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Identified auditory neurons in the cricket *Gryllus rubens*: temporal processing in calling song sensitive units

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Abstract

This study characterizes aspects of the anatomy and physiology of auditory receptors and certain interneurons in the cricket *Gryllus rubens*. We identified an 'L'-shaped ascending interneuron tuned to frequencies >15 kHz (57 dB SPL threshold at 20 kHz). Also identified were two intrasegmental 'omega'-shaped interneurons that were broadly tuned to 3–65 kHz, with best sensitivity to frequencies of the male calling song (5 kHz, 52 dB SPL). The temporal sensitivity of units excited by calling song frequencies were measured using sinusoidally amplitude modulated stimuli that varied in both modulation rate and depth, parameters that vary with song propagation distance and the number of singing males. Omega cells responded like low-pass filters with a time constant of 42 ms. In contrast, receptors significantly coded modulation rates up to the maximum rate presented (85 Hz). Whereas omegas required ~65% modulation depth at 45 Hz (calling song AM) to elicit significant synchrony coding, receptors tolerated a ~50% reduction in modulation depth up to 85 Hz. These results suggest that omega cells in *G. rubens* might not play a role in detecting song modulation per se at increased distances from a singing male.
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Keywords: Sinusoidal amplitude modulation; Temporal modulation transfer function; Omega cells; Afferents; INT-1; AN2; Ultrasound; *Gryllus bimaculatus*; *Teleogryllus oceanicus*; Calling song

1. Introduction

In most species of crickets (Gryllidae), males produce calling songs that function as sexual advertisement signals allowing conspecifics to discriminate among singing males (Walker, 1957; Alexander, 1960). In addition to song carrier frequency (Popov and Shuvalov, 1977) and relative song intensity (Farris et al., 1997), one of the most important parameters for discriminating songs is the temporal structure of a song's amplitude modulation (AM). Indeed, phonotactic decisions which are known to be ultimately correlated to species recognition and character displacement (Walker, 1974; Shaw and Herlihy, 2000) have been shown to be based on the AM rate of calling songs (Walker, 1957; Doherty, 1985; Popov and Shuvalov, 1977; Ulagaraj and Walker, 1975; Doolan and Pollack, 1985). In the field, however, variance in song AM is not limited to AM rate. As a song propagates through a complex environment, the AM structure can be distorted due to reverberations (i.e., multipaths), frequency-dependent absorption and the addition of sounds produced by other animals (see Forrest, 1994; Römer, 1998 for review). In the time domain, such environmental filtering smears the AM structure of a calling song and effectively removes the intervals between song pulses (Simmons, 1988).

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Abbreviations: hfAN, high-frequency ascending neuron; ON1, ON2, omega neuron 1, 2; SAM, sinusoidal amplitude modulation; TMTF, temporal modulation transfer function; INT-1, interneuron 1; AN2, ascending neuron 2

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Compared to frogs (i.e., species that produce repetitive or cricket-like song; Rose and Capranica, 1985), however, there are relatively few data for the effects of variance in song modulation depth on the physiological coding or behavioral attractiveness of calling songs in insects in general (Machens et al., 2001, Prinz and Ronacher, 2002) and crickets in particular; most studies of temporal sensitivity in acoustic insects have been concerned only with sensitivity to AM rate (i.e., pulse duration, inter-pulse interval, etc.), while foregoing tests on AM depth. Although several studies have indirectly assayed the effects of temporal distortion by measuring responses to either the simultaneous presentation of multiple songs (i.e., two stimuli with ideal pulsed or "DC-on" and "DC-off" structures; Schildberger, 1984; Doherty, 1985; Pollack, 1986; Römer and Krusch, 2000) or the presentation of songs at various distances (Römer and Bailey, 1986), these experimental paradigms do not systematically measure the limits of detection or discrimination of changes in modulation depth.

From a psychophysical point of view, a more general measure of temporal sensitivity can be derived from measuring a system's temporal modulation transfer function (TMTF). Considered a systems analysis approach, the TMTF explains the amount of modulation necessary for the detection of modulation at each modulation frequency (Viemeister, 1977, 1979). Although the structure of the AM stimuli used to measure TMTFs may vary across experiments [e.g., sinusoidal AM (SAM), pulsed AM and square AM] the independent variables are always the modulation frequency and depth. In this paper, we used SAM stimuli that varied in modulation frequency and depth, to measure the TMTF of identified auditory neurons in the cricket, *Gryllus rubens* (Gryllidae, Gryllinae). The calling song of *G. rubens* consists of a trill (i.e., sound pulses repeated at a constant interval, Römer, 1998) of 11 ms pulses of 4.5 kHz at 45 pulses/s at 22 °C (Walker, 1962; Bentley and Hoy, 1972; Doherty and Callos, 1991). Although no prior studies have characterized the anatomy and physiology of individual auditory units in *G. rubens*, the rationale for using this cricket to measure temporal sensitivity is related to its trilled calling song. Without the added complexity of chirps (i.e., more than one interpulse interval), the trilled song of *G. rubens* is ideal for examining sensitivity to amplitude modulation at one time scale only, the temporal structure of the pulses in the trill.

Although the stimuli used in previous studies of temporal sensitivity in crickets almost certainly contain more modulation than that found for songs in the field, the sinusoidally modulated stimuli used here may represent the other extreme in that there are no true 'offs' to the stimulus. The amplitude of the SAM stimulus is always changing and only instantaneously goes to 0 when 100% modulated. Thus, whereas the artificial stimuli used in most previous studies represent a best case scenario for signal transmission, the SAM stimuli used here hypothetically represent signal transmission that is less than ideal.

2. Methods

2.1. Subject animals

The breeding colony of *G. rubens* was started in 1997 from individuals sound trapped in Alachua County, Florida, reared under a 14 L/10 D h. light schedule and fed Cat Chow™ ad libitem. This project adhered to the Cornell University guidelines for animal care and use.

2.2. Acoustic stimuli

All stimuli were generated using Tucker Davis Technologies (TDT) 16 bit, digital-to-analog converters and custom written software (6 and 10 μs sampling period for tuning curve and SAM stimuli, respectively). Stimuli were amplified using a Harman/Kardon HK6150 integrated amplifier and broadcast from Radio Shack Super tweeters (cat. no. 40-1310b) located 30 cm from the preparation and positioned 0° normal to the longitudinal axis of the cricket. Stimulus amplitude was adjusted using TDT PA4 programmable attenuators. The stimuli were calibrated at the position of the test animal using online comparisons of the r.m.s. voltage of the stimuli to that generated by a B&K 4220 pistonphone calibrator (125 ms duration samples). The calibration system included a B&K 4135 1/4 inch microphone (0° angle of incidence), B&K 2639 preamp and a B&K 5935 microphone power supply. All sound pressure levels (dB SPL) are referenced to 20 μPa. The onset and offset ramps of pulses used to measure tuning curves are raised cosine. Total harmonic distortion of the system was determined to be <1% (−40 dB) using a Hewlett–Packard 3562A signal analyzer.

2.3. Neurophysiological recordings

The experimental procedures used here are similar to those in Mason et al. (1998). Briefly, cold-anethesized female crickets were mounted on a platform ventral side up in a foam lined Faraday cage that reduced acoustic and electrical noise. The prothoracic legs were extended laterally and waxed at the tarsi to stainless steel bars. After exposing the prothoracic ganglion by removing the ventral cuticle, the ganglion was supported on a stainless steel "spoon" that also served as the ground electrode. Electrical activity in

auditory units in the prothoracic ganglion was recorded using thin-walled (1.0 mm o.d.) borosilicate glass, micropipette electrodes filled at the tip with 2.5% Lucifer Yellow (Sigma) and backfilled with 0.1 mol l^{-1} LiCl. Electrode resistance varied from 30 to 120 MΩ . The search stimulus used to locate auditory units consisted of a pair of 20 ms pulses of 5 and 40 kHz presented asynchronously (25 ms interpulse interval). After amplification (AM systems model 1600 DC followed in some experiments by a custom built AC amplifier), the neural responses were digitized (100 μs sampling period) using a TDT AD3 and System II Array Processor. By injecting hyperpolarizing current (0.2–1.2 nA) after recording, cells were stained with Lucifer Yellow for anatomical identification. After staining, the ganglia were dissected and fixed (16–24 h) in 4% paraformaldehyde (not buffered), dehydrated in an ascending ethanol series and cleared using methyl salicylate. Stained cells were photographed and digitized as whole mounts using a Leitz Dialux 20 and BioRad MRC-600 confocal microscopes, respectively. No cells were included in the study unless staining provided adequate identification.

2.4. Experimental protocols

After penetrating a cell, we measured the temporal sensitivity of auditory units excited by 5 kHz by presenting a series of sinusoidal amplitude modulated (SAM) tones (5 kHz, 90 dB SPL) that varied in modulation rate and depth. The SAM-series consisted of five replicates of four modulation frequencies (25, 45, 65, 85 Hz) at five modulation depths (100%, 75%, 50%, 25%, 0%) to yield a total of 100 stimuli. Each stimulus consisted of ten cycles of modulation (i.e., stimulus duration varied from 118 to 400 ms). Although neural adaptation is likely to occur throughout the durations of these gated stimuli (i.e., stimulus duration is potentially shorter than the neural adaptation time and thus spike-train analysis includes onset responses; Givois and Pollack, 2000; Gleich and Klump, 1995), we chose the 10-cycle SAMs for the following reasons: (1) stimuli are at least twice the number of cycles required to elicit phonotaxis in gryllids and are thus behaviorally relevant (Nolen and Hoy, 1986a); (2) best measures of temporal processing are not necessarily made while auditory units are in a more adapted state (Epping, 1990; Prinz and Ronacher, 2002) and longer duration stimuli can reduce sensitivity to amplitude modulation (Smith and Brachman, 1980; Smith et al., 1985; see Frisina, 2001 for review); (3) psychophysical studies in humans comparing continuous and gated stimuli show gating only reduces thresholds at the slowest modulation rates without systematically affecting the low-pass cutoff frequency (Yost and Sheft, 1997); (4) use of gated stimuli facilitates comparisons with data from numerous other studies of temporal processing in crickets and other insects (e.g., Mason et al., 1998; Horseman and Huber, 1994; Schildberger, 1984; Tougaard, 1998); (5) it is not known whether the conditions for adaptation are met in the field. To reduce the spectral splatter associated with decreased modulation depth, 0.25 ms ramps (i.e., longer than one wavelength) were applied to the onsets and offsets of the SAM stimuli. Cells which exhibited spontaneous activity well after penetration (>30 s) were not included.

Behavioral and neural detection thresholds are determined by temporal integration of pressure and/or power (Heil and Neubauer, 2001; Gollisch et al., 2002; Farris and Hoy, 2000). The average power of a SAM stimulus varies with modulation depth as $(1 + \text{mod depth}^2/2) \times I_0$, where I_0 is the average power when mod depth = 0 (Viemeister, 1979). To ensure that the variance in neural discharges was due to the cells' ability to follow changes in amplitude modulation and not overall stimulus power (Frisina et al., 1990; Pollack and El-Feghaly, 1993), SAM stimulus amplitude was corrected online so that each SAM stimulus had the same energy (i.e., the integral of power) per modulation cycle (90 dB re. 20 μPa tone). Furthermore, this correction facilitated comparison of the cells' ability to detect sinusoidal amplitude modulation with the ability to detect changes in the amplitude of single pulses (see below). Spike times were measured from the digitized voltage traces using a custom written window discriminator of voltage level or voltage change (derivative). The SAM series was presented once per preparation.

Following the presentation of the SAM series, the frequency tuning of a cell was determined by measuring the minimum sound pressure level (±3 dB) required to elicit at least one action potential in 3/5 stimulus presentations. Stimuli were pure tones that varied in frequency from 3 to 65 kHz (10 ms pulses, 1 ms ramps, 400 ms ISI) and were presented in ascending order. Using the responses recorded during measurement of the tuning curve, input/output (I/O) functions at 5 kHz were measured by calculating the mean number of spikes elicited for the five repetitions presented at each intensity step.

2.5. Analysis

Methods used to assess the amount of modulation in the voltage response of cells sensitive to 5 kHz are modeled after those in Rees and Palmer (1989). Analysis of response modulation was accomplished by measuring the vector strength (r) (i.e., length of the mean vector) of the spike times, which is calculated by the following equation:

$$r = \sqrt{\left(\frac{\sum_i^n \cos(a_i)}{n}\right)^2 + \left(\frac{\sum_i^n \sin(a_i)}{n}\right)^2},$$

where a is the angular conversion of the spike times relative to the modulation period and n is the number of spikes (Rees and Palmer, 1989; but see Zar, 1999). The vector strength coefficient, r is inversely correlated to the variance in the spike times and can vary from 0 to 1; values near 0 represent the case in which there is no modulation in the spike train, whereas values close to 1 represent the opposite case in which spike times are perfectly concentrated at some phase in the modulation frequency. Statistical significance of r (assessing whether the spike times are uniformly distributed in the modulation cycle) was calculated using a Rayleigh test for circular uniformity (Zar, 1999). Vector strength values were also used to calculate the modulation gain (in dB) in the spike train using the following equation:

$$\text{Gain} = 20 \times \log \frac{\text{Percentage of modulation depth spike train}}{\text{Percentage of modulation depth stimulus}},$$

where the modulation depth of the spike train is defined as $200r$; because a spike train with 100% sinusoidal modulation produces a vector strength of 0.5, conversion of r to percent modulation depth requires a factor of 200 (Rees and Palmer, 1989; Gleich and Klump, 1995). This conversion of vector strength to gain (in dB) facilitates the analysis of the filter shape (Gleich and Klump, 1995; Frisina et al., 1990; Kim et al., 1990) and thus calculation of time constants. Furthermore, this conversion quantifies the signal-to-noise ratio received by cells post-synaptic to the recording site independent of spike number (Rees and Palmer, 1989; Frisina et al., 1990; but see Palmer, 1995 for review). Comparisons of modulation gain between cell classes were done using a t test, which conservatively assumes unequal variances (Welch's approximate t; Zar, 1999).

We used the slope of the I/O curves at 5 kHz (spikes/single 10 ms pulse) to assess the relationship between AM detection and intensity discrimination (Wojtczak and Viemeister, 1999). I/O responses were normalized to those for the 90 dB SPL pulses, the power of the SAM stimulus, to control for any variance in intensity discrimination threshold that may be correlated to the power of the carrier (Farris et al., 1997; McGill and Goldberg, 1968; Long and Cullen, 1985; Kohlrausch, 1993; Wojtczak and Viemeister, 1999). Subsequently, we solved for each cell's relative I/O slope by finding the least-squares solution of the following logistic equation:

$$\text{Proportion of the response to 90 dB} = \frac{\exp(\text{slope} \times \text{dB stimulus} + \text{intercept})}{1 + \exp(\text{slope} \times \text{dB stimulus} + \text{intercept})},$$

which is appropriate for analyzing dependent variables bounded by 0 and 1. Differences in the mean I/O slopes

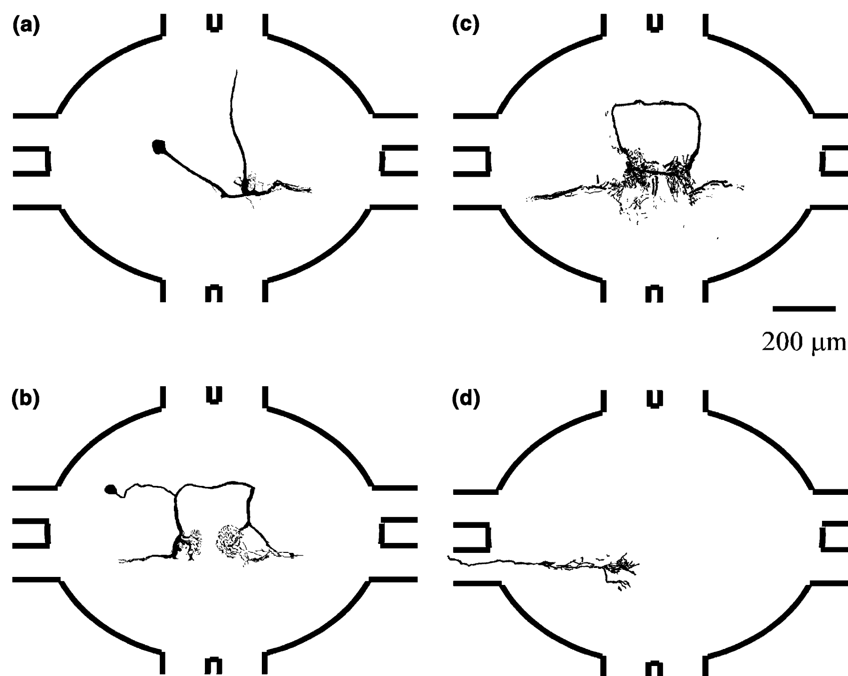

Fig. 1. Examples of the anatomy of auditory neurons recorded in the prothoracic ganglion (schematics; ventral view; top is anterior) of *G. rubens*. Sketches were produced by digitally tracing confocal micrographs. (a) hfAN (high-frequency ascending neuron). (b) ON1 (omega neuron 1). (c) ON2 (omega neuron 2). (d) Receptor neuron.

of different cell classes were tested using a two sample t test (two-tailed). Because one cell class had a small sample size (i.e., receptors, see Section 3) potentially affecting I/O slope variance, a variance ratio test was used to determine whether equal or unequal variance t tests were applied (Zar, 1999) and included only those slopes that were statistically significant ($P < 0.05$; but worst fit, $P = 0.006$).

3. Results

3.1. Characterized cells and frequency sensitivity

Three types of interneurons were recorded and stained in the prothoracic ganglion of *G. rubens* (Fig. 1). Nomenclature for these cells is based on their similarity to auditory units well known in other gryllines (Casaday and Hoy, 1977; Wohlers and Huber, 1982; Stiedl et al., 1997). The high-frequency ascending neuron (hfAN; Fig. 1(a)), characterized by its laterally extending dendritic processes, ascending axon and contralateral cell body is sensitive to frequencies >15 kHz with best sensitivity at 20 kHz (57 dB SPL, $N = 3$, Fig. 2(a)). In addition to this neuron's excitatory response to ultrasound, frequencies from 3 to 6 kHz elicited inhibition in some preparations (Figs. 2(a) and 3). The anatomy and physiology of this cell are indistinguishable from that of AN2 and Int-1 in *Gryllus bimaculatus* and *Teleogryllus oceanicus*, respectively (Moiseff and Hoy, 1983; Nolen and Hoy, 1986b; but see Schildberger et al., 1989; Hennig, 1988 for review).

We also recorded from several omega-shaped intrasegmental neurons ($N = 10$, Fig. 1(b) and (c)). Cells of this shape have been previously described in other ensiferan Orthoptera (Casaday and Hoy, 1977; Popov et al., 1978; Wohlers and Huber, 1982; Römer et al., 1988; Mason et al., 1998) and are broadly tuned to sounds with frequencies from 3 to 65 kHz, with a sensitivity peak at 5 kHz (~52 dB SPL; Fig. 2(b) and (c)). As in previous studies, we have sorted omega neurons into two subclasses, omega neurons 1 and 2 (ON1 and ON2). Both cells display the classic omega shape with bilateral arborizations connected by a large arched process that crosses the midline of the ganglion (Wohlers and Huber, 1982). The cell body extends laterally from the end of the arched midline process. Although based on only one preparation which did not include staining of the cell body, we have distinguished a second omega cell class (ON2) due to the presence of a large process that crosses the midline of the ganglion between the area of greatest arborization (Fig. 1(c)). This second large contralateral process is also the distinguishing character for ON2 in *G. bimaculatus* (Wohlers and Huber, 1982).

Auditory receptors were characterized by the presence of an axon extending laterally from the prothoracic ganglion down the tympanal nerve (Fig. 1(d)). Receptor tuning ($N = 4$) varied from frequencies <10 kHz to broad tuning across the entire stimulus range (Fig. 4). Although this pattern of variable tuning appears common for receptors (Nocke, 1972, Esch et al., 1980; Hutchings and Lewis, 1981; Imaizumi and Pollack, 1999), the presence of units tuned below 4 kHz raises the possibility that they originate in the subgenual rather than the tympanal organ [i.e., similar to the low

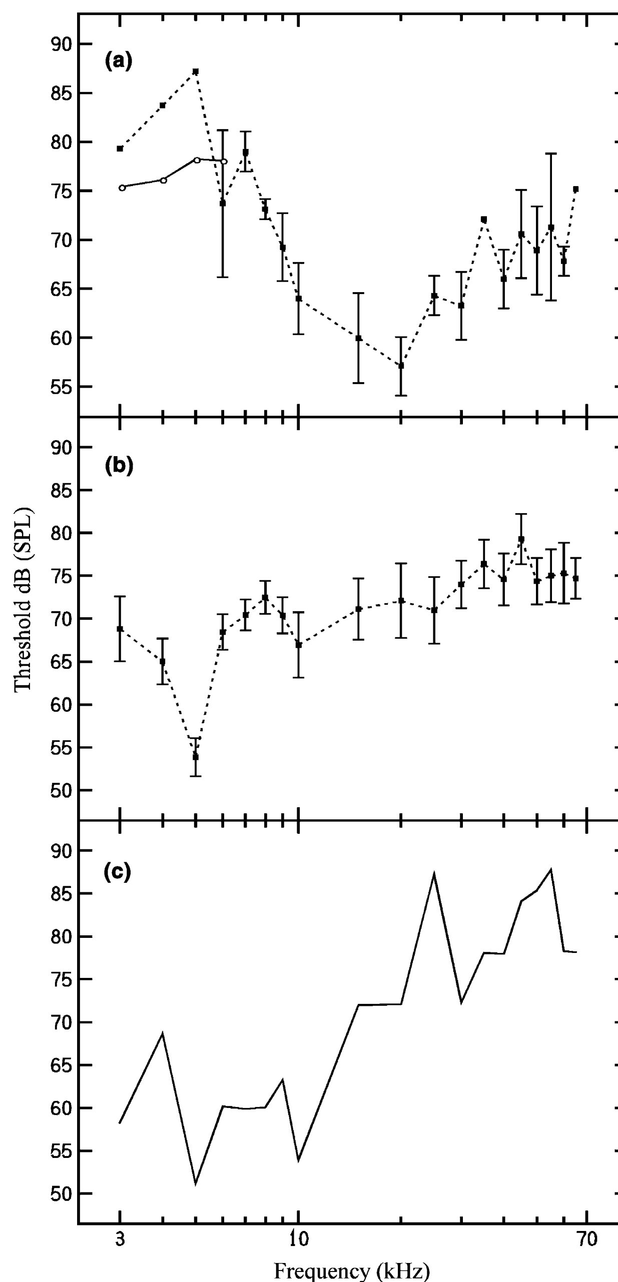

Fig. 2. Frequency tuning of interneurons. Squares are the mean thresholds (±SE) necessary to elicit an action potential in 3/5 presentations (10 ms duration, 1 ms ramps). Sample sizes were 3, 9 and 1 for hfAN (a), ON1 (b) and ON2 (c), respectively. Open circles in (a) show the mean thresholds for eliciting inhibitory post-synaptic potentials in certain preparations of hfAN ($N = 2$) (Fig. 3).

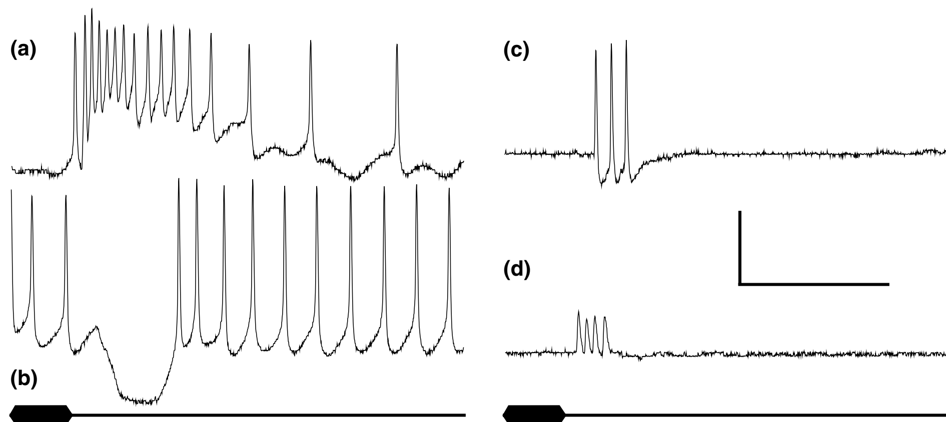

Fig. 3. Voltage traces of responses to single pulse stimuli of 10 ms duration (stimulus example in bottom trace). (a) Response of hfAN to 20 kHz (87 dB SPL). (b) Inhibition of spontaneous activity in hfAN by 4 kHz (74.7 dB SPL). The spontaneous activity, which was only initially encountered upon penetrating this cell, is displayed here to illustrate the scale of the IPSP. (c) Response of ON1 to 5 kHz (81.2 dB SPL). (d) Response of a receptor to 5 kHz (81.2 dB SPL). Calibration scale: 10 mV and 25 ms.

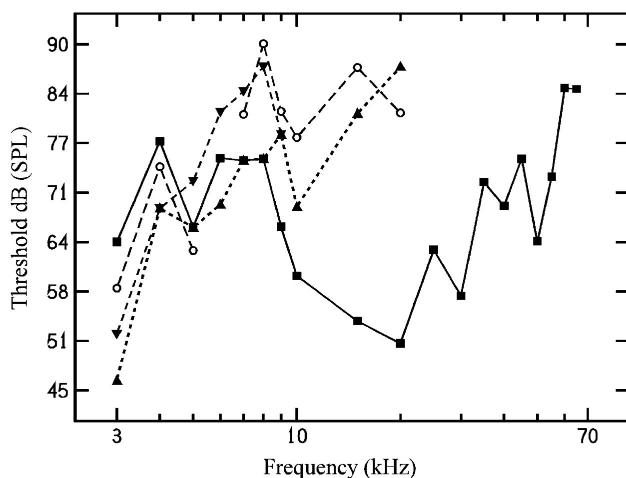

Fig. 4. Frequency tuning of four receptors. Symbols are the thresholds necessary to elicit an action potential in 3/5 presentations (10 ms duration, 1 ms ramps).

frequency receptor recorded by Nocke (1972)]. Nevertheless, their anatomical projections into the auditory neuropile as well as their sensitivity to short duration (10 ms) pulses of 5 kHz (i.e., ~72.5 dB SPL) suggest that they could detect and process the calling songs of gryllines (Nocke, 1971, Kavanagh, 1987, Nolen and Hoy, 1986a). Thus, we have included this small sample in the analysis of temporal processing below.

3.2. *Temporal sensitivity*

A complete SAM series was recorded for 10 ONs (including one ON2) and four receptors (data include one ON recording in which a frequency tuning curve was not measured). Example voltage traces and peri-stimulus time histograms (PSTHs) of the responses to the SAM series in the two cell classes are shown in Figs. 5–7, respectively. Whereas periodicity at the modulation

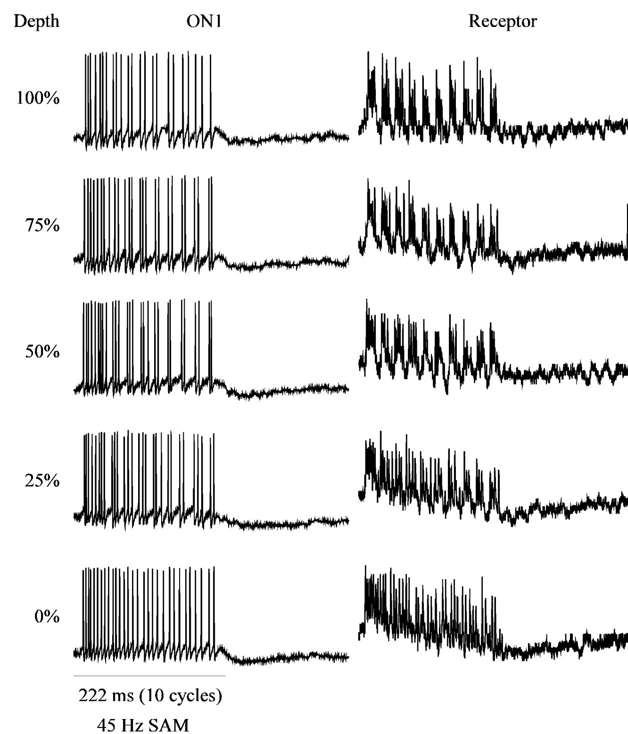

Fig. 5. Voltage trace responses of an ON1 and a receptor to a single SAM series at 45 Hz AM rate. Peri-stimulus time histograms of the responses of these same cells to the entire SAM series are shown in Figs. 6 and 7.

frequency in the receptor histograms (Fig. 7) is noticeable across all of the modulation frequencies, modulation in the PSTH of the exemplar ON1 response (Fig. 6) is displayed only up to 45 Hz. Drawing from convention in the field of psychoacoustics, TMTFs for ONs and receptors are plotted in Fig. 8 as the modulation depth necessary to elicit a spike train with statistically significant ($\alpha = 0.05$) modulation (at the stimulus modulation frequency) as a function of the modulation frequency of the stimulus. ONs demonstrate a low-pass filter response

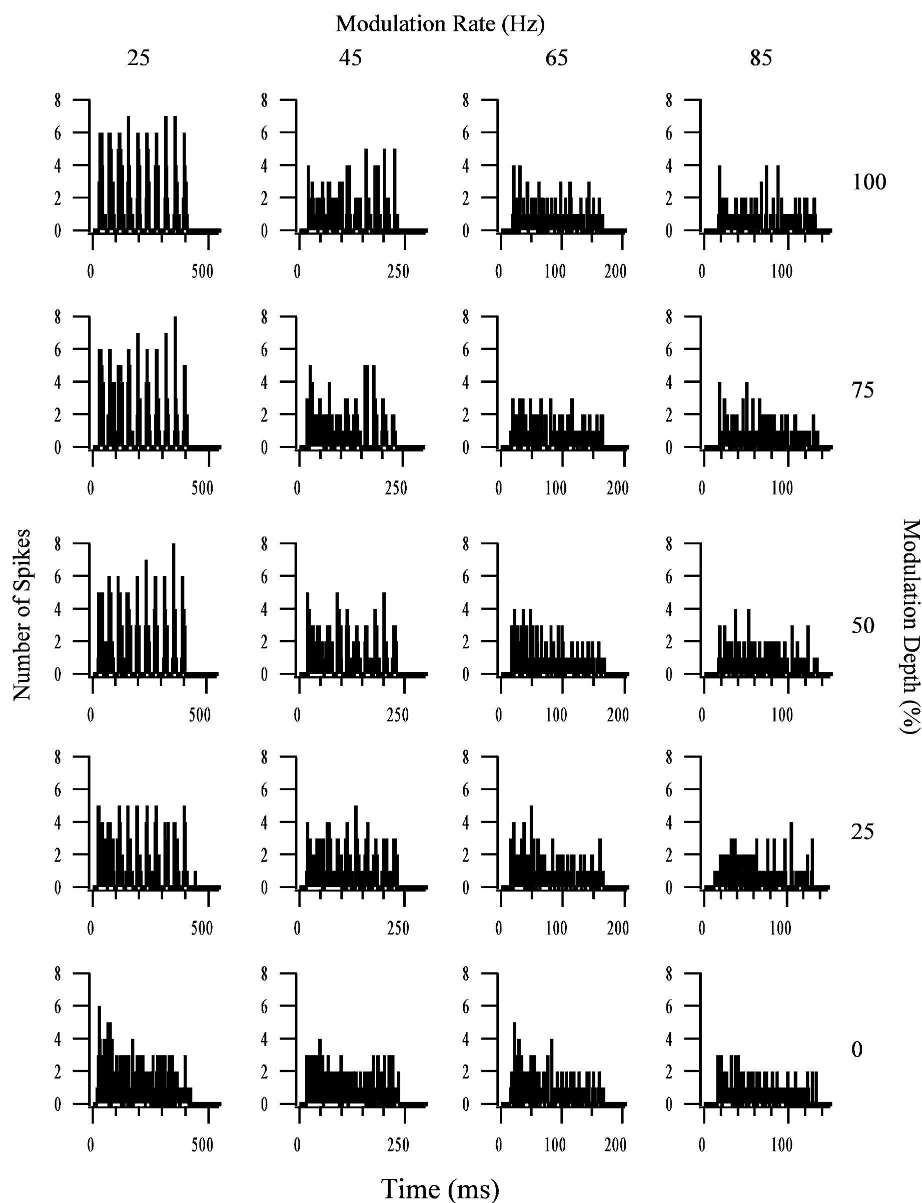

Fig. 6. Peri-stimulus time histograms of the response of an ON1 to the 20 different modulation stimuli. Each frame shows the summed response to five replicates of the 10-cycle stimulus. Note that for these histograms, binwidth equals 0.1 times the modulation period (e.g., binwidth at 25 Hz is 0.1 times 40 ms) which gives the appearance of variation in spike number between the tones.

whereas receptors show an all-pass filter response (Fig. 8). Whereas vector strength values (r) that were not significantly clumped (i.e., when $P > 0.05$) were thrown out for the TMTF in Fig. 8, the values still represent the relative distribution of spikes in the modulation cycle period. In order to make use of all of the r values to assess the response characteristics of the two classes of neurons, we calculated the amount of modulation gain (see Section 2) in the spike train (Fig. 9). These calculations reveal that in contrast to receptors, in which modulation is enhanced in the neural code at most modulation frequencies, modulation in the spike trains of omegas is reduced at all but the slowest rates. Furthermore, across all four modulation frequencies, gain in receptor spike trains was significantly greater than that for omegas at all depths except 25% (Table 1).

The low-pass like responses of omegas were modeled as one-pole low-pass filters using a least-squares fit of the following equation for each modulation depth except 0% (tones):

$$\text{Modulation gain}(f) = 20\log\sqrt{\frac{1}{1+f^2\tau^2}} + C,$$

where C is a constant shifting the filter above 0 (as the name implies, measures of gain may reveal increases in

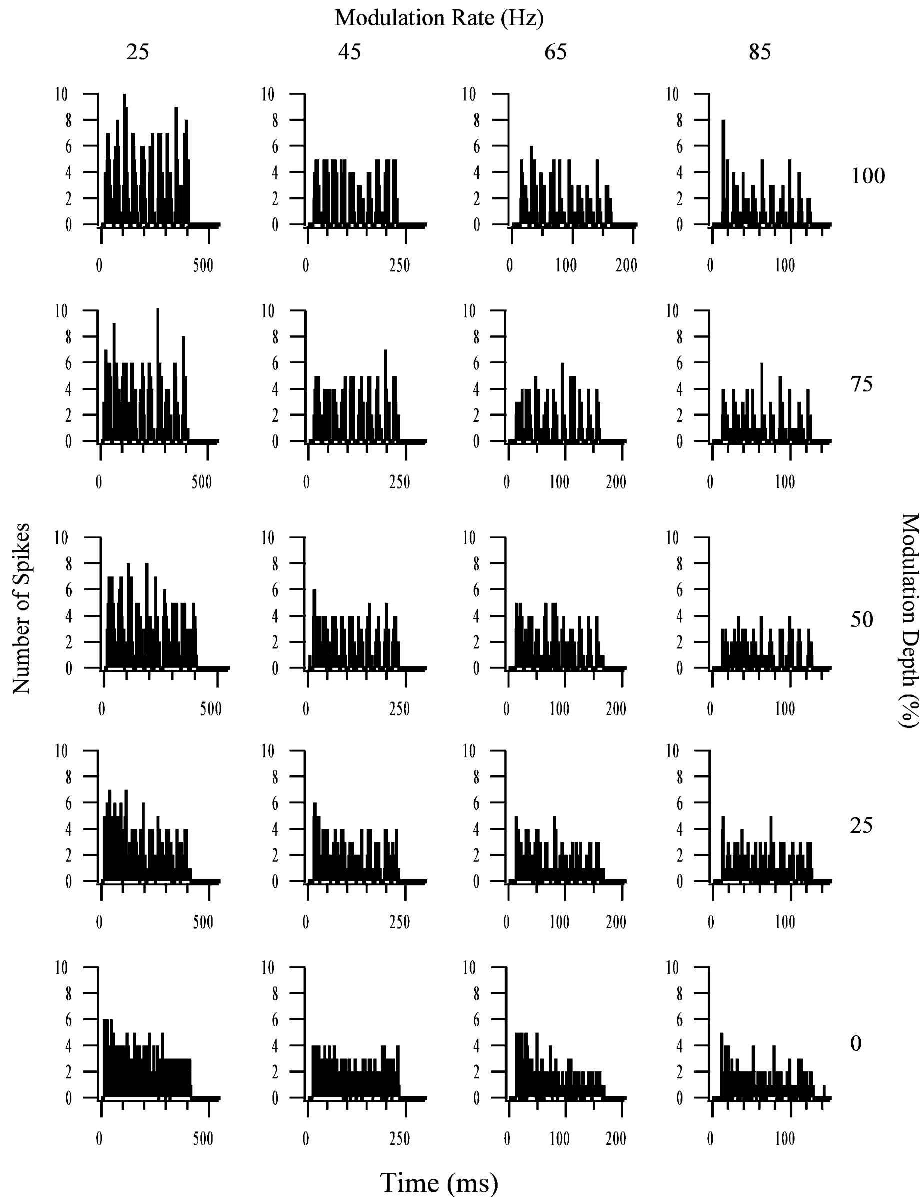

Fig. 7. Peri-stimulus time histograms of the response of a receptor to the 20 different modulation stimuli. Each frame shows the summed response to five replicates of the 10-cycle stimulus. Note that for these histograms, binwidth equals 0.1 times the modulation period (e.g., binwidth at 25 Hz is 0.1 times 40 ms) which gives the appearance of variation in spike number between the tones.

modulation), and f and τ are the modulation frequency and time constant of the filter, respectively. Although a simple linear regression of modulation gain verses log modulation frequency showed slopes slightly below that expected for a simple low-pass filter (i.e., −6 dB/octave; Table 2), the low-pass filter model explained a significant proportion of the variance in the responses of omega neurons at all depths except 25%. The mean cutoff frequencies calculated from our sample of omega cells using gain functions is ~24 Hz (varying with modulation depth; Table 2), slower than that for receptors ($\tau = 15$ ms, $1/\tau = 67$ Hz, $R^2 = 0.365$, $P = 0.013$).

3.3. Input/output at 5 kHz

Whereas no difference was found between the omega and receptor I/O slopes measured on an absolute scale (Fig. 10(a), mean $\mathrm{ON_{slope}} = 0.132$, $N = 8$, mean $\mathrm{receptor_{slope}} = 0.296$, $N = 4$, $P = 0.324$), the relative I/O slopes differed significantly (mean $\mathrm{ON_{slope}} = 0.115$, $N = 8$, mean $\mathrm{receptor_{slope}} = 0.307$, $N = 4$, $P = 0.024$; due to either an incomplete intensity series or a statistically insignificant I/O slope, two ONs were not included in these comparisons). This means that in comparison with omegas, this sample of receptors require smaller changes in intensity (from the 90 dB car-

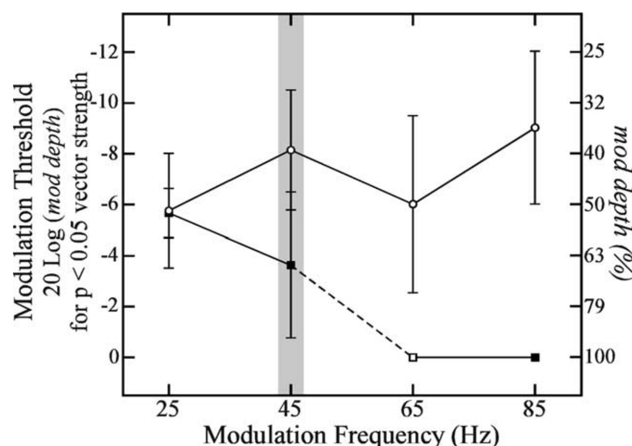

Fig. 8. Average temporal modulation transfer functions showing modulation threshold as a function of stimulus modulation frequency. Circles and squares are the mean (±SE) modulation index [i.e., 20 × log(mod depth)] necessary for significant ($P < 0.05$) modulation in the spike trains at the four modulation frequencies for receptors ($N = 4$) and omega neurons ($N = 10$), respectively. There were no omega preps which displayed statistically significant modulation at 65 Hz (open square) and only one prep at 85 Hz. Right axis shows the modulation index in percent depth. Shaded area marks the calling song modulation frequency at 22°.

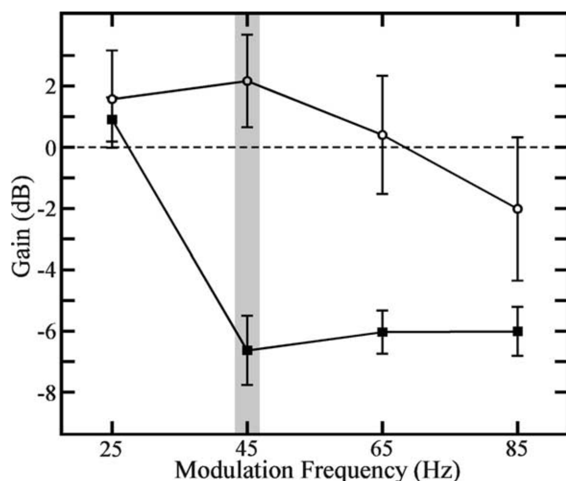

Fig. 9. Average modulation gain functions when calculated across all modulation depths for receptors (circles) and omegas (squares). Whereas points above the dashed line (positive gain) represent cases in which stimulus modulation is enhanced in the spike train, negative values represent distortion of the stimulus AM. Overall modulation gain is significantly greater in receptors than omegas (Table 1). Unlike Fig. 8, when expressed as modulation gain, the TMTF of receptors could be fit with a low-pass filter ($\tau = 15$ ms, $1/\tau = 67$ Hz, $R^2 = 0.365$, $P = 0.013$). Shaded area marks the calling song modulation frequency at 22°.

rier level) to produce greater relative changes in spike number, a result consistent with the greater AM sensitivity demonstrated above (Fig. 10(b)).

4. Discussion

4.1. Ultrasound sensitivity in hfAN

In a previous study with *G. rubens*, extracellular recordings in the neck connectives revealed at least one auditory unit sensitive to ultrasound (Farris et al., 1998). The results from the present study suggest that this high-frequency sensitivity could be mediated by hfAN, as the frequency response in the neck connectives recorded by Farris et al. (1998) is similar to those measured here in intracellular recordings of hfAN (Fig. 2). Candidate homologues to hfAN, units of the AN2 type occur in at least three other species of gryllids and are indistinguishable from hfAN in both anatomy and physiology (Casaday and Hoy, 1977; Wohlers and Huber, 1982). For example, like hfAN, Int-1 in *T. oceanicus* is most sensitive to sounds with frequencies from 15 to 30 kHz (~58 dB SPL, Moiseff and Hoy, 1983) and inhibited by frequencies from 3 to 8 kHz (65 dB SPL at 6 kHz, Nolen and Hoy, 1986a,b). Int-1 has been shown to mediate negative steering responses to bat-like ultrasound (Moiseff and Hoy, 1983; Nolen and Hoy, 1984). In *G. rubens*, pulsed ultrasound broadcast simultaneously with a calling song has been shown to reduce that song's relative attractiveness (Farris et al., 1998). Based solely on the similarity between the anatomy and physiology of Int-1 in *T. oceanicus* and hfAN in *G. rubens*, our results suggest that hfAN could play a similar role to that of Int-1 by mediating the repulsive effect of ultrasound in the context of anti-predator behavior.

4.2. Temporal sensitivity in omega neurons

Cells of the omega class have been characterized across several families of the ensiferan Orthoptera (see Mason et al., 1998). With respect to the true crickets (Gryllidae), ONs have been shown to be broadly tuned with best sensitivity at frequencies near that of the calling song carrier (Popov et al., 1978; Schildberger et al., 1989). Such a pronounced sensitivity peak was evident in ON1 and ON2 and suggests a specialization for the processing of calling songs. Unlike the sharp spectral tuning at 5 kHz, however, previous studies have found no evidence to suggest such a bias in the temporal tuning of omegas for stimuli (i.e., 100% modulated) with pulse rates like those found in the songs of conspecific males (Wohlers and Huber, 1982). Rather, omega cells have been shown to respond to a broad range of stimuli including heterospecific songs with temporal structures different from those in conspecific songs (Pollack, 1986). In *T. oceanicus* for example, omega neurons are able to code pulse rates of ~15–32 Hz, encompassing the range

Table 1

	Modulation depth (%)				
	25	50	75	100	All depths
P value receptor gain vs. omega gain	0.0920	0.0255	0.0014	0.0002	<0.0001

Modulation gain in receptors is greater than that in omegas at depths >25% (Welch's approximate *t*; Zar, 1999).

Table 2

Mod depth (%)	τ (s)	$1/\tau$ (Hz)	Filter constant (dB)	R^2	$P_{low\text{-}pass}$	Gain slope (dB)/octave	P_{octave}
100	0.049	20.40	2.024	0.274	<0.001	−4.287	<0.001
75	0.057	17.54	4.165	0.255	<0.001	−4.397	<0.001
50	0.042	23.81	3.568	0.192	<0.005	−4.046	<0.005
25	0.02	50.0	2.165	0.092	0.057	−2.836	0.055

Columns 1–6 are parameters for the least squares fit of a one-pole low-pass filter to the ON modulation gain functions at each modulation depth. Columns are the: modulation depth (%), filter time constant (τ), filter cutoff frequency (Hz), filter constant (*C*), R^2 and *P* values for the model. Columns 7 and 8 are the slopes (dB) and *P* values for a linear regression of modulation gain vs. octave modulation frequency.

found across the *T. oceanicus* and *T. commodus* songs (Pollack, 1986; Bentley and Hoy, 1972). Pollack (1986) did not test, however, whether the amount of temporal coding was correlated to the variance in the modulation rates found in the complex songs of *Teleogryllus* spp. In voltage traces shown by Wohlers and Huber (1982), temporal coding of stimulus modulation in the response of ON1 in *G. bimaculatus* appears to be lost at modulation frequencies between 29 and 66 Hz, frequencies just above those typical for the male calling song (20–29 Hz, Doherty, 1985).

In addition to changes in modulation rate, changes in modulation depth due to reverberations and overlap with multiple singers can temporally distort a calling song (Forrest, 1994). Investigations of the response of omega cells to the presentation of multiple songs have been conducted in the context of examining the ability of these cells to code song directionality as well as the mechanisms underlying omega cell adaptation (Sobel and Tank, 1994; Pollack, 1998; Römer and Krusch, 2000). For example, when presented with two songs, the biphasic response of ONs results in the 'selective attention' of ONs for the song with the greatest relative intensity, which may be produced either by the auditory system's directionality (e.g., ~20 dB difference at 90°) or the power output of the songs themselves (Pollack, 1986; but see Pollack, 1998 for review). Excitatory responses to the louder song subsequently elicit an outward hyperpolarizing current which adapts the cell and prevents responses to quieter songs. For the adapted cell, the quieter song is no longer above threshold as the I/O curve is shifted to higher intensities (see below). While the directionality of the auditory system and omega cell adaptation work in concert to selectively code certain sounds coming from different directions, the effect of these mechanisms is reduced as the sources move together (Pollack, 1986). Thus, probes of selective attention in omega neurons using two songs broadcast from different directions are not tests of temporal sensitivity per se (variance in coding is not correlated to a stimulus' temporal structure, but to its direction and thus relative intensity). The two-song experiments more closely resemble tests of directional biases in loudness and/or the position of the modulating stimulus on the cells I/O curve (which we address below for our data). Building on these experiments (e.g., Pollack, 1986), we examined the coding of temporally distorted stimuli that would hypothetically model songs emanating from the same direction. Under this experimental condition, the directional mechanisms mentioned above for coding only one song in many should be eliminated and thus allow us to measure the limits of temporal distortion tolerated by omega cells.

In *G. rubens*, the response of an omega neuron to SAM stimuli resembles that of a low-pass filter with a time constant (τ) of ~42 ms (Table 2). The TMTF (Fig. 8) shows that whereas only ~50% AM depth is required to elicit a significantly modulated response to 25 Hz AM, a ~65% modulation depth is necessary at the faster AM rates typical of male calling songs (45 Hz; Walker, 1962; Bentley and Hoy, 1972; Doherty and Callos, 1991). These results suggest that ON1 and ON2 might not play a role in the detection of song modulation structure at longer distances from a singing male where temporal distortion is greatest (Forrest, 1994; Simmons, 1988).

Omega neurons are likely important to ascending temporal processing, however. From a comparative point of view, the aspects of the central auditory circuitry involved in the processing of song AM have been characterized in a congener of *G. rubens* (Schildberger, 1984). At least one role for ON's is to lateralize and

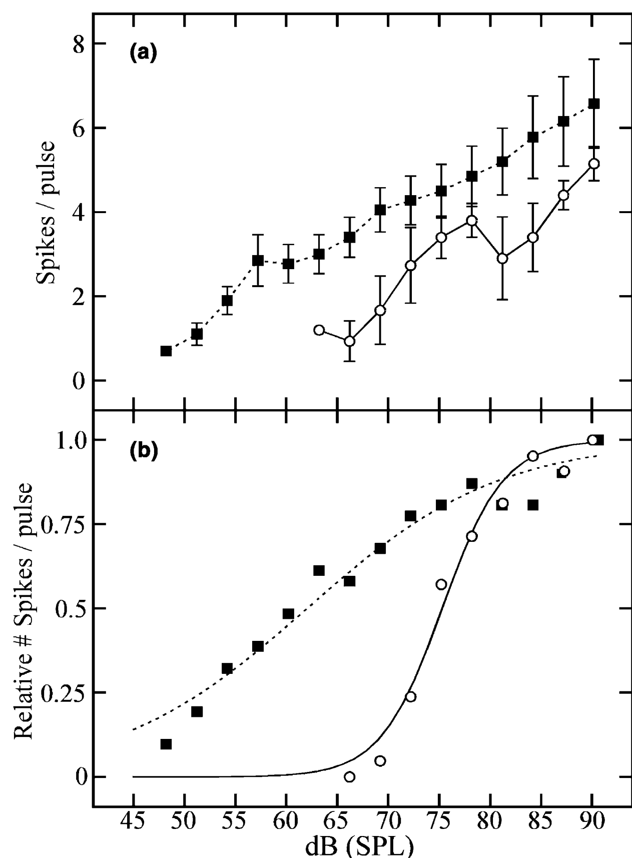

Fig. 10. Input/output (I/O) functions at 5 kHz (10 ms duration, 1 ms ramps). (a) Circles and squares are the mean number of spikes per stimulus as a function of stimulus amplitude (dB SPL) for receptor and omega neurons, respectively. (b) Curves represent the least-squares-fit of the exponential model to the normalized I/O responses (re. 90 dB SPL) of single omega and receptor cells (symbols same as in (a)). The exponential slopes for these two particular cells (omega = 0.106, receptor = 0.336) are similar to the sample means, which are significantly different (mean $ON_{slope} = 0.115$, mean $receptor_{slope} = 0.307$, $N = 12$, $t = 2.654$, $P = 0.024$). Note, the mean relative response is not shown in (b) because the mean of sigmoidal responses with different intercepts does not illustrate the mean slope.

modulate input to prothoracic ascending neurons (AN1) (Horseman and Huber, 1994), which are likely one synapse from two classes of brain neurons with temporal sensitivity matched to behavior. Thus, unlike vertebrates in which identification of post-synaptic cells is difficult, our measures of modulation gain in cricket ON spike trains allow us to consider the signal-to-noise ratio impinging on identified ascending units and assess whether subsequent stages of processing are improving, degrading or following ascending information. For example, Schildberger (1984) measured spike synchronization to 100% modulated pulsed stimuli in AN1 and two classes of brain neurons (BNC1&2) in *G. bimaculatus*. AN1 responded like a low-pass filter with a cutoff frequency of ~50 Hz, an octave higher than the ON's measured here. Assuming a common response for ON's in *G. rubens* and *G. bimaculatus*, such modulation rates would generate signals pre-synaptic to AN1 with degraded modulation (Fig. 9). Although several reasons could invalidate this comparison between studies (e.g., the criteria used to determine significant sychronization are less strict for the pulsed stimuli in Schildberger's study), our use of modulation gain to describe ON's response is important because it suggests that AN1 must either amplify the incoming AM and/or receive input from faster cells, such as receptors.

4.3. Temporal sensitivity and intensity coding in receptors

In contrast to ON1 and ON2, there was much greater sensitivity to amplitude modulation in the small sample of receptors. Over the range of modulation rates presented, on average <50% modulation depth was required to elicit a significantly modulated response in receptors, which is comparable to that found in the analogous receptors of locusts (Prinz and Ronacher, 2002). Note, however, that because Prinz and Ronacher (2002) use a linear extrapolation between responses at different modulation rates to estimate statistically significant temporal coding (even though the change in response vs. percent depth does not appear linear), their study may be methodologically biased to faster responses than those shown in Fig. 8. Although we found no evidence in receptor responses for any specialized bandpass tuning to the AM rates in calling songs, receptors must play a role in the temporal processing of song AM, as all temporal information available to the system must go through them.

In order to explore the difference in temporal processing in receptors and omegas, we examined their capabilities for coding intensity. Because they both require the detection of changes in stimulus amplitude, intensity discrimination and the detection of AM are thought to be related (Wojtczak and Viemeister, 1999). In humans for example, there is a positive correlation between the just-noticeable-difference for intensity discrimination and the amount of modulation required for the detection of AM (Wojtczak and Viemeister, 1999). From a physiological perspective, assuming that stimulus intensity corresponds to a certain point on a cell's I/O curve (e.g., the intensity that elicits a 50% response), steeper I/O functions should produce greater intensity resolution. By measuring I/O slopes relative to 90 dB (the power of the SAM stimulus) at 5 kHz for auditory receptor (high-cutoff TMTF) and omega (low-cutoff TMTF) cells, we compared the intensity resolution of these two 'fast' and 'slow' cell classes, respectively. On an absolute response scale, the change in spike number with stimulus intensity was similar between receptors and omegas (Fig. 10(a)). On a relative response scale, however, the I/O slopes of receptors are significantly steeper than those of omegas. In the context of detection of AM, this means that smaller AM depths are

necessary to elicit a change in receptor spike number than those for omegas. Consistent with the psychoacoustic relationship between increment and modulation detection (Wojtczak and Viemeister, 1999), we consider this result to be an independent confirmation of the results (i.e., faster and more sensitive receptors and slower omegas) collected in the SAM series.

In summary, the present study reveals several important points. First, from a comparative point of view, we found that the anatomy and physiology of identifiable auditory units in *G. rubens* are similar to those in other gryllines. Second, using a systems analysis approach, we have shown that temporal distortion affects the physiological detection of AM in the omega cells and receptors of *G. rubens*. This effect varied with cell type, however, as receptors could detect smaller modulation depths than omegas. It is important to note that our results suggest nothing about which AM rates are attractive, only which rates are effectively coded by a sample of auditory units.

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References

Alexander, R.D., 1960. Sound communication in Orthoptera and Cicadidae. In: Lanyon, W.E., Tavolga, W.N. (Eds.), Animal Sounds and Communication. AIBS Publication No. 7, Washington, DC, pp. 38–92.

Bentley, D.R., Hoy, R.R., 1972. Genetic control of the neuronal network generating cricket (*Teleogryllus oceanicus*) song patterns. Anim. Behav. 20, 478–492.

Casaday, G.B., Hoy, R.R., 1977. Auditory interneurons in the cricket *Teleogryllus oceanicus*: physiological and anatomical properties. J. Comp. Physiol. 121, 1–13.

Doherty, J.A., 1985. Trade-off phenomena in calling song recognition and phonotaxis in the cricket, *Gryllus bimaculatus* (Orthoptera, Gryllidae). J. Comp. Physiol. A 156, 787–801.

Doherty, J.A., Callos, J.D., 1991. Acoustic communication in the trilling field cricket, *Gryllus rubens* (Orthoptera: Gryllidae). J. Ins. Behav. 4, 67–82.

Doolan, J.M., Pollack, G.S., 1985. Phonotactic specificity of the cricket *Teleogryllus oceanicus*: intensity-dependent selectivity for temporal parameters of the stimulus. J. Comp. Physiol. A 157, 223–233.

Epping, W.J., 1990. Influence of adaptation on neural sensitivity to temporal characteristics of sound in the dorsal medullary nucleus and torus semicircularis of the grassfrog. Hear. Res. 45, 1–13.

Esch, H., Huber, F., Wohlers, D.W., 1980. Primary auditory neurons in crickets: physiology and central projections. J. Comp. Physiol. 137, 27–38.

Farris, H.E., Forrest, T.G., Hoy, R.R., 1997. The effects of calling song spacing and intensity on the attraction of flying crickets (Orthoptera: Gryllidae: Nemobiinae). J. Insect. Behav. 10, 639–653.

Farris, H.E., Forrest, T.G., Hoy, R.R., 1998. The effect of ultrasound on the attractiveness of acoustic mating signals. Physiol. Entomol. 23, 322–328.

Farris, H.E., Hoy, R.R., 2000. Ultrasound sensitivity in the cricket, *Eunemobius carolinus* (Gryllidae, Nemobiinae). J. Acoust. Soc. Am. 107, 1727–1736.

Forrest, T.G., 1994. From sender to receiver: propagation and environmental effects on acoustic signals. Am. Zool. 34, 644–654.

Frisina, R.D., 2001. Subcoritcal neural coding mechanisms for auditory temporal processing. Hear. Res. 158, 1–27.

Frisina, R.D., Smith, R.L., Chamberlain, S.C., 1990. Encoding of amplitude modulation in the Gerbil cochlear nucleus: 1. A hierarchy of enhancement. Hear. Res. 44, 99–122.

Givois, V., Pollack, G.S., 2000. Sensory habituation of auditory receptor neurons: implication for sound localization. J. Exp. Biol. 203, 2529–2537.

Gleich, O., Klump, G.M., 1995. Temporal modulation transfer functions in the European Starling (*Sturnus vulgaris*): II. Responses of auditory-nerve fibers. Hear. Res. 82, 81–92.

Gollisch, T., Schutze, H., Benda, J., Herz, A.V.M., 2002. Energy integration describes sound-intensity coding in an insect auditory system. J. Neurosci. 22, 10434–10448.

Heil, P., Neubauer, H., 2001. Temporal integration of sound pressure determines thresholds of auditory-nerve fibers. J. Neurosci. 21, 7404–7415.

Hennig, R.M., 1988. Ascending auditory interneurons in the cricket *Teleogryllus commodus* (Walker): comparative physiology and direct connections with afferents. J. Comp. Physiol. 163, 135–143.

Horseman, G., Huber, F., 1994. Sound localisation in crickets. 1. Contralateral inhibition of an ascending auditory interneuron (AN1) in the cricket *Gryllus bimaculatus*. J. Comp. Physiol. A 175, 389–398.

Hutchings, M., Lewis, B., 1981. Response properties of primary auditory fibers in the cricket *Teleogryllus oceanicus* (Le Guillou). J. Comp. Physiol. 143, 129–134.

Imaizumi, K., Pollack, G.S., 1999. Neural coding of sound frequency by cricket auditory receptors. J. Neurosci. 19, 1508–1516.

Kavanagh, M.W., 1987. The efficiency of sound production in 2 cricket species, *Gryllotalpa australis* and *Teleogryllus commodus* (Orthoptera, Grylloidea). J. Exp. Biol. 130, 107–119.

Kim, D.O., Sirianni, J.G., Chang, S.O., 1990. Responses of DCN-PVCN neurons and auditory nerve fibers in unanethetized decerebrate cats to AM and pure tones: analysis with autocorrelation/power spectrum. Hear. Res. 44, 95–113.

Kohlrausch, A., 1993. Comment on Temporal modulation transfer functions in patients with cochlear implants [J. Acoust. Soc. Am. 91, 2156–2164]. J. Acoust. Soc. Am. 93, 1649–1650.

Long, G.R., Cullen, K., 1985. Intensity difference limens at high frequencies. J. Acoust. Soc. Am. 78, 507–513.

Machens, C.K., Stemmler, M.B., Prinz, P., Krahe, R., Ronacher, B., Herz, A.V.M., 2001. Representation of acoustic communication signals by insect auditory receptors neurons. J. Neurosci. 21, 3215–3227.

Mason, A.C., Forrest, T.G., Hoy, R.R., 1998. Hearing in mole crickets (Orthoptera: Gryllotalpidae) at sonic and ultrasonic frequencies. J. Exp. Biol. 201, 1967–1979.

McGill, W.J., Goldberg, J.P., 1968. Pure tone intensity discrimination and energy detection. J. Acoust. Soc. Am. 44, 576–581.

Moiseff, A., Hoy, R.R., 1983. Sensitivity to ultrasound in an identified auditory interneuron in the cricket: a possible neural link to phonotactic behavior. J. Comp. Physiol. 152, 155–167.

Nocke, H., 1971. Biophysik der schallerzeugung durch die vorderflugel der grillen. J. Comp. Physiol. 74, 272–314.

Nocke, H., 1972. Physiological aspects of sound communication in crickets (*Gryllus campestris* L.). J. Comp. Physiol. 80, 141–162.

Nolen, T.G., Hoy, R.R., 1984. Initiation of behavior by single neurons: the role of behavioral context. Science 226, 992–994.

Nolen, T.G., Hoy, R.R., 1986a. Phonotaxis in flying crickets. I. Attraction to the calling song and avoidance of bat-like ultrasound are discrete behaviors. J. Comp. Physiol. A 159, 423–439.

Nolen, T.G., Hoy, R.R., 1986b. Phonotaxis in flying crickets. II. Physiological mechanisms of two-tone suppression of the high frequency avoidance steering behavior by the calling song. J. Comp. Physiol. A 159, 441–456.

Palmer, A.R., 1995. Neural signal processing. In: Moore, B.C.J. (Ed.), Hearing. Academic Press, New York, pp. 75–121.

Pollack, G.S., 1986. Discrimination of calling song models by the cricket, *Teleogryllus oceanicus*: the influence of sound direction on neural encoding of the stimulus temporal pattern and on phonotactic behavior. J. Comp. Physiol. 158, 549–561.

Pollack, G.S., 1998. Neural processing of acoustic signals. In: Hoy, R.R., Popper, A.N., Fay, R.R. (Eds.), Comparative Hearing: Insects. Springer, New York, pp. 139–196.

Pollack, G.S., El-Feghaly, E., 1993. Calling song recognition in the cricket *Teleogryllus oceanicus*: comparison of the effects of stimulus intensity and sound spectrum on selectivity for temporal pattern. J. Comp. Physiol. A 171, 759–765.

Popov, A.V., Markovich, A.M., Andjan, A.S., 1978. Auditory interneurons in the prothoracic ganglion of the cricket, *Gryllus bimaculatus* deGeer. J. Comp. Physiol. 126, 183–192.

Popov, A.V., Shuvalov, V.F., 1977. Phonotactic behavior of crickets. J. Comp. Physiol. 119, 111–126.

Prinz, P., Ronacher, B., 2002. Temporal modulation transfer functions in auditor receptor fibres of the locust (*Locusta migratoria* L.). J. Comp. Physiol. A 188, 577–587.

Rees, A., Palmer, A.R., 1989. Neuronal responses to amplitude-modulated and pure tone stimuli in the guinea pig inferior colliculus, and their modification by broadband noise. J. Acoust. Soc. Am. 85, 1978–1994.

Römer, H., 1998. The sensory ecology of acoustic communication in insects. In: Hoy, R.R., Popper, A.N., Fay, R.R. (Eds.), Comparative Hearing: Insects. Springer, New York, pp. 63–96.

Römer, H., Bailey, W.J., 1986. Insect hearing in the field II. Male spacing behaviour and correlated acoustic cues in the bushcricket *Mygalopsis marki*. J. Comp. Physiol. A 159, 627–638.

Römer, H., Krusch, M., 2000. A gain-control mechanism for processing of chorus sounds in the afferent auditory pathway of the bushcricket *Tettigonia viridissima* (Orthoptera; Tettigoniidae). J. Comp. Physiol. A 186, 181–191.

Römer, H., Marquart, V., Hardt, M., 1988. Organization of a sensory neuropile in the auditory pathway of two groups of Orthoptera. J. Comp. Neurol. 275, 201–215.

Rose, G.J., Capranica, R.R., 1985. Sensitivity to amplitude modulated sounds in the anuran auditory nervous system. J. Neurophysiol. 53, 446–465.

Schildberger, K., 1984. Temporal selectivity of identified auditory neurons in the cricket brain. J. Comp. Physiol. A 155, 171–185.

Schildberger, K., Huber, F., Wohlers, D.W., 1989. Central auditory pathway: neuronal correlates of phonotactic behavior. In: Huber, F., Moore, T.E., Loher, W. (Eds.), Cricket Behavior and Neurobiology. Cornell University Press, Ithaca, NY, pp. 423–458.

Shaw, K., Herlihy, D.P., 2000. Acoustic preference functions and song variability in the Hawaiian cricket *Laupala cerasina*. Proc. R. Soc. Lond. B 267, 577–584.

Simmons, L.W., 1988. The calling song of the field cricket, *Gryllus bimaculatus* (De Geer): constraints on transmission and its role in intermale competition and female choice. Anim. Behav. 36, 380–394.

Smith, R.L., Brachman, M.L., 1980. Dynamic response of single auditory-nerve fibers: some effects of intensity and time. In: van den Brink, G., Bilsen, F.A. (Eds.), Psychophysical, Physiological and Behavioural Studies in Hearing. Delft University Press, The Netherlands, pp. 312–319.

Smith, R.L., Brachman, M.L., Frisina, R.D., 1985. Sensitivity of auditory-nerve fibers to changes in intensity: a dichotomy between decrements and increments. J. Acoust. Soc. Am. 78, 1310–1316.

Sobel, E.C., Tank, D.W., 1994. In vivo Ca^{2+} dynamics in a cricket auditory neuron: an example of chemical computation. Science 263, 823–826.

Stiedl, O., Stumpner, A., Mbungu, D.N., Atkins, G., Stout, J.F., 1997. Morphology and physiology of local auditory interneurons in the prothoracic ganglion of the cricket *Acheta domesticus*. J. Exp. Zool. 279, 43–53.

Tougaard, J., 1998. Detection of short pure-tone stimuli in the noctuid ear: what are temporal integration and integration time all about? J. Comp. Physiol. A 183, 563–572.

Ulagaraj, S.M., Walker, T.J., 1975. Response of flying mole crickets to three parameters of synthetic songs broadcast outdoors. Nature 253, 1278–1279.

Viemeister, N.F., 1977. Temporal factors in audition: a systems analysis approach. In: Evans, E.F., Wilson, J.P. (Eds.), Psychophysics and Physiology of Hearing. Academic Press, London, pp. 419–428.

Viemeister, N.F., 1979. Temporal modulation transfer functions based upon modulation thresholds. J. Acoust. Soc. Am. 66, 1364–1380.

Walker, T.J., 1957. Specificity in the response of female tree crickets (Orthoptera: Gryllidae: Oecanthinae) to calling songs of the males. Ann. Entomol. Soc. Am. 50, 626–636.

Walker, T.J., 1962. Factors responsible for intraspecific variation in the calling songs of crickets. Evolution 16, 407–428.

Walker, T.J., 1974. Character displacement and acoustic insects. Am. Zool. 14, 1137–1150.

Wohlers, D.W., Huber, F., 1982. Processing of sound signals by six types of neurons in the prothoracic ganglion of the cricket, *Gryllus campestris* L. J. Comp. Physiol. A 146, 161–173.

Wojtczak, M., Viemeister, N.F., 1999. Intensity discrimination and detection of amplitude modulation. J. Acoust. Soc. Am. 106, 1917–1924.

Yost, W.A., Sheft, S., 1997. Temporal modulation transfer functions of tonal stimuli: gated versus continuous conditions. Audit. Neurosci. 3, 401–414.

Zar, J.H., 1999. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, NJ.