Single Neuron Dynamics —
Models Linking Theory and Experiment

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Auditory Receptor Neurons of Locusts
6.1 Introduction

Models of the generation of spikes and of spike-frequency adaptation were introduced in the first part of this thesis. These single-compartment models are tested on real neurons in this and the following chapter. Auditory receptor cells of grasshoppers are well suited to apply the proposed models, since they lack a dendritic tree and intermediate synapses. The signal-transmission properties of the auditory receptor cells are of great interest for investigations on subsequent stages of auditory processing.

The auditory system of grasshoppers plays an important role in their reproduction. With their legs and/or wings they produce calling songs to attract their mates. The receiver has to recognize a con-specific song among other songs, assess it, and respond or move towards it. This behavior has been studied extensively (von Helversen, 1997).
The auditory system of grasshopper is therefore well suited for studying how this pattern-recognition task is implemented on the neural level.

Acridid grasshoppers have their ears at the abdomen (see Fig. 1.2). A sound wave excites the oval tympanum (Michelsen, 1971; Michelsen & Rohrseitz, 1995), which is exposed to the outside world. The auditory ganglion (Müller’s organ) is attached to the inner surface of the tympanum (Gray, 1960; Breckow & Sippel, 1985). The ganglion contains the auditory receptor neurons, which pick up the oscillations of the tympanum (Hill, 1983a,b; Oldfield & Hill, 1986). The receptor neurons translate the sound wave into a sequence of spikes. The axons of the receptor cells form the auditory nerve and make contact with auditory interneurons in the metathoracic ganglion (Halex et al., 1988; Boyan, 1999; Jacobs et al., 1999).

Electrophysiological recordings from auditory receptor cells of adult *Locusta migratoria* are analyzed in this chapter. Onset, steady-state and adapted *f-I*-curves, and adaptation time constants were measured, in order to quantify spike-frequency adaptation observed in the receptor cells (Michelsen, 1966). These findings are compared with the expectations of the theoretical considerations about adaptation from chapter 4. Phase-resetting curves were measured to determine whether the receptor neurons are class-I or class-II neurons (see chapter 2), and to choose an appropriate phase-oscillator model from chapter 3. Finally, implications on the transmission of calling songs are discussed.

### 6.2 Methods

**Animals**

All experiments were performed on adult *L. migratoria*. The animals were obtained from commercial cultures.

**Preparation**

The legs, the wings, head and gut were removed and the animal was waxed ventral side uppermost on a platform. Then the dorsal part of the thorax was cut open to expose the metathoracic ganglion and auditory nerve. The auditory nerve was fixed with a special forceps mounted on a micromanipulator.

**Recordings**

Receptor cells were recorded intracellularly in the auditory nerve with standard glass micro-electrodes (borosilicate, 50 – 110 MΩ resistance), filled with 1 M KCl solution. Neural responses were amplified (NPL, BRAMP-01) and recorded by a data acquisition board (National Instruments, PCI-MIO-16E-1) with a sampling rate of 10kHz. If not stated otherwise, the animals were heated to a temperature of about 35°C. The phase-resetting curves were measured at room-temperature (about 20°C). The experiments were performed by Hartmut Schütze, Olga Kolesnikova, Tim Gollisch, and myself.
Figure 6.1: Measuring the threshold-curve of an auditory receptor neuron. A An $f$-$I$-curve calculated from the mean spike count evoked by 40 ms long stimuli with carrier frequency 3.5 kHz. A line is fitted into the dynamic range (dashed line) to determine the threshold intensity (dot at 50 dB). B The threshold intensities $I_{th}$ for different carrier frequencies $f_c$ of the stimulating sound waves. The threshold-curve shown is typical for a low-frequency receptor. The cell shown here is most sensitive at about 3.5 kHz. The data were recorded at room temperature.

Online stimulus generation and data analysis

For online stimulus generation and data analysis together with Christian Machens I developed the OEL-software (Online-Electrophysiology-Laboratory). It is written in C++ under Linux and uses the Qt-library (Troll Tech) for graphical user interface. Two character-device drivers were implemented as loadable kernel modules for the communication with the data-acquisition board (National Instruments, PCI-MIO-16E-1) and the attenuator of the acoustic signal (Chrrystal semiconductors, CS3310). OEL handles the output of stimuli, reads continuously the recorded voltage trace, and detects the spikes, using an adaptive threshold of the first derivative of the voltage trace. So called RePros (Research Programs) can be written in C++ and added to OEL, which analyze the spike trains and then generate the next stimulus. Stimuli were sampled with 200 to 240 kHz. Their intensity is measured in decibel sound pressure level (dB SPL).

$f$-$I$-curves

First, the carrier frequency for which the cell is most sensitive was determined (Fig. 6.1). For that purpose stimuli of 40 ms duration and 1 ms ramps were used. Each stimulus with a particular carrier frequency and intensity was repeated two times. The response of the cell was measured as the mean spike count of the two trials during stimulation. If a stimulus evoked a response, the next stimulus was applied after a pause of 260 ms. Otherwise the next stimulus followed after 5 ms. An $f$-$I$-curve was measured at each carrier frequency for intensities ranging from 20 to 100 dB SPL. Starting with a resolution of 24 dB, which then was successively divided by two, the response to different intensities down to a resolution of 6 dB was measured. Intensities below a higher intensity, which did not evoke any response, were skipped. The response to intensities spaced by 3 dB was only determined for intensities, were the slope of the already measured $f$-$I$-curve was larger than 5 Hz/dB. With this procedure the measurement of the $f$-$I$-curve focused on its dynamic range. A line was fitted into the dynamic range and its intersection with the
intensity-axis was taken as the threshold intensity of the receptor neuron. The \( f \)-\( I \)-curves were measured at maximal 21 different carrier frequencies between 1.25 and 40 kHz. The carrier frequencies were spaced by a factor of \( \sqrt[2]{2} \approx 1.1892 \), i.e. the carrier frequencies were 1.25, 1.49, 1.77, 2.1, 2.5, …, 40 kHz. The carrier frequency of the lowest threshold intensity was taken as the best carrier frequency of the receptor cell. All stimuli of the following experiments used this frequency for the carrier sound wave.

Next, a more detailed \( f \)-\( I \)-curve was measured using stimuli of 300 ms duration, which were repeated six times. The minimum resolution of intensities was 1.5 dB. The peak of the trial-averaged time course of the firing frequency (equation (5.5) on page 105) was taken to construct the onset \( f \)-\( I \)-curve \( f_0(I) \). The steady-state \( f \)-\( I \)-curve \( f_\infty(I) \) was calculated from the mean response between 240 and 290 ms after stimulus onset (Fig. 6.3). A single exponential was fitted into the time course of the firing frequency to obtain the effective adaptation time-constant \( \tau_{eff} \) (Fig. 6.4).

For a parameterization of the onset \( f \)-\( I \)-curve the positive part of a hyperbolic tangent

\[
f_0(I) = \begin{cases} 
  f_{max} \tanh'(k(I-I_{th})) & ; I > I_{th} \\
  0 & ; I \leq I_{th}
\end{cases}
\]

(6.1)

was used, where \( f_{max} \) is the maximum firing frequency and \( I_{th} \) is the threshold intensity (see Fig. 6.2 A). \( k \) determines the initial slope and the exponent \( r > 0 \) distorts the hyperbolic tangent. The slope \( s_0 \) at the turning point of (6.1) is

\[
s_0 = 2f_{max}k \frac{r}{r+1} \sqrt{\frac{r-1}{r+1}}^{r-1}.
\]

(6.2)

The width of the dynamic range of the sigmoidal is approximately \( f_{max}/s_0 \).

For the steady-state \( f \)-\( I \)-curve the Boltzmann function was fitted on the data

\[
f_\infty(I) = f_{max} \left( \frac{1}{1 + e^{-k(I-I_{th}/2)}} \right)^r
\]

(6.3)
I_{1/2} is the intensity where the firing frequency is one half of the maximum frequency $f_{\text{max}}$, provided $r = 1$. The slope at the turning point is

$$s_{\infty} = f_{\text{max}} k \left( \frac{r}{1 + r} \right)^{1 + r}.$$  

(6.4)

The time constant of recovery from adaptation was measured as described in Fig. 4.10. A 200 ms stimulus was used to adapt the cell. The test stimuli of 40 ms duration were repeated eight times. Between test stimulus and the next adaptation stimulus was a pause of one second.

To measure the adapted $f$-$I$-curves, a special stimulus as described in Fig. 6.5 was used. Appropriate background intensities were calculated online based on the onset and steady-state $f$-$I$-curves measured before.

**Phase-resetting curves**

The latency $\lambda$ of the system (transduction of the sound wave, transformation and time of the spikes traveling down the axon to the microelectrode; the time of the sound wave to reach the ear was already subtracted from the spike data) was determined with perturbations of 12 dB strength and one millisecond duration on top of a background intensity that evoked a firing frequency of approximately 100 Hz. After 400 ms to let the cell adapt to its steady-state firing frequency, the perturbations were randomly spaced from 30 to 60 ms. The entire stimulus was two seconds long, contained 35 perturbations and was repeated five times. The latency of the system was determined as the time shift that maximized the number of spikes, which were directly initiated by the perturbations, i.e. a spike occurred within 0.5 ms after the time of the perturbation plus the time shift.

Phase-resetting curves were measured with stimuli similar to the one used to determine the latency of the system. The perturbations were smaller (3–9 dB) and shorter (0.3 and 0.6 ms) and were randomly spaced from 50 to 100 ms. These stimuli were repeated 20 times. The period $T_0$ of the unperturbed oscillation was calculated as the mean interspike interval before the first perturbation. The time of a perturbation $t_p$ plus the latency of the system $\lambda$ minus the time of the preceeding spike $t_i$ normalized by $T_0$ is the phase of the perturbation

$$\varphi = \frac{t_p + \lambda - t_i}{T_0}, \quad t_i < t_p + \lambda \leq t_{i+1}.$$  

(6.5)

The phase shift $\Delta \varphi$ is the difference of the unperturbed period $T_0$ and the interspike interval $t_{i+1} - t_i$ over the perturbation

$$\Delta \varphi = \frac{T_0 + t_i - t_{i+1}}{T_0}, \quad t_i < t_p + \lambda \leq t_{i+1}.$$  

(6.6)

The phase shifts were binned in $\psi_{10}$ phases and the median as well as the second and third quartile was calculated for each bin to get a phase-resetting curve.
Adaptation model

With the following adaptation model the firing frequency to arbitrary stimuli $I(t)$ was predicted:

$$f(t) = f_0(I - A)$$
$$\tau A = \frac{I - f_0^{-1}(f_\infty(I))}{f_\infty(I)} f(t) - A.$$  \hspace{1cm} (6.7)

In this equation $A$ is the state of adaptation and $\tau$ is the adaptation time-constant. The model (6.7) is a variant of the model for encoder adaptation (4.22), which can be applied to neurons with saturating $f$-$I$-curves. The original model for encoder adaptation (4.22) is not applicable, since the inverse function of the steady-state $f$-$I$-curve is not defined for firing frequencies greater than its maximum firing frequency. To overcome this problem, the steady-state adaptation strength in (6.7) is forced to be proportional to the firing frequency $f(t)$. All deviations from this proportionality are handled by the intensity dependence of the proportionality factor. See discussion for a validation of this model.

The firing frequency from the model (6.7) was smoothed by applying the non-leaky phase oscillator (3.5) in the following way: At a given time $t$ a time interval $T$ centered over $t$ is determined such that

$$t + \frac{T(t)}{2} \int_{t - \frac{T(t)}{2}}^{t + \frac{T(t)}{2}} f(t') dt' = 1.$$  \hspace{1cm} (6.8)

The resulting firing frequency $v(t) = 1/T(t)$ was compared with the experimentally measured firing frequency $f_{\text{exp}}(t)$.

The prediction error

$$p.e. = \frac{\sigma^2_{\text{diff}}}{\sigma^2_{\text{exp}}}$$  \hspace{1cm} (6.9)

relates the mean squared difference

$$\sigma^2_{\text{diff}} = \frac{1}{T_s} \int_0^{T_s} (f_{\text{exp}}(t) - v(t))^2 dt$$  \hspace{1cm} (6.10)

of the experimentally measured firing frequency $f_{\text{exp}}(t)$ and the firing frequency $v(t)$ of the model to the variance $\sigma^2_{\text{exp}}$ of $f_{\text{exp}}(t)$ over the entire duration of the stimulus $T_s$. For a perfectly predicted firing frequency the mean squared difference $\sigma^2_{\text{diff}}$ equals zero and the prediction error $p.e.$ becomes zero.

To test the performance of the models, two types of stimuli were applied: (i) the envelope of a calling song of a male of the grasshopper *Chorthippus biguttulus*. (ii) white noise stimuli of ten seconds duration with an amplitude distribution similar to the grasshopper song and different cut-off frequencies (Machens et al., 2001). All these stimuli were filled with a sine wave with their frequency set to the neurons best frequency.

### 6.3 Results

Data from still ongoing experiments are presented in the following.
Figure 6.3: \( f-I \)-CURVES AND LATENCIES OF LOW-FREQUENCY RECEPTORS. The animals were heated to 35°C. Each stimulus was 300 ms long and was repeated \( n = 6 \) times. A The onset \( f-I \)-curve \( f_0(I) \) and the steady-state \( f-I \)-curve \( f_\infty(I) \) of a low-frequency receptor with its best carrier-frequency at 2.5 kHz. Errorbars denote the standard error of the tuning frequency. The dashed line is the fit of the hyperbolic tangent (6.1) used for the onset \( f-I \)-curve. The solid line is the fit of the Boltzmann function (6.3) used for the steady-state \( f-I \)-curves. B Latencies to the first spike with standard errors in comparison with the mean interspike interval \( 1/f_0(I) \) of the onset \( f-I \)-curve. Note that also the (unknown) latency of the system \( \lambda \) contributes to the latencies shown here, which has to be subtracted from the data. C The onset \( f-I \)-curve of the cell shown in A compared with the onset \( f-I \)-curve of a different low-frequency receptor with best frequency at 2.97 kHz. D Comparison of the steady-state \( f-I \)-curves of the same cells from C. E The dependence of the slopes of both the onset (open circles) and the steady-state \( f-I \)-curves (filled circles) on the intensity of their turning point. The values of the two cells from C & D are indicated by the arrows. The dashed line is the expected slope (6.14) of the onset \( f-I \)-curves, if they all would have the same width in terms of sound-wave amplitudes. A fit on the data revealed a value of 507 for the ratio \( \Delta p/p_0 \) of the amplitude interval \( \Delta p \) to the reference sound pressure \( p_0 = 20 \mu \text{Pa} \). F Linear parts of \( f-I \)-curves with different threshold intensities as expected from the dashed line in D.
Table 6.1: Properties of f-I-curves of low-frequency receptor neurons.

<table>
<thead>
<tr>
<th>f-I-curve</th>
<th>$f_{\text{max}}$ (Hz)</th>
<th>$s$ (Hz/dB)</th>
<th>$\Delta I$ (dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>onset</td>
<td>570 ± 38</td>
<td>58 ± 32</td>
<td>13 ± 7</td>
</tr>
<tr>
<td>steady-state</td>
<td>370 ± 50</td>
<td>18 ± 4</td>
<td>22 ± 4</td>
</tr>
</tbody>
</table>

Summarized are the averaged maximum firing frequency $f_{\text{max}}$, slope $s$, and width $\Delta I$ of the dynamic range of $n = 11$ f-I-curves measured in seven different receptor cells. The slopes were calculated by means of (6.2) and (6.4).

**Spike-frequency adaptation**

To characterize spike-frequency adaptation the onset and the steady-state f-I-curve of a neuron is needed (see chapter 4). A typical example of f-I-curves of a low-frequency receptor is illustrated in Fig. 6.3 A. On average (see tab. 6.1) the onset f-I-curves had a maximum firing frequency of 570 Hz. The maximum firing frequency of the steady-state f-I-curves was 370 Hz. This is about two third of the onset f-I-curves ($f_0/f_\infty = 1.6 \pm 0.1$). The percentage of adaptation $F_{\text{adap}} = \frac{f_0/f_\infty}{f_0}$ (Wang, 1998) is 56 ± 12 %. Both the slope of the onset f-I-curves (mean 58 Hz/dB) and the width of their dynamic range (mean 13 dB) vary strongly. As demonstrated in Fig. 6.3 C, D & E, the onset f-I-curves become steeper with higher threshold intensity. In contrast, the slopes of the steady-state f-I-curves and the widths of their dynamic range are independent of their threshold intensity (18 Hz/dB and 22 dB, respectively). On average the slopes of the onset f-I-curves are three times larger than the slopes of the steady-state f-I-curves ($s_0/s_\infty = 3.1 \pm 1.2$). Consequently, the width of the dynamic range of the onset f-I-curves is smaller than the width of the steady-state f-I-curves ($\Delta I_0/\Delta I_\infty = 0.6 \pm 0.2$).

The Boltzmann function (6.3) is a good model for a parameterization of the steady-state f-I-curves. It fits them for the whole range of intensities fairly well. The situation is more complicated for the onset f-I-curves. A slight spontaneous activity of the neurons implicates that there is even for subthreshold stimuli an enlarged activity somewhere during the stimulus, which is taken as the onset response. Therefore for low intensities the onset f-I-curves level out at a non-zero frequency, and it is not clear, whether the onset f-I-curves start smoothly or as a straight line with finite slope. However, they show a wide linear range, which is better fitted by the hyperbolic tangent (6.1).

Latencies to the first spike after stimulus onset are of the order of magnitude of the corresponding interspike interval from the onset f-I-curve. The latencies become very large close to the threshold as can be seen in Fig. 6.3 B.

The time courses of the firing frequency to constant stimuli with different intensities are shown in Fig. 6.4 A. A fit with a single exponential reveals the effective time constant of adaptation. The effective time constants for different stimulus intensities are plotted in comparison with the f-I-curves in Fig. 6.4 C. For intensities near threshold the time constants of adaptation are small (about 10 ms). They increase with stimulus intensity and approach on average a maximum value of $\tau_{\text{eff}} = 75 \pm 12 \text{ ms}^1$.

In $n = 6$ cells the time constant of recovery from adaptation was measured (see [Preliminary results from experiments performed at room temperature reveal similar maximum time constants of adaptation of about 80 ms.])
Figure 6.4: **Adaptation Time-constants of a Low-frequency Receptor.** Same cell as in Fig. 6.3. A Three traces of the trial averaged firing frequency \((n = 6)\) evoked by constant stimuli with different intensities as indicated are shown (carrier frequency 2.5 kHz, duration 300 ms). Single exponentials were fitted into the traces (dashed lines) to determine the effective adaptation time-constant \(\tau_{\text{eff}}\). B The firing frequencies evoked by test stimuli of 40 ms duration at time \(t\) after an initial adaptation stimulus of 200 ms duration with intensity \(I = 51.2\) dB SPL. A fit with a single exponential (dashed line) revealed a time constant from recovery of adaptation of \(\tau = 80\) ms. C The dependence of the effective adaptation time-constants (dots) from \(f-I\)-curves from Fig. 6.3 are superimposed. The triangle marks the time constant of recovery from adaptation from B. The errorbars denote the uncertainty of the fits.

Fig. 6.4 B). On average the time constants were \(\tau = 50 \pm 17\) ms. They were smaller than the corresponding maximum effective time constants of adaptation, but clearly larger than the time constants measured close to threshold (Fig. 6.4 C).

Adapted \(f-I\)-curves were measured successfully in \(n = 9\) cells. Three examples are illustrated in Fig. 6.6. With increasing background intensity the adapted \(f-I\)-curves are shifted appropriately to higher intensities. In six cells the slope of the adapted \(f-I\)-curves remained unchanged, while in three cells the slope decreased a little at high background intensities. The maximum firing frequency of the adapted \(f-I\)-curves decreased with increasing background intensity towards the maximum firing frequency of the steady-state \(f-I\)-curve. The adapted \(f-I\)-curves shifted not beyond the saturation of the onset \(f-I\)-curve. The maximum difference between the threshold intensity of the onset \(f-I\)-curve and the threshold intensity of an measured adapted \(f-I\)-curve was about 10 dB.
6.3 RESULTS

Figure 6.5: MEASURING AN ADAPTED f-I-CURVE. A Time course of the firing frequency $f(t)$ evoked by the stimulus $I(t)$ shown in B. First the neuron was adapted to the background intensity $I_b$ (dotted line in B). Beginning at $t = 300$ ms the response (filled circles in A) to different intensities in a descending order (triangles in B) was tested. Directly after the test stimuli above background intensity the stimulus was set to zero (arrows in B) in order to minimize the total activity of the neuron, since very often the neuron’s response to the background intensity (upper dotted line in A) between the test stimuli decreased slowly during the entire stimulus. The duration of the test stimuli was 30 ms for $I > I_b$ and 60 ms otherwise. The lower dotted line in A is the reciprocal of the 60 ms stimuli, which is approximately the lowest firing frequency that can be measured (see also Fig. 4.12 in chapter 4).

Phase-resetting curves

Phase-resetting curves were measured successfully in $n = 5$ cells. The data from one example cell are illustrated in Fig. 6.7. The latency $\lambda$ of the system was on average $6.4 \pm 0.6$ ms. The phase-resetting curves for the weak perturbations were always positive, except at very late phases, which is an effect of noise (see Fig. 6.7 B for an explanation). The response function of the $\theta$-model (3.20) corresponds well with the measured phase-resetting curves of the receptor neurons in that both are positive (at least for phase angles $< 0.7$) and both have a single peak approximately at intermediate phases. Even perturbations, which consist only of a single oscillation of the carrier sound-wave, still had an effect on the following spikes.

Performance of the models

To test the models, first the onset and the steady-state $f-I$-curves of the receptor cell were measured. Then the cell was stimulated with the calling song and the white-noise stimuli. The evoked firing frequency was then compared with the prediction of the proposed adaptation model (6.7), whose output firing-frequency $f(t)$ was smoothed with the non-leaky phase oscillator (3.6).

The model reproduced the measured firing frequency with high accuracy (see Fig. 6.8,
Figure 6.6: ADAPTED f-I-CURVES OF RECEPTOR NEURONS. A, B, C & D Adapted f-I-curves $f(I,A)$ of a low-frequency receptor with best frequency at 2.97 kHz. Four different background intensities $I_b$ as indicated (vertical lines) were used. For comparison the onset f-I-curve $f_0(I)$ and the steady-state f-I-curve $f_\infty(I)$ of the same cell are drawn. At background intensity the adapted f-I-curves are expected to intersect the steady-state f-I-curve. Increasing background intensity shifts the adapted f-I-curves and decreases their saturation level towards the one of the steady-state f-I-curve. D The adapted f-I-curve at the background intensity is below the steady-state f-I-curve. This may be caused by additional very slow (> 1 s) processes, which further weaken the response of the cell. E Some adapted f-I-curves in comparison to the onset and steady-state f-I-curves of a low-frequency receptor with best frequency at 3.54 kHz. With increasing background intensity the slope of the adapted f-I-curves is slightly decreasing. F Adapted f-I-curves of a high-frequency cell with best frequency at 40 kHz. In this cell the slope of the adapted f-I-curves does not change.
Figure 6.7: PHASE-RESETTING CURVES OF A LOW-FREQUENCY RECEPTOR. A The latency $\lambda$ of the system was measured using strong perturbations of $\Delta I = 12$ dB and $\Delta t = 1$ ms duration (a part of the sound wave is sketched in the inset). Such perturbations force almost immediately the generation of a spike. For the calculated system latency of 6.7 ms the phase shifts line up very closely to the $1 - \phi$-line (dashed line). This line indicates spikes which were generated at the same time as the perturbation. B, C & D To measure the phase-resetting curves, shorter and weaker perturbation stimuli as indicated were used. B The resulting phase shifts are very noisy (dots), since the timing of the spikes is very irregular even without perturbations. However, the medians of the data binned into ten bins reveal a clear phase-resetting curve. The errorbars denote the second and third quartile. The phase-resetting curve is always positive, except at very late phases. The negative values of the data there is an effect of the noisy spikes. The data cannot be higher than the $1 - \phi$-line (dashed line) to ensure causality. Therefore they only can fluctuate below this line. C Phase-resetting curves (median) evoked by perturbations of 0.6 ms duration. This corresponds to two oscillations of the carrier frequency of 3.54 kHz used for the stimuli (inset). The strength $\Delta I$ of the perturbations was varied as indicated. D Same as in C, but with the duration of the perturbation of only 0.3 ms, which corresponds to one oscillation of the sound wave (inset). Superimposed is an appropriately scaled response function of the $\theta$-model (dotted line).

Fig. 6.9, and Fig. 6.10). A model without adaptation, i.e. a mapping of the stimulus directly through the neuron’s onset $f-I$-curve, clearly failed to reproduce the experimentally measured firing frequency (Fig. 6.8 A). To the white-noise stimuli the cell adapted quickly to an approximately constant state of adaptation (Fig. 6.8 C and Fig. 6.9 C). The intensity of the calling song slowly increases with time. The state of adaptation followed this slow component of the stimulus and kept the output firing frequency of the cell at a nearly constant level (Fig. 6.10 B & D).

The $f-I$-curve of the neuron is shifted dynamically by the state of adaptation $A(t)$. 
The varying threshold of the adapted $f$-$I$-curve is given by the threshold of the onset $f$-$I$-curve $I_{th}$ plus the state of adaptation $A$. Only parts of the stimulus above the current threshold are transmitted by the neuron. As can be seen in the examples of Fig. 6.8 C, Fig. 6.9 C, and Fig. 6.10 B & D, adaptation keeps most of the stimulus below the neuron’s threshold. Subtracting the state of adaptation $A(t)$ from the stimulus $I(t)$ results in an effective stimulus, which is then transmitted via the onset $f$-$I$-curve, provided the slope of the adapted $f$-$I$-curves does not change. In Fig. 6.11 the original amplitude distribution of a white-noise stimulus is compared with the amplitude distribution of the effective stimulus. Without adaptation most of the original stimulus is above threshold. Due to adaptation, however, for the receptor cell the stimulus becomes softer and is shifted to lower intensities from the point of view of the onset $f$-$I$-curve. Finally one half of the stimulus remains sub-threshold.

The strong effect of the non-leaky phase oscillator (6.8) on the output firing frequency of the adaptation model (6.7) is illustrated in Fig. 6.9 A & B. The adaptation model (6.7)
only maps the input $I(t)$ through the onset $f-I$-curve. Therefore it transmits the stimulus independently of how fast it fluctuates (panel C), resulting in firing frequencies which can vary much faster than the observed firing frequency of the real receptor cell. In fact, the adaptation models are high-pass filters (see page 88). However, fluctuations of the stimulus between two succeeding spikes in general cannot be resolved from the firing frequency (see Fig. 3.7 on page 37). The simplest way to account for this effect is to generate spikes out of the output firing-frequency of the adaptation model (6.7), and compute the final firing frequency from these spikes (such spikes are shown in Fig. 6.10 E). A more continuous way to implement this procedure is the filter (6.8), which is based on the non-leaky phase oscillator (3.2). The fluctuations of the resulting firing frequency are then on a similar time scale as the measured firing frequency.

In Fig. 6.10 E the spike trains simulated by the non-leaky phase oscillator (3.2) and the $\theta$-model (3.22) are directly compared with spike trains of a receptor cell. These are preliminary data and still have to be analyzed quantitatively. Both models produce spike patterns, which match the experimentally measured spikes quite well.
Figure 6.10: PERFORMANCE OF THE ADAPTATION MODEL FOR CALLING SONGS. The stimulus is the envelope of the calling song of a male of the grasshopper *Chortippus biguttulus*, filled with a 5 kHz carrier sound-wave. The song starts at $t = 0$ and lasts 2.7 s. A The response of the same receptor cell as in Fig. 6.8 (dashed line) to the song with its mean intensity during the loudest syllables at 84 dB SPL. The measured firing frequency is predicted with high accuracy by the firing frequency of the adaptation model (6.7) smoothed by (6.8). The prediction error (6.9) for the entire song is 11 %. Without including adaptation into the model, i.e. just mapping the stimulus through the onset $f_I$-curve, the prediction error is 278 %. B The stimulus (solid line) in comparison with the threshold of the adapted $f_I$-curve (dashed line). The actual threshold follows the slow increase of the stimulus intensity. C & D Same as in A for the song with mean intensity 78 dB SPL, which was more closely to the neuron’s threshold at $I_{th} = 69$ dB SPL. The prediction error of the model is 14 %. Without adaptation it is 351 %. E The five spike trains obtained experimentally from the receptor cell (lower five rows) and the spike trains simulated with the non-leaky phase oscillator (3.2) and the $\theta$-model (3.22).
6.4 Discussion

Receptor neurons of locusts were subject of many studies during the past five decades. The work of Suga (1960), Michelsen (1966), Römer (1976), and Sippel & Breckow (1984) already showed that these neurons exhibit spike-frequency adaptation to constant stimulation. However, this was never examined quantitatively from a dynamical point of view. A test for class-I or class-II dynamics of a spiking cell was so far never addressed with the measurement of phase-resetting curves.

Class-I or class-II neuron?

To decide whether a neuron is a class-I or a class-II neuron, the properties of its f-I-curve, latencies, and phase-resetting curve can be used as summarized in chapter 2. All these properties measured in the auditory receptor neurons support that they are class-I neurons. The receptor cells can fire with arbitrary low firing frequencies. However, for two reasons this is not a strong argument. First, it is hard to measure arbitrary low firing frequencies experimentally. Very long stimuli are needed to test for frequencies below about 10 Hz. Spontaneous activity observed in some cells sets a lower limit to firing frequencies. Second, even a class-II neuron can exhibit class-I f-I-curves, if the discontinuity of its f-I-curve is smeared out by noise. Indeed, the spike pattern of auditory receptor cells to constant stimuli is very noisy. For intermediate firing frequencies the CV (standard deviation of interspike intervals divided by their mean) measured during steady-state is high (about 0.5).

Latencies and phase-resetting curves are much stronger cues. The latencies of the first
spike after stimulus onset can be arbitrary long for stimulus intensities close to threshold (Fig. 6.3 B). Furthermore, the latencies are of the order of magnitude of the corresponding interspike interval of the onset $f$-$I$-curve. Note that from the measured latencies the latency of the system has to be subtracted, i.e. the time of the transduction process plus the time the spikes need to travel down the axon to the microelectrode. For receptor neurons at room temperature this system latency is about 6 – 7 ms. For receptor neurons heated to 35 °C the system latency was not measured. Since the minimal latencies measured at this temperature were about 4 ms, the system latency has to be shorter to ensure causality. Without knowing the exact value of the system latency it cannot be decided whether the latencies are longer or shorter than the corresponding interspike intervals. This is an interesting issue for the performance of the phase oscillators from chapter 3.

Due to the high variability of the interspike intervals even in the steady-state (after more than 200 ms) the resulting data of the phase-shift measurements were very noisy, too (Fig. 6.7 B). However, the median values reveal a monophasic phase-resetting curve of a class-I neuron. Phase-resetting curves were measured at room temperature, to have a steady-state firing frequency that is not too high. The shortness of the perturbations is limited by the period of the carrier sound wave. For low-frequency receptors stimulated at their best frequency the duration of one oscillation is in the range of 0.2 – 0.4 ms. Perturbations consisting of two oscillations make about one twentieth of an interspike interval at 100 Hz firing frequency. Therefore the firing frequency should not be higher. On the other hand, the variability (CV) of the interspike intervals increases with decreasing firing frequency (not shown). Thus, measuring the phase-resetting curves at room temperature and firing frequencies of about 100 Hz seemed to be a good compromise.

**Properties of $f$-$I$-curves**

In previous electrophysiological studies of receptor cells, $f$-$I$-curves were measured as the mean spike-count per the entire duration of the stimulus (ranging from 10 to 1000 ms). If short stimuli of less than about 20 ms were used, such $f$-$I$-curves are close to the onset $f$-$I$-curve. The longer the stimuli are, the more these $f$-$I$-curves are dominated by the steady-state $f$-$I$-curve. However, the distinction between onset and steady-state $f$-$I$-curves is necessary to quantify the properties of spike-frequency adaptation and to distinguish between transducer and encoder adaptation (see chapter 4).

The onset $f$-$I$-curve $f_0(I)$ is the result of the transduction of the intensity $I$ of the sound wave into a conductance change $g(I)$ of the receptor membrane and of the processes generating spikes:

$$f_0(I) = f(g(I)),$$  \hspace{1cm} \tag{6.11}

where $f(g)$ is the dependence of the firing frequency on the conductance $g$, which is a property of the spike generator. The transduction of the sound wave $g(I)$ most probably is a sigmoidal function. At some intensity $I$ equal to or below the threshold of the onset $f$-$I$-curve, $g(I)$ activates and it saturates at a higher intensity. Since the receptor cells are class-I neurons, the transformation of the conductance into spikes $f(g)$ is expected to be approximately a square-root function

$$f(g) \approx c \sqrt{g - g_{th}}.$$  \hspace{1cm} \tag{6.12}

Important is that $f(g)$ does not saturate for arbitrary high conductances $g$. The $f$-$I$-curves of different neurons measured with current injections indeed do not saturate at plausi-
ble firing frequencies (Madison & Nicoll, 1984; Lanthorn et al., 1984). Therefore the observed saturation of the onset $f$-$I$-curves has to be attributed to the transduction process $g(I)$. Note also, that the $f$-$I$-curves of the receptor neurons are remarkable similar to the simple model for encoder adaptation shown in Fig. 4.8, where such an saturating transduction is implemented.

As shown in Fig. 6.3 D the slope of the onset $f$-$I$-curves increases with increasing threshold intensity. Imaizumi & Pollack (2001) reported similar findings in auditory receptor neurons of the cricket *Teleogryllus oceanicus*. This might be an effect of the logarithmic decibel scale. Sound waves are pressure waves with an amplitude $p$. The intensity measured in dB SPL (decibel sound pressure level) is defined as

$$I_{\text{dB SPL}} = 20 \log_{10} \frac{p}{p_0},$$

(6.13)

where $p_0$ is the reference pressure of $p_0 = 20 \, \mu\text{Pa}$, which corresponds to the human hearing threshold at 1 kHz. Assume the transducer properties of all receptor cells to be similar except for their sensitivity, i.e. the spike generation $f(g)$ is the same as well as the shape of the transduction function $g(I)$. Thus, the width of the dynamic range of the transduction $g(I)$ is assumed to be the same for all receptor cells. However, is this width measured in decibel, pressure amplitude, or squared pressure-amplitude? Obviously the data in Fig. 6.3 C & D cannot be explained with a constant width measured in decibel.

Alternatively, the width of the transduction $g(I)$ could be a constant difference in sound pressure $\Delta p$. Then the width $\Delta I$ measured in decibel is

$$\Delta I = 20 \log_{10} \left( 1 + \frac{\Delta p}{p_0} 10^{-\frac{I_0}{20}} \right),$$

(6.14)

which depends on the threshold intensity $I_0$ of the transduction. The corresponding slope $s = f_{\text{max}}/\Delta I$ with $f_{\text{max}} = 570 \, \text{Hz}$ was fitted into the data of Fig. 6.3 D. It describes the data well, but more data are needed to verify this relation especially at high threshold intensities. In Fig. 6.3 E $f$-$I$-curves resulting from this relation are sketched. A similar relation can be obtained for a constant width measured in squared pressure amplitudes. The resulting function is very similar to (6.14) and the data do not allow to distinguish between these two alternatives. In a companion work, Gollisch et al. (2001) showed that the variance of the sound pressure-wave, i.e. the squared pressure amplitude is the relevant signal for the receptor cells.

An other hint concerning the time scales of the transduction process comes from the measurements of phase-resetting curves. Perturbation stimuli much shorter than the time constant of the transduction process may be filtered out and may have no or only a little effect on the spike generator, since the tympanum (Schölten et al., 1981; Breckow & Sippel, 1985) and the transduction process constitute a low-pass filter. However, even perturbations as short as one oscillation of the carrier sound-wave (0.3 ms) still have an effect. Therefore, the cut-off frequency of this low-pass filter cannot be much lower. This is in good agreement with the work of Schölten et al. (1981), who measured the damping time constant of the tympanum to be 90 $\mu\text{s}$. The fact that even a single oscillation of the sound wave has an effect on the following spike does not imply that the spikes lock on the oscillations of the sound wave. The timing of the spikes is only slightly advanced by the oscillations. Suga (1960) and Hill (1983a) already showed that the spikes of auditory receptor neurons of locusts do not lock on the oscillation of the stimulating sound wave.
Adaptation mechanisms

There are mainly two possibilities to explain the spike-frequency adaptation of the auditory receptor cells. First, the transducer process of the sound wave may adapt in dependence on the sound intensity (transducer adaptation). Second, additional ionic currents adapt the spike generator in dependence on the output firing frequency (encoder adaptation).

The decrease of the effective adaptation time-constants for decreasing intensities and the time constant of recovery from adaptation in the order of the maximum adaptation time constants (Fig. 6.4 C), is a strong hint for encoder adaptation. For transducer adaptation such a strong dependence of the effective time constants on intensity in relation to the \( f-I \)-curves is not expected (recall Fig. 4.11 on page 82).

The decreasing maximum firing frequency of the adapted \( f-I \)-curves (Fig. 6.6), and the fact that their dynamic range is always below the intensity, where the onset \( f-I \)-curves saturates, is in accordance with the view of equation (6.11), where encoder adaptation acts subtractive on the transducer conductance \( g \) and not on the stimulus. Encoder adaptation does not influence the transduction of the sound wave into a change of a conductance. Therefore, the intensity, where the onset and the adapted \( f-I \)-curves saturate, remains the same, independently of the state of adaptation. See also Fig. 4.8 on page 76 for an illustration of this issue.

In summary, spike-frequency adaptation in auditory receptor cells of locusts on timescales below 100 ms is caused by encoder adaptation. Various mechanosensory receptor cells of invertebrates were investigated using current injections. By this direct method encoder adaptation was found to be the main contribution to the observed spike-frequency adaptation, too (French, 1989b,a; Purali & Rydqvist, 1998; Torkkeli et al., 2001).

There is an additional adaptation process, which was not investigated in this study, acting on much slower time scales of several seconds (Michelsen, 1966). This type of adaptation seems to shift the adapted \( f-I \)-curves below the saturation of the onset \( f-I \)-curve, as can be seen for example in Fig. 6.4 D. Thus, this slow adaptation may take place in the transducer process.

Model for spike-frequency adaptation

Encoder adaptation acts subtractive on the input current, which is approximately proportional to the input conductance \( g(I) \), as derived in chapter 4. Thus,

\[
f(t) = f(g(I) - A)
\]

would be the right equation for encoder adaptation in the receptor cells. However, the exact form of \( g(I) \) is not known, but it is very likely that the function \( g(I) \) is approximately linear near the threshold of the onset \( f-I \)-curve. In this linear regime adaptation acts also subtractive on the input directly

\[
f(t) \approx f_0(I - A).
\]

The model (6.7) used here is a variant of the original model (4.22) of encoder adaptation. It accounts phenomenologically for the observed spike-frequency adaptation. The assumption of this model that \( A_\infty(I) \) is proportional to the firing frequency \( f \) is at least satisfied for the linear part of the steady-state \( f-I \)-curve, since such linear adaptation keeps
linear $f$-$I$-curves linear as shown in section 4.3.2 on page 85. However, the model (6.7) does not reproduce the decreasing saturation of the adapted $f$-$I$-curves. It is valid only for stimuli which are close to the receptor’s threshold. This restriction is not a problem for the examples shown in Fig. 6.8, Fig. 6.9, and 6.10, since these stimuli stay in the lower part of the $f$-$I$-curve.

**Adaptation and the distribution of stimulus amplitudes**

By means of the adaptation model (6.7) the time course of the state of adaptation, which shifts the neuron’s $f$-$I$-curve, for particular stimuli was accessible. The effective stimulus for a specific neuron has been calculated by subtracting the state of adaptation from the stimulus intensity (Fig. 6.11). Therefore, it was possible to compare the distribution of stimulus amplitudes with and without adaptation with the neuron’s onset $f$-$I$-curve. Adaptation made the stimuli for the neuron effectively softer without changing the shape of the amplitude distribution too much. Thus, spike-frequency adaptation only compensates for the mean intensity of a stimulus. It does not change the slope of the $f$-$I$-curve in order to adapt to the standard deviation of the stimulus, as proposed for modulatory mechanisms (Shin et al., 1999). Note that these results depend on the time scales of the stimulus in comparison to the adaptation time constant. The stimuli of the examples shown have most of their power in frequency components which are much faster than the adaptation process. The situation is different for stimuli which vary on a time scale which is comparable to the spike-frequency adaptation.

**Song recognition**

What is the role of spike-frequency adaptation in auditory receptor neurons for the transmission of calling songs of the grasshoppers? Adaptation shifts the neuron’s $f$-$I$-curve according to the mean intensity of the stimulus. First, this keeps the louder parts of the stimulus in the dynamic range of the receptor neuron. Thus, the substructure of syllables of the songs can be encoded in different firing frequencies. Second, gaps in the signal become more distinct since softer parts of the stimulus are effectively shifted below the neuron’s threshold. This helps to suppress the influence of background noise on the detectability of gaps (c.f. chapter 4). Third, the neuron recovers from adaptation during pauses in the signal. Thus, the onset of the next syllable is more pronounced. However, the capabilities of the receptor neurons to adjust their $f$-$I$-curve according to the mean intensity of a stimulus are limited. Their $f$-$I$-curves can only be shifted over about 10 dB as shown in the Results. Römer (1976) investigated the distribution of threshold intensities of receptor neurons. The thresholds of low-frequency receptors are distributed in three clusters over a range from 20 up to 60 dB. Therefore, it is likely that there are always some receptors which are stimulated in their dynamic range. It would be interesting to explore how an interneuron which receives input from the receptor neurons uses them. Response properties of such an interneuron are investigated in a cricket species in the next chapter.
6.5 Summary

- Response properties of auditory receptor neurons were studied in *Locusta migratoria*.

- Spike-frequency adaptation in receptor neurons has a maximum effective time constant of about 80 ms.

- The dependence of the effective time constants on stimulus intensity and the time constant of recovery from adaptation, as well as the shape of the adapted $f$-$I$-curves in relation to the onset $f$-$I$-curve show that spike-frequency adaptation is mainly caused by intrinsic mechanisms of the spike encoder.

- A variant of the model for encoder adaptation (6.7) predicts the firing frequency for arbitrary stimuli with high accuracy.

- The width of the onset $f$-$I$-curves decreases with increasing threshold intensity. This probably implies that receptor neurons perceive the amplitudes of sound waves not in logarithmic units.

- Auditory receptor neurons are class-I neurons:
  - They can fire with arbitrary low frequencies.
  - Latencies to the first spike are of the order of magnitude of the corresponding interspike interval from the onset $f$-$I$-curve and can be arbitrary long.
  - Perturbations always advance the following spike. The resulting phase-resetting curves are similar to the one of the $\theta$-model.

- A single stronger oscillation of the carrier sound-wave is sufficient to advance the following spike.