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Discrimination of Acoustic Communication Signals by Grasshoppers (*Chorthippus biguttulus*): Temporal Resolution, Temporal Integration, and the Impact of Intrinsic Noise

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A characteristic feature of hearing systems is their ability to resolve both fast and subtle amplitude modulations of acoustic signals. This applies also to grasshoppers, which for mate identification rely mainly on the characteristic temporal patterns of their communication signals. Usually the signals arriving at a receiver are contaminated by various kinds of noise. In addition to extrinsic noise, intrinsic noise caused by stochastic processes within the nervous system contributes to making signal recognition a difficult task. The authors asked to what degree intrinsic noise affects temporal resolution and, particularly, the discrimination of similar acoustic signals. This study aims at exploring the neuronal basis for sexual selection, which depends on exploiting subtle differences between basically similar signals. Applying a metric, by which the similarities of spike trains can be assessed, the authors investigated how well the communication signals of different individuals of the same species could be discriminated and correctly classified based on the responses of auditory neurons. This spike train metric yields clues to the optimal temporal resolution with which spike trains should be evaluated.

Keywords: neural encoding, temporal resolution, intrinsic noise, spike distance metric, sexual selection

A prominent feature of acoustic communication signals in insects, anurans, birds, and mammals is their richness in amplitude fluctuations, and often it is the temporal structure of the signal's envelope that conveys the most relevant information (e.g., Shannon et al., 1995; Smith et al., 2002; Kroodsma & Miller, 1996; Gerhardt & Huber, 2002; Joris et al., 2004). Hence, the ability to process such temporal patterns, and, in particular, to resolve rapid amplitude changes, is crucial for the recognition and interpretation of acoustic signals. Signal recognition is, however, not only impeded by various kinds of extrinsic noise that may mask and distort the temporal pattern of signals, but also by "intrinsic noise" of the receiver: additional variability of the neuronal signals is caused by stochastic events acting on various stages of neuronal processing (e.g., Borst & Theunissen, 1999; Ronacher et al., 2004). This intrinsic noise may constrain the resolution of weak amplitude modulations and the discrimination of similar acoustic signals. To distinguish between similar signals is of particular relevance in the broad context of sexual selection and mate choice, where the task is to extract information about a signaler's quality from subtle variations of the common species-specific signal pattern—usually a much more demanding problem than only to reject signalers belonging to different species. Here, we will relate these questions to the general problems of temporal resolution and temporal integration in sensory systems, using the example of acoustically communicating grasshoppers.

Features of the Grasshopper Communication System and Problems Related to Signal Recognition

Acoustic communication signals play a major role in the life of many grasshopper species: they serve to attract sexual partners and thus contribute directly to the mating success and the production of offspring—the basic currency in evolution. Acridid grasshoppers produce their stridulation signals by moving their hind legs in a species-specific temporal pattern against a vein on their forewings (see Figure 1). Both signal production and signal recognition by the receiver are innate behaviors and differ between species, thus constituting a major barrier against hybridization (Elsner, 1974; Gottsberger & Mayer, 2007; Stumpner & von Helversen, 1994; von Helversen & von Helversen, 1975, 1994). Behavioral experiments with song models revealed that it is mainly the temporal pattern of amplitude modulations (AM) that conveys the essential cues for signal recognition (Hennig et al., 2004; Stumpner & von Helversen, 1992, 2001; von Helversen & von Helversen, 1997, 1998).

Grasshoppers and other orthopteran insects offer important experimental advantages for studies of sensory processing: (1) they allow a rather direct connection between behavioral experiments and neurophysiological data (e.g., Machens et al., 2003; Ronacher & Krahe, 2000; cf. also Marsat & Pollack, 2006; Schildberger, 1984; Schildberger & Hörner, 1988); and (2) as in other invertebrates many neurons of these insects can be identified as individ-

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uals, on the basis of their characteristic morphology (see Figure 2), which is revealed by dye injection during intracellular recordings (e.g., Römer & Marquart, 1984; Stumpner & Ronacher, 1991). This makes possible repeated recordings from a well-defined neuron type. In addition, grasshoppers possess relatively few neurons, at least compared to vertebrate model systems for auditory processing, such as owls, gerbils, or cats. Therefore, one can hope to unravel a neuronal network that is specifically designed for the processing of AM patterns (Hennig et al., 2004).

In the context of mate finding the basic task is to recognize the acoustic signals of a potential conspecific partner, and to avoid the erroneous acceptance of signals from individuals belonging to a different species (Stumpner & von Helversen, 2001; von Helversen & von Helversen, 1994). In addition to determining species identity, it may also be important for these animals to extract from the heard signals some hints to the quality of the sender, for example indications of the sender's health and condition (see below). In theory, the decision about species identity does not seem a difficult one, since the signal patterns usually differ considerably between species (Elsner & Popov, 1978; Stumpner & von Helversen, 1994). In practice, however, several factors aggravate the problems of signal identification: (1) The acoustic signals will often be degraded on their way through the biotope by attenuation, reflections, reverberations and so forth, and masked by ambient noise and by signals produced by conspecific signalers (for reviews of various factors see, e.g., Dusenbery, 1992; Greenfield, 1994, 2005; Römer, 1998, 2001). In the following, we will subsume all these factors as *extrinsic noise*. (2) In addition to extrinsic noise, signal recognition may be impeded by *intrinsic noise* sources that result from stochastic processes within the animal's nervous system, for example, during signal transduction in the sensory neurons, during synaptic transmission and spike generation. The impact of this intrinsic noise will be the main focus of this report (see below). (3) Another factor that can impede signal recognition is *temperature*. Because grasshoppers are heterothermic animals, that is, they cannot keep their body temperature constant, changes in ambient temperature strongly influence the speed of the stridulatory movements and as a consequence the temporal pattern of their songs. For a receiver with different body temperature this may render the signals incomprehensible. How the animals cope with this "time-warp" problem of their signaling is, however, beyond the scope of this report.

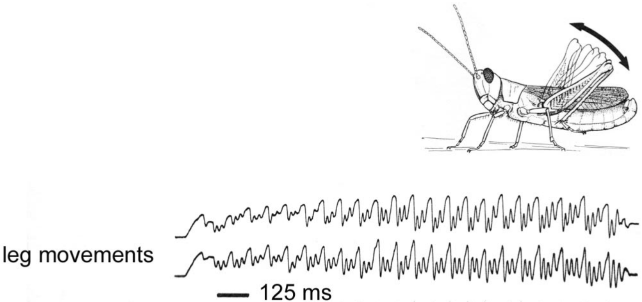

Figure 1. Stridulation of a male *Chorthippus biguttulus*. Lowest trace: sound pattern; upper traces, movements of left and right hind legs, recorded with an optoelectronic camera. Courtesy of Otto von Helversen.

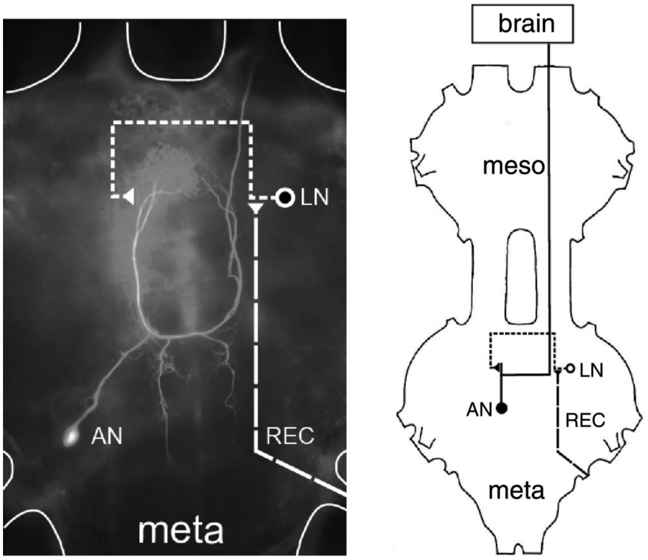

Figure 2. Sketch of the information flow in the auditory pathway of grasshoppers (right), and example of an ascending auditory neuron stained with Lucifer Yellow (left). Meta, meso indicate the third and second thoracic ganglion, respectively (the proper metathoracic ganglion is fused with the first three abdominal ganglia). REC: receptor neurons, LN: local neurons, AN: ascending neurons. For further details see text.

Intrinsic Noise May Constrain in Particular the Discrimination of Similar Signals

All information about the external world must be conveyed to the brain in the form of spike trains, that is, sequences of action potentials, provided by the sensory neurons, via higher order neurons. Both, the variations of the signals arriving at the sense organ and the additional noise generated within the nervous system contribute to the overall variability of spike trains, which is crucial for any signal recognition task (see, e.g., Borst & Theunissen, 1999; Grewe et al., 2003; Ronacher et al., 2004). Intrinsic noise becomes evident if one records from a neuron while repeatedly presenting an identical signal (see Figure 3). In the grasshopper auditory system, the variability of spike trains tends to increase from the periphery to higher-order interneurons (Figure 3; Vogel et al., 2005). It should be noted, however, that the effect of intrinsic noise depends on several factors, for example, the sound intensity (Schaette et al., 2005), the spike rate (Vogel et al., 2005; see also Gabbiani & Koch, 1998), and in particular the structure of the stimulus envelope. As concerns the latter, the depth and rate of change of AM is important. Very precise spiking behavior is observed in response to steep amplitude rises, in particular in receptor neurons (Machens et al., 2001; Rokem et al., 2006).

In the next Section we will first discuss temporal integration as a means to reduce intrinsic and extrinsic noise problems, and provide some behavioral results on the size of the processing time window. Temporal integration, however, cannot be considered independently of, and must be weighted against, temporal resolution. In the following Section we therefore investigate the temporal resolution properties of neurons at different stages of the grasshoppers' auditory pathway, by determining modulation transfer functions. Finally, in the last Section, we focus on the problem of

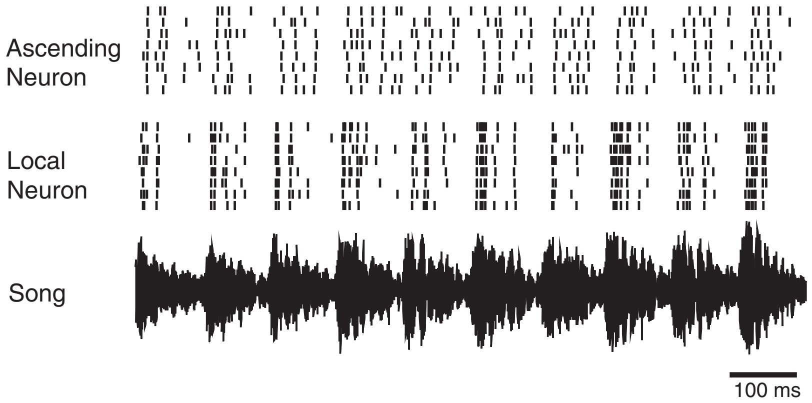

Figure 3. Spiking responses of two auditory neurons of *C. biguttulus* to a segment of the species-specific song. Spikes are shown as raster plots, that is, each spike is represented by a small vertical line. Shown are responses of a local neuron (TN1) and an ascending neuron (AN11) to eight stimulus repetitions. S. Wohlgemuth, unpublished results.

how well subtle differences between similar acoustic signals can be discriminated on the basis of spike trains—the only information a central nervous system has about the external world. To assess the discriminability of acoustic signals we apply a recently developed method to quantify the similarity of spike trains, a spike train metric (van Rossum, 2001).

Temporal Integration and the Size of the Processing Time Window

There are two principal ways to cope with signal distortions and spike train variability: temporal integration and "spatial" summation. Reliability, and hence signal recognition, can be improved by averaging neuronal responses over a longer time segment, or by averaging over the responses of many similar neuronal elements (see, e.g., Rieke et al., 1996; Shadlen & Newsome, 1998). However, considering as an example the processing of visual information, the respective major drawbacks of the two strategies become obvious: by averaging the input from many photoreceptors sensitivity is enhanced, but at the cost of reduced spatial resolution, as is evident in the rod pathway of mammals. Temporal integration, on the other hand, implies the risk of missing fast events (Warrant, 1999). Temporal integration therefore entails a particularly delicate problem in the acoustic modality, where rapid AM often convey important information. Indeed, there is an ample psychoacoustic literature devoted to the so-called integration-resolution paradox (de Boer, 1985; Green, 1985; Tougaard, 1998). This term refers to a strong discrepancy between the time constants found in different experimental paradigms. In a detection task near threshold one usually observes a trade-off between duration and intensity: the detection threshold decreases with increasing stimulus duration, up to 100 or 200 ms, and the product of threshold intensity and duration is roughly constant, indicative of energy integration (Green, 1985; Tougaard, 1998). Given the time scales of AM in acoustic communication signals, however, it is quite obvious that no auditory system could afford to sacrifice temporal resolution to such a degree (see Gollisch & Herz, 2005). Indeed, in different experimental paradigms, such as double-click and gap-detection experiments, one finds much shorter time constants in the order of a few ms (e.g., Franz & Ronacher, 2002; Joris et al., 2004; Prinz & Ronacher, 2002; Tougaard, 1996; Viemeister & Plack, 1993). As a possible solution for this integration-resolution paradox, Viemeister and Wakefield (1991) have proposed a 'multiple-look model.' According to this model, sensory input is initially sampled at high rate and then analyzed with different integration times depending on the task and the context of processing. To our knowledge, however, a physiological implementation of this model has not yet been demonstrated in higher vertebrates (cf. however, Alder & Rose, 1998, 2000; Edwards et al., 2002). Whether grasshoppers employ a multiple-look solution is currently unclear. However, it might be beneficial for them to analyze sounds with a range of integration times to improve information extraction from distorted or masked signals. The highly repetitive character of grasshopper songs, which consist of many stereotyped subunits (Figures 1, 3), seems to directly invite to use temporal integration as a means to alleviate the problems of signal degradation. Therefore, we asked how strongly signal recognition in these animals depends on the repetitive structure of their songs.

Behavioral Experiments to Determine Lower and Upper Bounds of the Processing Time Window

Method

Animals and test procedures. Behavioral experiments were performed on males of the grasshopper *Chorthippus biguttulus*, caught as adults or last instar larvae in the field. We took advantage of a behavior that reliably indicates whether or not an animal can identify modified signals as conspecific communication sounds. *C. biguttulus* males react very specifically to the response song of a female with a conspicuous turning movement, which is the first step of the phonotactic approach toward the female (von Helversen, 1997). This phonotactic turning response is shown with high reliability and exclusively to the female song, and is therefore an excellent indicator that a male has classified a sound as a female song signal. Tests took place in a reflection-attenuated room, by stimulating a single male with female song models of different durations via a loudspeaker situated lateral of the animal, and recording the phonotactic turning responses of males. The standard song model consisted of 12 identical subunits and had a duration of 1,015 ms; the durations of the 5, 4, 3-subunit stimuli were 420, 335, and 250 ms, respectively. Following von Helversen and von Helversen (1997), the signal level was set at 50 dB re $2*10^{-5}$ Nm^{-2} (dB SPL; rms-value, which corresponded to peak levels of 64.5 dB SPL). Broadband noise (main energy between 3 and 10 kHz) could be delivered by a speaker situated 65 cm above the animal. For further details of the experimental procedure see Ronacher, Krahe, and Hennig (2000).

Results and Discussion

Using this reliable behavior as a tool we found, without continuous noise added from above, that a 250-ms signal, consisting of only three subunits, elicited a mean response rate of ∼90% (median 100%; Ronacher & Krahe, 1998; see also arrow in Figure 4A). Hence, a surprisingly small segment of the female signal (the natural duration is ∼1 seconds) suffices for recognition. With an even shorter signal (two subunits; corresponding to 165 ms) still one-third of the tested males (18 out of 53) responded to more than 50% of the stimulus presentations, indicating an even lower limit of the processing time window (Ronacher & Hennig, 2004). It should be emphasized that a single presentation of such shortened signals suffices to elicit the male's turning response; hence, this is a system capable of one-shot recognition, within a few hundred milliseconds!

These data indicate a *lower bound* for the signal duration that is necessary to enable recognition (for further controls see Ronacher & Krahe, 1998, and Ronacher & Hennig, 2004). To obtain an estimate for the *upper bound* of the evaluation time window (or integration time window) we performed similar tests in the presence of ambient noise. The experimental approach was analogous to measuring the integration time window in signal detection experiments (see above and Green, 1985). We tested each *C. biguttulus* male with a standard female signal consisting of 12 subunits while increasing the noise level until the phonotactic response dropped to below 50% (Figure 4A, filled symbols). We then repeated this experiment using shorter signals and measured again the noise level at which the response dropped to 50%. With the three-subunit stimulus the animals ceased to respond at somewhat lower noise levels (double arrow in Figure 4A). The long 12-subunit signal was less susceptible to noise by ∼2 dB, indicating that the evaluation of a longer segment of the signal yielded a—moderate—improvement in signal detectability. This improvement, however, dropped to almost zero when a 5-subunit stimulus was compared to the long standard stimulus (Figure 4B). The regression line intersects with zero at 441 ms. In other words, an extension of the signal beyond this "critical duration" of ∼450 ms does not yield any further improvement in the detection of a masked signal. Thus, temporal integration, as a means for improving signal recognition, seems to be restricted to a time window of maximally 450 ms (Ronacher et al., 2000). In summary, these experiments revealed that *C. biguttulus* males can recognize an undisturbed signal as short as 2 to 3 song subunits, but do not exploit more than five subunits for signal recognition—even in the presence of noise.

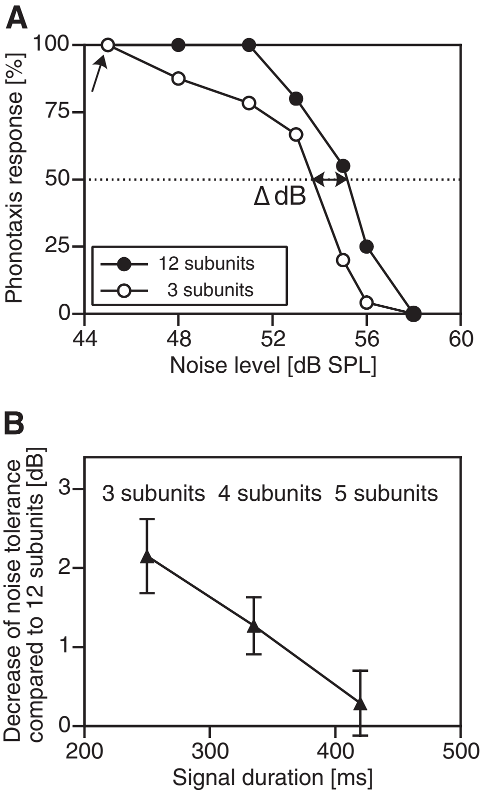

Figure 4. (A) Behavioral responses of a *C. biguttulus* male to female signals of different durations. Filled symbols: phonotactic response to a long stimulus (1015 ms; 12 subunits), open symbols: response to a short 3-subunit stimulus (250 ms). Abscissa: noise level, ordinate: phonotactic turning responses as percentage of stimulus presentations. The open symbol at 100% at low noise levels (arrowhead) indicates that even with the shortened signal this male responded to every stimulus presentation. If males do not recognize a signal as a female song the turning response does not occur, yielding 0%. Double pointed arrow indicates the shift of the 3-subunit curve to lower noise levels and the determination of the ΔdB value. Each percentage value in Figure 4A is based on between 15 and 30 stimulus presentations. (B) ΔdB values determined for different signal durations of the short signal (3 subunits, 4 subunits, and 5 subunits; always in comparison to the 12-subunit stimulus). $N = 11$, 13, and 10 males tested, for the 3-, 4-, and 5-subunit stimuli, respectively; bars indicate 95% confidence intervals. Modified from Ronacher et al., 2000.

Layout and Temporal Resolution Properties of the Auditory Pathway

As emphasized above, the ability to resolve rapid amplitude changes is crucial for signal recognition in grasshoppers. Therefore, we aimed at measuring the upper limits of temporal resolution at various stages of the auditory pathway. In brief, the layout of the auditory pathway is as follows (see Figure 2): The ears of these grasshoppers are located in the first abdominal segment, directly beneath the wing base. The axons of auditory receptor neurons (approximately 50 to 80 per ear) enter the metathoracic ganglion complex, which houses a first important stage of processing auditory information (Römer & Marquart, 1984; Stumpner & Ronacher, 1991; Stumpner & von Helversen, 2001). Because receptors exhibit rather broad tuning in only two frequency ranges

(Jacobs, Otte, & Lakes-Harlan, 1999), there is only little capacity for a fine analysis of carrier frequencies; grasshoppers probably can discriminate only between a low frequency and a high frequency range (Stumpner & von Helversen, 2001; von Helversen & von Helversen, 1997). Receptors connect to local neurons, mainly in the third thoracic ganglion (see Figure 2). A set of ca. ten to 15 local neurons receives input from the receptor neurons und transmits information to ascending neurons (ca. 15–20), which in turn project to the brain (Boyan, 1992; Marquart, 1985; Stumpner & Ronacher, 1991). The final decision whether a song signal is acceptable or not takes place within the brain circuits (Bauer & von Helversen, 1987; Ronacher, von Helversen, & von Helversen, 1986). Obviously, the set of ascending neurons represents a bottleneck in forwarding the information to those circuits.

Measuring the Temporal Resolution Capacities of Auditory Neurons by Modulation Transfer Functions (MTF)

A standard paradigm to investigate the temporal resolution of sensory systems, or their parts, is the use of modulation transfer functions (MTF: Krishna & Semple, 2000; Joris et al., 2004; Rhode & Greenberg, 1994; Viemeister & Plack, 1993). The stimuli used consist of a carrier (in our case broadband noise) that is sinusoidally amplitude modulated at different modulation frequencies (Figure 5A). Applying this MTF paradigm to neurons, there are two ways to evaluate the neuronal responses: (1) to count the spikes in a larger time window, or (2) to quantify the locking of spikes to the stimulus envelope. In the first case, this results in rate-MTF (*rMTF*), in the second in temporal-MTF (*tMTF*; see Figure 5. Obviously, these two evaluation procedures relate to the above mentioned resolution, integration paradox. On the other hand, from a different point of view, to the question of whether a nervous system encodes information by a *rate code* or a *temporal code* (e.g., Borst & Theunissen, 1999; Rieke et al., 1996). The upper limit of temporal resolution is characterized by the corner frequency, that is, the modulation frequency at which the response measure drops by a certain amount (Krishna & Semple, 2000; Rhode & Greenberg, 1994). In psychophysical experiments, the corner frequency characterizes the upper limit of AM frequencies that the system can still discriminate from an unmodulated stimulus.

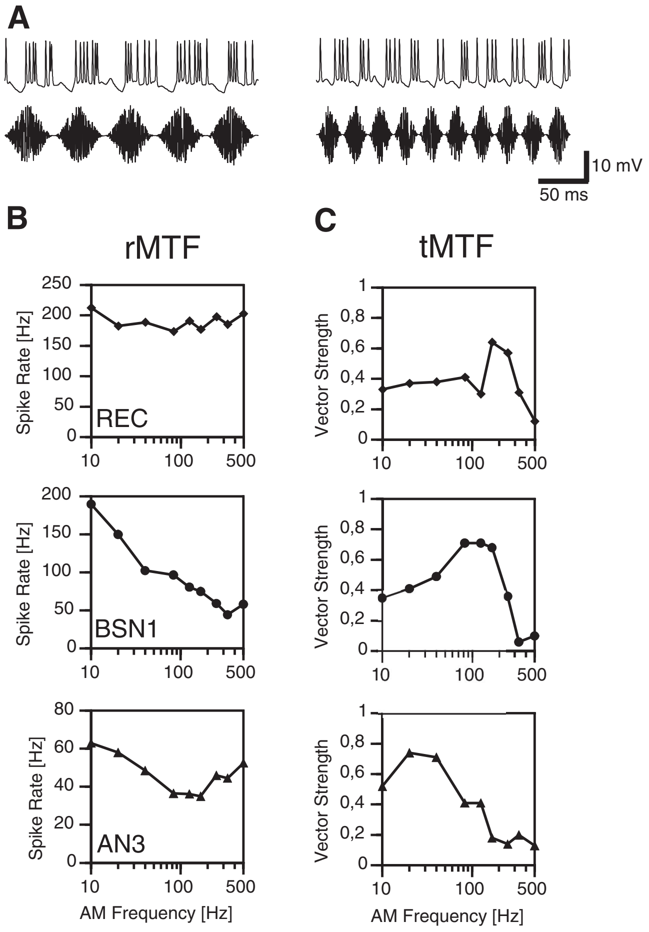

Figure 5. (A) Response of a local neuron (BSN1) to broadband noise that was sinusoidally amplitude modulated at 20 and 40 Hz (100% modulation depth); scale bars: 10 mV, 50 ms. (B, C) Modulation transfer functions (MTFs) for a receptor neuron, a local and an ascending neuron (from top to bottom). (B) rate-MTFs in which spike rates are plotted against modulation frequency; (C) temporal MTFs (based on an evaluation of vector strength; for details of the evaluation procedure see Franz, 2004, and Wohlgemuth & Ronacher, 2007).

Method

Animals and electrophysiology. To determine modulation transfer functions for auditory neurons we used adult locusts (*Locusta migratoria*), which were obtained from a commercial supplier. The larger locust is often used as a model system for the smaller *C. biguttulus*, by virtue of the very similar properties of the auditory pathway in both species (Stumpner, 1988; Stumpner & Ronacher, 1991; Wohlgemuth, Neuhofer, & Ronacher, 2007). The electrophysiological methods were conventional and are described in detail in Vogel et al. (2005). During the experiments the preparation was kept at a constant temperature of 30 ± 2 °C. Auditory neurons were recorded intracellularly and identified by means of dye injection (Lucifer Yellow) after completion of the physiological experiments (see Figure 2A as an example). After amplification, neural responses were digitized at a sampling rate of 20 kHz. The experimental protocols complied with the German laws governing animal care.

Acoustic stimulation. The stimuli for the modulation transfer functions consisted of a broad-band noise carrier (0.5–30 kHz) that was sinusoidally amplitude modulated at nine frequencies (10 Hz, 20 Hz, 40 Hz, 83 Hz, 125 Hz, 167 Hz, 250 Hz, 333 Hz, and 500 Hz; modulation depth 100%). To ensure that each neuron was in a well-defined adaptation state, each stimulus was preceded by a 200-ms segment of unmodulated noise, followed by a 1-s segment of constant modulation frequency and depth, and another segment of 200 ms of unmodulated noise. The stimuli were repeated four times with stimulus intervals of 300 ms. Acoustic stimuli were broadcast via one of two speakers (D-28/2, Dynaudio, Skanderborg, Denmark), situated laterally at a distance of 30 cm from the preparation. All acoustic stimuli were stored digitally and delivered by custom-made software (Labview, National Instruments), via a 100-kHz D/A-conversion (PCI-MIO-16E-1, National Instruments, München, Germany), an amplifier (Mercury, 2000, Jensen, Pulheim, Germany), and an attenuator (PA5, Tucker-Davis Technologies, Gainesville, FL). Sound intensities were calibrated with a Brüel and Kjær (*N*ærum, Denmark) 1/2 in. microphone, positioned at the site of the preparation, a Brüel & Kjær (*N*ærum, Denmark) measuring amplifier (Type 2209), and are given in dB re $2*10^{-5}$ Nm^{-2} (dB SPL). The sound intensity of test stimuli was adjusted to ~20 dB above a neuron's threshold.

Data analysis. From the digitized recordings, the spike times were extracted by means of a voltage threshold criterion. Rate modulation transfer functions, (*rMTF*) and temporal modulation transfer functions (*tMTF*) based on vector strength were determined according to Krishna and Semple (2000). In short, for the *rMTF* (compare Figure 5B) mean spike rates were computed for the modulated part of the stimuli. For the computation of the t*MTF,* period histograms (divided into 18 bins, i.e., 20°) were obtained. From the period histograms the vector strengths (VS) were calculated according to:

$$VS = \sqrt{\left(\frac{1}{n}\sum_{i-1}^{n}\cos\alpha_{i}\right)^{2} + \left(\frac{1}{n}\sum_{i-1}^{n}\sin\alpha_{i}\right)^{2}} \tag{1}$$

where α_i is the timing of spike *i* defined as the phase of the modulation waveform and *n* is the number of spikes. Vector strength can vary from a minimum of zero to a maximum of 1, the latter indicating a "perfect" phase locking to the stimulus envelope, that is, all spikes falling in the same bin. From the resulting *tMTF* curves, *corner frequencies* were determined following Krishna and Semple (2000) as the frequency where the curve dropped to the 90% point from the maximal vector strength value (Figure 5C).

Results and Discussion

Figure 5 shows *rMTF* curves (left) and *tMTF* curves (right) for typical neurons of the three processing levels mentioned above. Receptor neurons generally show all-pass *rMTF* curves, that is, their spike rates do not change markedly with AM frequency (Figure 5B, top). Note that it is not the carrier frequency tuning, but the frequency of the amplitude modulations, the signal's envelope, that matters in this context. A quite similar behavior is found also in some local interneurons with primary-like responses (not shown). *rMTF*s of other local neurons, however, tend to show low pass behavior, see BSN1 as an example (Figure 5B). This selectivity is increased at the next processing level, where we find low-pass, high-pass and band-stop filters (see AN3 in Figure 5B for an example of a weak band-stop filter). In their *temporal MTF*s (Figure 5C) receptor neurons usually exhibit a band pass (Figure 5C top, and Prinz & Ronacher, 2002), while among local and ascending interneurons often low-pass or bandpass filters are found. The corner frequencies, however, differ conspicuously between processing levels. Among the receptors and primary-like local neurons we found the highest corner frequencies, up to >150 Hz, indicating a precise locking of spikes to the stimulus envelope up to relatively high AM frequencies. This high temporal resolution is markedly reduced to corner frequencies mostly below 80 Hz at the next processing level, in ascending neurons (Figure 5C, see also Franz, 2004; Wohlgemuth & Ronacher, 2007). Because large differences in spike rate can influence vector strength values we checked whether the observed differences in *tMTF* corner frequencies could have been because of spike rate differences. However, the corner frequencies did not depend significantly on mean spike rate for receptor neurons (Prinz & Ronacher, 2002), nor for auditory interneurons (Weschke & Ronacher, in press). Rather, the differences between the *tMTF* curves in Figure 5C reflect differences in the precise locking of spikes to the stimulus envelope.

Rate and temporal modulation transfer functions can provide information about the temporal limits of a sensory system. However, they do not necessarily yield an answer to the more specific question of how well different stimuli can be *discriminated.* We will pursue this question in the next section.

Discrimination of Similar Patterns in the Context of Sexual Selection

Why should grasshopper females discriminate between different conspecific songs? Apart from their basic function in assisting to avoid heterospecific pairings, grasshopper calling songs probably serve an additional function: they may be used as indicators of a potential mate's quality (Kriegbaum, 1989). Because grasshopper females invest heavily into large eggs while the males provide only "cheap" sperm, we find these animals in a situation that is typical for sexual selection (e.g., Andersson, 1994; Andersson & Simmons, 2006). Females in particular should be choosy and try to find a high quality male, or at least avoid mating with a low quality male, to optimize their investments. Exactly this has been shown for the grasshopper *C. biguttulus*: females consistently prefer the songs of certain males, indicating that the song signals yield important clues for discriminating among different conspecific males (Kriegbaum, 1989; see also Balakrishnan et al., 2001).

The central nervous system of a grasshopper must extract information about differences between song signals from the spike trains provided by auditory neurons. We tried to solve the same problem and asked how well the acoustic signals produced by different individuals of one and the same species can be *discriminated* based on the spike trains produced by single grasshopper neurons. Evidently, the repeatability of neuronal messages, which is limited by the intrinsic noisiness of spike trains, will impose constraints on the discrimination of similar signals. A prerequisite for the correct classification of two sensory stimuli is that the spike trains elicited in repeated presentations of stimulus X are sufficiently different from those caused by stimulus Y– or, in other words, spike trains of Type X exhibit higher similarity among each other than compared with spike trains of Type Y. To quantify similarities between pairs of spike trains we used a spike train metric introduced by van Rossum (2001).

Metric Similarities of Spike Trains as a Basis for Stimulus Discrimination

Method

The basic idea of van Rossum's method is to replace each spike of a pair of spike trains by a filter function (Equation 2) and then to subtract the two resulting waveforms (Figure 6A). The difference is then squared and the integral calculated, yielding a measure for the (dis)similarity of the two examined spike trains, f and g. The basic rationale is as follows: if two spike trains share many coincident

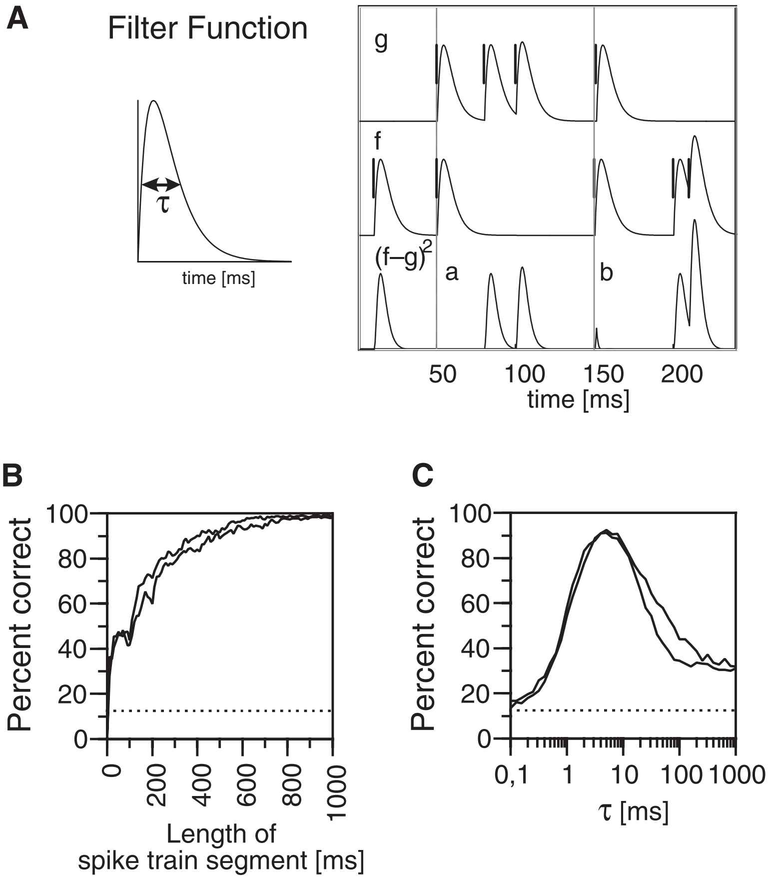

Figure 6. Classification of natural stimuli based on metric distances between spike trains. (A) Procedure to determine spike train distances (after van Rossum, 2001). To compare two spike trains, f and g, each spike (vertical line) is first replaced by a filter function. Then the two resulting functions are subtracted, and the difference is squared, to remove negative values (bottom). The integral over the resulting function is a measure for the distance between the two spike trains. The contribution of spikes that occur at the same or nearly the same time in f, g (coincident spikes) cancels out (see a, b). Two spike trains that contain many coincident spikes therefore yield a low distance value, which corresponds exactly to their common sense similarity. By varying the width of the filter function, different emphasis can be laid upon how much the timing of spikes influences the metric distances. See text for further information. (B) Classification success depends on the length of the spike train segments that are evaluated. As described in Machens et al., 2003, songs of eight individual males of *C. biguttulus* were presented and their discriminability based on spike train distances was evaluated. Data of two specimens of the local neuron TN1 of *C. biguttulus*. Note the high classification success that is possible with longer signal durations. Dotted line indicates chance level (12.5%); τ was set at 5 ms. (C) Classification success as a function of the temporal resolution parameter τ (same data as in B). (A: modified after van Rossum, 2001, and Machens et al., 2003; B,C: Wohlgemuth, unpublished results).

spikes, and are therefore similar, their contribution to the integral is cancelled out by the subtraction process (see a, b in lowest trace of Figure 6A). This results in a small value of the integral, which is indicative of a high similarity or a small distance (see van Rossum, 2001; Machens et al., 2003, for details of the procedure).

$$f(t) = t{*}\exp(-\alpha\, t) \text{ for } t > 0 \qquad (2)$$

$$f(t) = 0 \text{ for } t <= 0$$

The time course of the filter function can be considered as mimicking an excitatory postsynaptic potential (EPSP) in a hypothetical downstream neuron. The width of the filter function is defined by the temporal resolution parameter τ ($\tau \approx 2.45/\alpha$). This free parameter τ can be varied to study the influence of the temporal resolution with which spike trains are evaluated: if τ is large (>200 ms), it is mainly the difference in average firing rates that contributes to the distance, whereas for small τ values the metric distance depends on differences in spike timing. Once the distances between all spike trains of a certain neuron were computed, a cluster algorithm was applied to quantify the discrimination performance. Clustering was examined by randomly picking a spike train as a "template" for each stimulus. The remaining spike trains were classified by assigning them to that template to which they exhibited the smallest distance. This procedure was repeated

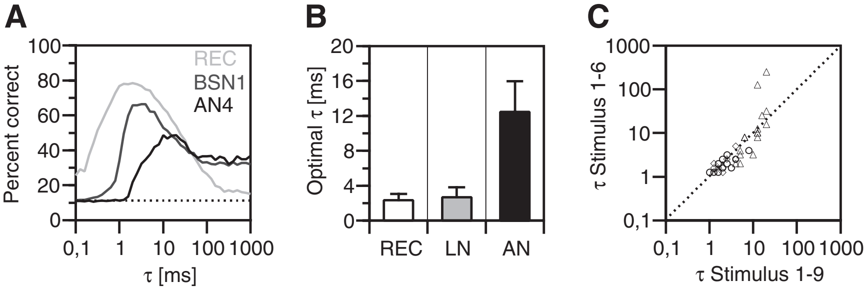

Figure 7. Discrimination of sinusoidally amplitude modulated stimuli (compare Figure 5A), based on spike train distances of neurons at different processing levels. Nine modulation frequencies between 10 Hz to 500 Hz served as stimuli (see below). (A) Percentage of correct classification as a function of temporal resolution τ for a receptor neuron (REC), a local neuron (BSN1), and an ascending neuron (AN4) (length of spike train segments evaluated: 500 ms; chance level is 11.1%). (B) Optimal temporal resolution τ (derived from the peak values of curves as in A) for neurons of the three processing levels ($N = 8, 11, 17$). Values of receptors and local neurons are not significantly different, while both are significantly different from the data of ascending neurons ($p < .001$); bars indicate 95% confidence intervals. (C) Correlation between optimal τ values measured for different stimulus sets. Stimulus set 1–9: 10, 20, 40, 83, 125, 167, 250, 333, and 500 Hz modulation frequency; Stimulus set 1–6: 10–167 Hz modulation frequency. Modified after Wohlgemuth & Ronacher, 2007.

for all possible template permutations, resulting in an average probability of correct classification (for details of the procedure see Wohlgemuth & Ronacher, 2007). Because the classification success depends on the resolution parameter τ (see Figure 6C), for each cell its optimal τ value was determined. These values ranged between 1 and 30 ms.

Results

We investigated auditory neurons of the grasshopper *C. biguttulus* and determined, with this metric, how well a set of acoustic stimuli can be classified and discriminated on the basis of the similarities of spike trains these neurons produce in response to different stimuli. Focusing on the responses of auditory receptor neurons and using a set of natural songs of eight different *C. biguttulus* males as stimuli, a surprisingly high discrimination success was found by Machens et al. (2003): ~90% of these songs were classified correctly on the basis of single spike trains from a single receptor neuron!

Using the same stimuli as Machens et al. (2003), we here extended this kind of analysis to the next stages of the auditory pathway. A similarly high classification success, as with receptor neurons, was found for a local neuron with primary-like response type (Figure 6B). Shown in Figure 6B is how the classification success develops with the length of the spike train segment evaluated. The curves exhibit a steep rise within the first 200 ms and then level off. At a spike train length of 250 ms (i.e., that is the signal duration that allows for reliable signal recognition in males, see above, Figure 4A) a discrimination level of ~80% is reached. In view of the difficult discrimination task, this high classification success based on single spike trains of a single neuron is quite remarkable.

What Determines the Optimal Time Constant for Temporal resolution?

The classification shown in Figure 6B was performed with the temporal resolution parameter τ set to 5 ms, which yielded the optimal results for these two neurons. Figure 6C shows how the classification success depended on the resolution parameter τ. The curves are based on a spike train length of 500 ms (which corresponds to the upper limits of temporal integration derived from Figure 4B). Performance was maximal with τ between 3 and 10 ms; with larger τ, that is, reduced temporal resolution, the curves fell quickly until they reached a constant level of ~30% at τ >200 ms. We can conclude that at the input level of the auditory pathway the necessary information to reliably discriminate between the signals of different conspecifics is present, provided that the spike trains are evaluated with a high temporal resolution of τ ≈5 ms (see also Machens et al., 2003).

In this context immediately the question arises of whether the optimal time constant of approximately 5 ms reflects properties of the auditory neurons under study or whether it was imposed by the specific temporal structure of the particular set of grasshopper songs used? To answer this question we used a set of stimuli with reduced complexity, that is, the same nine stimuli with sinusoidal AM as used for the modulation transfer functions (see above and Figure 5A). We calculated spike train distances and determined the percentages of correct discrimination based on the distances between the respective spike trains. This was done for neurons of the first three levels of processing, that is, receptor neurons, local interneurons and ascending interneurons in the migratory locust (Wohlgemuth & Ronacher, 2007).

Figure 7A shows the percentages of correct classifications as a function of the temporal resolution parameter τ of the spike metric (compare Figure 6C) for representatives of the three processing levels. For this simplified stimulus ensemble, the maximal classification success of the auditory receptor neuron was around 80% (which is still high, taking into account that the stimulus set encompassed a large proportion of high modulation frequencies, see figure legend). For the auditory receptor neuron the optimal classification was achieved with a temporal resolution of 2 to 3 ms. For the local neuron the optimal τ was in the same range, but the classification success was somewhat reduced (compare Figure 7B). A marked decline in maximal classification success was found for ascending neurons in combination with a shift of the optimal τ to values mostly above 10 ms (Figure 7A, B).

To further test for possible influences of the stimulus ensemble on τ, we compared the optimal temporal resolution for different subsets of this stimulus ensemble. A likely hypothesis was that the very small optimal τ values ($<$5 ms) found for auditory receptors and local neurons were influenced by the prevalence of high modulation frequencies in our stimulus set (4 stimuli above 150 Hz). We controlled for this by restricting the data evaluation to a reduced stimulus set (6 stimuli between 10 Hz and 167 Hz). The exclusion of the three highest modulation frequencies, however, had virtually no effect on the optimal τ values (Figure 7C). Thus, the small optimal τ values found for the receptor and local neurons were obviously not enforced by the large proportion of stimuli with high modulation frequencies but appear to reflect a time constant characteristic for a given neuron or processing level.

Discussion

An important property of the spike-train metric method is its focus on single spike trains. Thus, the trial-to-trial variability of neuronal responses is taken into account, which is highly relevant for the operation of nervous systems—but is ignored in many experimental paradigms, for example, by the MTF procedures introduced in Figure 5. Another advantageous feature of the spike train metric is the possibility to change the temporal resolution with which spike trains are compared, by varying the free parameter τ of the filter function used (inset in Figure 6A). Choosing a very large τ ($>$200 ms) implies that the timing of spikes is virtually neglected and only *spike count differences* contribute to the discrimination, whereas with a smaller τ more and more emphasis is laid on the exact *timing of spikes*. Hence, this method allows to explore the temporal resolution with which a spike train should be evaluated to obtain an optimal classification and discrimination of external stimuli, and may give clues to the encoding principles used at different stages of neuronal processing.

The data presented in Figure 7 suggest that the optimal τ of the spike train metric does reflect the properties of the investigated neurons. This leads to questions about the encoding of information within the auditory pathway. Traditionally, two coding principles are contrasted, a rate code, where the information is encoded in the average number of spikes measured over a longer time segment, and a temporal code, for which the timing of spikes contributes to the information content.

How Is Temporal Information Encoded at Different Processing Levels?

The sample curves in Figure 7A reveal three messages: first, they indicate a substantial reduction in the overall classification success at the level of ascending neurons. This impression is supported by a quantitative analysis: receptor neurons (N = 8) allowed for an average 78.8 ± 4.5% classification success (mean and 95% confidence intervals), whereas the mean of N = 17 ascending neurons was 48.9 ± 5.2% (all values for a spike train length of 500 ms). Local neurons with primary-like responses achieved similar values as receptor neurons (81.6 ± 3.4%; N = 5), while another local neuron, BSN1 was intermediate (60.9 ± 9.9%; N = 6). A similar trend to reduced classification success at higher processing levels was observed with natural songs as stimuli (not shown).

Second, there was a clear shift of the optimal τ between local and ascending neurons (Figure 7A,B). While the optimal τ values for receptors and local neurons were very small (and not significantly different, p = .866), the values for ascending neurons were distinctly larger ($p < .001$; Kruskal Wallis test and Games Howell post hoc test). This indicates a reduction of temporal precision at higher levels of the auditory pathway, which is in accord with the observation of increased spike train variability in ascending neurons of the locust (Vogel et al., 2005).

A third message can be extracted from Figure 7A by comparing the classification success at the optimal τ and at τ = 1000 ms. Remember that for $\tau > 200$ ms the metric procedure virtually ignores the timing of spikes, and, hence, any classification success must be based solely on spike count differences. Seen that way, the curve for the receptor neuron perfectly reflects the fact that the rMTF-curves are flat (see Figure 5B): the curve approaches chance level for large τ values. To turn the argument around, this shows that the receptor's high classification success (Figure 7A) is based almost exclusively on the timing of spikes. With the ascending neuron the picture changes: at τ = 1000 ms we find a classification success around 30%, which must be attributed to spike count differences between stimuli. The best classification success obtained for this neuron, at $\tau \approx 20$ ms, was around 50%, a value to which both spike count differences and spike timing information contributed. However, the improved classification success at τ = 1, 000 ms for ascending neurons reveals that the relative contribution of spike timing is reduced, compared to the situation in the receptor and local neurons, and approximately equals that of spike count. Hence, this kind of data evaluation suggests a quantitative shift of coding strategy that takes place between the most peripheral and more central stages of processing, that is, a gradual change from a code based predominantly on exact spike timing toward a rate code. It also emphasizes that evaluation of spike count and spike timing information are not mutually exclusive coding principles, but might be combined with varying weights (cf. Borst & Theunissen, 1999).

General Discussion

This account focuses on the question of how auditory neurons of insects process signals whose information is contained in the temporal pattern of amplitude modulations. Signal recognition is impaired by two different types of noise, extrinsic noise that degrades the signals arriving at the receiver's ears, and intrinsic noise that introduces additional variability in the spike trains of receptor neurons as well as auditory neurons at higher processing stages. The problems caused by noisy spike trains could, in principle, be alleviated by averaging across a set of similarly reacting neurons or by temporal integration. In the auditory domain, however, temporal integration may interfere with the necessity to resolve fast amplitude modulations. The upper limits of temporal resolution (as measured by the corner frequencies of the *tMTF*s) were high for receptor neurons and a local neuron with receptor-like responses, whereas they decreased markedly in ascending neurons (Figure 5C, see also Franz, 2004; Wohlgemuth & Ronacher, 2007). Parallel to this decrease in corner frequencies, the *rMTF* curves changed from all-pass to (mostly) low-pass, band-pass or band-stop behavior between receptors and ascending neurons (Franz, 2004).

These limits of temporal resolution can be directly related to the behavioral performance of grasshopper females in experiments that used song models in which the frequency composition of the envelopes was systematically varied. Using this approach and starting from parameters of natural songs, von Helversen and von Helversen (1998) demonstrated that a periodic envelope composed of five frequencies (10, 20, 30, 40, and 50 Hz) was sufficient to elicit a strong response of *C. biguttulus* females. However, much higher frequencies, up to 150 Hz, can cause the rejection of song models by females indicating that these frequencies are perceived by the animals. *C. biguttulus* females respond to neither natural songs nor song models if these contain tiny gaps of only 2–3 ms duration (von Helversen & von Helversen, 1997). Such gaps are characteristic for the songs of males that have lost one hind leg, and in the field females exert a very strong sexual selection against these males, which have only a 10% mating success compared to intact males (Kriegbaum, 1989). To perceive these gaps, higher envelope frequencies, up to 150 Hz, are needed (von Helversen & von Helversen, 1998). At first glance this seems to be inconsistent with the finding of a reduced temporal resolution (Figure 5C), and larger τ values (Figure 7 B), at the level of ascending neurons. However, at least in some ascending neurons a shift of the encoding scheme seems to occur by which this discrepancy is resolved. A neuron (AN4) has been described that exhibits a low corner frequency in the *tMTF*, but does nevertheless respond highly selectively to stimuli containing gaps (Ronacher & Stumpner, 1988). This selective response, which results in a band-stop filter characteristic of the *rMTF*, is caused by a preceding inhibition that is triggered by each new stimulus onset after a small gap (Franz, 2004; Franz & Ronacher, 2002). The gap detection capacity of this neuron depends, however, on the high temporal resolution of receptor neurons. This example demonstrates that a high temporal resolution capacity of neurons, while obviously essential at the level of auditory receptor neurons must not necessarily persist up to higher processing levels, a feature of the auditory pathway of grasshoppers that is shared with vertebrates.

By applying a metric that assesses the similarities between spike trains, we were able to make predictions about the limits of discriminability of similar signals. The discrimination of similar signals is a prerequisite for sexual selection processes, where the task is to assess a signaler's condition or health from often-subtle deviations from a common species-specific pattern. We found that the spike trains of receptor neurons convey surprisingly much information allowing for an excellent discrimination success, provided that the spike trains are evaluated with high temporal resolution. In other words, the information basis for female choice is already present in the responses of single neurons, if the timing of spikes is taken into account at a 5-ms scale. At this point, however, a critical remark seems in order: the high classification success found with the spike train metric paradigm describes the situation of an *ideal observer*. Experiments are under way to test to what degree the central nervous system of grasshoppers is able to exploit the information content present in the spike trains of receptor neurons. The reduced classification success found for the ascending neurons (Figure 7A) may be taken as an indication that the ultimate goal of these animals probably is not a most sophisticated discrimination of individuals but rather to find high quality males or to reject males of inferior song quality. Interestingly, females seem to differ somewhat in their ideas of what is a good song (Balakrishnan et al., 2001).

The alpha function used in the metric paradigm can be interpreted as mimicking a postsynaptic potential in a down-stream neuron. Thus, the optimal τ values found in this kind of data evaluation would reflect the temporal resolution of the respective next processing stages.

The spike train metric thus offers the important advantage of providing information about the optimal temporal resolution with which spike trains should be evaluated and yielding clues to possible encoding principles. The shift found among ascending neurons to larger optimal τ values, that is, a reduced temporal resolution, complements the results of the MTF paradigm. It seems to be a general trend in sensory systems to shift from encoding of the stimulus pattern by the phase locking of spikes in the periphery, to a feature-extraction and feature-evaluation process at more central stages (e.g., Gabbiani et al., 1996). A loss of temporal resolution as observed here may be a necessary corollary of such a feature extraction process. Alternatively, an optimal temporal resolution of around 10 to 20 ms may reflect a compromise between the necessities to preserve information about the signal's temporal structure, and, on the other hand, to reduce noise introduced by neuronal jitter (Narayan et al., 2006; Wohlgemuth & Ronacher, 2007). This consideration fits the observation of increased spike train variability in ascending neurons of the locust (Vogel et al., 2005). The observation that at the level of ascending neurons the classification success of single neurons was clearly reduced as compared with more peripheral neurons, may be an additional indication that ascending neurons are specialized to extract different signal features, and that the information about these features is distributed among a set of neurons.

The steps of processing and extracting information performed by auditory neurons of grasshoppers appear to follow the same scheme as in vertebrates. Auditory nerve fibers of vertebrates generally exhibit all-pass rate-*MTF*s, and therefore the information about amplitude modulations must be present in the temporal placement of spikes (Alder & Rose, 2000; Joris et al., 2004; Rhode & Greenberg, 1994; Rose & Capranica, 1985). At higher stations of the auditory pathway, however, the exact phase locking of spikes tends to disappear, and a variety of rate filters, or even a filter bank for amplitude modulation frequencies is found (Joris et al., 2004; Langner, 1992).

Insects and higher vertebrates that rely on acoustic communication face similar problems of signal recognition in a noisy world. However, insects are much more limited in their neuronal hardware that is devoted to these tasks. The rather small number of auditory neurons implies that the intrinsic noise of spiking responses may become an ultimate hurdle for signal recognition and discrimination. The obvious solution for reducing the impact of intrinsic noise (to average over large numbers of similar elements) appears to be ruled out for most insects. Notwithstanding the intrinsic spike train variability, our spike metric analysis showed that a high classification performance is in principle possible even based on spike trains of single neurons (see Figure 6). This could be an indication that the communication signals of this species have been shaped by sexual selection in a way to elicit highly reliable responses in the auditory system of the female receivers.

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