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Adaptation in pheromone-sensitive trichoid sensilla of the hawkmoth *Manduca sexta*

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Summary

In extracellular tip recordings from long trichoid sensilla of male *Manduca sexta* moths, we studied dose–response relationships in response to bombykal stimuli of two different durations in the adapted and the non-adapted state. Bombykal-responsive cells could be distinguished from non-bombykal-sensitive cells in each trichoid sensillum because the bombykal-responsive cell always generated the action potentials of larger initial amplitude. The bombykal cell, which was recorded at a defined location within a distal flagellar annulus, can resolve at least four $\log_{10}$-units of pheromone concentrations but is apparently unable to encode all stimulus durations tested. Parameters of the amplitude-modulated sensillar potential and the frequency-modulated action potential responses were examined in different states of adaptation. Evidence is presented for the existence of several mechanisms of adaptation, which affect distinct steps of the transduction cascade. After adapting pheromone stimuli, the sensillar potential rises to a lower amplitude and declines faster compared with the non-adapted response. In addition, the frequency of the adapted action potential response is reduced. Only the time of rise of the sensillar potential is differentially affected by adapting pheromone stimuli of different duration. The time of rise does not increase after short, but only after long, adapting stimuli. Both short and long adapting stimuli shift the dose–response curves of the sensillar potential amplitude, as well as the initial slope of its rising phase, to higher stimulus concentrations by approximately one $\log_{10}$-unit. The shift in the dose–response curve of the action potential response is larger than for the sensillar potential response, suggesting that an additional adaptation mechanism acts at the level of action potential generation. Furthermore, a faster decline of the sensillar potential after short and long adapting stimuli suggests that the resting potential of the olfactory receptor neuron is stabilized.

Key words: *Manduca sexta*, insect olfaction, adaptation, pheromone sensillum, tip recording, bombykal, action potential, sensillar potential.

Introduction

While the transduction cascade of pheromone-sensitive olfactory receptor neurons (ORNs) in insects has been thoroughly investigated *in vitro* (Breer et al., 1988, 1990; Boekhoff et al., 1990, 1993; Stengl et al., 1992; Stengl, 1993, 1994; Wegener et al., 1993, 1997), little is known about olfactory adaptation and the mechanisms involved. Adaptation is a universal characteristic of receptor cells of all sensory modalities (Burkhardt, 1961). There are different definitions for the term ‘adaptation’ but implicit in any definition is a decrease in sensitivity due to the influence of a previous (conditioning) stimulus (Zack, 1979).

Moths distinguish pheromone mixtures according to the concentration ratios of the different pheromone components. Thus, differentiation of pheromone concentrations is very crucial for recognition of prospective mates. Turbulences and wind velocities determine the structure of the pheromone filaments that stimulate the antenna of a flying moth. Thus, adapting and non-adapting pheromone stimuli of variable concentrations and stimulus durations reach different parts of the antenna at various time intervals. It is still unknown how the moth can recognize relevant pheromone ratios in various states of adaptation. In our study of pheromone-sensitive ORNs, we examine how adapting pheromone stimuli affect the encoding of different pheromone concentrations (quantity coding) in the intact moth. We distinguish the rapidly (within seconds to minutes) reversible reduction of sensitivity due to prior stimulation (short-term adaptation) from the decline in excitation, as seen during a phasic–tonic response to a stimulus of long duration (desensitization; Zufall and Leinders-Zufall, 2000). Thus, we compare quantity coding in response to short (50 ms) and long (1000 ms) pheromone stimuli, as possibly encountered during flight to the calling female. We do not examine the more slowly occurring (within several minutes to hours) reduction of sensitivity due to

previous strong stimulation as seen during long-term (i.e. long-lasting) adaptation (Ziegelberger et al., 1990; Marion-Poll and Tobin, 1992; Boekhoff et al., 1993; Stengl et al., 2001; Dolzer, 2002).

Quantity coding in response to adapting pheromone stimuli has been studied in extracellular tip recordings in saturniid moths, in which the different terms 'short-term' *versus* 'long-term' adaptation were coined for insect olfaction (Zack, 1979; Zack-Strausfeld and Kaissling, 1986; Kaissling et al., 1986, 1987). Concerning ORNs of the hawkmoth *Manduca sexta*, however, there is only one study about olfactory adaptation in temporal coding of pheromone pulse trains (Marion-Poll and Tobin, 1992) and a few studies on quality odour coding (Kaissling et al., 1989; Kalinová et al., 2001).

Moths detect the pheromones using specialized ORNs, which innervate long multiporous trichoid sensilla on the antenna in pairs (Keil, 1989). It has been shown that in each trichoid sensillum, one of the ORNs (the bombykal cell) responds to bombykal, the main component in the conspecific pheromone blend (Starratt et al., 1979; Tumlinson et al., 1989). The second cell (the non-bombykal cell) is tuned to other different pheromone components in different sensilla. While the study by Kaissling et al. (1989) examined the coding of different odour qualities, not much attention was paid to the coding of odour concentrations. Quantification of pheromone responses in *M. sexta* was not reliable, because at that time it could not be consistently distinguished whether an action potential originates from the spontaneously active non-bombykal cell or from the bombykal cell. A more recent study of trichoid sensilla in *M. sexta* could distinguish two different nerve impulse classes, termed the small and the large action potential classes, by amplitude (Dolzer et al., 2001). However, it was still not completely resolved whether the bombykal cell always generates the large or the small action potentials.

Thus, the present study first examines which action potential class the bombykal cell from a specific region of a distal flagellar annulus belongs to, since a clear distinction of both cells is a prerequisite for an unequivocal quantification of the bombykal responses. Then, we examine how a previous strong stimulus (a conditioning stimulus) can adapt and thus change the coding of bombykal concentrations at the level of the sensillar potential and the action potential response and whether these stimuli affect flight behaviour. In dose–response curves to short (short-term adaptation) and long (desensitization) bombykal stimuli, we show that the bombykal cell can resolve four $\log_{10}$-units of pheromone concentrations but is apparently unable to encode stimulus duration with all parameters tested. Finally, we present evidence for the presence of several functionally distinct mechanisms of short-term adaptation, which affect the rising phase of the sensillar potential, its decline and the action potential response. In addition, we show at least one additional mechanism of desensitization. None of these bombykal stimuli affected flight behaviour.

Materials and methods

Animals and preparation for electrophysiological recordings

Manduca sexta (Johannson) moths (Lepidoptera: Sphingidae) were raised from eggs, feeding on an artificial diet (modified from Bell and Joachim, 1976). The animals were kept under a long-day photoperiod (17 h:7 h L:D; lights off at 8.00 h) at 24–27°C and 40–60% relative humidity. Male pupae were isolated one day before emergence, gently cleaned with 70% ethanol and allowed to hatch without contact with pheromone. During their second dark phase, the adults were carefully fixed in a teflon holder without the necessity for anaesthesia. The flagellum of the right antenna was immobilized with dental wax (Boxing wax; Sybron/Kerr, Romulus, MI, USA), and the 15–20 most-apical annuli of the flagellum were clipped off. A glass electrode filled with haemolymph Ringer (Kaissling, 1995) was inserted into the flagellar lumen and sealed with electrocardiogram electrode gel (PPG; Hellige, Freiburg, Germany). The tips of long trichoid sensilla from the apical row on the 2nd–10th remaining distal annulus were removed using sharpened forceps. The recording electrode, filled with sensillum lymph Ringer (Kaissling, 1995), was slipped over one sensillum. To minimize contributions of the electroantennogram, we recorded from an annulus close to the tip of the haemolymph electrode. The connection to the amplifier inputs was established with Ag/AgCl wires immersed in the electrolytes. Signals were amplified approximately 200-fold in a custom-built amplifier (0 Hz–2 kHz; input impedance $10^{12}\ \Omega$) and passed through a 2 kHz anti-aliasing filter (900C/9L8L; Frequency Devices, Haverhill, MA, USA). Flight activity of the animals was monitored using a piezo-electric element (PI Ceramic, Lederhose, Germany) placed at the thorax. For data acquisition, a Digidata 1200 B digitizer (Axon Instruments, Union City, CA, USA) and pCLAMP software (versions 6–8) from the same manufacturer were used. The electrophysiological signal and a highpass-filtered equivalent (cut-off frequency 2 Hz or 5 Hz), as well as the piezo signal, were continuously recorded on a strip chart recorder (WindoGraf or EasyGraf, Gould, Valley View, OH, USA). Sections of the recordings were additionally stored on FM tape (Store 4 D; RACAL, Bergisch Gladbach, Germany) or digital audio tape (DTR-1202, Bio-Logic, Claix, France). Voltage polarity is given with the sensillum lymph electrode in reference to the haemolymph electrode. After the recordings, >90% of the moths were in sufficiently good condition to be returned to the flight cage.

Cartridge stimulation

All recordings were performed at room temperature (18–23°C). Charcoal-filtered and moistened air was permanently blown over the preparation through a glass cartridge ($13\ l\ min^{-1}$). The airstream could be redirected through cartridges containing a piece of filter paper (approximately $1\ cm^2$) loaded with synthetic bombykal (E,Z-10,12-hexadecadienal) generously provided by T. Christensen (Tucson, AZ, USA). Bombykal doses are always given in

$\log_{10}$-unit intervals; for example, in Fig. 9, –6 $\log_{10}$ bombykal dose (μg) means 10^{-6} μg bombykal. The cartridges were placed with the outlet in a distance of 4.5–6 cm from the recording site. The airstream was switched between the cartridges using solenoid valves (JFMH-5-PK and MFH-5-1/8; FESTO, Esslingen, Germany) controlled by the computer. Airstream velocity was monitored with a thermistor (BC32L1; Fenwal, Framingham, MA, USA) placed near the recording site and connected to a custom-built anemometer. Limited by the specified switching time of the solenoids (15 ms), and verified by the anemometer recording, the shortest applicable stimulus duration was approximately 50 ms. Doses between 10^{-6} μg and 100 μg bombykal dissolved in n-hexane (Merck, Frankfurt, Germany) were applied to the filter papers (10 μl or 100 μl per paper), and the solvent was allowed to evaporate. Stimulus intensity is always given in terms of the bombykal dose applied to the filter paper. Stimulus durations between nominally 10 ms and 1000 ms were employed. Due to the switching times of the solenoid valves, stimulus durations below 50 ms are less clearly defined than above this duration and are only included for completeness.

A suction tube of 10 cm diameter was placed below the animal to rapidly remove the pheromone after the stimulations and to avoid uncontrolled stimulation by bombykal leaking out of the stimulus cartridges, which were put in place as short as possible (5–30 s) prior to stimulation. Between the recording sessions, the cartridges were stored at –80°C in individual glass scintillation vials. Control cartridges loaded with hexane alone were prepared and treated the same way. When stored together with the vials containing the bombykal cartridges, the control cartridges elicited weak responses after several recording sessions, indicating a cross contamination that is also likely to have occurred for the lower stimulus doses (Figs 7–9). This effect is presumably enhanced by molecules of the highly hydrophobic pheromone component adhering to parts of the electrophysiological set-up. These contaminations influence the baseline of the dose–response curves, as well as the peri-stimulus-time histograms at low pheromone doses, but they do not affect the main conclusions of our study. A set of stimulus cartridges was used for 5–20 recording sessions; the control cartridges were replaced more frequently.

Local stimulation

While cartridge stimulation, as described above, was used in most of the experiments, a small subset of the sensilla was stimulated locally. In these experiments, the pheromone was applied to a piece of thread, which was then inserted into a glass pipette with an opening of approximately 40 μm (Kaissling, 1995). The stimulation pipette was placed below the recorded sensillum, and the stimulus airstream was redirected through the pipette for stimulation. The only dose tested with local stimulation was 1 μg bombykal; stimulus durations were between 20 ms and 2000 ms. Of the data presented here, only the recordings shown in Fig. 10 were obtained using local stimulation; all other data and results were obtained with cartridge stimulation.

Acquisition protocols and data analysis

In the beginning of each recording, a series of 5 mV calibration pulses was applied to the haemolymph electrode, which was otherwise grounded. The pheromone responses were recorded in sweeps of 3 s duration at sampling frequencies of 5 kHz and 1.67 kHz (Clampex, episodic stimulation mode with a gear shift after 1000 ms). The solenoids were controlled by a digital output signal switched to ‘high’ 20 ms after the sweep start. The stimulus airstream, as monitored by the anemometer, arrived at the recording site approximately 50 ms after the trigger signal.

The recordings were evaluated using macros in Clampfit 6, Microsoft Excel, versions 7 and 8, and Automate 4 (Unisyn Software; Los Angeles, CA, USA) (Dolzer, 2002). For the analysis of the sensillar potential, the responses were lowpass-filtered at a cut-off frequency of 50 Hz or 70 Hz (Clampfit, Gaussian filter). The evaluated parameters of the sensillar potential, as illustrated in Fig. 1A,B, were: (1) the overall amplitude (SP amplitude), (2) the initial slope between the onset of the sensillar potential and the half-maximal SP amplitude (slope), (3) the half-time of the rising phase ($t_{1/2\ \mathrm{rise}}$) and (4) the half-time of the declining phase ($t_{1/2\ \mathrm{decline}}$). The half-times of the rising and declining phases were analyzed to compare our results with studies on silkmoths (*Antheraea* sp.; Zack, 1979; Kaissling et al., 1987; Kodadová, 1993; Kodadová and Kaissling, 1996), and the initial slope was analyzed as an important parameter describing the kinetics of the sensillar potential.

For the analysis of the action potentials (Fig. 1C), the lowpass-filtered trace was subtracted from the original response. This pseudo-highpass filtering procedure, in contrast to actual highpass filters, does not distort the shape of the action potentials and therefore allows the analysis of their amplitude and waveform (Dolzer, 2002). The action potential response was characterized by: (1) the peak frequency computed from the first five interspike intervals (AP frequency; Fig. 1C) and (2) the latency between the beginning of the sensillar potential and the first action potential (AP latency; Fig. 1B). In the background activity, action potentials of two amplitude classes were recorded (Dolzer et al., 2001). Therefore, when action potentials of two classes, distinct by amplitude or by frequency and latency, were observed during the response, they were analyzed separately. Because responses of trichoid sensilla from different intact moths varied greatly in amplitude (Table 1), it was impossible to quantify these variable data sets without normalization. Thus, for the quantitative analysis, the response parameters were normalized to the highest response during the recording from the same sensillum. The highest response was defined as the largest value of those parameters that were positively correlated to the stimulus intensity and as the smallest value of parameters, which were negatively correlated to the stimulus intensity. Thus, the normalized responses were computed from: $\mathrm{Response}_{\mathrm{norm}} = \mathrm{Response}/\mathrm{Response}_{\mathrm{max}}$ and $\mathrm{Response}_{\mathrm{norm}} = \mathrm{Response}_{\mathrm{min}}/\mathrm{Response}$, respectively. The sensillar potential amplitude, the initial slope of the SP, $t_{1/2\ \mathrm{decline}}$ and the action

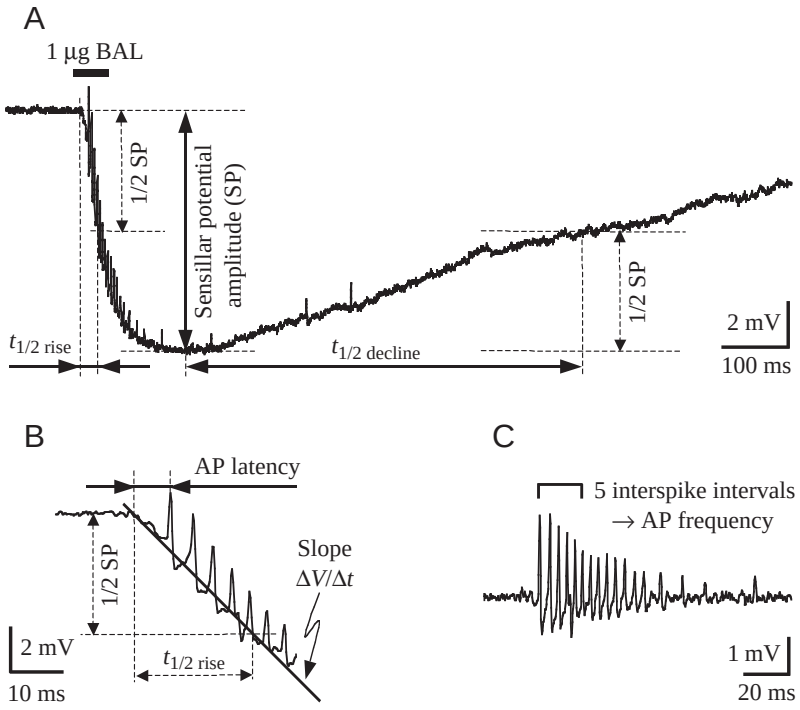

Fig. 1. The pheromone response is characterized by six parameters. (A) An unfiltered response to a 50 ms stimulus of 1 µg bombykal (BAL). Action potentials are superimposed on the negative deflection of the transepithelial potential, the sensillar potential (SP). The maximal SP amplitude is measured between the baseline before the response and the negative peak during the response. The half-time of the rising phase ($t_{1/2\ rise}$) is determined between the onset of the SP and the time the potential has reached 50% of SP. The half-time of the decline phase ($t_{1/2\ decline}$) is measured between the end of the response, which at short stimulus duration coincides with the negative peak, and the time the potential has decayed to 50% SP. For the analysis of all parameters describing the SP, the responses are lowpass-filtered at 50 Hz or 70 Hz. (B) The initial phase of the response at an enlarged time scale. The initial slope is determined by dividing 0.5×SP by $t_{1/2\ rise}$. The action potential (AP) latency is measured between the onset of the SP and the peak of the first AP. (C) For the analysis of APs, the lowpass-filtered response is subtracted from the original trace, yielding a straight baseline. The initial AP frequency is computed from the first five interspike intervals.

Table 1. *Variability of unadapted responses to strong stimulation*

	Mean	Median	S.D.	S.E.M.	Min.	Max.
DC						
SP amplitude (mV)	10.10	10.07	3.45	0.53	3.92	17.02
Initial slope (µV ms^{-1})	157	164	78	12	50	412
$t_{1/2\ rise}$ (ms)	34	35	9	1	13	58
$t_{1/2\ decline}$ (ms)	897	788	344	53	435	1847
AC						
AP frequency (Hz)	278	296	81	12	99	423
AP latency (ms)	33	27	15	2	5	72

N=73 responses of 43 sensilla. Bombykal dose, 10 µg; stimulus duration, 50 ms.

DC, variables of the sensillar potential (SP); AC, variables of the action potential (AP); $t_{1/2\ rise}$, half time of the rising phase of the sensillar potential; $t_{1/2\ decline}$, half-time of the decline phase of the sensillar potential.

potential response were normalized to the largest value during each recording from the same sensillum. Since $t_{1/2\ rise}$ and AP latency were negatively correlated with stimulus strength, both were normalized to the smallest value during one recording. The inverse normalization of the negatively correlated parameters ($t_{1/2\ rise}$ and AP latency) allowed us to focus on responses in the physiological range. Direct normalization of, for example, AP latency, either to the largest or smallest value of the data set, exaggerates long latencies in response to low stimulus intensity and obscures differences in the response to physiological stimuli of a higher dose. For the investigation of desensitization, stimuli of 1000 ms duration were applied with 60 s between consecutive stimulations. The action potential response characteristics were analyzed with peri-stimulus-time histograms to evaluate changes in the action potential frequency over time. In time windows (bins) of 10 ms, action potential responses were added up and plotted over time, with *t*=0 being the start of the DC response.

Stimulation protocols

In total, 1462 stimulations (all doses including controls) were applied to 70 sensilla of 42 animals. Since the experiments were done with a whole-animal preparation, no rundown of the responses was observed, even during recordings of more than 3 h with repetitive stimulation.

Non-adapted responses were obtained by applying either 50 ms stimuli or 1000 ms stimuli of increasing bombykal dose, separated by 60 s (dose ramp). Control stimuli with only hexane on the filter paper were applied before each dose ramp. In some recordings, additional control stimuli were applied.

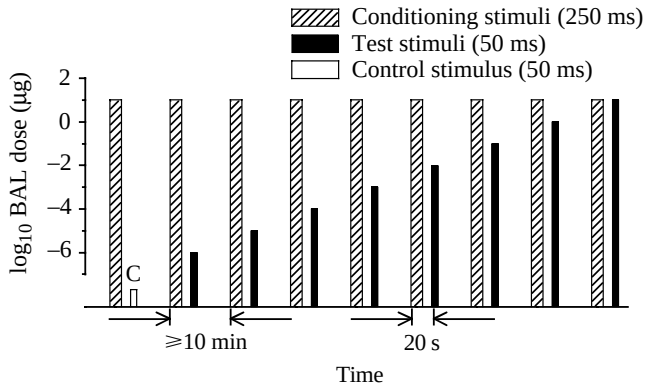

Fig. 2. The adaptation protocol for short-term adaptation. A conditioning stimulus of 10 µg bombykal (BAL) and 250 ms duration was applied 20 s before each test/control stimulus of 50 ms duration. Test stimuli of various BAL doses (measured in $\log_{10}$-units; filled columns) or the control (C; open columns) were applied in increasing order. To avoid accumulative adaptation, stimulus pairs were separated by at least 10 min. The time axis is not drawn to scale. For test stimuli of 1000 ms duration, a similar stimulation protocol was used. The conditioning stimuli were 1000 ms long, however, and applied 60 s before the test stimuli.

Adapted responses were achieved by conditioning the sensilla with 10 µg stimuli of 250 ms duration, applied 20 s before the test stimuli of 50 ms duration (Fig. 2). For the 1000 ms stimuli, the sensilla were adapted by 10 µg stimuli of 1000 ms, applied 60 s before the test stimuli. To ensure identical conditions, each test stimulus was preceded by its own conditioning stimulus. The sensilla were allowed to recover for at least 10 min between every two stimulus pairs to avoid accumulative adaptation. This interval between the stimulus pairs was chosen because pilot experiments had suggested the complete recovery from adaptation by a stimulus as used in the adaptation protocol after approximately 5 min (Dolzer, 1996). To avoid contamination of our short-term adaptation experiments by long-term adaptation (which lasts for hours), we did not employ pheromone doses higher than 10 µg bombykal for the 50 ms stimuli and we used a maximal dose of 100 µg for the stimuli of 1000 ms duration.

Results

In extracellular tip recordings from long trichoid sensilla, we investigated odour quantity coding by measuring the electrical responses to stimulation with bombykal at different doses. Slow, amplitude-modulated bombykal-dependent sensillar potentials with superimposed, fast, frequency-modulated action potentials were quantified. We wanted to know whether the previous exposure to a conditioning stimulus or the duration of bombykal stimuli affect quantity coding and influence flight behaviour. The responses of, in total, 70 sensilla of 42 animals to bombykal doses between 10^{-6} µg and 100 µg were characterized and compared in different states of adaptation. Most experiments were only performed on a subset of the sensilla.

General parameters of the pheromone response

Bombykal application causes depolarizing sensillar potentials in olfactory receptor neurons (ORNs), which elicit action potential responses that are carried along the axons of the ORNs to the brain to excite postsynaptic antennal lobe neurons. In extracellular tip recordings, the sensillar potential (SP) in response to pheromone stimulation is measured as a negative deflection of the transepithelial potential. According to previous practice and because the SP is assumed to reflect the depolarizing receptor potential of the ORNs, its increase to maximal amplitude is described as the SP's rising phase. Its repolarization to the steady-state potential is described as its decline. Superimposed on the SP, action potentials can be recorded (Figs 3–6). The amplitude of action potentials decreased in response to strong stimulation (Figs 1C, 3–5). When action potentials of two classes occurred during a response (Figs 3B, 4A,B), they are referred to as small and large action potentials according to the amplitude of the first action potential, i.e. before the stimulus-correlated amplitude reduction took place. This terminology was introduced previously in

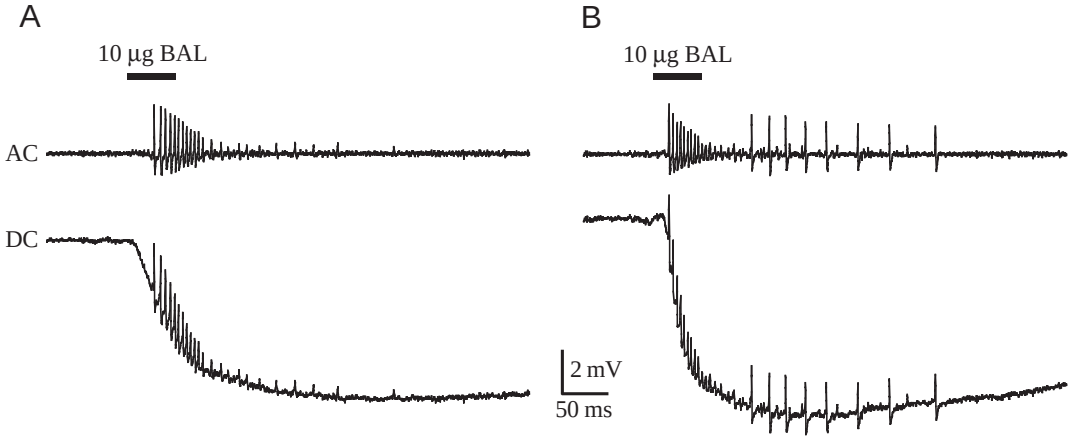

Fig. 3. Cross-excitation of the non-bombykal cell occurred in response to a subset of the 10 µg bombykal (BAL) stimuli of 50 ms duration (see Table 2). While in (A) BAL stimuli elicited only large action potentials, in (B) small action potentials were also elicited at a lower frequency and after a longer latency. Responses of small action potentials were not elicited at BAL doses below 10 µg. The terms 'large' and 'small' action potentials refer to the amplitude of the first action potential of each class, before the stimulus-correlated amplitude reduction took place. AC: pseudo-highpass-filtered traces, obtained by subtracting lowpass-filtered responses (50 Hz Gaussian filter) from the original signals (DC).

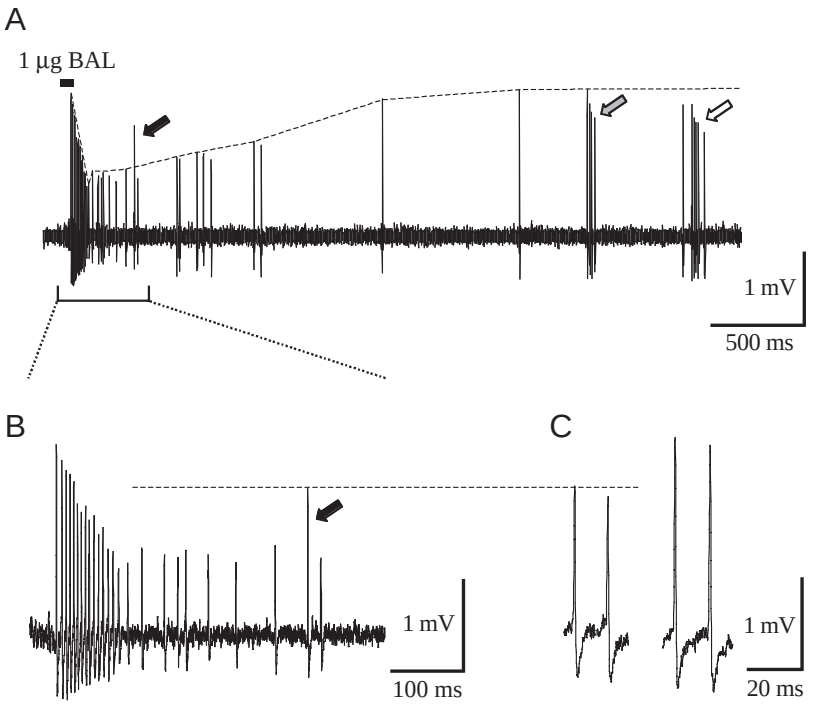

Fig. 4. At doses below 10 µg bombykal (BAL), only action potentials of the large class were affected by the stimulus-correlated amplitude reduction. (A) Response to a 50 ms stimulus of 1 µg BAL, pseudo-highpass-filtered as in Fig. 3. The amplitudes of the large action potentials were reduced after strong bombykal stimuli and regained their original amplitude in the course of several seconds (as illustrated by the broken line). Occasionally, spontaneous small action potentials occurred during (filled arrow) or after (open arrow) the response. The reduction of the amplitude during bursts of large (shaded arrow) and small (open arrow) action potentials was reported previously (Dolzer et al., 2001). (B) Enlarged view of the marked section in A. (C) Two small and two large spontaneous action potentials recorded before the stimulation, plotted at the same amplitude scaling as in B. The small action potential during the response was of the same amplitude as the small spontaneous action potentials.

recordings of spontaneously active ORNs (Dolzer et al., 2001). Action potentials of two amplitude classes were also observed between the stimulations. All action potentials, whether spontaneous or in a pheromone response, were recorded with their positive phase first. There was a large variability in both the sensillar potential and action potential responses among individual sensilla from different moths (Table 1). Sixty-nine of the 70 sensilla tested responded to bombykal. The only non-responding sensillum had no spontaneous action potential activity, indicating that it was damaged.

To determine whether stimulation with the different stimuli of the main pheromone component bombykal alone elicits flight activity in the intact, tethered moth, flight activity was recorded with a piezo-electric element placed at the thorax. In addition, since a correlation between flight activity and transepithelial potential oscillations was observed previously (Dolzer et al., 2001), the transepithelial potential was continuously monitored between the stimulations. In 18 of 22 animals analyzed, bombykal stimulation did not elicit flight activity. In three cases, the animals exhibited continuous flight activity. In only one animal, flight activity occurred immediately after strong stimulation, whereas the animal was silent before. Oscillations of the transepithelial potential were observed in the recordings from 16 sensilla. In no case did bombykal stimulation suppress or detectably influence the oscillations.

Specificity of the ORNs

Because the two ORNs per trichoid sensillum can be clearly distinguished according to the amplitude of their action potentials, we wanted to know whether the bombykal cell always produces the large or the small action potentials. Unless the stimulus dose exceeded 10 µg, only action potentials of the large amplitude class were observed during the bombykal responses. When small action potentials occurred at these dosages, they could not be distinguished from spontaneously occurring action potentials by their frequency or reduction of the amplitude. At doses of 10 µg bombykal, however, the small action potentials occurred at a frequency that could not be considered spontaneous in 22 of 68 stimuli of 50 ms duration (Fig. 3; Table 2). Apparently, the non-bombykal cell was ‘cross-excited’. In the presence of small action potentials, the SP amplitude was significantly larger (10.85±0.64 mV, N=22) than in responses with only large action potentials (8.75±0.50 mV; means ± S.E.M.; P<0.01, N=46; Student’s t-test). The action potential frequency of the small action potentials was significantly lower and the action potential latency was significantly longer than for the large action potentials of the bombykal cell (Table 3).

When cross-excitation occurred during a response to 10 µg bombykal, the stimulus-correlated amplitude reduction of the small action potentials was less prominent than that of the bombykal cells (Fig. 3B). At lower doses, the amplitude and frequency of small action potentials occurring during the response was the same as during spontaneous activity (Fig. 4).

Dose dependence

After distinction of the action potential responses of the bombykal and the non-bombykal cells, we measured dose–response relationships of the bombykal cell in response to 50 ms bombykal stimuli to determine the threshold, range of resolution and saturation (Figs 5, 7). The bombykal responses varied considerably among individual sensilla and among individuals (Table 1). Therefore, for a quantitative analysis it was necessary to normalize the response parameters

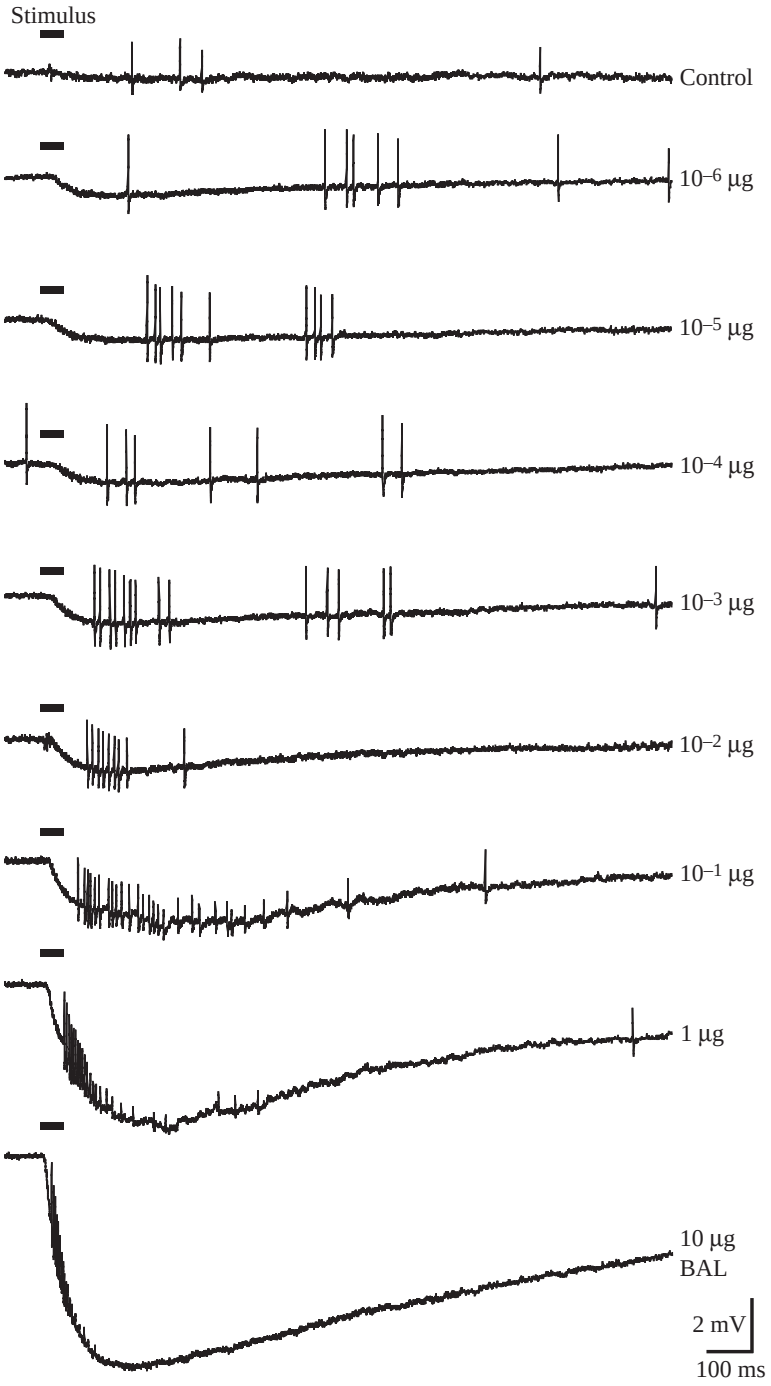

Fig. 5. Pheromone responses are dose-dependent. Stimulation with a solvent-loaded filter paper (control) did not elicit a measurable sensillar potential. The action potentials of the small class occurred at random. Stimulations with increasing doses of bombykal (BAL) elicited sensillar potentials of increasing amplitude, together with large action potentials at increasing frequency and shorter latency. At stimulus loads of $\geq 10^{-2}$ μg, the reduction of the action potential amplitude during the response became obvious. The stimulus duration was 50 ms and stimuli were applied in increasing order, separated by intervals of 60 s (dose ramp).

(see Materials and methods; Fig. 7). The SP amplitude, the initial slope of the SP and the half-time of the decay phase ($t_{1/2\ decline}$), as well as the action potential frequency and latency, but not the half-time of the rising phase ($t_{1/2\ rise}$), were dose-dependent (Figs 5–7). With higher pheromone concentrations, the amplitude of the SP increased, its initial slope became steeper and its decline to baseline became slower (Fig. 7). The action potential frequency increased, and the latency to the first action potential was shortened. The threshold was between 10^{-3} μg and 10^{-2} μg bombykal for all these variables. The ORN was able to resolve at least four $\log_{10}$-units of odour concentrations. The $t_{1/2\ rise}$ exhibited no dose dependence (Fig. 7C).

Short-term adaptation

To evaluate changes in quantity coding after previous pheromone experience, the sensilla were short-term adapted. Short-term adaptation is defined here as a rapidly (within seconds to minutes) reversible reduction of sensitivity due to prior stimulation with a conditioning stimulus. Short-term adaptation is distinguished from long-term adaptation, which lasts for hours and involves rises of intracellular cyclic GMP (cGMP) concentrations (Stengl et al., 2001). When the sensilla were adapted by a strong conditioning stimulus (10 μg bombykal; 250 ms duration) 20 s prior to the test stimulus, the SP amplitude and the action potential frequency were reduced (Figs 6, 7A,E). In addition, the decay of the sensillar potential was accelerated (Figs 6B, 7D), as characterized by a faster $t_{1/2\ decline}$. The dose–response curves of the SP amplitude and the initial slope were shifted to higher stimulus intensities by approximately one $\log_{10}$-unit (Fig. 7A,B), which means that 10 times more pheromone was needed to elicit the same response amplitudes. The $t_{1/2\ rise}$ was virtually dose-independent at a stimulus duration of 50 ms and was not significantly altered by the adapting stimulus (Figs 6B, 7C). The dose–response curve of the action potential frequency was shifted by two $\log_{10}$-units, and the shift of the dose–response curve of the latency between the onset of the SP and the first action potential was even larger (Fig. 7E,F). For none of the variables was there an obvious difference in the slopes of the adapted and the non-adapted dose–response curves.

Desensitization

Desensitization is the decline in excitation as seen during a phasic–tonic response to a stimulus of long duration and, thus, is distinct from short-term adaptation (Zack, 1979; Zufall and Leinders-Zufall, 2000). To determine whether the stimulus duration affects coding of pheromone quantity in the non-adapted and adapted state, we employed pheromone stimuli of 20 times longer durations. With stimulations of 1000 ms duration in the absence of a preceding adapting stimulus (N=129), the action potential response was phasic–tonic at all

Table 2. *Cross-excitation of the non-bombykal cell*

Bombykal dose (μg)	1		10	
Duration (ms)	50	250	50	250
No. of responses	108	14	68	69
With non-BAL APs	0	0	22	29
Only BAL APs	108	14	46	40

BAL, bombykal; APs, action potentials.

Table 3. *Frequency and latency of large and small action potentials*

Action potentials	Large		Small
Frequency (Hz)	319±10	–**–	105±10[a]
AP latency (ms)	23±2	–**–	79±5

Data are means ± S.E.M.; stimulus duration, 50 ms; bombykal dose, 10 μg; N=22 [except [a], where N=18: in four responses, the action potential (AP) frequency over five interspike intervals could not be determined, since <6 small action potentials occurred]. **P<0.01 (Student's t-test).

doses between 10^{-6} μg and 100 μg bombykal (Fig. 8). The phasic component was more prominent with increasing pheromone dose. Thus, this stimulus duration was not reliably encoded by the phasic part of the action potential response. After the phasic peak, the action potential frequency declined to a tonic plateau with a time constant of approximately 150 ms. Thresholds were between 10^{-2} μg and 10^{-1} μg bombykal for all variables, and approximately four $\log_{10}$-units of bombykal concentrations were resolved (Fig. 9).

In contrast to short stimuli of 50 ms duration, in response to 1000 ms stimuli, the $t_{1/2\ rise}$ was dose-dependent at higher doses of bombykal in the non-adapted state as well as in the adapted state (Fig. 9C; Dolzer, 1996). As for short stimuli, after a preceding adapting stimulus of 10 μg bombykal, the amplitude of the sensillar potential was decreased, the initial slope was less steep and the frequency of the action potential response was reduced (Fig. 9A,B,E). In contrast to short stimuli, the $t_{1/2\ rise}$ was further prolonged after an adapting stimulus, the decline not further accelerated at all stimulus concentrations and the action potential latency not further increased at all high pheromone concentrations (Fig. 9C,D,F).

Encoding stimulus duration

After testing responses to different stimulus concentrations, we examined how the bomykal cell can encode different stimulus durations. The stimulus duration of 50 ms was neither encoded by the sensillar potential nor by the duration of the phasic or tonic part of the action potential response (Figs 5, 10). When different stimulus durations from 20 ms to several seconds were tested (at a dose of 10 μg with an interstimulus interval of 60 s), none of the parameters of the sensillar potential reliably encoded all stimulus durations (Figs 10, 11). Only stimulus durations of >100 ms were distinguished by $t_{1/2\ decline}$ and by the total number of action potentials, but not by the SP amplitude, $t_{1/2\ rise}$ and action potential frequency (i.e. the first five interspike intervals; Fig. 11).

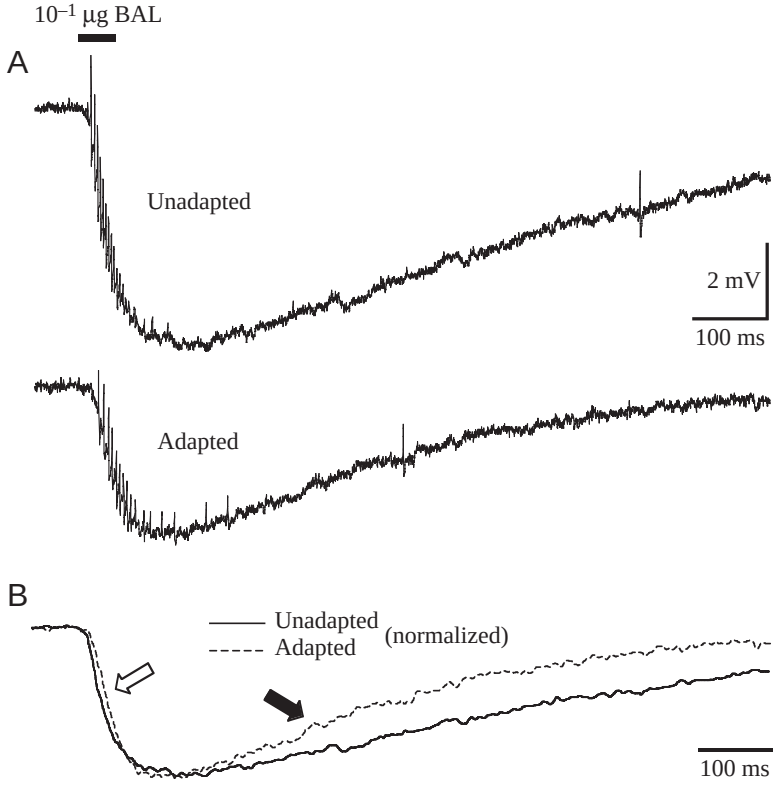

Fig. 6. A strong conditioning stimulus caused adaptation of the sensillar potential and action potential responses. (A) Compared with a response without a preceding strong stimulus (upper trace), the sensillar potential amplitude and the action potential frequency were reduced (lower trace) after an adapting stimulus. (B) After lowpass-filtering and normalizing both responses to the same maximal amplitude, the faster decline (filled arrow) to baseline of the sensillar potential in the adapted state (broken line) became obvious. But the half-time of the rising phase ($t_{1/2\ rise}$; open arrow) was less affected. Stimulus duration was 50 ms. BAL, bombykal.

Discussion

In extracellular tip recordings of long trichoid sensilla, we investigated the electrical responses to stimulation with bombykal, the main component in the conspecific pheromone blend. While previous studies on pheromone sensilla in *M. sexta* focused on the specificity of the olfactory receptor neurons (ORNs; Kaissling et al., 1989; Kalinová et al., 2001) and on temporal coding in the action potential response (Marion-Poll and Tobin, 1992), we characterized and compared the dose dependence of the sensillar

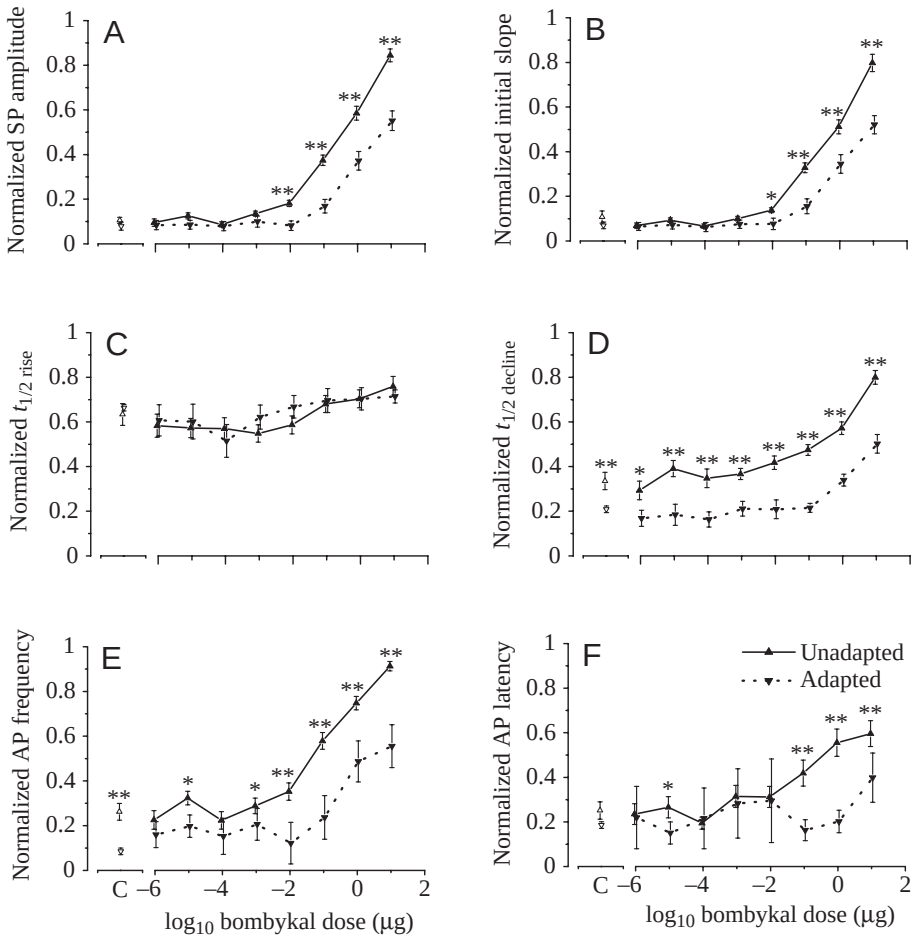

Fig. 7. Short-term adaptation of responses to short (50 ms) stimuli shifted the dose–response curves to higher stimulus intensities. While the sensillar potential (SP) amplitude (A) and the initial slope (B) encoded concentration changes at doses $\geq 10^{-2}$ µg bombykal, the half-time of the rising phase ($t_{1/2\ rise}$; C) showed no significant dose dependence. The decline of the SP back to baseline ($t_{1/2\ decline}$; D) was relatively slow, even at low doses. After adapting stimuli (dotted lines), the amplitude of the sensillar potential and its initial slope were reduced, while $t_{1/2\ rise}$ was not affected. Adaptation accelerated the decline of the SP and reduced the action potential frequency computed from the first five interspike intervals (AP frequency), while the action potential latency (AP latency) increased. After adapting stimuli, the dose–response curves of those parameters that describe the initial phase of the SP were shifted to the right by approximately one $\log_{10}$-unit. Thus, 10 times more pheromone was needed to elicit the same response (A,B). The decline, in addition to a right-shift by more than one $\log_{10}$-unit, was accelerated even in the baseline region of the dose–response curve (D). The AP frequency and the AP latency were shifted by more than one $\log_{10}$-unit (E,F). The data were normalized to the highest response during each recording, which is the largest numerical value for those variables positively correlated to the stimulus intensity. The action potential latency and $t_{1/2\ rise}$, which are negatively correlated to the intensity, were inversely normalized to the smallest numerical value to focus on responses in the physiological dose range. C, control. Data represent means ± S.E.M. The stimulus duration was 50 ms. N=31 (A–D; unadapted), N=24 (E,F; unadapted) and N=10 (A–F; adapted). Asterisks indicate significant differences between the adapted and non-adapted state (*P<0.05; **P≤0.01; Student's t-test).

potential and action potential responses in different states of adaptation. Because the bombykal cell in each sensillum always produced the action potentials of larger initial amplitude, distinct quantification of bombykal responses was possible. Thus, it could be shown that different mechanisms of adaptation occur in *M. sexta* at different levels of the transduction cascade: at the generation of sensillar potentials and at the transformation into action potentials. In responses to short and long pheromone stimuli, a previous strong bombykal stimulus decreased the amplitude and the initial slope of the sensillar potential and accelerated its decline. In addition, the initial action potential frequency was reduced even further than the parameters of the sensillar potential, suggesting that an additional adaptation mechanism acts at the level of action potential generation. At least one adaptation mechanism is stimulus-duration-dependent, because for long, but not short, pheromone stimuli the same adapting stimulus slowed $t_{1/2\ rise}$ at higher stimulus concentrations. Despite the fact that stimulus duration strongly affects flight behaviour when freely flying *M. sexta* moths are stimulated with the native pheromone blend (Vickers et al., 2001), none of the bombykal stimulus protocols changed flight activity of the moth. Whether these different adaptation mechanisms have a distinct molecular basis and resemble the mechanisms described in vertebrate olfaction (reviewed in Zufall and Leinders-Zufall, 2000) remains to be examined.

In vertebrate olfaction, three stages of adaptation are distinguished in current recordings *in vitro* by their effects and the underlying mechanisms: short-term adaptation, desensitization and long-lasting adaptation (Zufall and Leinders-Zufall, 2000). In vertebrates, short-term adaptation reduces the amplitude of brief odour-dependent currents after previous stimuli, is Ca^{2+}-dependent and cGMP-independent, and declines within several seconds. By contrast, desensitization, which reduces the amplitude and slows down the kinetics of the odour response during maintained stimulation, is Ca^{2+}-dependent and calcium/calmodulin-dependent protein kinase (CaM kinase)-dependent and lasts longer than short-term adaptation. Long-lasting adaptation, however, is triggered by stronger stimulation, is Ca^{2+}-, CO_2- and cGMP-dependent

and lasts many minutes. In the following, we will discuss the general characteristics of the bombykal cell in *M. sexta* and the different mechanisms of adaptation in moths in the context of known olfactory signal transduction pathways, to look for common features between insects and vertebrates and to suggest a testable hypothesis of olfactory adaptation in insects.

Specificity of the olfactory receptor neurons and behavioural aspects

As described previously (Dolzer et al., 2001; Kalinová et al., 2001), but in contrast to other studies in *M. sexta* (Kaissling et al., 1989; Marion-Poll and Tobin, 1992), two classes of action potentials, which can probably be assigned to the two ORNs in each sensillum (Keil, 1989), could be distinguished by their amplitude in most of the recordings. Our experiments showed that in *M. sexta*, as in other moth species investigated, the ORN that fires the action potentials with the larger amplitude responds to the main component of the conspecific pheromone blend (*Bombyx mori*, Kaissling et al., 1978; *Antheraea polyphemus* and *A. pernyi*, Zack, 1979; *Mamestra suasa*, Lucas and Renou, 1989; *Mamestra brassicae*, Renou and Lucas, 1994). Only at very high pheromone doses was the cell with the smaller action potential amplitude excited as well. Such cross-excitation was also described in *A. polyphemus* (Kodadová and Kaissling, 1996; Kodadová, 1993).

Interestingly, none of the sensillar potential parameters and neither the phasic nor the tonic part of the action potential response encoded all stimulus durations tested. Only longer stimulus durations were distinguished by the overall action

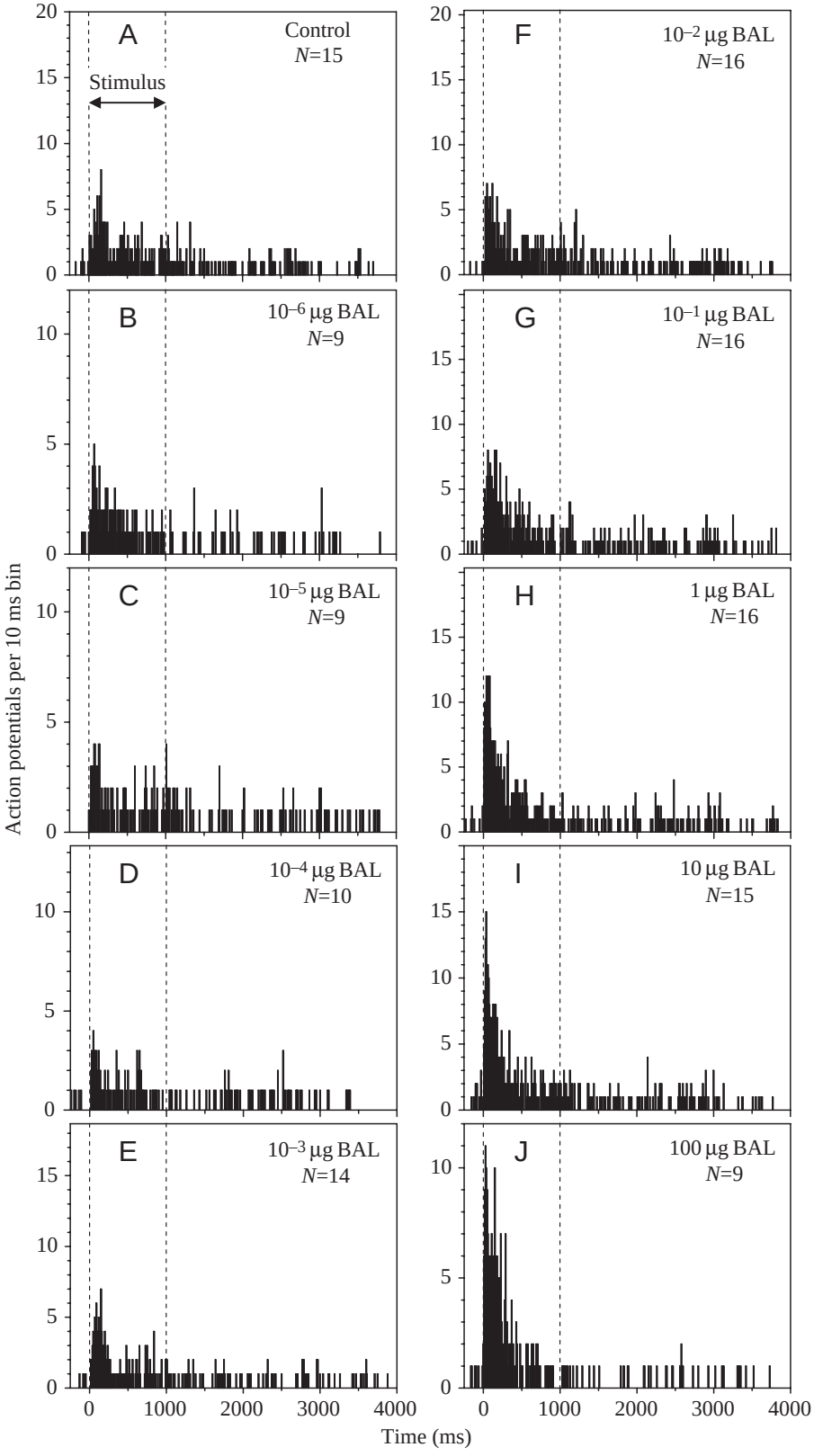

Fig. 8. (A–J) Desensitization of pheromone responses during long pheromone stimuli. The action potential response to long bombykal (BAL) stimuli became more phasic with increasing stimulus intensity. Peri-stimulus-time-histograms (PSTHs) at a bin width of 10 ms were calculated for action potentials elicited by 1000 ms stimuli of BAL, which are indicated by the broken lines. While phasic–tonic responses occurred at all stimulus intensities, the phasic component was more prominent at high intensities. This indicates the presence of desensitization. Since the sample sizes (*N*) differed for the individual doses, all *y*-axes were scaled to 4/3×*N*. The considerable responses elicited by control stimulations with solvent-loaded filter papers (A) are discussed in the text.

potential frequency but were not reflected by the duration of the action potential response. From analysis of the odour plume and from antennal lobe recordings, it is known that odour intensity and duration vary rapidly in nature and can be resolved by *M. sexta* antennal lobe neurons on a millisecond time scale (Christensen et al., 1996, 1998; Vickers et al., 2001). Thus, in their natural environment, moths have to resolve rapid changes in stimulus strength and duration but might not need to respond reliably to ongoing odour stimulation over a period of seconds to minutes.

In wind-tunnel assays, Tumlinson et al. (1989) found that both bombykal and (E,E,Z)-10,12,14-hexadecatrienal are required to elicit a sequence of anemotaxis and mating behaviour. They did not test for activation, however. Our results suggest that bombykal alone is not sufficient to activate the moths. Neither did bombykal stimulation elicit flight activity nor suppress oscillations of the transepithelial potential, as would have been expected if activation is assumed to be correlated with octopamine release (Roeder, 1999; Dolzer et al., 2001). Although the experiments were performed during the active phase of the nocturnal moths (reared in an inverse culture), we cannot exclude influences of the preparation procedure or the unnatural situation during the recordings. Thus, this finding needs to be confirmed in experiments with freely behaving moths. Finally, it remains to be tested in behavioural experiments how the different forms of adaptation affect recognition of the species-specific pheromone blend.

Adaptation of the rising phase and the amplitude of the sensillar potential

In our study, the rising phase of the sensillar potentials (which in the extracellular recording appears as a negative deflection from baseline to the maximal amplitude) was characterized using two different parameters, the initial slope and $t_{1/2\ rise}$. The initial slope, whether determined by a straight line fit (not shown) or computed from $t_{1/2\ rise}$ and half the SP amplitude, exhibited a clear dose dependence in correlation with the SP amplitude (Figs 7B, 9B). In contrast to studies with pheromone sensilla of *A. polyphemus* and *Antheraea pernyi* (Zack, 1979; Kaissling et al., 1987; Kodadová, 1993; Kodadová and Kaissling, 1996; Pophof, 1998) and benzoic acid-sensitive sensilla of *Bombyx mori* (Kodadová, 1993), in our experiments with short pheromone stimuli, $t_{1/2\ rise}$ showed virtually no dose dependence and was not influenced by adaptation (Fig. 7C). The major difference in the regimens of stimulation between our study and the studies mentioned above is the stimulus duration (50 ms *versus* 2 s or 5 s). Thus, we suspect that the longer stimulus duration, which resulted in a larger total pheromone amount applied, produced desensitization and possibly also long-term adaptation, which might be reflected by a change in the kinetics of the rising sensillar potential phase. This assumption is supported by our recordings in *M. sexta* at stimulus durations of 1000 ms (Figs 8, 9). These recordings showed a dose dependence and prolongation of $t_{1/2\ rise}$ after adapting pheromone stimuli

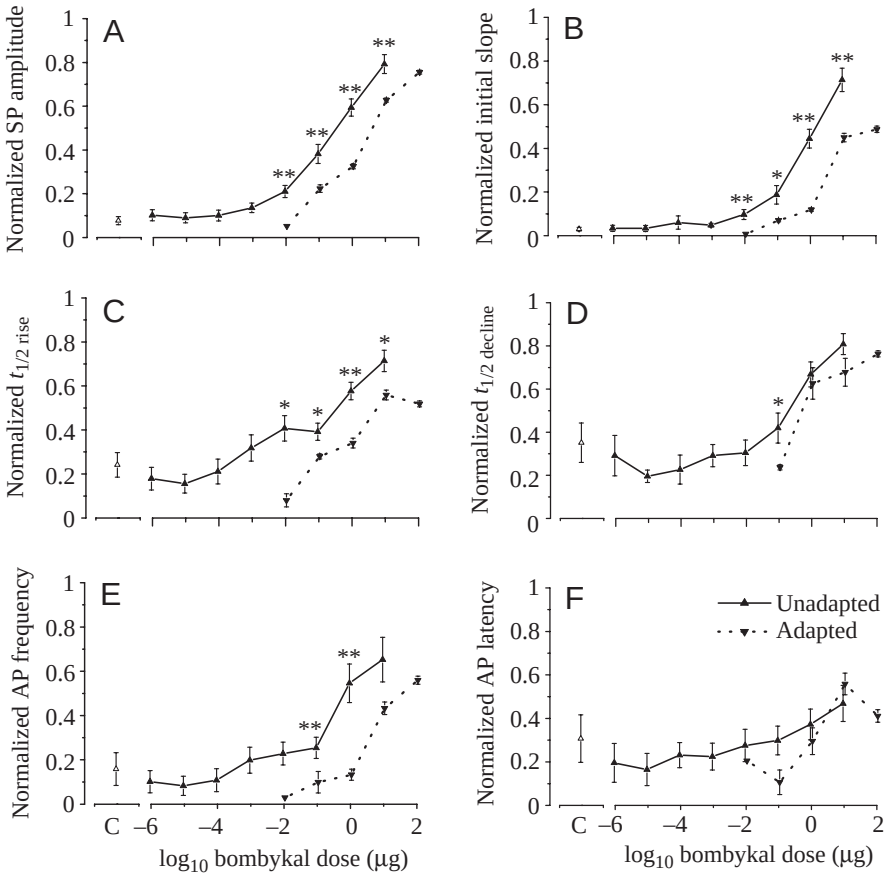

Fig. 9. Adaptation of responses to long stimuli. At a stimulus duration of 1000 ms, the sensillar potential (SP) amplitude (A), the initial slope (B), the half time of the rising phase of the SP ($t_{1/2\ rise}$; C), the half time of the decline of the SP ($t_{1/2decline}$; D) and the action potential (AP) frequency (E) and latency (F) were dose-dependent (solid lines). After adaptation by a 1000 ms stimulus applied 60 s before the test stimuli, these dose–response curves were shifted to higher stimulus intensities (broken lines). As with stimuli of 50 ms duration (Fig. 7), the shift was larger for the AP response than for the SP response. Responses in the adapted state were recorded to a maximal dose of 100 μg bombykal. The data were normalized to the highest response during each recording, which is the largest numerical value for those variables positively correlated to the stimulus intensity. The AP latency and $t_{1/2\ rise}$, which are negatively correlated to the intensity, were inversely normalized to the smallest numerical value to focus on responses in the physiological dose range. C, control. Data represent means ± S.E.M. Sample sizes (N) range between 2 (10^{-2} μg; adapted) and 22 (1 μg; non-adapted). Asterisks indicate significant differences between the adapted and non-adapted state (*P<0.05; **P<0.01; Student's t-test).

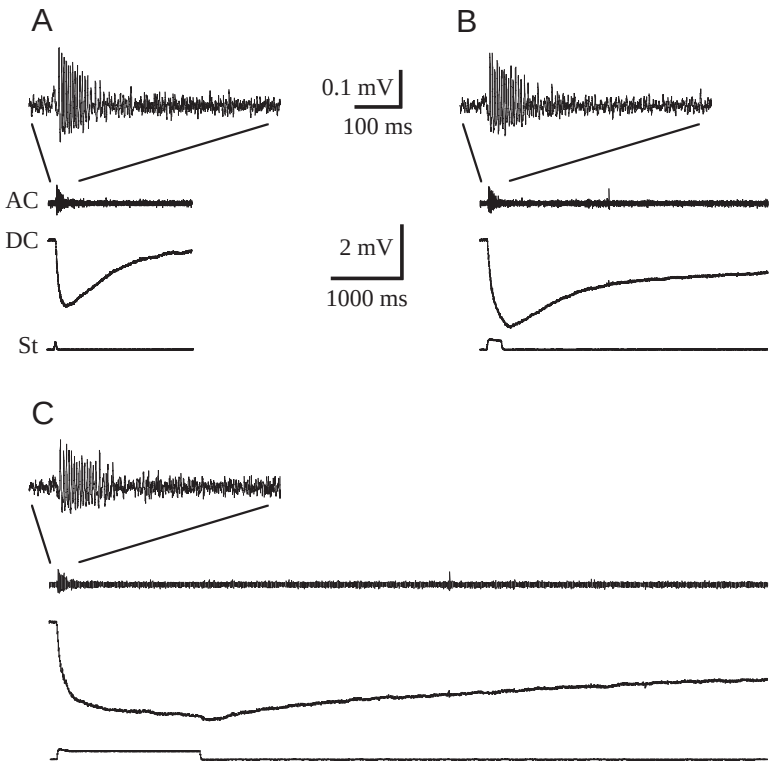

Fig. 10. Encoding of stimulus duration [20 ms (A), 200 ms (B) or 2000 ms (C)] in the bombykal cell. Neither the evaluated parameters of the sensillar potential nor the action potential response appeared to encode stimulus durations of 20 ms to 2 s. At long stimulus durations of 2000 ms, the duration of the plateau of the sensillar potential matched the stimulus duration without being reflected in the duration of the action potential response. The sensillum was stimulated locally with a dose of 1 μg bombykal. DC, unfiltered recording, AC, pseudo-highpass-filtered signal, St, stimulus signal, recorded by a pressure sensor. Above the AC traces, the initial portions of the action potential responses are shown as inserts at enlarged scale.

(Fig. 9C), as was also found in *A. polyphemus* (Zack, 1979; Kaissling et al., 1987). This might indicate that a stimulus-duration-dependent mechanism of adaptation exists that occurs during desensitization and possibly also during long-term adaptation, which affects the amplitude and the kinetics of the rising phase of the sensillar potential in different ways.

Based on the assumption that the sensillar potential predominantly reflects the receptor potential of the adequately stimulated ORN, the waveform of the sensillar potential is probably governed by the complex processes of the chemo-electrical transduction cascade (reviewed by Stengl et al., 1998). From patch-clamp studies on cultured ORNs of *M. sexta* we know that after application of a very low dose of bombykal, inositol (1,4,5)-trisphosphate (IP_3)-dependent Ca^{2+} channels open, causing a very transient rise in the intracellular Ca^{2+} concentration (Stengl, 1994). This rapid increase of intracellular Ca^{2+} then opens Ca^{2+}-dependent cation channels, and possibly also Ca^{2+}-dependent Cl^- and K^+ channels, which, as counteracting currents, may determine the kinetics of the rising phase and the amplitude of the sensillar potential (Stengl, 1993, 1994; Stengl et al., 1992, 1998). Because two of these channel types (which share properties with *trp*- and *trpl*-like channels) are permeable to Ca^{2+} and because both are closed in a Ca^{2+}-dependent manner, it is very likely that intracellular Ca^{2+} rises caused by these channels are involved in short-term adaptation, as has been shown in *Drosophila* (Störtkuhl et al., 1999; Montell, 2001). Biochemical and physiological evidence suggests the involvement of cGMP (Ziegelberger et al., 1990; Boekhoff et al., 1993; Stengl et al., 2001; Dolzer, 2002) together with Ca^{2+} concentration rises (Stengl, 1993, 1994; Dolzer et al., 1999) in long-term adaptation in moths. Thus, it is likely that Ca^{2+}- and cGMP-dependent mechanisms affect the rising phase of the sensillar potential in response to long and very strong pheromone stimuli.

Adaptation of the declining phase of the sensillar potential

The faster decline of the sensillar potentials in the short-term-adapted state in response to short stimuli, as well as to long stimuli (Figs 6B, 7D, 9D), suggests the presence of an additional adaptation mechanism, acting *via* stabilization of the resting potential. Thus, we would expect that either K^+ efflux or Cl^- influx is increased *via* short-term adaptation, possibly together with a faster closure of depolarizing ion channels. Since preliminary measurements with Ca^{2+}-sensitive dyes (M. Stengl and B. Lindemann, unpublished results) suggest that short-term adaptation is caused by prolonged rises in the intracellular Ca^{2+} concentration while long-lasting adaptation is also due to cGMP-dependent mechanisms, it is likely that opening of Ca^{2+}-dependent K^+ channels (Zufall et al., 1991), and possibly also Ca^{2+}-dependent Cl^- channels (Dolzer, 2002), is responsible for the faster decline of the sensillar potential. We are currently testing whether large Cl^- channels or non-specific cation channels, which are probably Ca^{2+}- and/or cGMP-dependent (Dolzer and Stengl, 1998; Dolzer, 2002), might be responsible for this stabilization of the resting potential.

Adaptation of the action potential response

In accordance with findings in *A. polyphemus* (Kaissling et al., 1987), in *M. sexta* the dose–response relationship of the action potential response to short and long bombykal stimuli after an adapting stimulus underwent a larger shift to higher stimulus intensities than did the relationships of the SP amplitude and the initial slope (Figs 7, 9). This suggests the presence of at least one additional mechanism of adaptation, acting on the transformation of receptor potentials into action potentials. This transformation process probably takes place in the soma or axon hillock region, morphologically and electrically remote from the origin of the receptor potentials (de Kramer et al., 1984; Kodadová, 1993), which favours the assumption of a functionally distinct adaptation mechanism.

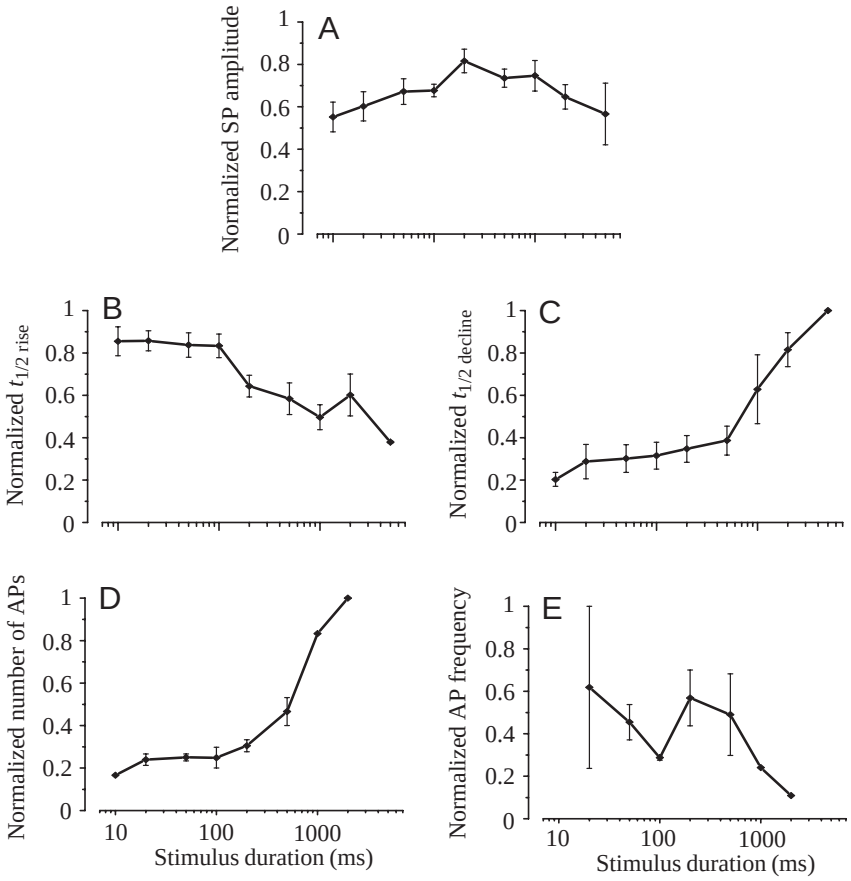

Fig. 11. Encoding of stimulus duration in the bombykal cell was tested with cartridge stimulations at a dose of 10 µg bombykal (*N*=7). The sensillar potential (SP) amplitude (A), the half time of the rising phase of the SP ($t_{1/2\ rise}$; B), and the action potential (AP) frequency (E) did not encode stimulus durations from 20 ms to 5 s. But the declining phase of the SP, as reflected by $t_{1/2\ decline}$ (C), and the total number of APs (D) distinguished stimulus durations between approximately 100 ms and 5 s.

We will test whether cGMP- (Zufall et al., 1991; Zufall and Hatt, 1991; Stengl et al., 1992, 2001; Dolzer, 2002) or a Ca^{2+}-dependent phosphorylation of ion channels (Zufall and Hatt, 1991; Stengl, 1993) are involved in the adaptation of the action potential response.

In *A. polyphemus*, a change in the slope of the dose–response curves of the action potential frequency, as well as the SP amplitude and $t_{1/2\ rise}$, was found after adapting stimulation (Kaissling et al., 1987). Responses to weak stimuli were further reduced compared with responses to strong stimuli, leading to a steeper slope of the dose–response relationship in the adapted state. In *M. sexta*, however, the dose–response curves were only shifted to higher stimulus intensities but were not obviously altered in their slope. Our study aimed to investigate stimuli in a physiological range. Thus, we did not routinely exceed a stimulus dose of 10 µg and did not statistically analyze whether there is a change in the slope. In the study by Kaissling et al. (1987), applied pheromone doses were corrected for a nonlinearity in the ratio of the pheromone dose loaded onto the filter paper and its release, as found by studies on radioactively labeled pheromone (Kaissling, 1995). Because no radioactively labeled bombykal is available to date, we do not know whether the same nonlinearity exists for bombykal. Nevertheless, we assume that the differences in the results are rather due to differences in the stimulus protocols used to adapt the sensilla. Kaissling et al. (1987) used the strongest stimulus of a protocol that resembles our dose ramp as the only adapting stimulus for a sequence of test stimuli that were applied in increasing order successively. Thus, the time interval between the adapting stimulus and the test stimulus was longer for the test stimuli of higher doses, giving the sensillum more time to recover. In our current study, however, the intervals between conditioning and test stimuli were kept constant (Fig. 2).

Desensitization

The phasic–tonic action potential response observed with long stimulations (Fig. 8) indicates the presence of desensitization (Zufall and Leinders-Zufall, 2000). Desensitization quickly stops the response to a stimulus and thus allows for resolving temporally patterned odour stimuli. The phasic–tonic pattern of the action potential response cannot be completely due to a nonlinearity in the stimulus concentration, because the plateau of the sensillar potential lasts longer than the phasic part of the action potential response (Fig. 10; Kaissling and Priesner, 1970). Since rises in the cGMP concentration after adapting pheromone doses are very slow (Boekhoff et al., 1993), it is likely that mostly Ca^{2+}- and/or phosphorylation-dependent mechanisms and, to a lesser extent, also cGMP-dependent mechanisms are involved in desensitization. Because after long pheromone stimuli intracellular Ca^{2+} rises closed IP_3-dependent Ca^{2+} channels and Ca^{2+}-dependent cation channels but opened protein kinase C-dependent cation channels (Stengl 1993, 1994), it is likely that these Ca^{2+}-dependent mechanisms also underlie desensitization. We assume that after long, strong pheromone stimuli the population of the more slowly activating protein kinase C-dependent cation channels, which are less Ca^{2+}-permeable and which are not blocked by Ca^{2+}, dominates the late phase of the sensillar potential and underlies the tonic depolarization of ORNs. Future studies will examine which ion channels and second messenger cascades are involved in the different states of adaptation to challenge our hypotheses.

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