Differential Temporal Coding of Rhythmically Diverse Acoustic Signals by a Single Interneuron

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Marsat, G. and G. S. Pollack. Differential temporal coding of rhythmically diverse acoustic signals by a single interneuron. J Neurophysiol 92: 939–948, 2004; 10.1152/jn.00111.2004. The omega neuron 1 (ON1) of the cricket Teleogryllus oceanicus responds to conspecific signals (4.5 kHz) and to the ultrasonic echolocation sounds used by hunting, insectivorous bats. These signals differ in temporal structure as well as in carrier frequency. We show that ON1’s temporal coding properties vary with carrier frequency, allowing it to encode both of these behaviorally important signals. Information-transfer functions show that coding of 4.5 kHz is limited to the range of amplitude-modulation components that occur in cricket songs (<32 Hz), whereas coding of 30-kHz stimuli extends to the higher pulse rates that occur in bat sounds (~100 Hz). Nonlinear coding contributes to the information content of ON1’s spike train, particularly for 30-kHz stimuli with high intensities and large modulation depths. Phase locking to sinusoidal amplitude envelopes also extends to higher AM frequencies for ultrasound stimuli. ON1’s frequency-specific behavior cannot be ascribed to differences in the shapes of information-transfer functions of low- and high-frequency-tuned receptor neurons, both of which are tuned more broadly to AM frequencies than ON1. Coding properties are nearly unaffected by contralateral deafferentation. ON1’s role in auditory processing is to increase binaural contrast through contralateral inhibition. We hypothesize that its frequency-specific temporal coding properties optimize binaural contrast for sounds with both the spectral and temporal features of behaviorally relevant signals.

INTRODUCTION

Sensory information is often conveyed by the temporal structures of signals. This is particularly evident for acoustic signals. Speech, bird song, and communication sounds of many other species are characterized by stereotypic rhythms of modulation of frequency and/or amplitude. Behavioral tests have shown that these temporal features carry much of the signal’s information (e.g., Ghazanfar et al. 2001; Shannon et al. 1995).

Crickets communicate using songs composed of rhythmical sequences of sound pulses. Both the carrier frequency of songs and their temporal structures are important cues for signal recognition. Sounds with spectral and temporal properties similar to those of cricket song elicit orientation and locomotion toward the sound source (reviewed in Pollack 1998). Crickets also hear the echolocation calls of hunting, insectivorous bats; such sounds elicit evasive responses (Nolen and Hoy 1986).

Cricketsongs and bat calls differ both in spectrum and in temporal pattern. Songs of the cricket species studied here, Teleogryllus oceanicus, have a dominant carrier frequency of ca. 4.5 kHz with higher harmonics that are lower in intensity by ≥20 dB. The sound pulses that comprise the songs are 20–35 ms in duration and occur at rates from ca. 7 to 32 pulses/s (Balakrishnan and Pollack 1996). Bat calls are ultrasonic (>20 kHz) and consist of series’ of brief sound pulses (typically 1–20 ms) occurring at rates ≥200 pulses/s (Wimsalt 1970).

The auditory system of T. oceanicus is specialized to detect sound frequencies similar to those that occur in cricket songs and bat calls. This is apparent at both the receptor-neuron and interneuron levels, where individual neurons respond most strongly to either cricket- or bat-like frequencies (Atkins and Pollack 1987; Imaiizumi and Pollack 1999). The identified interneuron omega neuron 1 (ON1) is unusual in that it is dually tuned with enhanced sensitivity to both ranges of sound frequency (Atkins and Pollack 1986). Each ON1 receives excitatory input mainly from the receptors of one ear and inhibits neurons that receive input from the opposite ear, thus enhancing binaural contrast and, presumably, facilitating sound localization (Faulkes and Pollack 2000; Horsemann and Huber 1994; Selverston et al. 1985).

Work in several systems has shown that the temporal coding properties of neurons are matched to the structures of behaviorally important signals (Machens et al. 2001; Nagarajan et al. 2002; Rieke et al. 1995; Theunissen et al. 2000). ON1 is unusual among auditory neurons in that this single neuron processes two different classes of signal, cricket songs and bat calls, that differ in carrier frequency, temporal structure, and behavioral consequence. ON1 is specialized for the differing carrier frequencies of these signals. Here we show that it is also specialized to encode their differing temporal structures.

MATERIALS AND METHODS

Electrophysiology

Teleogryllus oceanicus were reared in the laboratory. Virgin females were used for experiments at age 10–21 days after the final molt. They were mounted on a support ventral-side uppermost with the front legs (the site of the ears) held flexed against the pronotum in a position similar to that adopted during flight. The prothoracic ganglion was exposed by ventral dissection, supported on a metal platform, and bathed in physiological saline (Strausfeld et al. 1983). ON1 was recorded extracellularly from its soma-contralateral processes in the prothoracic ganglion using blunt glass microelectrodes filled with 1M NaCl (resistance, 5–10 MΩ). ON1 can be identified unambiguously in this recording configuration by its increased sensitivity to electrode-contralateral sound (threshold lower by 10–20 dB) (see Pollack 1986 for further details) as well as by its greater...
sensitivity, and longer latency, to 4.5-kHz stimuli than to ultrasound (Faulkles and Pollack 2000). Receptor neurons were recorded intracellularly at the entrance of the leg nerve to the prothoracic ganglion. Threshold for recorded neurons at 4.5 and/or 30 kHz was defined as the minimum sound level (accuracy of ± 1 dB for ON1, ±2.5 dB for receptors) that consistently evoked >1 spike per 30-ms sound pulse. Recordings were digitized (16 bits, sampling rate: 10 kHz; Digidata 1320A, Axon Instruments) and analyzed off-line.

Sound stimuli

Stimuli were either 30-ms sound pulses (including 5-ms linear onset and offset ramps) presented at 2-pulse/s, 15-s tones (4.5kHz or 30 kHz) the amplitude of which was modulated sinusoidally (100% modulation depth, amplitude-modulation (AM) rates: 1–140 Hz; sinusoidal amplitude modulation: SAM) or 15- to 30-s tones that were modulated in amplitude by multiplication with a low-pass-filtered Gaussian signal (<200 Hz) with SD of 3 dB except where otherwise noted (random amplitude modulation: RAM). Stimuli were produced by National Instruments AD/DA boards with 12 bits of resolution, at a sampling rate of either 100 or 200 kHz. Sounds were broadcast from loudspeakers situated perpendicular to the cricket’s longitudinal axis, ipsilateral to the ear driving the recorded neuron. For ON1, 30- and 4.5-kHz stimuli were presented in alternation; low- and high-frequency receptor neurons were stimulated with 4.5- or 30 kHz stimuli, respectively. Stimuli were followed by silent recovery periods of 45 s (15-s stimuli) or 90 s (30-s stimuli). Sound level was calibrated using Brüel and Kjaer instruments (4135 microphone, 2610 sound-level meter).

Data analysis

INFORMATION-TRANSFER FUNCTIONS. Temporal coding properties of neurons can be described in terms of the information contained within their spike trains about changes in a stimulus. We used two approaches to compute information-transfer functions. We derived the maximum information content of the spike train from the variation between responses to repeated presentations of an identical RAM stimulus, yielding an upper-bound estimate of information transfer (although, in our experiments, this corresponds to the actual information-transfer rate; see following text). We also constructed estimates of the stimulus based on an optimal linear filter relating the neuron’s spike train to preceding stimulation (reverse reconstruction) and derived a lower bound on information transfer from the difference between the actual and estimated stimulus.

We used two methods to compute the upper bound on information transfer. First, we described the neuron’s response to each trial, \( r_i(t) \), as firing rate in successive 0.5-ms-wide bins, i.e., firing rate per bin either 0 or 2,000 Hz. These arrays were averaged across trials, yielding \( \bar{r}(t) \). \( \bar{r}(t) \) represents the component of the response that is determined by variations in stimulus amplitude. Deviations from \( \bar{r}(t) \) on individual trials were assumed to be due to additive noise, \( n_i(t) \), and were calculated as \( r_i(t) - \bar{r}(t) \). Signal-to-noise ratio as a function of AM frequency, \( SNR(f) \) was calculated as \( PNR(f)/PN(f) \), where \( PNR(f) \) and \( PN(f) \) are, respectively, the power spectrum of \( \bar{r}(t) \) and the mean of the power spectra of \( n_i(t) \) (Haag and Borst 1997). Information transfer was calculated as \( I(f) = \log_2 (1 + SNR(f)) \) (Borst and Theunissen 1999). This method was applied to five ON1s with the RAM stimulus repeated 30 times. Second, spike timing was compared between pairs of responses by calculating response-to-response coherence (Roddey et al. 2000). Each individual response, expressed as firing rate, \( \bar{r}(t) \), was separated into overlapping, Hanning-windowed, 300-ms segments and converted to the frequency domain \( (R(f)) \) using the fast Fourier transform. The coherence of a pair of responses \( i \) and \( j \) was calculated as:

\[
Coh = \sqrt{R(i,f)R(j,f)R(i,f)^*R(j,f)^*}/R(i,f)^2R(j,f)^2R(i,f)^*R(j,f)^*
\]

where * indicates complex conjugation and ( ) indicates averaging across the 300-ms response segments. For each neuron, coherence was calculated for all pair wise combinations of responses to the same stimulus and averaged across response pairs, yielding Coh. Information was calculated as \( I(f) = -\log_2 (1 - Coh) \) (Borst and Theunissen 1999).

The two methods of calculating upper-bound information-transfer functions were compared for five ON1 neurons, and the results were indistinguishable. Here, we present results from the second method. Receptor neurons can be recorded for only a few minutes (Inaizumi and Pollack 1999), and we were able to repeat RAM stimuli only two to three times. The first method cannot be applied with such small sample sizes, but these are sufficient data for the second method (Roddey et al. 2000); thus only the second method was used for receptors.

If signal power and noise power both have Gaussian probability distributions, the upper bound on information transfer equals the actual information transfer rate (Borst and Theunissen 1999). For the subset of ON1s that were tested with 30 stimulus repetitions, we confirmed that these requirements were met by comparing the distributions of signal (in this case, \( R(f) \) and noise \( N(f) \) for all i) with Gaussian distributions having the same mean ± SD (Kolmogorov-Smirnov test; range of \( P \) values, 0.11–0.6).

Lower-bound information transfer functions, reflecting linearly coded information, were derived from reconstructions of the stimulus envelope based on an optimum linear filter describing the relationship between the stimulus and ON1’s firing rate (Clague et al. 1997; Theunissen et al. 1996). Arrays representing firing rate, \( r(t) \) and the stimulus envelope, \( s(t) \), were expressed as variations around their respective means, yielding \( r'(t) \) and \( s'(t) \). These arrays were segmented and Fourier-transformed as described above, yielding \( R'(f) \) and \( S'(f) \). The frequency-domain representation of the linear reverse filter for response \( i \) was calculated as:

\[
H_i(f) = (R_i'(f)S'(f))/\overline{R(f)}S'(f)\overline{R(f)}
\]

where \( \overline{R(f)} \) was converted to the time domain, \( h_i(t) \), using the inverse Fourier transform. The result of these operations is equivalent to the cross-correlation of \( r'(t) \) and \( s'(t) \), divided by the autocorrelation of \( r'(t) \) (Press et al. 1992). \( h_i(t) \) was convolved with \( r(t) \) to reconstruct the neuron’s estimate of the stimulus, \( est_i(t) \). Noise, \( n_i(t) \), was computed as \( s_i(t) - est_i(t) \). Signal-to-noise ratio, \( SNR_i(f) \), was computed as the power spectrum of \( est_i(t) \) divided by that of \( n_i(t) \). \( SNR_i(f) \) was averaged for all \( i \) and information transfer was calculated from \( SNR_i(f) \) as in the preceding text.

Firing rate adapts markedly during the first two seconds of the response, yet an assumption behind our calculations is that the response statistics are stationary over time (Rieke et al. 1997). To restrict our analysis to the stable, i.e., adapted, portion of the response, the first 5 s were excluded.

We summarize information-transfer functions by the maximum rate of information transfer, the AM rate at which information transfer is maximum, and the half-width, defined as the (possibly noncontiguous) range of AM frequencies at which information rate is greater than half the maximum.

SYNCHRONIZATION COEFFICIENT. Synchronization coefficient was calculated for responses to SAM stimulus as \( \sqrt{\sum x_1^2 + \sum y_1^2}/n \), where \( x_1 \) and \( y_1 \) are, respectively, the cosine and sine of the phase of the sinusoidal AM envelope at which the \( i \)th spike occurs, and \( n \) is the number of spikes included in the analysis (Goldberg and Brown 1969). Synchronization coefficient ranges from 0 to 1 and reflects both the extent of spike clustering during each cycle of AM and the consistency of response phase from cycle to cycle.

Calculations were performed using programs written for Scilab (www.scilab.org). Statistical tests were performed using Statistica 5.5 (Statsoft, Tulsa, OK).
RESULTS

Dependence of temporal coding on carrier frequency

ON1 shows enhanced sensitivity to both cricket-like (4.5 kHz) and ultrasonic (>20 kHz) frequencies, but threshold is lower for the former frequency range (4.5 kHz: 53.6 ± 3.5 dB SPL; 30 kHz: 65.8 ± 4.6, n = 15) (cf. Atkins and Pollack 1986). To compare coding in these two ranges, we presented stimuli at equivalent intensities relative to threshold (20 dB above threshold unless otherwise stated, denoted as +20 dB).

Figure 1A shows excerpts of the responses of a single ON1 to repeated presentations of RAM stimuli with the identical modulation envelope but with carrier frequency of either 4.5 or 30 kHz. The spiking patterns differ for the two stimuli. This is most obvious in the second half of the response segments shown, where ON1’s spike train more precisely reflects features of the stimulus envelope for the 30-kHz stimulus.

We characterized ON1’s coding of the stimulus envelope by comparing responses to repeated presentation of the identical RAM stimulus (see METHODS). The result of this analysis, the information-transfer function, quantifies, as a function of the frequency of AM, the information content of the neuron’s spike train pertaining to changes in stimulus amplitude. These functions vary with carrier frequency (Fig. 1A). For 4.5-kHz stimuli, the rate of information transfer drops markedly for AM frequencies >25 Hz; at AM frequencies >50 Hz, little or no information about the AM envelope is coded in the neuron’s spike train. For 30-kHz stimuli, information rate drops more gradually, approaching 0 only at AM rates >100 Hz. This is reflected by the half-width of the curve, which is greater for 30 kHz (4.5 kHz: 31.9 ± 1.3 Hz; 30 kHz: 69.7 ± 2.9 Hz; paired t-test, P < 10−5). Information transfer reaches higher peak rate for 4.5 kHz (1.9 ± 0.1 bits/s) than for 30 kHz (1.1 ± 0.1; P < 10−5), but the frequency at which the peak occurs is similar for the two carrier frequencies (4.5 kHz: 12.2 ± 1.6 Hz; 30 kHz: 18.2 ± 4.1; P > 0.1).

Effects on coding of stimulus intensity and modulation depth

The curves shown in Fig. 1B were derived from responses to stimuli presented at equivalent sound levels relative to threshold (+20 dB), with envelope SD of 3 dB. Under these conditions, mean firing rate is lower for 30-kHz stimuli (24.4 ± 1.8 vs. 46.2 ± 2.3 spikes/s, paired t-test, P < 10−5). However, this does not account for the difference in shape of the information transfer functions, which is apparent over a range of stimulus intensities and firing rates (Fig. 2, A and B). For example, firing rates are similar for responses to 4.5 kHz, 20 dB above threshold (Fig. 2A, 46.2 ± 2.3 spikes/s) and 30 kHz, 30 dB above threshold (Fig. 2B, 43.7 ± 3.3 spikes/s; P = 0.5), yet half-width is still greater for 30 kHz (81.7 ± 2.9 vs. 31.9 ± 1.3 Hz for 4.5 kHz; P < 10−5). Mean firing rates are also similar for the two carrier frequencies even at the same mean stimulus intensity (+20 dB) when modulation depth is greater (see following text), and again the information-transfer curves maintain their frequency-specific shapes (Fig. 2, C and D).

For 4.5-kHz stimuli, information-transfer curves change only slightly, though significantly, with changes in stimulus intensity (Fig. 2A). Peak information rate varies slightly (though not monotonically) with intensity (ANOVA: P = 0.04), frequency at which the peak occurs increases (ANOVA: P < 10−5), and half-width increases (ANOVA: P < 10−4). For 30 kHz, peak information rate increases markedly with increasing intensity (ANOVA: P < 10−5), but other features of the curves are unchanged (ANOVA: frequency at which information rate is maximal, P = 0.6; half-width, P = 0.6).

Coding is strongly affected by changing the depth of modulation, i.e., the SD of the stimulus envelope, even though overall (rms) sound level is constant (+20 dB; Fig. 2, C and D). For both carrier frequencies, the peak information-transfer rate increases with modulation depth (ANOVA: P < 10−5 for 4.5 kHz, P < 10−4 for 30 kHz), but other parameters of the information-transfer curves are unchanged. For 4.5 kHz, mean firing rate does not change significantly with modulation depth (range: 41.3 ± 3.2 to 42.5 ± 2.8 spikes/s ANOVA: P = 0.98), although it does increase for 30-kHz stimuli (Fig. 2D, inset; ANOVA: P = 0.03). For envelope SD of 7 dB, mean firing rate is similar for the two carrier frequencies (paired t-test, P = 0.27) although, as stated earlier, the information-transfer curves clearly differ in shape (half-widths: 37.3 ± 1.9 Hz for 4.5 kHz; 108.7 ± 6.2 for 30 kHz, paired t-test, P = 0.0008).

ON1’s coding of information about changes in stimulus amplitude could, in principle, be accounted for by a linear relationship between amplitude and spike rate. Alternatively, this relationship might also include nonlinear components. Linear and nonlinear coding (if present) both contribute to the information-transfer functions in Figs. 1 and 2. We estimated how much information was transmitted with a linear code by reconstructing the stimulus envelope using a linear reverse filter (see METHODS). Time-domain representations of the reverse filters (i.e., the filter impulse functions) are shown in Fig.
The impulse function derived from responses to 30-kHz stimuli is narrower than that for 4.5 kHz, and latency between its peak and ON1’s action potential is shorter by several milliseconds (cf. Faulkes and Pollack 2000). The stimulus estimate derived from the response to 4.5 kHz reflects mainly slower changes in stimulus amplitude, whereas that derived from the 30-kHz response captures more rapid amplitude changes (Fig. 3B).

Linear estimates of information transfer are compared with the actual information-transfer rates in Fig. 3C. For the conditions illustrated, envelope SD of 7 dB, stimulus intensity 30 dB above threshold, the linear estimates capture only a portion of the information in the spike train. Nevertheless, the information-transfer functions based on linear estimates retain their characteristic carrier-frequency-specific shapes (half-width: 33.3 ± 1.1 vs. 86.7 ± 12.4 Hz; frequency of maximum information: 15.3 ± 3.1 vs. 28.6 ± 0.8 Hz; maximum information rate: 1.57 ± 0.06 vs. 0.75 ± 0.02 bits/s; 4.5 vs. 30 kHz, respectively; \( P < 0.001 \) for all comparison).

As shown in Fig. 2, the information transfer rate increases with increasing stimulus intensity and envelope SD. Total information transfer can be partitioned into linear and nonlinear components (Fig. 3D). For 4.5-kHz stimuli, both linear and nonlinear components increase in parallel, but for 30-kHz stimuli, the increase in information is due predominantly to a marked increase in the nonlinear component. For 30-kHz stimuli with high intensity and SD, linear coding accounts for <20% of the overall information content of ON1’s spiking response.

Responses to SAM stimuli

For computational reasons, our analysis of information transfer excluded the initial few seconds of the response (see METHODS), but behavioral responses (avoidance of ultrasound; attraction to cricket song) occur with short latency [avoidance: 25–70 ms (Nolen and Hoy 1986) attraction: 300–400 ms (Pollack 2003)]. Another way to assess temporal coding, which can be applied even to the early portion of a response, is to examine responses to SAM stimuli. For low AM frequencies, ON1’s initial response captures the temporal structures of SAM stimuli with both 4.5- and 30-kHz carrier frequencies; however, for high AM frequencies, the spike train reflects the amplitude envelope only for the 30-kHz stimuli (Fig. 4).

We examined coding of SAM stimuli quantitatively by plotting the relationships between synchronization coefficient and AM frequency (synchronization-coefficient modulation transfer function: scMTF) and between firing rate and SAM frequency (firing-rate modulation transfer function: frMTF). To analyze synchronization during the initial portion of the response, we computed synchronization coefficients for responses to the first four cycles of AM. Initial synchronization is best at lower AM frequencies for both 4.5- and 30-kHz stimuli (Fig. 5A). However, synchronization to high AM frequencies is better for ultrasound stimuli (ANOVA, \( P < 0.0001 \); Tukey test, \( P < 0.05 \) for AM rates 45, 65, and 100 Hz), resulting in a less sharply tuned scMTF (half-widths: 36.2 ± 11.4 for 4.5 kHz and 63.7 ± 30.5 for 30 kHz; \( P = 0.008 \)). We measured initial firing rate as the maximum instantaneous rate (i.e., inverse of the smallest interspike interval) during the response to the first cycle of AM. This measure thus reflects sensitivity to the rate of change of amplitude rather than to the repetition rate of AM cycles. For both carrier frequencies, instantaneous firing rate was low for low AM frequencies and increased to an approximate plateau (Fig. 5B). For rates >25 Hz, however, firing rate was higher for ultrasound stimuli, (ANOVA, \( P < 0.0001 \); Tukey test, \( P < 0.05 \) for all AM frequencies >25 Hz).

We also examined coding of SAM stimuli after adaptation was established, by computing MTFs for the response during the last 10 s of a 15-s stimulus. As for the initial response, synchronization at high AM frequencies was greater for ultra-
sound stimuli (Fig. 5C; ANOVA, \( P < 0.0001 \); Tukey test, \( P < 0.05 \) for AM rates \( > 25 \) Hz). However, the frMTF differed markedly from that at the onset of the response. Whereas instantaneous firing rate at response onset is greater for 30 kHz, during the adapted portion of the response, mean firing rate is lower for 30 kHz (ANOVA, \( P < 10^{-6} \); Fig. 5D). The frMTFs for the two carrier frequencies also differ in shape. Firing reaches a peak at higher SAM frequencies for 30 kHz carrier than for 4.5 kHz (median frequency: 45 Hz for 30 kHz, 18 Hz for 4.5 kHz, Wilcoxon test, \( P = 0.002 \)).

**Coding properties of auditory receptor neurons**

ON1’s coding properties might simply mirror the coding properties of auditory receptor neurons. We examined this possibility by recording receptor responses to RAM stimuli. Based on frequency sensitivity, receptors fall into three groups, which are most sensitive to the low frequencies that occur in cricket song (low-frequency receptors), to ultrasound, or to a mid-range of frequencies (Imaizumi and Pollack 1999). Most of the latter also respond strongly to ultrasound; here, we consider these last two groups together as high-frequency receptors. Low-frequency receptors comprise two distinct groups that differ in anatomy as well as in a number of physiological parameters, including threshold (Imaizumi and Pollack 2001). Based on anatomy, low-frequency receptors are either MT types (midline-terminating) or BC types (bifurcating arbors). The former type has lower threshold. Receptors with threshold, at 4.5 kHz, \( \leq 55 \) dB SPL were classed here as low-threshold (putative MT types), and those with thresholds \( \geq 65 \) dB SPL as high-threshold (putative BC types). Six receptors of each threshold class were stained with Lucifer yellow; in all cases, their structures matched their putative anatomical designation. We also stained four high-frequency receptors; their structures also matched that described previously (Imaizumi and Pollack 2001) for mid-frequency and ultrasound receptors (which are anatomically indistinguishable).

ON1’s frequency-specific coding properties differ from those of receptors. Information-transfer functions of receptors (Fig. 6) peak at higher AM frequencies and are broader than the corresponding functions for ON1 (i.e., low-frequency receptors vs. ON1’s response to 4.5-kHz carrier, high-frequency receptors vs. ON1’s response to 30-kHz carrier; see figure legend for statistics). Surprisingly, for the stimulus conditions we used (10–15 dB above threshold, envelope SD of 3 dB), only 4 of the 10 low-threshold, low-frequency receptors that we recorded showed statistically significant coding of the stimulus envelope as assayed by jackknife resampling (Efron and Tibshirani 1993); Fig. 6A includes only these four receptors. All of the high-threshold, low-frequency receptors significantly coded the stimulus envelope (Fig. 6B). Information-transfer rates of both groups of low-frequency receptors are

**FIG. 3.** Nonlinearity of ON1’s coding. A: impulse responses of ON1’s linear reverse filters for RAM stimuli with 4.5- or 30-kHz carrier frequency (intensity +20 dB, envelope SD: 3 dB). Thin lines represent individual neurons (n = 10); thick lines are their means. The vertical arrow indicates the timing of ON1’s spike. B: estimates, for a representative ON1, of stimulus modulation envelopes determined by linear reverse reconstruction. C: comparison of information-transfer curves representing actual information and information coded linearly. Curves are means of five neurons. Stimulus intensity (rms): 30 dB above threshold; SD of the modulation envelope: 7 dB. D: total information rate (summed over all AM frequencies) for stimuli with different mean intensities, modulation depths (i.e., envelope SDs), and carrier frequencies. Information transmission that cannot be accounted for by linear coding (white) is attributed to nonlinear coding (gray). Data are means ± SE of 5 neurons.

**FIG. 4.** Responses to sinusoidally amplitude-modulated (SAM) stimuli vary with carrier frequency. Top: SAM stimuli, middle: ON1 spike trains, and bottom: instantaneous firing rate, i.e., inverse of inter-spike interval for low- and high SAM frequencies (left and right, respectively), for stimuli with 4.5- and 30-kHz carriers (top and bottom), at rms intensity 20 dB above threshold.
lower than that of ON1. By contrast, information rates of high-frequency receptors (all of which coded significantly) are higher than that of ON1 (Fig. 6C).

As for ON1 (see Fig. 3D), coding by receptors includes both linear and nonlinear components (Fig. 6D). For 4.5-kHz stimuli of equivalent intensity and modulation depth (envelope SD, 3 dB), the proportion of the total information transfer ascribed to nonlinear coding is similar for receptor neurons and ON1 (low-frequency, low-threshold receptors versus ON1, 10 dB above threshold: \( P > 0.3 \); low-frequency, high-threshold receptors versus ON1, 30 dB above threshold: \( P > 0.15 \)). For 30-kHz stimuli, however, nonlinear coding is more pronounced in ON1 than in receptors (high-frequency receptors vs. ON1, 10 dB above threshold: \( P < 0.02 \)).

**Frequency-specific coding characteristics do not require contralateral inhibition**

Wiese and Eilts (1985) suggested that ON1’s temporal selectivity is determined by the dynamics of reciprocal inhibition between the left and right ON1s. We tested this hypothesis by comparing information-transfer functions before and after cutting the contralateral auditory nerve, thus removing input from the contralateral ear. Information-transfer curves were largely unaffected by unilateral deafening (Figs. 7A) although, for 4.5-kHz stimuli, there was a slight increase in peak information-transfer rate and decrease in the frequency at which information is maximal (paired \( t \)-test; \( P = 0.03 \) in both cases). Responses to SAM stimuli were also similar before and after removing contralateral input. As expected, mean firing rate increased when contralateral inhibition was removed, but the shapes of the frMTFs were unchanged (Fig. 7B; no difference in SAM frequency of maximal firing rate: Wilcoxon test, \( P > 0.5 \) for 4.5 kHz and \( P > 0.3 \) for 30 kHz). Similarly, the shapes of the scMTFs were unaffected (Fig. 7C; no difference in half-width, paired \( t \)-test, \( P > 0.5 \) for both carriers).

**Coding properties and natural stimuli**

ON1’s frequency-specific temporal coding properties are well suited to behavioral requirements. In Fig. 8A, we compare ON1’s information-transfer curve for cricket-like carrier with the temporal structure of the species’ calling song. It is clear that ON1’s coding properties are well matched to the song’s structure. We also show behavioral selectivity for stimuli consisting of sound pulses presented at different rates (Pollack and El-Feghaly 1993). These stimuli differ substantially from the RAM stimulus used to derive the information-transfer function, and so behavioral and neural curves are not directly comparable. Nevertheless, the behavioral curve is centered on only one peak in the song spectrum, whereas ON1’s coding includes the entire song spectrum. Thus ON1’s coding properties do not appear to account for behavioral selectivity.

Figure 8B shows ON1’s information-transfer function for bat-like stimuli, along with the ranges of pulse rates found in the calls of several species of bat. ON1’s enhanced coding of high AM frequencies encompasses much of the range of pulse rates in these bat sounds. In addition, bat pulses may be very brief (<2 ms) (Jones 1999; Jones and Corben 1993) and thus rapidly modulated in amplitude. Such stimuli elicit strong, phase-locked responses in ON1 (see Fig. 5, A and B).

**Discussion**

**Coding characteristics**

The temporal characteristics of ON1’s response differ for cricket- and bat-like sound frequencies. Information in ON1’s spike train about stimuli with cricket-like carrier frequency is restricted to a narrow range of AM frequencies below ca. 32 Hz, similar to the range of temporal components of intraspecific communication signals. The drop off of information is more gradual for bat-like carrier frequencies, extending ON1’s coding into the higher range of AM frequencies that occur in bat echolocation signals (Fig. 8). ON1’s differential coding of signals with cricket- and bat-like carriers is also apparent in its ability to synchronize to SAM stimuli, as well as in its firing rates to such stimuli. Thus these two approaches to studying temporal coding lead to qualitatively similar conclusions.

A limitation of the information-theoretic approach is that it cannot be used to characterize coding during the initial, nonstationary portion of the response, even though it is during this
period that behavioral responses are initiated. Avoidance responses to ultrasound, in particular, are initiated after only a few spikes in AN2, occurring with high instantaneous rate (Nolen and Hoy 1984, 1986). We characterized coding by ON1 during this period using responses to SAM stimuli. Like AN2, ON1 responds strongly at the onset of ultrasound stimuli, with instantaneous firing rate approaching 500 spikes/s. Moreover, maximum instantaneous firing rate is highest for stimuli with rapid rates of AM (Fig. 5B), a characteristic of bat sound pulses. Thus ON1's initial response to ultrasound is well suited to influence even the onset of ultrasound-elicited behavioral responses. Moreover, neither ultrasound-avoidance responses nor attraction to cricket songs are restricted to only the first few milliseconds after stimulus onset. Positive phonotaxis to cricket-song stimuli persists for minutes (e.g., Pollack and El-Feghaly 1993). Recent infrared movies of bat-insect encounters show that these may continue for several seconds as the bat follows its intended prey through successive evasive maneuvers (Simmons 2003). Although ON1's responses to SAM stimuli change as it adapts, its temporal coding characteristics remain well matched to the structures of cricket songs and bat calls (Fig. 5, A and C).

Nabatian et al. (2003) also studied temporal selectivity of ON1 of another cricket species, Gyrillus bimaculatus. Their stimuli were trains of sound pulses with cricket-like carrier frequency presented at different pulse rates. They found that maximum instantaneous firing rate per pulse increased with increasing pulse period, reaching a plateau at the pulse period that occurs in the species' song. They present a model in which the number of spike-rate peaks per chirp varies in a band-pass manner with pulse period, mirroring the behavioral effectiveness of the stimuli. Because of differences in the design of stimuli, we cannot compare our results directly with theirs. However, the coding properties that we describe do not appear to account for behavioral selectivity to temporal pattern (Fig. 8A).

Schildberger (1984) described neurons in the brain that respond selectively to behaviorally effective sound-pulse rates. Anatomical evidence suggests that the source of input to these brain circuits is the ascending neurons, AN1 and AN2. If ON1 (which, in mature adults, is a local, prothoracic neuron) (Atkins and Pollack 1986) affects brain recognition circuits, it must do so indirectly, through its inhibition of the ascending neurons.

ON1’s coding properties include both linear and nonlinear components. The nonlinear component is most evident for responses to higher intensities and larger envelope SDs. The underlying cause may be high-intensity in both cases; because rms intensity was held constant, peak stimulus intensity increased with envelope SD (see Fig. 2C, inset). The nonlinear contribution to coding was much more pronounced for 30-kHz

![Diagram](http://jn.physiology.org/download/doi/10.1152/jn.00878.2003/medial.png)
stimuli. Other features of our data also point to nonlinear response properties for this carrier frequency. Mean firing rate increases at greater-than-linear rate with increasing intensity of 30-kHz RAM stimuli over the range of intensities 10–30 dB above threshold, whereas the increase for 4.5-kHz stimuli is approximately linear (see Fig. 2B, inset). Moreover, when stimulus intensity was held constant, mean firing rate increased with envelope SD (and thus peak stimulus intensity), but only for 30-kHz stimuli.

Increasingly nonlinear coding with increasing stimulus intensity has been described previously. In the visual system of flies, linear coding accurately describes the responses of motion-sensitive interneurons, provided that the changes in velocity of the stimulus are small. For larger changes in velocity, nonlinear coding accounts for a substantial fraction of the information. As for ON1, the shape of the function describing coding as a function of stimulus frequency is similar whether or not nonlinear coding is taken into account (Haag and Borst 1997). Nonlinear coding also increases with stimulus intensity in wind-sensitive sensory neurons of cricket cerci (Roddey et al. 2000). In this case, however, nonlinearity is most prominent for stimulus frequencies that are poorly coded at low intensities. As a result, selective coding of particular frequencies is lost as intensity increases.

Enhanced coding of behaviorally relevant features of natural stimuli, as we have shown for ON1, has been described previously. For example, in auditory receptors of bullfrogs and grasshoppers, the rate of information transmission is highest for signals with temporal structures similar to those of conspecific communication signals (Machens et al. 2001; Rieke et al. 1995). Similarly, coding of AM by electoreceptors of weakly electric fish improves as the bandwidth of the stimulus envelope is restricted to match the range of AM frequencies found in natural signals (Wessel et al. 1996). Recently, it has been shown that neurons in the brains of electric fish also exhibit different coding properties specific to signal geometries characteristic of signals from prey and conspecifics (Chacron et al. 2003). Like ON1, these neurons have different coding properties for different behaviorally relevant signals.
Possible mechanisms

Receptor neurons implement a first stage of temporal filtering, with broad selectivity to low AM rates (Fig. 6). Selectivity of ON1 for ultrasound stimuli is only slightly sharper than that of high-frequency receptors. For 4.5-kHz sound frequencies, this second stage of filtering is much sharper. The difference in the range of AM frequencies coded by ON1 for the two carrier frequencies cannot be explained by sharper filtering by low-frequency receptors compared with ultrasound receptors.

Wiese and Eltis (1985) proposed that temporal selectivity of ON1 might result from inhibitory interactions with its contralateral partner. In this case, the characteristics of contralateral inhibition would be expected to differ for low- and high-carrier frequencies. Indeed, the timing of contralateral inhibition does differ for these two frequencies (Faulkes and Pollack 2000). However, we found nearly no difference in ON1’s temporal coding properties, either in information transfer functions or in the MTFs, when its contralateral partner was deafenated. In our experiments, stimuli were presented from an azimuth of 90° on the neuron’s most sensitive side, a location that would only weakly stimulate the contralateral ON1 (Pollack 1986). We cannot rule out the possibility that, for stimuli arising closer to the midline, the resulting stronger contralateral inhibition might influence temporal coding. Nevertheless, the fundamental coding properties that we describe do not require contralateral inhibition.

Mechanisms contributing to temporal filtering in other systems include synaptic plasticity (Fortune and Rose 2000), passive filtering, active conductances (Fortune and Rose 1997), and network structure (Grothe 1994; see Pollack 2001 for review). If passive filtering were to explain ON1’s properties, receptor neurons tuned to low and to high sound frequencies would be expected to terminate at different regions within ON1’s dendritic tree. One subset of low-frequency receptors (MT) and ultrasound receptors terminate in nearly the same region of auditory neuropil, overlapping with ON1’s dense dendritic branches (Imaizumi 2000; Imaizumi and Pollack 2001). It seems unlikely that passive filtering would differ greatly for inputs from these two groups of receptor neurons. The other subset of low-frequency receptors (BC) terminate lateral and posterior of ON1’s dense dendritic arbor and probably make few if any direct connections with ON1 (Imaizumi and Pollack 2001; Pollack and Imaizumi 1999).

Nevertheless, the nature of input from low-frequency and ultrasound receptors to ON1 differs markedly. Ultrasound receptors elicit large, monosynaptic, unitary excitatory postsynaptic potentials (EPSPs) (Faulkes and Pollack 2001), whereas discrete, unitary EPSPs are not seen for low-frequency stimuli; rather, a smooth, graded depolarization occurs (Pollack 1994). This might result from summation of small, monosynaptic EPSPs from many low-frequency receptors. Consistent with this, low-frequency receptors outnumber high-frequency receptors by a factor of 3–4 (Imaizumi and Pollack 1999, 2001). Moreover, the information content of responses of single low-frequency receptors is less than that of ON1 (Fig. 6), suggesting that pooling of inputs from many receptors may be required to account for ON1’s coding properties. By contrast, information content of single high-frequency receptors exceeds that of ON1, suggesting that pooling might not be required in this case.

Another possible explanation for the difference in EPSPs elicited by low and high frequencies is that low-frequency input to ON1 may be mainly polysynaptic (Faulkes and Pollack 2001). If the putative intervening neuron(s) were nonspiking, then a smooth, graded EPSP might be expected in ON1. In any case, low-frequency-elicited EPSPs in ON1 rise and decay more slowly than those evoked by ultrasound, and it is possible that this difference in EPSP shape contributes to ON1’s temporal properties. Of course, if ON1’s low-frequency input is primarily polysynaptic, then its filtering properties for low-frequency stimuli may be imposed by the intervening circuitry.

Behavioral significance

ON1 enhances binaural contrast through contralateral inhibition, thus facilitating sound localization (Atkins et al. 1984; Horsemann and Huber 1994; Schildberger and Hörner 1988). Among ON1’s contralateral targets are the ascending neurons AN1 and AN2, which carry information about cricket songs and bat calls, respectively, to the brain. The timing and strength of ON1’s response to a temporally patterned stimulus will thus influence its inhibition of its targets (Faulkes and Pollack 2000).

We hypothesize that ON1’s frequency-specific coding of temporal pattern affects the efficiency of contralateral inhibition so as to maximize bilateral differences among its targets specifically for stimuli with both the carrier frequencies and temporal structures of behaviorally relevant signals. This would enhance localization cues precisely for those stimuli that must be localized, whereas less relevant sounds would be perceived as spatially more diffuse. Thus the coding properties we describe might implement a type of spatially selective attention.

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