Single auditory neurons rapidly discriminate conspecific communication signals

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Animals that rely on acoustic communication to find mates, such as grasshoppers, are astonishingly accurate in recognizing song patterns that are specific to their own species[1,2]. This raises the question of whether they can also solve a far more complicated task that might provide a basis for mate preference and sexual selection: to distinguish individual songs by detecting slight variations around the common species-specific theme. Using spike-train discriminability to quantify the precision of neural responses from the auditory periphery of a model grasshopper species, we show that information sufficient to distinguish songs is readily available at the single-cell level when the spike trains are analyzed on a millisecond time scale.

To investigate whether information about variations between conspecific songs is available for discrimination, we made electrophysiological recordings in *Chorthippus biguttulus* females and recorded spike trains from single auditory receptors in response to songs from different conspecific males (**Fig. 1a** and **b**). To eliminate simple discrimination cues such as variations in syllable length, we also recorded responses to songs that were rescaled to have a common syllable duration and carrier spectrum. To obtain these song variants, the original amplitude modulations were calculated using the Hilbert transform[3], rescaled in time, and then combined with the spectrum of one of the songs (**Fig. 1c**). The resulting mean firing rates are indistinguishable (**Fig. 1d**).

Successful discrimination would demand that spike trains elicited by repeated presentations of the same song be more similar to each other than spike trains elicited by different songs (**Fig. 1d**). To compute the similarity between two spike trains, each spike was replaced by a function $f(t)$ that mimics the time course of an excitatory postsynaptic potential (EPSP) in a hypothetical downstream neuron of the grasshopper's auditory system (**Fig. 1e**). A distance between the two spike trains was then defined as the mean square distance between their EPSP-convolved traces[4]. By varying the width (τ) of the EPSP function, effects of the temporal resolution can be studied: if τ is large, only differences in the average spike rate contribute to the distance measure; if τ is small, even small differences in spike timing matter.

We analyzed responses to songs from eight *Ch. biguttulus* males. For each song, one spike train was arbitrarily chosen as a template.
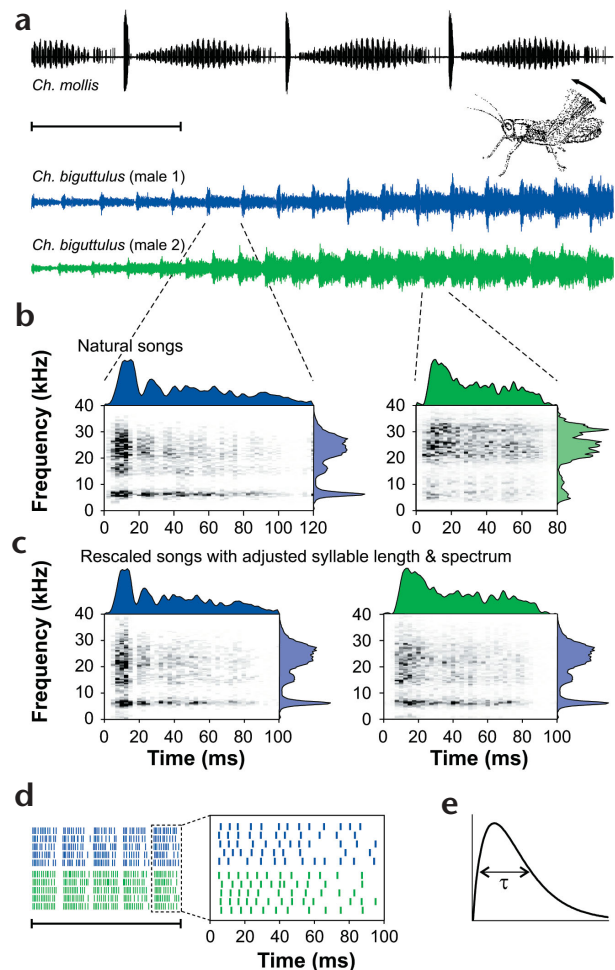

Fig. 1. Acoustic communication signals, artificial songs and sample responses. (**a**) Grasshoppers produce species-specific sound patterns by rasping their hindlegs across their forewings. Shown are songs generated by one *Chorthippus mollis* and two *Chorthippus biguttulus* males. Each song consists of many repetitions of a basic pattern, termed 'syllable'. Scale bar, 500 ms. (**b**) Within each syllable, the amplitude of the high-frequency broadband carrier is strongly modulated in time as illustrated by the two spectrograms. (**c**) Equalizing the song spectra and rescaling the length of each syllable to 100 ms yields artificial songs that differ only in their amplitude modulation. (**d**) Spike raster plots of responses from a single auditory receptor to five presentations of the two songs shown in (**c**). (**e**) The EPSP function $f(t) = t*\exp(-\alpha t)$ used to compute spike-train distances. The full width at half maximum, τ, is $\tau \approx 2.45/\alpha$. Details of the experimental protocols, which complied with German law governing animal care, are described elsewhere[5]. Stimulus intensities were 50–70 dB SPL, yielding spike rates between 100 and 200 Hz; temperature during the measurement was 33–35 °C. Responses to at least six repetitions of the rescaled songs were recorded from $n = 18$ receptors; for three of these neurons, responses to the original songs were also obtained.

The remaining spike trains were classified by assigning each one to the closest of the eight templates. Averages were then computed by permuting all possible template choices, yielding classification matrices (**Fig. 2a**). The diagonal elements of the matrices correspond to correctly classified spike trains, and the off-diagonal elements to misclassified spike trains. When insisting on a very high time resolution ($\tau \leq 1$ ms) or when largely neglecting temporal information ($\tau \geq 100$ ms), the songs cannot be discriminated well, as
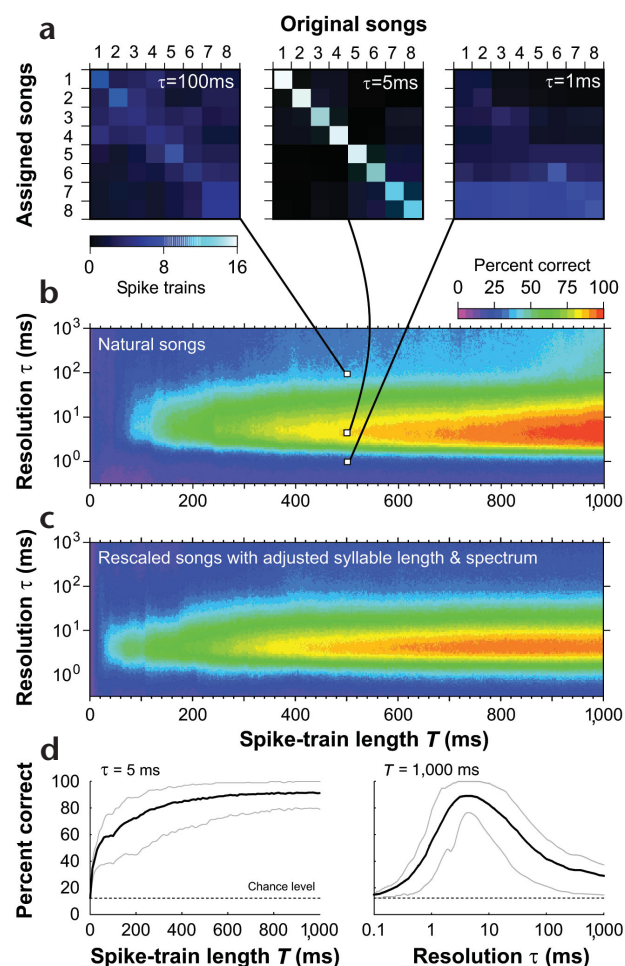
Fig. 2. Analysis of single neuron responses. (**a**) Discrimination matrices computed at *T* = 500 ms for τ = 1 ms, τ = 5 ms and τ = 100 ms; the parameter *T* denotes the spike-train length used for analysis, and τ measures the width of the EPSP function. Rows and columns of the matrices represent the consecutively numbered songs. The matrix entries *N*(*a*, *b*) give the number of spike trains that were elicited by song *b* (horizontal axis) and classified as belonging to song *a* (vertical axis). A spike train is correctly classified if *a* = *b* (diagonal elements). (**b**) Discrimination performance as a function of duration *T* and resolution τ. Within the red area, at least 95% of the spike trains are classified correctly; the width of the EPSP-like function used to filter the spike trains is then within the range of τ = 3–10 ms. (**c**) Discrimination performance for artificial song variants. (**d**) Summary of responses from 18 cells to the artificial songs. As a function of the spike-train length *T*, means and standard deviations of the discrimination performance are depicted on the left for τ = 5 ms. On the right, the discrimination performance is shown as a function of the resolution τ for *T* = 1,000 ms. All results are quite robust: cost-based metrics[8] as well as several supervised and unsupervised cluster algorithms yielded similar results.

indicated by the many off-diagonal entries in the corresponding matrices. For τ ≈ 5 ms, however, most spike trains are correctly assigned, suggesting that this time scale corresponds to unique song features that auditory receptors accurately encode. Signal variations on a scale of a few milliseconds are thus well suited for song evaluation; downstream neurons with much longer EPSPs will hardly be able to process the information relevant for discrimination.

To investigate how the discrimination ability unfolds in time, we computed how many spike trains are correctly classified as a function of both the spike-train length *T* and EPSP width τ (contour plot in **Fig. 2b**). Already at *T* = 400 ms, after just a few syllables or roughly 50 action potentials, the songs are correctly recognized in over 80% of all trials. Differences in the songs' amplitude modulations, syllable length and carrier spectrum all contribute to this surprisingly rapid and accurate discrimination. Notably, variations in spectral composition often lead to different firing rates, thereby facilitating discrimination.

Successful discrimination of the rescaled songs with equalized carrier spectrum and uniform syllable length, however, must be based solely on differences in the detailed structure of amplitude modulations within a syllable. Nevertheless, the neural performance does not decrease substantially (**Fig. 2c** and **d**). For small *T* or very small τ, discrimination may even slightly improve as the adjusted spectra lead to higher firing rates and lower trial-to-trial spike time variability for some of the songs. Together, our results show that auditory receptor neurons react to rapid amplitude modulations with high temporal accuracy, so even slight differences among stimuli can be recovered. This finding coincides with the observation that quickly varying stimuli lead to greater precision in receptor spike timing than slowly varying stimuli[5], just as neocortical neurons respond more precisely to fluctuating current injections than to constant currents[6].

Our data indicate that short segments of single spike trains from one auditory receptor suffice to rapidly discriminate the songs of conspecific grasshoppers, provided that a time resolution of a few milliseconds is maintained. With 40–50 receptors per ear, even more information is available for spike-time based computations by downstream neurons. That the female grasshopper's auditory system could, in principle, distinguish individual male songs does not prove, however, that female grasshoppers use this capability. But if near-perfect discrimination is possible, then so is the easier task of preferring certain male songs over others, which female grasshoppers do[7]. The precise role of song discriminability in sexual selection remains an open question for further investigation.

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