not correlated with the onset of the stimulus (Fig. 4C).

Cessation of singing and song pausing occur only when an ultrasonic stimulus arrives during the window of silence between stridulatory syllables (Figs. 5, 6, and 8). The simple explanation is that the katydid's own song – which is loud – temporarily deafens or masks it's auditory system, thus limiting signal detection to the final 60% of the syllable period. Because hearing is suppressed in some orthopterans during the production of stridulatory syllables (e.g., Wolf and von Helversen 1986; Hedwig 1990), Greenfield (1990) in his review on acoustic communication in the genus *Neoconocephalus* proposed that the reason why chirps and interrupted buzzes arose in male calling song was to provide "acoustic windows" through which singing males could monitor the songs of conspecifics, and if necessary, respond by adjusting their own temporal pattern. While our data do not refute this hypothesis, they do, however, provide a more general explanation for the evolution of acoustic windows – namely to allow singers to listen for *any* exogenous sounds, including those made by predators.

A potential criticism of this study is that because our experiments were conducted on isolated males singing in an anechoic environment, a situation that occurs infrequently in nature, the phenomenon observed may not occur if males were subjected to realistic levels of hetero- and conspecific background noise. Background noise is undoubtedly a problem for any hearing organism because it lowers sensitivity and masks the detection of signals of interest (but see the phenomenon of stochastic resonance; Braun et al. 1994). However, this argument does not invalidate the present findings; background noise may alter thresholds, but the best opportunity for predator detection will still occur during the window of silence between stridulatory pulses. It is also possible that the statistical criterion we used to define a response was too conservative to detect subtle phase-response effects; however, acoustic startle is not a subtle behavior.

Our field observations revealed that katydids are extremely sensitive to sounds and vibrations in their environment, and startle thresholds measured in the laboratory revealed a sensitivity to ultrasound that is behaviorally and ecologically relevant. Male *N. ensiger* regularly ceased calling and dropped to the ground during our approaches to collect them. This was particularly true in the early part of the breeding season when competition for females was presumably most severe. Apparently, the costs associated with temporarily suspending advertising for females until a male can climb back up to his singing perch are small when compared to the costs of predation at this time (i.e., no further chance of reproduction). Late in the breeding season males also ceased singing when disturbed, but were reluctant to drop from their perch unless we were nearby. Perhaps katydids combine information from their auditory and vibration receptors (Dambach 1989) to assess the proximity and thus potential threat of a

stimulus before taking further evasive action (e.g., dropping to the ground) that would, out of necessity, introduce delays in their bouts of mate-calling. Such delays may be relatively costly late in the breeding season when life expectancy is short. Alternatively, males may opt for a higher risk of predation when expected future reproduction benefits are low. And while cessation of singing is an inapplicable behavior to *N. ensiger* females and nymphs, by no means are they free from the selection pressures associated with terrestrial ultrasound. Indeed, females and nymphs possess sensitive ultrasound hearing (Faure 1999), and when disturbed they too are capable of rapid escape – dropping to the ground, jumping, and active flight (adult females only).

Comparison with other insect ASRs

Cessation of singing is a robust behavior and should therefore be added to the growing list of insect ASRs. Table 2 compares the context, frequency, behavioral threshold, and response latency for all insect orders known to possess ASRs. Startle thresholds are quite similar across all groups, lying between 60 dB SPL and 80 dB SPL in most instances. Note that the data for singing *N. ensiger* are well within the values reported for other insects. That startle latencies are so rapid is not surprising given their role in early warning and/or escape. Although tympanal hearing has evolved independently in each of the orders (Hoy and Robert 1996), regardless of behavioral context, acoustic startle is exclusively ultrasound induced. Furthermore, all of the insects in Table 2 are at least partially nocturnal, which reinforces the notion that bat echolocation was and continues to be an important driving force in the evolution of insect hearing.

Cessation of flight versus cessation of song: the role of behavioral context

Singing *N. ensiger* had their lowest startle thresholds for frequencies between 30 kHz and 100 kHz and were reluctant to startle at behaviorally relevant amplitudes for frequencies < 17 kHz (Fig. 3). Although we did not test katydids using playbacks of conspecific song, males singing beside one another in the field or in the laboratory never appear to startle each other. Because the calling song of *N. ensiger* contains both sonic and ultrasonic frequencies (Fig. 2, Table 1), it seems reasonable to speculate that sonic frequencies may act to inhibit any startle behaviors that might otherwise be triggered by ultrasound within the song. If true, then neurons involved in the ASR should show reduced or inhibitory responses to sonic frequencies (Faure 1999). Temporal features of calling song would also be important for discriminating signaling neighbors (conspecific and heterospecific katydids) from natural enemies,

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especially in katydids with purely ultrasonic signals (e.g., Morris et al. 1994).

N. ensiger also has the classic bat-avoidance ASR – cessation of flight. Within 50 ms of receiving a pulse of ultrasound, flying *N. ensiger* alter their posture and wingbeat frequency resulting in the closure of all four wings (Libersat and Hoy 1991). Like cessation of singing, cessation of flight is a robust, ultrasound-tuned behavior (80% of individuals responded in both studies). Figure 8 compares the tuning curves for both ASRs. Although startle thresholds were determined by different researchers using different protocols, and the frequency range tested for flying *N. ensiger* was narrower than that used in this study, the behavioral audiograms are strikingly similar. The average best frequencies and best thresholds were 45 kHz and 30 kHz, and 72 dB SPL and 63 dB SPL, and the Q_{10dB} for the flying and singing ASRs were 1.13 and 1.25, respectively. While the context is seemingly very different (flying versus singing), the correspondence in tuning strongly suggests that a single mechanism underlies both behaviors. This hypothesis is plausible given that in crickets and katydids many of the muscles, motor neurons, and neural networks involved in the production of song are also used for flight (Josephson and Halverson 1971; Elder 1971; Bentley and Hoy 1974); thus by extension, the mechanism responsible for cessation of flight should be similar to that for cessation of song.

Figure 8 also shows the average threshold-tuning curve for an auditory interneuron located in the prothoracic ganglion of *N. ensiger* – the T-cell (Faure 1999). The cellular morphology of this neuron gives it a T-shaped appearance (e.g., Schul 1997). Even though T-cell thresholds are considerably lower than ASR thresholds (mean $\Delta dB \approx 35$ dB), the similarity in shape between the behavioral and neural tuning curves is
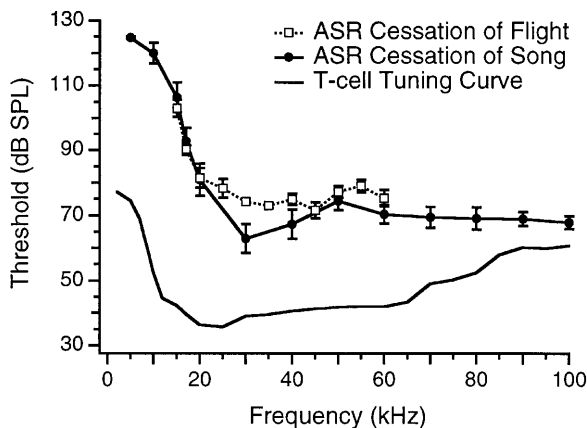
obvious. Indeed, correlations between the physiology of the T-cell and the in-flight ASR have been suggested (Libersat and Hoy 1991). Recently we have shown that the physiology of the T-cell makes it well suited to function as a bat detector neuron (Faure 1999; P.A. Faure and R.R. Hoy, unpublished observations). Additional data and arguments for the involvement of the T-cell in the ASRs of volant and non-volant *N. ensiger* – in both nymphs and adults – is the subject of future papers.

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Fig. 8 Comparison of ASR thresholds in two behavioral contexts: cessation of flight and cessation of song. Shown are the mean ± SE behavioral (startle) audiograms for *N. ensiger* males measured during tethered flight (*open squares*; Libersat and Hoy 1991) and while mate-calling (*filled circles*; Fig. 3). Also shown is the average threshold tuning curve for a prominent ultrasound-sensitive interneuron in *N. ensiger* – the T-cell (loudspeaker position = 90°)

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