Behaviorally Relevant Burst Coding in Primary Sensory Neurons

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Sabourin P, Pollack GS. Behaviorally relevant burst coding in primary sensory neurons. J Neurophysiol 102: 1086–1091, 2009. First published June 17, 2009; doi:10.1152/jn.00370.2009. Bursts of action potentials in sensory interneurons are believed to signal the occurrence of particularly salient stimulus features. Previous work showed that bursts in an identified, ultrasound-tuned interneuron (AN2) of the cricket *Teleogryllus oceanicus* code for conspicuous increases in amplitude of an ultrasound stimulus, resulting in behavioral responses that are interpreted as avoidance of echolocating bats. We show that the primary sensory neurons that inform AN2 about high-frequency acoustic stimuli also produce bursts. As is the case for AN2, bursts in sensory neurons perform better as feature detectors than isolated, nonburst, spikes. Bursting is temporally correlated between sensory neurons, suggesting that on occurrence of a salient stimulus feature, AN2 will receive strong synaptic input in the form of coincident bursts, from several sensory neurons, and that this might result in bursting in AN2. Our results show that an important feature of the temporal structure of interneuron spike trains can be established at the earliest possible level of sensory processing, i.e., that of the primary sensory neuron.

**METHODS**

**Electrophysiology**

*T. oceanicus* were reared in the laboratory. Virgin females were used for experiments 10–20 days after the final molt. They were anesthetized by chilling and mounted on a support ventral side uppermost. The prothoracic ganglion was exposed by ventral dissection, supported on a metal platform, and bathed in physiological saline (Strausfeld et al. 1983). The cricket’s front legs (which bear the ears) were held flexed against the pronotum in a position similar to that assumed during flight.

Receptors were recorded intracellularly in the leg nerve slightly lateral to its junction with the prothoracic ganglion using glass microelectrodes filled with 1.5 M potassium acetate (Kac; resistance, >30 MΩ). Previous work (Imaizumi and Pollack 2005) identified four types of receptor neuron: two anatomically distinct types of low-frequency-tuned receptors, denoted MT (for medially terminating) and BC (bifurcating), a mid-frequency-tuned group, and a group tuned to ultrasound. Both of the latter groups respond robustly to ultrasound stimuli, and we combine these here with the designation HF (for high frequency). These were identified in the present study by their greater sensitivity to 30- than to 4.5-kHz stimuli. HF receptors terminate in the same region of neuropil as the dendrites of first-order auditory interneurons, including AN2. In this paper, we consider only the MT type of low-frequency-tuned receptor because, based on anatomy, only these have terminal boutons within the main region of auditory neuropil (Imaizumi and Pollack 2005). MT types were differentiated from BC types by their lower threshold to 4.5-kHz stimuli (≤50 dB SPL for MT type and ≥60 dB SPL for BC type (Imaizumi and Pollack 2001); in our data set, thresholds were: MT type, 43.3 ± 1.9 (SE) dB SPL and BC type, 64.5 ± 2.0 dB SPL) and, when possible, through visual inspection after fluorescent staining (ALEXA 568; 3 MT-type receptors were successfully stained and visual inspection of their morphology confirmed the identification based on auditory threshold). Typically, only one receptor per animal was recorded.

AN2 was recorded extracellularly with a saline-filled suction electrode on the cut cervical connective, where it produces large, easily recognized spikes (Moiseff and Hoy 1983; also see Fig. 3A).

**Stimuli**

Stimuli were generated by a National Instruments (Austin, TX) A/D/D/A board (PCI-6251; 12 bits of resolution; sampling rate 100 kHz) driven by custom Matlab programs (Rel 2008a; The Mathworks, Natick, MA). Sound was broadcast from a loudspeaker ipsilateral to the recorded axon, positioned perpendicular to the cricket’s longitu-
dinal axis. Sound level was adjusted with a custom built programmable attenuator and calibrated using Bruel & Kjaer (Naerum, Denmark) instruments (4135 microphone; 2610 sound-level meter).

Stimuli were 5-s tones, the amplitude of which was modulated through time (random amplitude modulation: RAM) by multiplication with a low-pass filtered Gaussian signal (5th-order Butterworth, cut-off fre-
frequency: 200 Hz) that defined the stimulus envelope. The modulation depth (in dB) of the RAM was defined as the SD of this envelope (3, 5, or 7 dB). Before multiplication with the carrier sine (4.5 or 30 kHz), the envelope was converted from dB to linear scale using the formula $\text{STIM}_{\text{envelope}} = 10^{\text{ENVELOPE}_{\text{dB}}/20}$. The intensity of the RAM was defined as the RMS intensity and, except where otherwise noted, took values of +5, +15, or +25 dB (+ sign means that intensity is relative to the receptor’s threshold, defined as the lowest sound level (+2 dB) that reliably elicited one to two spikes for each presentation of a 30-ms sound pulse).

The shape of the RAM signal was “frozen,” meaning that the same RAM envelope was used throughout the experiment and only the carrier frequency, intensity, and modulation depths were varied. Stimuli were preceded by silent periods of 15 s.

**Analysis**

Responses of auditory receptors adapt markedly. To ensure that analyses were performed when response statistics were stationary, the first 2 s of the responses, when most of the adaptation occurs, were discarded.

Interspike intervals (ISIs) were constructed using bins that were equal in width on a logarithmic scale (Reich et al. 2000; Selinger et al. 2007). Criterion ISI for identifying bursts in receptors was determined visually from the ISI histograms (see Fig. 1, A and B). All spikes separated by an interval <5 ms were considered part of a burst. A similar burst criterion was arrived at by inspecting the autocorrelation function of the spike train (Eyherabide et al. 2008; data not shown). If two or more ISIs of <5 ms were consecutive, then all of these spikes were considered to be part of a single burst. Burst criterion in AN2 was 6 ms, in accordance with previous work (Marsat and Pollack 2006).

The ability of receptors to detect features in a stimulus was assessed through standard feature-detection analysis (Metzner et al. 1998). Spike trains were first “binarized” by setting the time points at which events of interest occurred (either isolated spikes or the 1st spikes of bursts) to 1 and all other points to 0. For each time point $t$ of a binarized neural response $r$ (in the case of bursts, the timing was considered to be the time of occurrence of its 1st spike), a 50-ms section of the stimulus $s$ preceding that point was extracted and assigned to one of two ensembles, event-triggered (ET) or no-event-triggered (NET), depending on the occurrence or nonoccurrence of an event (spike or burst) at time $t$. A feature vector, $f$, was defined as $f = \text{ETA} - \text{NET}$, where ETA (event-triggered average) and NET (non-event-triggered average) are obtained by averaging all the stimulus vectors in ET and NET, respectively. The difference between the two sets in stimulus space was measured through a linear classifier

$$h(\theta, s) = f^T \cdot s - \theta$$

where the Euclidean feature vector $f$ is projected on the stimulus vector $s$ ($T$ indicates transposition). $\theta$ is the designated threshold for similarity of feature to stimulus, so that a positive value of $h$ will be interpreted as the presence of a feature in the stimulus. The probability of detection, $P_D$, was defined as the probability of a feature preceding an event

$$P_D(h(\theta, s) > 0) = \text{ETF}$$

and the probability of failure, $P_F$, as the probability that the absence of an event is preceded by a feature

$$P_F(h(\theta, s) > 0) = \text{ETF}$$

The receiver-operating characteristic curve (ROC) was obtained by plotting $P_F$ against $P_D$ for all values of the threshold $\theta$. The minimum error was derived from the ROC

$$\text{Minimum error} = \min\left(\frac{1}{2} P_F + \frac{1}{2} (1 - P_D)\right)$$

where the minimum error is the minimum of all the errors derived from all pairs of $P_F$ and $P_D$ in the ROC. The minimum error is in fact the average of two types of error: that due to undetected features (feature but no event) and that due to erroneously detected features (event but no feature).

**FIG. 1.** Interspike interval histograms and firing rates. A: interspike interval (ISI) histogram of high frequency (HF, gray) and MT (black) receptors (see METHODS). The vertical dotted line indicates the 5-ms bursting threshold that was estimated by visual inspection. There is a distinct peak of short ISIs, corresponding to ISIs within bursts, for HF receptors, but not for MT receptors. ISI histograms were computed for each individual neuron, normalized, and averaged for each type of receptor. Intensity = +15 dB; modulation depth = 5 dB, $n = 18$ for each receptor type. B: ISI histograms for 5 individual MT (top, black) and HF (bottom, gray) receptors. C: effect of modulation depth on firing rate and bursting (intensity = 15 dB above threshold). Numbers in lower portions of the bars indicate the proportions of spikes within bursts. Both firing rate and proportion of spikes in bursts (means ± SE) are significantly higher in HF receptors. Proportion of spikes in bursts increases with modulation depth [see Supplemental Fig. S2 for complete data set; 3-way ANOVA (factors: intensity, modulation depth, receptor type) on complete data set, modulation-depth effect, $P < 0.001$, $n = 15–18$. D: effect of intensity on firing rate and bursting (modulation depth = 5 dB). Numbers in lower portions of the bars indicate the proportions of spikes within bursts. Firing rate and proportion of spikes in bursts increase with stimulus intensity and are significantly higher for HF than for MT receptors at +5 and +15 dB [3-way ANOVA on complete data set (factors: intensity, modulation depth, receptor type), intensity effect, $P < 0.001$; post hoc $t$-test at +5 and +15 dB, $P < 0.01$].
We assessed the impact of coincident isolated spikes and bursts in HF receptors on AN2 bursting (Fig. 4) using the following procedure. Binarized receptor spike trains, drawn from experiments where receptors and AN2 were recorded simultaneously (n = 9), were separated into trains containing either isolated spikes or bursts; for the latter, only the first spike of each burst was retained as a marker of the burst’s occurrence. Composite trains were then computed by summing pointwise all of the responses. The composite trains were then filtered using a running sum (window width, 5 ms) to yield a signal that, at any time point (resolution, 1 ms), indicates the number of events (bursts or isolated spikes) in all receptors in the 5 ms following that time point. Isolated spikes occurring <5 ms after the last spike in the summing window were also included in the sum to mimic the situation that might occur with bursts, where only the first spike of the burst falls within the 5-ms window. The probability of bursting in AN2 at times surrounding the analysis window was computed by extracting bursts from the binarized AN2 spike trains and by averaging the burst trains point-wise. This procedure is illustrated graphically in Supplemental Fig. S1.1

Data analysis, including statistical tests, was done using Matlab (Rel. 2008a, The Mathworks).

RESULTS

Bursting in receptor neurons

As ultrasound-elicited bursting in interneurons is known to be important for behavior (Marsat and Pollack 2006, 2007), we asked whether this might have its origins in the structure of afferent spike trains. Indeed, HF receptors often produce bursts of spikes interspersed with nonburst or isolated spikes (e.g., see Fig. S3). This is reflected in the distribution of interspike intervals, where a distinct peak of short intervals, corresponding to the intervals between spikes within a burst, is evident (Fig. 1, A and B, bottom). By contrast, a distinct peak of short intervals is absent for MT receptors (Fig. 1, A and B, top). Using the interspike-interval histogram of Fig. 1A as a guide, we take 5 ms as the threshold interspike interval for identifying spikes within bursts. For consistency, we identify as bursts series of MT action potentials separated by ≥5 ms, even though for this group of receptors, these brief intervals represent one end of a unimodal distribution rather than a distinct peak. The tendency to produce bursts varies with both mean stimulus intensity and modulation depth, but is consistently higher in HF receptors than in MT receptors (Fig. 1, C and D). Mean firing rate is also higher in HF receptors; however, a comparison of burst rates and firing rates across a range of modulation depths and stimulus intensities demonstrates that higher firing rate does not by itself account for the increased tendency for bursting in HF receptors. For example, mean firing rate of MT receptors for +15 dB intensity and modulation depth of 3 dB (Fig. 1C) is greater than that for HF receptors stimulated at +5 dB intensity and modulation depth of 5 dB (Fig. 1D), yet, the proportion of spikes in bursts is nearly three times as large for HF receptors. Additional examples can be found in Supplemental Fig. S2, which presents the complete data set.

Feature detection by bursts and isolated spikes

Figure 2A shows, for both MT and HF receptors, the mean stimulus amplitude occurring prior to the onsets of bursts. Bursts in HF receptors tend to occur following a conspicuous...
increase in stimulus amplitude that is preceded by a smaller decrease, similar to the burst-evoking feature that was described previously for AN2 (Marsat and Pollack 2006). For MT receptors, the increase in amplitude preceding high-rate spike sequences is less steep than for HF receptors, and the preceding decrease in amplitude is less pronounced. The amplitude profile preceding isolated spikes is similar for both receptor types and is both briefer and smaller than that preceding bursts (Fig. 2B).

These preevent averages formed the basis for feature-detection analysis (see METHODS). Previous work showed that for interneurons, bursts perform better than isolated spikes as feature detectors (Marsat and Pollack 2006, 2007). This is the case for receptor neurons as well (Fig. 2C), both for HF receptors (in which bursts occur as distinct events) and for MT receptors (for which short, burst-like interspike intervals form one end of a continuum; see Fig. 1). Performance is quantitatively similar for bursts in both receptor types, although isolated spikes of HF receptors perform slightly better than those of MT receptors. This is summarized in Fig. 2D, which shows minimum error values (reflecting both failure to detect a feature when 1 occurs, and erroneous reporting of a feature when there is none). For both bursts and isolated spikes, minimum errors increased with higher intensities and smaller modulation depths (see Supplemental Fig. S3).

**Bursts are correlated between HF receptors and AN2**

The occurrence of bursting in HF receptors, which provide input to AN2, raises the possibility that this might be responsible for the bursting that is known to occur in AN2. Indeed visual inspection of simultaneous recordings from HF receptors and AN2 suggests that bursts in receptors and AN2 occur at roughly the same time (Fig. 3A). This is confirmed in Fig. 3B; AN2 bursts are most probable following receptor bursts. There is a weaker correlation between the timing of isolated spikes and AN2 bursts. Note in Fig. 3B, that some AN2 bursts occur before the burst in the receptor that is recorded. This is not unexpected, because other receptors, which are not recorded, may produce bursts earlier than the recorded receptor. Indeed, bursts are loosely correlated across HF receptors (Fig. 3C). In the set of spike trains recorded from separate animals, for each burst in each HF receptor, a burst occurs in at least one other HF receptor within ±5 ms. A similar argument applies to the weaker correlation between the timing of isolated spikes and AN2 bursts seen in Fig. 3B; isolated spikes in one receptor are likely to coincide with bursts in one or more other receptors, although this correlation is weaker than that between bursts in different receptors (Fig. 3C). In our data set, for 53% of isolated spikes in reference HF spike trains, a burst occurs in at least one other spike train within ±5 ms of the reference isolated spike.

Even if isolated HF spikes are ineffective in eliciting AN2 bursts, it is conceivable that closely spaced spikes carried by different receptor neurons (i.e., “distributed” bursts) might be effective. We examined this by scanning through our data set of spike trains from simultaneous recordings for instances when two, four, six, or eight spikes occurred within the same 5-ms window (see METHODS). As Fig. 4A shows, there is no relationship between distributed receptor bursts and bursts in AN2. By contrast, the probability of bursting in AN2 increases systematically with the number of coincident “true” bursts in different receptor neurons (Fig. 4B).

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**FIG. 3.** Correlation between isolated-spike and burst events in receptors and AN2. A: example of simultaneous recordings. **Top:** stimulus; **middle:** HF receptor response; **bottom:** AN2 response. Bursts are highlighted in black. **B:** probability of bursting in AN2 following a spike or a burst in a HF receptor (from simultaneous paired recordings). There is a higher probability of bursting in AN2 at times close to a receptor burst than at times close to a nonburst spike. **C:** probability of receptor bursts, averaged over 8 HF receptors (recorded in different animals) at times surrounding a burst (gray) or nonburst spike (black) in a reference high-frequency receptor.
DISCUSSION

Our results show that bursting, a behaviorally relevant feature of the spike train of an interneuron, can be accounted for by the temporal characteristics of the spike trains of receptor neurons. This differs from previously described systems, where bursts are generated at higher levels of processing. Bursts in pyramidal cells in the electrosensory lateral-line lobe of weakly electric fish result from electrotonic interactions between long-lasting dendritic action potentials and the neuron’s axonal spike-initiation zone (Lemon and Turner 2000). In the thalamus, bursting is produced by activation of a Ca$^{2+}$ conductance in the bursting neuron (Jahnsen and Llinás 1984). Other ion currents, intrinsic to the bursting neuron, may operate in other systems (see Krahe and Gabbiani 2004 for review). Bursting has, however, been described previously at the sensory periphery (e.g., Amir et al. 2002; Gallar et al. 2003; Iggo et al. 1985).

In two cases, electrorceptors of weakly electric fish (Chacron et al. 2004) and auditory receptors of locust (Eyherabide et al. 2008), a possible function for afferent bursts, feature detection, has been proposed. However the present case is, to our knowledge, the only example in which bursting of sensory neurons can, through their correlations with bursts in an identified interneuron, AN2, be compellingly related to a behavioral function, namely predator avoidance (Marsat and Pollack 2006).

Bursts, as distinct events, occur only in HF receptors. Although MT receptors may reach instantaneous firing rates similar to those achieved by HF receptors during bursts, these do not, in MT receptors, form a distinct set of events; rather, short interspike intervals are at one end of a continuous, unimodal distribution. HF and MT receptors also differ in the amplitude trajectory that most often elicits bursts. For HF receptors, bursts are elicited by a considerable amplitude decrease followed by a sharp and even larger amplitude increase. The earlier decrease in amplitude might serve to allow recovery from adaptation, which is particularly pronounced in HF receptors (Sabourin 2008) (Supplemental Fig. S4), permitting high-rate firing in response to a subsequent large and rapid amplitude increase. Indeed instantaneous firing rate at stimulus onset is higher for HF than for MT receptors (Imaizumi and Pollack 2001; Sabourin 2008), and the functions relating firing rate to stimulus intensity are steeper (Imaizumi and Pollack 2001) (Supplemental Fig. S4).

Bursts in HF receptors, but not isolated spikes, are effective in eliciting bursts in AN2. Interestingly, the lack of effectiveness of isolated spikes holds even when these are closely spaced, but in different receptor neurons (“distributed bursts”). The apparent requirement that closely spaced spikes occur in the same receptor terminals suggests that homosynaptic facilitation may be one mechanism contributing to bursting in AN2. An important caveat here is that the analysis that leads to this suggestion (Fig. 4) is based on the assumption that the pattern of spikes in receptors recorded in different animals is representative of the pattern across receptors of a single animal. Determining whether this is true requires simultaneous recordings from multiple receptor axons, which has not yet been done.

Another identified interneuron, ON1, is dually tuned with enhanced sensitivity to both cricket- and bat-like sound frequencies. Anatomical data suggest that ON1 receives direct input from both MT and HF receptors (Imaizumi and Pollack 2005). Interestingly, in response to RAM stimuli, ON1 pro-
duces bursts only for ultrasound stimuli (Marsat and Pollack 2007). Ultrasound-elicited bursts in ON1 are statistically indistinguishable from those in AN2, possibly because bursts in both neurons are driven by input from the same set of bursting HF receptors. The absence of bursting in ON1 for low-frequency stimuli suggests that MT receptors, even when firing at high rate, are ineffective in driving postsynaptic bursting. There are at least two possible reasons for this. First, whereas unitary excitatory postsynaptic potentials in ON1 resulting from single HF spikes are relatively large, those from single MT spikes are probably small (Pollack 1994). Second, temporal correlation among spike trains of different HF receptors is higher than that among MT receptors (unpublished data), favoring strong, coincident input to postsynaptic neurons.

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REFERENCES


