Neural Correlations Increase Between Consecutive Processing Levels in the Auditory System of Locusts

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Vogel A, Ronacher B. Neural correlations increase between consecutive processing levels in the auditory system of locusts. J Neurophysiol 97: 3376–3385, 2007. First published March 14, 2007; doi:10.1152/jn.00796.2006. Nervous systems may encode information about sensory stimuli using the temporal relations in spiking patterns between neurons. By conducting simultaneous intracellular recordings on pairs of auditory neurons we measured the strength of correlations between elements of the first three processing levels in the metathoracic auditory network of the locust. We quantified the degree of spike synchronization and rate covariations that occur among auditory neurons during acoustic stimulation. In addition to the acoustic stimulation, current pulses were injected into both neurons to study the connectivity within this network. Our findings support the view that the metathoracic auditory system is a hierarchically organized feedforward network. Strong synaptic connections were observed only between consecutive processing levels, whereas there was no indication for strong connections between elements of the same processing level. Both spike synchronization and rate covariations were increased among neurons on higher processing levels. We further investigated the consequences that correlations may have on the common estimates of neuronal variability. For example, rate covariations caused by strong synaptic coupling between two neurons may lead to an overestimation if the variability is measured trial by trial with respect to only single neurons. For the vast majority of cell pairs tested, however, no strong synaptic coupling could be demonstrated. Thus we could show that in most cases no serious errors are made if one determines variability by following the usual procedure on the basis of single-cell recordings.

INTRODUCTION

The task of sensory systems is to extract relevant information from a plethora of input signals, represented by spike trains in the nervous system. A meaningful interpretation of the environment usually requires the comparison of spiking responses from different neurons (e.g., Georgopoulos et al. 1986). The population’s response considered as a whole may represent sensory stimuli better than the sum of individual neuron response contributions (Oram et al. 1998; Reich et al. 2001; Warland et al. 1997). For a population code the temporal relations in the spiking patterns among elements are crucial (Krahe et al. 2002a). The investigation of such a coding scheme, therefore requires simultaneous recordings of at least two elements. Temporal interactions among neurons are commonly measured as spike synchronization or rate covariations between pairs of neurons (Averbeck and Lee 2004). It is mainly the timescale of evaluating the interactions that represents the difference between both measures. Rate covariances are derived from trial-to-trial variability in spike counts, thus representing interactions on long timescales. In contrast, spike synchronizations indicate the temporal relation between the spike times of two neurons on short timescales. They are often estimated on basis of the cross-correlation function (Aertsen and Gerstein 1985).

The consequences that correlations may have on neural coding constituted the subject of many studies (Abbott and Dayan 1999; König et al. 1996; Nirenberg et al. 2001; Shadlen and Newsome 1994, 1998; for review see Averbeck and Lee 2004). An important issue concerns the relationship between strength of correlations and neural variability (Mazurek and Shadlen 2002; Zohary et al. 1994). In general, variability of spiking responses is measured trial by trial for single neurons (Vogel et al. 2005). However, as a result of possible rate covariations between parts of a neuronal network this method may overestimate the variability that a nervous system faces at a given time (Ronacher et al. 2004). Thus the quantification of correlations across neurons is a way to obtain a more realistic estimate of relevant spike train variability.

To assess the magnitude and possible impact of intrinsic spike train variability in a model sensory pathway, we focused on the metathoracic auditory system of acridid grasshoppers—an intensively studied model system for investigating the processing of acoustic stimuli (e.g., Gollich and Herz 2005; Machens et al. 2001; Rokem et al. 2006; Schaette et al. 2005; Stumpner et al. 1991; Vogel et al. 2005). The metathoracic auditory network is characterized by a separation into two hemispheres and a hierarchical organization consisting of receptor neurons (RE) and both segmental interneurons (SN) and ascending interneurons (AN), respectively. Auditory neurons can be identified as individuals on the basis of their characteristic morphology and physiology. Figure 1 shows basic principles of information flow between different processing levels. More than 50 receptor neurons per ear converge onto 10–15 segmental interneurons that are connected to another 15–20 ascending interneurons (Jacobs et al. 1999; Römer and Marquart 1984; Stumpner and Ronacher 1991; Stumpner and von Helversen 2001). The latter transmit information to the brain where the final evaluation of acoustic information takes place (Bauer and von Helversen 1987; Ronacher et al. 1986). Thus all relevant information about the acoustic environment available to the brain must be encoded by the set of ascending
The preparation was placed in a Faraday cage lined with reflection-attenuating pyramidal foam and was acoustically stimulated by two loudspeakers (D2905/9700a, Scanspeak), situated laterally at a distance of 30 cm from the preparation. Sound intensities were calibrated with a Bruel & Kjær microphone (1/2 in.), positioned at the place of the animal, and a Bruel & Kjær measuring amplifier (type 2209). Intensities are given in decibels re 2 × 10⁻⁵ N/m² (dB SPL). All stimuli were stored digitally and delivered by custom software (LabVIEW, National Instruments) using a 100-kHz D/A-converter (PCI-MIO-16E-4, National Instruments).

Neurons were considered as auditory if their spike rate depended on acoustic search pulses. To obtain spike rate—versus—intensity curves, we used broadband noise stimuli (bandwidth: 0.5–30 kHz) of 100-ms duration including 2-ms ramps at intensities ranging from 30 to 70 dB increasing in 10-dB steps. The stimuli were separated by 300-ms interstimulus intervals and repeated 10 or 15 times at each intensity.

**Electrical stimulation**

After completion of the acoustical stimulation protocol, depolarizing current pulses were injected in either of both neurons to test for synaptic interactions. The rectangular current pulses increased from 1 to 4 nA in 1-nA steps. Just before each depolarizing pulse, which lasted 90 ms, the neuron was hyperpolarized for 10 ms at −0.5 nA. Depolarization did not elicit spikes in all neurons. However, as a consequence of the limited recording time it was not possible to apply extensive stimulation programs to increase the success rate. Analysis of the synaptic connectivity was performed off-line on the basis of spike-triggered superpositions of the voltage traces of both neurons (see Fig. 2). In general, only the first spike elicited by a current pulse was considered in the superposition because no previous spikes should have affected the postsynaptic potential. If this was not possible only spikes with a minimal interspike interval of 15 ms were accepted. In a few cases, in which the current injection failed to elicit spikes, we triggered on spontaneous spikes.

**Data analysis**

Spiking responses were digitized on-line with 0.05-ms precision (A/D-converter, PCI-MIO-16E-4, National Instruments). From the digitized recordings the spike times were determined off-line by means of a voltage-threshold criterion.

**Spike synchronization**

For the cross-correlation procedure spikes were binned in 1-ms classes such that in each trial we collected at most one spike per bin. Empty bins got a value of zero. To quantify the temporal correlation between two simultaneously recorded auditory neurons, we calculated the coherence function, based on the cross-correlation (see Kimpo et al. 2003; Rosenberg et al. 1989). The coherence of both cells during acoustic stimulation was calculated in a time window of 130 ms after stimulus onset. As an additional control, the coherence was assessed in the stationary part of the spiking response. For that purpose the first parts of the spike trains of both neurons were removed and a time window from 65 to 130 ms was analyzed.

In a first step we determined the cross-correlation function, which represents a basis to assess the presence of interactions between spike trains. The cross-correlation C(τ) of a spike train R_{i}(t) relative to a

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**Methods**

**Animals and electrophysiology**

Animals were adult female and male locusts (*Locusta migratoria*), obtained from a commercial supplier, held at room temperature (22–25°C). After removal of head, legs, and wings the animals were fixed with their dorsal side up onto a holder. The thorax was opened dorsally and the metathoracic ganglion was exposed and stabilized by a small NiCr platform. The whole torso was filled with locust Ringer solution (Pearson and Robertson 1981). The temperature of the preparation was adjusted by means of a Peltier element at 30 ± 2°C.

Intracellular recordings from auditory receptors and interneurons were obtained in the auditory nerve and the frontal auditory neuropil of the metathoracic ganglion, respectively. Because for some cells the recording site was far from the spike-initiation zone the spike amplitudes were rather small in those cases (see Figs. 2 and 3). For simultaneous recordings of two neurons we used standard electrophysiological equipment (Krahe and Ronacher 1993). After amplifying the intracellular voltage signal (Bramp-01, NPD) it was filtered through a 10-kHz low-pass filter. The tips of the glass microelectrodes (Clark Electromedical Instruments) were filled with a 3–5% solution of Lucifer yellow (Aldrich) in 0.5 M LiCl. This dye was injected after completion of the physiological recordings by applying hyperpolarizing current. After an experiment the thoracic ganglia were fixed in 4% paraformaldehyde, dehydrated, and cleared in methylsalicylate. Stained cells were identified under a fluorescence microscope based on their characteristic morphology (terminology after Römer and Marquart 1984). Although both neurons were filled with the same dye, an unambiguous identification was possible by combining the knowledge about response characteristics and recording sites.

**Acoustic stimulation**

The aim of our study was to investigate the strength of correlations across neurons and its relationship to neuronal variability in the metathoracic auditory network of *Locusta migratoria*, an acridid grasshopper. For that purpose we quantified the degree of spike synchronization and rate covariations among auditory neurons. Simultaneous intracellular recordings were performed within the first three processing levels (RE, SN, and AN) as well as between neurons of different levels to investigate whether correlations persist or develop across processing levels. The strength of correlations across neurons was evaluated during acoustic stimulation. To exclude—as far as possible—correlations that are induced by the stimulus we used simple rectangular modulated sound pulses. Those acoustic stimuli effectively activated the auditory neurons without introducing additional stimulus-related temporal information to the spiking responses; therefore any correlations across neurons should depend primarily on the properties of the network. In addition to the acoustic stimulation we injected current pulses in either of both neurons to study the connectivity within the auditory network.

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**FIG. 1.** Schematic diagram of the information flow in the auditory pathway of *Locusta migratoria*. Only one of the 2 mirror-image hemispheres is shown. More than 60 receptor neurons (RE) converge onto a much lower number of segmental (SN) or bisegmental interneurons (BSN, in the following also included with the SN), which then serve as presynaptic elements for ascending interneurons (AN). Only the ascending interneurons project to the brain; thus they represent a bottleneck in information transfer.
second spike train $R_i(t)$ as a function of $\tau$ (a time shift between the two spike trains) is given by the following expression

$$C(\tau) = \frac{1}{T} \sum_{t=0}^{T} R_i(t)R_i(t+\tau)$$  (1)

where $[T]$ is the duration of the signal being analyzed and $\langle \rangle$ indicates averaging over all trials.

Before considering a peak in this so-called raw cross-covrelogram to be relevant the covariations in firing rate of the two stimulated cells must be removed. The easiest way to “correct” for these stimulus-induced effects is the shuffle corrector (Brody 1999). The shuffle corrector (SC) results from a cross-correlation of the postsynaptic time histograms (PSTHs) of both neurons, where the PSTHs $\{R_i(t)\}$ are simply the spike trains averaged over trials

$$SC(\tau) = \frac{1}{T} \sum_{t=0}^{T} (R_i(t))(R_i(t+\tau))$$  (2)

The shuffle corrector eliminates coincidences that occurred by chance as the result of high spike rates. The shuffle corrector corresponds to a simplified version of the shift predictor, which results from the cross-correlation between all nonsimultaneously recorded trials (Aertsen et al. 1989).

After subtracting the shuffle corrector from the raw cross-covrelogram the resulting equation is called cross-covariance. The cross-covariance (CC) of neurons [i] and [j] is then defined by

$$CC(\tau) = \frac{1}{T} \sum_{t=0}^{T} (R_i(t))(R_j(t+\tau))$$  (3)

The temporal structure of firing may pose another possible source of cross-covariance between two neurons that does not reflect true neuronal interactions between these cells. Assuming that a spike in neuron i triggers a spike in neuron j and neuron [i] is a bursting neuron—which has a high probability of firing again after it has fired once—the second spike in the burst of neuron [j] will thus also be correlated to the spike in neuron [i], although the spike was actually triggered by the first spike in [i]. To correct for this type of correlation, we calculated the coherence function (\gamma) (Rosenberg et al. 1989). The coherence function is normalized by the autocovariance function of each of the two spike trains. This normalization takes into account bursting or other temporally structured behavior in either neuron [i] or neuron [j] (or both) that would result in additional or artificially large and wide peaks in the cross-covariance functions. In practice, the coherence is calculated in the frequency domain. The coherence is given by

$$\gamma(\omega) = \frac{CC(\omega)}{\sqrt{AC_i(\omega)AC_j(\omega)}}$$  (4)

where $CC(\omega)$ is the Fourier transform of the cross-covariance between the responses from [i] and [j] and $AC_i(\omega)$ and $AC_j(\omega)$ are the Fourier transforms of the autocovariance (AC, see Eq. 3) of activity from neurons [i] and [j], respectively. For plotting purposes, the coherence in the time domain is then calculated by taking the inverse Fourier transform of Eq. 4.

**Rate covariations**

To quantify the rate covariations (RC) between two simultaneously recorded neurons, covariations of spike count were measured on a trial-by-trial basis. Spikes were counted within a time window of the stimulus duration, to which the response latency was added. Rate covariations are given by the correlation coefficient

$$RC_{ij} = \frac{\langle c_i c_j \rangle - m_i m_j}{\sqrt{(c_i - m_i)^2(m_i - m_j)^2}}$$  (5)

where $[c_i, c_j]$ represent the spike count of cells [i] and [j] per trial, $\langle \rangle$ indicates averaging over all trials, and $[m_i, m_j]$ represent the mean spike counts of both neurons.

**FIG. 3.** Response characteristics of 2 pairs of simultaneously recorded auditory interneurons: a segmental interneuron (SN) with an ascending interneuron (AN) (same as in Fig. 2A) and the 2 ascending interneurons from Fig. 2B. A and B: spike traces of both cell pairs. Neurons were stimulated with a 100-ms rectangularly modulated noise pulse at 50 dB SPL. C and D: spike raster plots for 10 of the 15 stimulus repetitions at 50 dB. Spike traces in A and B correspond to the 10th (from bottom) (A) and the 3rd (B) stimulus repetition, respectively. E and F: postsynaptic time histograms (PSTHs) for each neuron of a pair. PSTHs are normalized to one stimulus repetition (gamma-scale), bin width 1 ms, $n = 15$ (A) and $n = 10$ (B). G and H: intensity response functions that illustrate mean spike rates and SDs at each intensity ($n = 15$).
**Rate covariations and variability**

In most investigations the spike count variability is measured for a single neuron based on several stimulus repetitions. However, nervous systems consist of many elements, which may also exhibit temporal correlations such as covariations of spike rate. In our analysis we tried to quantify the effect of such covariations on the commonly used measure of variability, by comparing the responses of two simultaneously recorded neurons instead of a single cell. First, we calculated the expected standard deviation (SDexpected) of spike count under the assumption that both neurons would respond in a stochastically independent manner. In this case the expected SD is calculated according to the law of error propagation (Sachs 1999)

\[
SD_{\text{expected}} = \sqrt{SD_i^2 + SD_j^2}
\]  

(6)

where \([SD_i]\) and \([SD_j]\) are the SDs of spike count for cell \([i]\) and \([j]\), respectively, derived from a trial-to-trial protocol.

As a second measure we calculated the actual SD (SDmeasured) of the differences in spike count between the two simultaneously recorded neurons. For that purpose the difference of spike counts \([c_i - c_j]\) for both cells was determined for each trial, after which the SD of this distribution was calculated

\[
SD_{\text{measured}} = \pm \text{SD of } (c_i - c_j)
\]  

(7)

In the case of strong covariations this difference remains roughly constant over different trials, yielding a rather small SDmeasured (see Fig. 9). As an additional control the SDmeasured was calculated after shuffling the response trials of cell 1 (see Fig. 9D).

**RESULTS**

A total of 43 paired recordings of identified auditory neurons were analyzed. We obtained responses from 13 pairs of receptor neurons (RE); nine pairs of receptor–segmental neurons (SN); five pairs of segmental–segmental interneurons; eight pairs of segmental–ascending interneuron (AN) combinations; and eight pairs of ascending interneurons (compare Fig. 1). Before analyzing spike time and spike count correlations within and between processing levels of the auditory network we will focus on its connectivity to facilitate the interpretation of further results.

**Connectivity within the neural network tested with electric stimulation**

Depolarizing currents were injected to explore synaptic connections between pairs of neurons. According to present knowledge, no synapses exist between receptor neurons from different sides nor between those from the same side (Marquart 1985b; Rehbein 1976). Of the remaining 30 cell pairs evidence for a strong synaptic contact was found in only four cases. Consistent with the information flow proposed in Fig. 1, these strong synaptic connections were observed between successive elements in the hierarchy. One connection was observed between a receptor and a segmental neuron (RE–SN) and two connections between a segmental and an ascending interneuron (SN–AN; one connection was observed twice with the same combination of individual neurons). Altogether 17 of 30 cell pairs consisted of successive elements.

In Fig. 2A the spike-triggered superpositions are shown for a cell pair, where an ascending neuron showed a reliable response to the activity of a segmental neuron. The top traces indicate the current-elicited action potentials of the presynaptic neuron and the bottom traces show the corresponding postsynaptic response. Sometimes a postsynaptic potential did not occur until the second or third presynaptic spike. The response latency was about 2.5 ms. In most recorded cell pairs, however, the spikes of one cell had no detectable effect in the other neuron—neither excitatory nor inhibitory. An example is illustrated in Fig. 2B for a pair of two ascending interneurons.

Although strong synapses were observed only rarely within the metathoracic auditory network, the existence of weak synaptic connections cannot be ruled out (see DISCUSSION). Weak connections, however, may be detected by an analysis of temporal correlations (see following text).

**Correlations between neuronal responses elicited by acoustic stimulation**

In the following we will analyze the correlations between simultaneously recorded neurons that were investigated with auditory stimuli instead of current pulses. The response characteristics of two simultaneously recorded cell pairs are illustrated in Fig. 3. Figure 3, left represents the synthaptically connected cell pair shown in Fig. 2A. The response characteristics of two ascending interneurons, for which no indication of a strong synaptic connection could be found, are illustrated in Fig. 3, right (compare Fig. 2B). Individual spike trains recorded at a stimulus intensity of 50 dB are shown in Fig. 3, A and B. While the spike raster plots below indicate the spike times for several stimulus repetitions (Fig. 3, C and D), the PSTHs show the average response to the acoustic stimulus for both cell pairs (Fig. 3, E and F). The intensity response functions illustrate the mean spike rate as a function of the stimulus intensity (Fig. 3, G and H).

**Spike synchronization**

Temporal correlations are observed if a spike occurring in one neuron is associated with an increased probability of a spike occurring in the other neuron. To quantify the temporal relations between the spike times of two simultaneously recorded cells, we calculated the coherence function (see METHODS). In Fig. 4 the coherence functions are illustrated for the same cell pairs as in Fig. 3. The cell pair that exhibited a strong synaptic connection (compare Fig. 2A) showed a pronounced peak at a time shift of about 3 ms in the coherence function, indicating that the AN neuron often spiked around 3 ms after the SN spike (Fig. 4A). Compared with Fig. 4A the peak in the coherence function in Fig. 4B is less highlighted from the baseline with respect to both its height and its width.

To quantify the strength of correlations across neurons we calculated the peak amplitude relative to the twofold standard deviation (SD) of the coherence function (shaded area) (see arrows in Fig. 4A). We choose the relative peak height as an indicator for cell-to-cell connections because a reliable temporal relationship between spikes should result in a peak that clearly rises above a noisy baseline (compare Fig. 4A). We were interested in temporal relations occurring on short time-scales and thus determined relative peak values within a time shift (τ) of ±25 ms. However, the SD of the coherence function was calculated over the entire function (±50 ms).

In Fig. 5 the mean relative peak values are summarized over all simultaneous recordings for positive (Fig. 5A) and negative
correlations (Fig. 5B). The cell pairs were classified with respect to the three processing levels mentioned earlier. The shaded area (value of 1.0) in Fig. 5 corresponds to the twofold SD of the coherence function. First, we will focus on positive correlations (Fig. 5A). Obviously, the spike time correlations were highest between segmental and ascending interneurons (mean value 1.8). This can be mainly attributed to the cell pairs that were synaptically coupled and thus increased the average value. For those cell pairs the peaks exceeded the chance level up to threefold (for values see legend of Fig. 5A). However, we also found significant correlations between receptors and segmental interneurons (bars in Fig. 5 indicate 99% confidence intervals). In both classes, RE–SN and SN–AN information is transferred to the next processing level. Rather unexpected was the increased correlation between pairs of ascending neurons because no strong synaptic connections were observed within this group (Fig. 5A, AN–AN). In contrast, correlations between two receptors or two segmental interneurons showed only small (although in the first case significant) deviations from chance level.

We further checked whether the increasing correlations persist if only the stationary part of the spiking response is analyzed. By restricting the data evaluation to a time window between 65 and 130 ms, possible transient responses at the stimulus onset were excluded. A comparison of the maximal relative peaks for the long and the short time window showed the same pattern of increasing correlations at higher processing levels (compare Fig. 5, A and C). However, differences between the classes became more pronounced in an analysis of a longer spike train segment.

In Fig. 5B the mean relative peaks are shown for negative values. Negative peaks in the coherence indicate an increased probability for the absence of a postsynaptic spike in response to a presynaptic one. In contrast to the results of Fig. 5A this analysis did not yield pronounced deviations from the chance level for any neuronal class (Fig. 5B). The 99% confidence interval exceeded the chance level in only two classes: RE–SN and AN–AN. Only minor deviations from chance level were found as well if the analysis was confined to the stationary part (65–130 ms) of the spiking response (Fig. 5D).

So far we considered only the peak amplitudes but not the time shift of the peak values or the peak width. If spike synchronization reflects synaptic interactions both should occur on short timescales. We restricted the investigation of peak positions and width on maximal peaks corresponding to positive correlations because only these showed strong deviations from chance (Fig. 5). The relative peak values were determined in a \( \tau \)-range of \( \pm 25 \) ms. The distribution of the absolute \( \tau \)-positions in Fig. 6A clearly shows an accumulation of maximal peaks within a time window of 4 ms (at \( \tau = 0 \)–0.99 ms: 10%; at \( 1 \leq \tau < 4 \) ms: 34% of all peaks). At \( \leq 10 \) ms the distribution is still dense (0 \( \leq \tau < 10 \): 65% of all peaks) and turns into a noisy distribution at \( > 10 \) ms. We therefore can assume that those peaks at \( \tau \)-values \( > 10 \) ms arise from a noisy baseline. The distribution of peak widths measured at half-amplitude is shown in Fig. 6B. In 73% the peak width was \(< 2 \) ms (median: 1.4 ms).

**Rate covariations**

Rate covariations of simultaneously recorded neurons indicate common input or comodulations of spike count rather than an exact relation of spike times. Noise correlations were derived from trial-to-trial variability of spike counts (see METHODS). For each stimulus presentation, the spike counts of both neurons were plotted against each other, as illustrated in Fig. 7 for the two cell pairs already shown in Figs. 2 to 4. The correlation coefficients are shown for each sound intensity. Significant correlations occurred over almost the whole intensity range if both neurons were synaptically connected [Fig. 7A, all correlations were significant (\( P < 0.01 \), except at 30 dB)], whereas high correlations between the spike counts of both cells were only rarely found for the other cell pair (Fig. 7B, all correlations n.s., except at 50 dB).

Similar as for the quantification of spike time correlations (Fig. 5) the mean values obtained for the different cell classes are shown in Fig. 8. For the spike time correlations in Fig. 5 the mean values were calculated separately for positive and negative peaks. In contrast, the correlation coefficients for spike count combine positive or negative correlations in a single expression (Fig. 8). Therefore the chance level is represented by a value of zero in this analysis. There was a gradual increase of spike count correlations between neurons at higher processing levels, but significant deviations from zero were found only in the classes SN–AN and AN–AN (see 99% confidence intervals). To demonstrate that rate covariations of simultaneously recorded neurons indicate common input or comodulations of spike count rather than stimulus-induced covariations we calculated the correlation coefficients after shuffling the response trials of one of both neurons (Fig. 8B). The mean correlation coefficients now fluctuate around zero for all cell classes. This indicates that the covariations observed in Fig. 8A were not caused by an influence of the stimulus onset.

**Impact of spike count correlations on estimates of variability**

A standard quantification of the variability of spike counts is to determine the variance of a spike count distribution obtained
from several stimulus repetitions, although this procedure entails a problem. As a rule, natural stimuli are not repetitive, which precludes the strategy to enhance reliability by averaging across many trials. Rather, nervous systems will be forced to exploit the information present in parallel neuronal elements with a single stimulus presentation. This difference between our usual experimental protocols—single-cell recording combined with many stimulus presentations and the one-shot information-processing capacities of nervous systems—may lead to a distorted picture of the “true” variability of neuronal responses. Consider, for example, the case where there are changes in general excitability of a neural network on time-scales of seconds to minutes. Then our usual experimental protocols will result in a massive overestimate of the variability that is relevant for the one-shot stimulus processing. The simultaneous recordings from two neurons applied in this study now enable us to assess the errors that will be introduced by the single-cell recording paradigm into our estimate of neuronal variability.

In Fig. 9 the spike count covariations are illustrated for both cell pairs that were introduced earlier (compare Fig. 7; 60 dB). Treating each cell independently as is the case in single-cell recordings, one can derive the expected SD (SDexpected) according to Eq. 6 (see METHODS). This prediction is shown in the left column in Fig. 9A (wide stripes). The right column (narrow stripes) reflects the measured SD (SDmeasured) under consideration of the rate covariations between two neurons. This measure was derived from the spike count differences between both neurons (see Eq. 7 in METHODS). A comparison of Fig. 9, A and B underlines the impact of correlations across neurons. In agreement with the weak covariations of spike count in Fig. 9B both variability measures yield similar values. In contrast, the strong covariations between two neurons would lead to an overestimation of the trial-to-trial variability in the case of single-cell recordings and repeated stimulation (Fig. 9A).

A compilation of all data is shown in Fig. 9C. The SDs expected from single-cell recordings and the respective SDs measured in simultaneous recordings are compared. Although the majority of points is grouped around the 45° line there seems to be a tendency toward slightly lower SDs than expected from single trials. The trial-to-trial method yields a substantial overestimation of the instantaneous variability mainly in those cases where the activity of two neurons correlates strongly (compare open stars in Fig. 9C, which correspond to the cell pair in Fig. 9A). This impact becomes obvious when comparing Fig. 9, C and D. In Fig. 9D the SDmeasured was calculated after shuffling trials and thus removing covariations. Data points of those cell pairs with strong correlations now clustered around the 45° line. A comparison of Fig. 9, C and D further shows that in most cases the trial-to-trial variability derived from single-cell measurements yields a reasonable estimate of the actual variability within this nervous system.

**D I S C U S S I O N**

The main goal of the present study was to elucidate the functional organization of the metathoracic auditory network of the locust by means of simultaneous intracellular recordings. This approach allowed us to cover three aspects: 1) to quantify the degree of correlations across neurons at the first three processing levels, 2) to obtain a measure of variability that takes into account correlations across neurons, and 3) to detect the existence and degree of synaptic connectivity between elements of this circuit.
Connectivity and general information flow within the metathoracic auditory network

The auditory pathway of Locusta migratoria represents a very well studied model system to investigate the processing of acoustic stimuli (Gollisch and Herz 2005; Machens et al. 2001; Schaeffer et al. 2005; Stumpner and Ronacher 1991; Vogel et al. 2005). Response characteristics of many individually identified thoracic auditory neurons are extensively documented (Franz and Ronacher 2002; Krahe et al. 2002b; Römer and Marquart 1984; Ronacher and Römer 1985; Stumpner and Ronacher 1991; Stumpner et al. 1991). However, only a few studies examined the connectivity within the auditory network (Boyan 1991, 1992, 1999; Marquart 1985a).

Our current injection protocols aimed at detecting synaptic connections. Of a total of 30 potentially connected cell pairs only four clear synaptic links were found. Without exception, these synapses were observed between elements of successive processing levels. In contrast, between elements of the same processing level we found no indication of strong synapses. However, a methodological remark is necessary. Although positive evidence, such as postsynaptic potentials (PSPs) or spikes that are time-locked to the spikes of another cell, demonstrates a strong synaptic connection between these cells, the reverse conclusion does not hold in the case of “negative” evidence. Weak synaptic contacts may have been undetected by this procedure because, for example, the recording sites are too far away to observe PSPs. Thus a “negative” result, as shown in Fig. 2B, cannot rule out the existence of weaker synaptic connections between a cell pair. This asymmetry must be kept in mind when considering possible causes for correlations across neurons.

In conclusion, our findings support the view of a hierarchically organized feedforward network (Fig. 1). Based on current evidence there appear to exist only few strong connections between processing levels. This evidence, however, does not rule out the possible existence of many weak synapses that contribute to auditory processing.

Increase of correlations at higher processing levels

Neuronal correlations may be caused by different sources: 1) the temporal structure of the external stimulus; 2) strong synaptic connections between neurons, by which mainly one cell drives the spike generation in another cell; or 3) common synaptic input that drives the activity of both simultaneously recorded neurons. The last case can be further separated into: 3a) both simultaneously recorded neurons are mainly driven by a single presynaptic neuron or 3b) they share a pool of many presynaptic elements.

By using rectangular modulated stimuli we tried to reduce stimulus-induced correlations as far as possible (compare also Figs. 8B and 9D). In accordance with source 2 we observed both spike synchronization and rate covariations between SN and AN (see values for cell pairs SN-AN(b) and (c) in legends of Figs. 5 and 8). In contrast, the spike count correlations for the synapse between RE and SN were very weak, indicating that the impact of this particular receptor neuron on the SN during acoustic stimulation was small compared with the amount of other synaptic inputs affecting this SN neuron.

At the level of ascending interneurons we observed an increase of spike synchronization and rate covariations which could be attributable to either source 2 or source 3. Rate covariations may result from interactions on a long timescale, which could result, for example, from sharing many presynaptic elements (source 3b). Spike synchronization, in contrast, reflects a distinct relationship between the spike times of two neurons probably caused by strong synapses (source 2 or source 3a).
source 3a). However, because we did not detect any strong synaptic connections between pairs of ascending interneurons the origin of spike synchronization at this level remains unclear. One could speculate that only a few elements of the lower processing level, the segmental interneurons, have a strong presynaptic impact on a large number of ascending neurons. Apart from AN(b) and (c) (see legends of Figs. 5 and 8), which appears to be driven mainly by a single SN, it seems unlikely that certain segmental neurons caused the higher correlations between ascending interneurons. The complex and highly diverse response patterns of ascending interneurons make this scenario improbable. The complex response patterns, in addition to the limited number of neurons within the metathoracic network and the increase of correlations across neurons, favor our former suggestion of synaptic crosslinks, which are strong only in exceptional cases (Fig. 2A). Thus the correlations between ascending interneurons could be caused by a sharing of several presynaptic elements (source 3b).

In a convergent network architecture correlations between elements of the same processing level can occur only if they already exist at the presynaptic processing level. In contrast, a divergent architecture supports the new appearance of correlations arising from common presynaptic elements. Which neural substrate is available for the appearance of correlations in the metathoracic network? Within the metathoracic network a substantial convergence exists between receptors and segmental interneurons, whereas between segmental and ascending interneurons the network is probably rather divergent (Fig. 1).

The rate covariations, which occurred between segmental interneurons, were not transmitted from the presynaptic level because the receptor neurons responded in an uncorrelated way (Fig. 8). Thus the correlations between segmental interneurons may have been caused by interconnections within this processing level. The situation is different at the level of ascending interneurons. In view of the relatively small number of presynaptic elements it is rather likely that the spike time corre-
Correlations and variability

The relevance of correlations across neurons for a neural code and its impact on neuronal variability still remains open for debate. Spike count correlations could, for example, induce a bias in the traditional methods that measure variability from many stimulus repetitions. In the metathoracic auditory system of grasshoppers we could show that such a bias leading to an overestimate of the “true” variability depended on the presence of high correlations and thus occurred only between cell pairs with a strong synaptic connection. For the majority of cell pairs tested (26 of 30) no strong synaptic coupling could be demonstrated and their correlation coefficients only rarely exceeded 0.4 (Fig. 8). This leads to the conclusion that in most cases no overly serious errors will be made if one estimates variability by the usual procedure, on the basis of many stimulus repetitions. In the metathoracic auditory system of locusts an increase of correlations across neurons was observed as well (Laurent 2002; Perez-Orive et al. 2002).

REFERENCES


