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OSCILLATIONS OF THE TRANSEPITHELIAL POTENTIAL OF MOTH OLFACTORY SENSILLA ARE INFLUENCED BY OCTOPAMINE AND SEROTONIN

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Summary

The biogenic amine octopamine is known to enhance the sensitivity of male moths to their species-specific pheromones in flight-tunnel experiments. This sensitization of pheromone-guided upwind flight is at least partly due to octopamine-dependent increases in the peak nerve impulse frequency of the pheromone response of olfactory receptor neurons. It is not known, however, whether octopamine exerts its effects directly on the electrical properties of the olfactory receptor neurons or indirectly, *via* modulation of the accessory cells of the sensillum.

In extracellular tip recordings of pheromone-dependent trichoid sensilla on the antennae of male *Manduca sexta* moths, we investigated the effects of octopamine and serotonin on the transepithelial potential, which is generated by the activity of V-ATPases in sensillar accessory cells. In addition, the action potential activity of unstimulated olfactory receptor neurons was examined in the presence of biogenic amines. Under constant environmental conditions, the transepithelial potential oscillated regularly with periods of 2–8 min and with a 1–25 mV peak-to-peak amplitude over periods of several hours. These oscillatory intervals were interrupted by periods of relatively stable transepithelial potential, correlated with flight activity by the moth. Octopamine reduced the amplitude of the transepithelial potential oscillation and decreased the resistance of the sensillum preparation in a dose-dependent manner. Serotonin altered the waveform of the transepithelial potential, but did not change the resistance of the preparation. Thus, both amines affect the accessory cells, but have different targets in the regulation of the transepithelial potential. Neither amine significantly influenced the spontaneous action potential activity of the olfactory receptor neurons.

Key words: *Manduca sexta*, pheromone sensillum, tip recording, transepithelial potential, oscillation, action potential, octopamine, serotonin.

Introduction

Octopamine, a biogenic monoamine structurally related to norepinephrine, is an invertebrate-specific neurotransmitter, neuromodulator and neurohormone (Roeder, 1999). It acts as a stress hormone, which is released from neurohaemal organs to cope with energy-demanding situations, such as pheromone-dependent flight in insects. In wind-tunnel experiments with various moth species, octopamine improved pheromone blend discrimination and orientation towards pheromone sources, possibly also *via* effects on antennal olfactory sensilla (Linn, 1997; Linn and Roelofs, 1986; Linn and Roelofs, 1992; Linn et al., 1992; Linn et al., 1996). Octopamine is released into insect antennae *via* secretion onto antennal hearts that circulate haemolymph into the antennae (Pass et al., 1988; H. Agricola, personal communication). In addition, 1–2 octopamine-immunoreactive neurons project into the antennal nerve of the moth *Manduca sexta* (U. Homberg, personal communication). Furthermore, putative receptors for octopamine and/or serotonin have been cloned from the antennae of the moths *Bombyx mori* and *Heliothis virescens* and localized in an unidentified cell type at the base of olfactory sensilla (von Nickisch-Rosenegk et al., 1996), suggesting an effect of octopamine on olfactory sensilla in moth antennae. In accordance with these observations, the peak nerve impulse frequency in response to pheromone was found to be increased specifically by octopamine in the moth *Antheraea polyphemus* (Pophof, 2000). It is still not known, however, whether octopamine exerts its effects directly on the olfactory receptor neurons (ORNs) or indirectly *via* effects on the accessory cells of the olfactory sensillum. The accessory cells generate a transepithelial potential (TEP), which has been assigned an important function in increasing the driving force for the ionic movements underlying the receptor potential, thus improving the sensitivity of the sensillum (Thurm, 1972; Thurm and Wessel, 1979). Slow oscillations in the TEP of moth pheromone sensilla are generated by unknown mechanisms (Zack, 1979), and these have also been found in the Malpighian tubules of the beetle *Onymacris plana* (Nicolson and Isaacson, 1987) and of the mosquito *Aedes aegypti* (Williams and

Beyenbach, 1984; Beyenbach et al., 2000). However, it is not clear whether these TEP oscillations are artefacts or have a functional significance.

In tip recordings of pheromone-dependent sensilla trichoidea, we tested whether the biogenic amines octopamine and serotonin affect the accessory-cell-dependent TEP in *Manduca sexta* antennae or the spontaneous action potential activity of the two pheromone-dependent ORNs found in each sensillum (Kaissling et al., 1989; Keil, 1989). Our experiments revealed no significant effects of the biogenic amines on the spontaneous activity of unstimulated ORNs, but showed that both amines, either directly or indirectly, affect different targets in the accessory cells that generate the TEP.

Materials and methods

Animals and preparation for electrophysiological recordings

Manduca sexta moths (Johannson) (Lepidoptera: Sphingidae) were raised from eggs, and larvae were fed on an artificial diet (modified after Bell and Joachim, 1976). The animals were kept under a long-day photoperiod (L:D 17 h:7 h, lights off at 08:00 h) at 24–27 °C and 40–60 % relative humidity. Male pupae were isolated 1 day before emergence, gently cleaned with 70 % ethanol and allowed to hatch without any contact with pheromone. During their second dark phase, the adults were fixed into a Teflon holder. The head capsule was pierced with a syringe needle approximately 1 mm dorsocaudal to the antennal base. The flagellum of the right antenna was immobilized with dental wax (Boxing wax, Sybron/Kerr, Romulus, Michigan, USA), and the 15–20 most apical annuli were clipped off. A glass electrode filled with haemolymph Ringer (Kaissling, 1995) was inserted into the flagellar lumen and sealed with ECG electrode gel (PPG, Hellige, Freiburg, Germany). The tips of long trichoid sensilla from the apical row on the third to tenth remaining annuli were clipped off using sharpened forceps. The recording electrode, filled with sensillum lymph Ringer (Kaissling, 1995), was slipped over one sensillum. A connection to the amplifier inputs was established with Ag/AgCl wires immersed in the electrolytes. In side-wall recordings, an electrochemically sharpened tungsten wire placed near the sensillar base was used as the recording electrode. Signals were amplified approximately 200-fold in a custom-built amplifier (direct current to 2 kHz, input impedance $10^{12}\,\Omega$) and passed through a 2 kHz anti-aliasing filter (900C/9L8L, Frequency Devices, Haverhill, Massachusetts, USA). Flight activity of the animals was monitored using a piezo-electric element placed on the thorax. A Digidata 1200 B digitizer (Axon Instruments, Union City, California, USA) and pCLAMP 8 software from the same manufacturer were used to acquire data. The electrophysiological signal and a highpass-filtered equivalent (cut-off frequency 2 or 5 Hz), as well as the piezo signal, were continuously recorded on a strip chart recorder (EasyGraf, Gould, Valley View, Ohio, USA). Sections of the direct-current-coupled recording and the piezo-electric signal were also stored on DAT (DTR-1202, Bio-Logic, Claix, France). After the recordings, more than 90 % of the moths were in sufficiently good condition to be returned to the flight cage.

Environmental conditions

All recordings were performed at room temperature (18–23 °C). The long-term recordings were performed in constant room light to exclude circadian effects. No part of the electrophysiological apparatus had been in contact with pheromone for at least 15 months. Charcoal-filtered and moistened air was blown continuously over the preparation ($13\,l\,min^{-1}$).

Drug application

Octopamine and serotonin (Sigma, Deisenhofen, Germany), dissolved in haemolymph Ringer, were injected through the hole in the head capsule with a glass capillary. We injected a minimum of 2 µl of a $0.5\,mmol\,l^{-1}$ octopamine stock solution and a maximum of 5 µl of a $500\,mmol\,l^{-1}$ solution, resulting in doses of 1–2500 nmol. The highest dose was of the same order of magnitude as in previous studies by Pophof (Pophof, 2000) and Grosmaitre et al. (Grosmaitre et al., 2001), who injected a maximum dose of 1 µl of a $100\,\mu g\,\mu l^{-1}$ solution ($527\,mmol\,l^{-1}$). Because an adult moth contains approximately 1 ml of haemolymph (J. Truman, unpublished observation), the final octopamine concentration in the haemolymph was between $1\,\mu mol\,l^{-1}$ and $2.5\,mmol\,l^{-1}$.

In addition, we injected a minimum of 2 µl of a $5\,mmol\,l^{-1}$ serotonin solution and a maximum of 15 µl of a $50\,mmol\,l^{-1}$ solution, resulting in a dose of 10–750 nmol and a haemolymph concentration of $10–750\,\mu mol\,l^{-1}$. In a set of pilot experiments, food dye injected at the same site was transported into the antenna with a delay of <1 to 3–5 min.

Polarity conventions

Voltage polarity is given for the sensillum lymph electrode with reference to the haemolymph electrode. Current flow is defined in terms of the movement of positive charge. The sign of current values was chosen to match the polarity of the corresponding voltage response. Therefore, current is termed positive if it flows out of the sensory hair into the sensillum lymph electrode, and negative if it flows the opposite way (see Redkozubov, 2000).

Data-acquisition protocols

At the beginning of each recording, a series of 5 mV calibration pulses was applied to the haemolymph electrode, which was otherwise grounded. The resistance of the preparation (R_{prep}) was then determined by injecting current pulses of –100 pA through the recording electrode (de Kramer, 1985). To improve the signal-to-noise ratio, 10 current steps were subsequently applied, and the voltage responses were averaged. For current injections, the input resistance of the amplifier (R_i) was reduced to $10^9\,\Omega$. Since the amplification factor changes, if R_i and R_{prep} are in the same range/of the same order of magnitude, an additional calibration step was performed for voltage measurements during current injection.

The correct calibration of the injected currents was regularly verified with a reference resistor connected to the amplifier.

Current step protocols were used to determine the current amplitude required to elicit action potentials electrically. Positive current pulses of 50 ms duration, incrementing by 25 pA, were injected, and the capacitative transients originating from the passive properties of the sensillum in combination with the capacitance neutralization circuit of the amplifier were eliminated by adding the voltage responses to two pre-pulses of half the amplitude and of opposite polarity (P/N leak subtraction). The current that elicited action potentials was determined for large and small action potentials separately using the averaged results of five sequential protocols. To account for spontaneously occurring action potentials, action potentials were only considered to be elicited if they were also present in at least the succeeding two sweeps (i.e. at a higher current amplitude). For the same reason, protocols with more than 36 action potentials in the baseline region (50 ms pre-step and 50 ms post-step for each sweep) were discarded. In these cases, the mean of the remaining protocols was analyzed.

For long-term recordings, the signals were acquired in segments of 10 min, at a sampling rate of 19.6 kHz (Clampex, fixed-length events). Each action potential triggered a sweep of 12.75 ms duration, and the highpass-filtered signal served as a trigger channel only. All analyses were performed using the direct-current-coupled signal. The mean voltage during the initial 2.5 ms was defined as the baseline and used to measure the TEP. Thus, the time course of the TEP was monitored with variable sampling intervals depending on the occurrence of action potentials. The baseline of all action potential sweeps was then adjusted to 0 mV (see Fig. 5A) to identify sweeps that were triggered by artefacts. The peak-to-peak amplitude of each action potential was measured and plotted *versus* the time of its occurrence (see Fig. 5B). In addition, amplitude distribution histograms were created (see Fig. 5C). A combination of these plots was used to determine the threshold for action potential sorting.

Action potential sorting

Action potentials separated by an interspike interval (ISI) of no more than 50 ms were defined as members of bursts. The decreasing amplitude of the action potentials within a burst (see Fig. 4C, Fig. 5B) did not allow a simple threshold-based procedure for action potential sorting. Instead, an MS-Excel macro was used that performed the following steps: if the interspike interval preceding the action potential under consideration was larger than 50 ms, only the peak-to-peak amplitude was used for action potential classification. Any action potential that occurred 50 ms or less after an action potential classified as large was also considered to be large. Since the amplitude only decreased, but never increased, during bursts, all action potentials with an amplitude above threshold were classified as large, even if they occurred within 50 ms of a small action potential. This could cause misclassifications, however, if a single large action potential were to occur within a burst of small ones, since the subsequent members of the burst would incorrectly be classified as large. The action potential sorting algorithm recorded such cases, and these action potentials were classified manually.

Bursting behaviour

After separating the action potentials into two classes, we determined the interspike intervals (ISIs) for each 10 min data segment. The variables computed to describe the spontaneous action potential activity and the bursting behaviour of the ORNs were (i) the mean action potential frequency, (ii) the percentage of action potentials that were members of bursts, (iii) the mean number of action potentials per burst and (iv) the coefficient of variation (CV) of the ISIs, where CV=S.D./mean. In a sequence of events that occur independently of preceding events, i.e. in a Poisson process, the CV of the intervals between every two successive events is equal to 1 (Rospars et al., 1994). Thus, a CV significantly different from 1 indicates that the action potentials are not randomly distributed.

Resistance and transepithelial potential

At the beginning of each recording, when typically no TEP oscillations were present, the TEP and the resistance of the preparation were measured. The measured TEP value was corrected for the electrode potential (–35.6±1.4 mV; median ± S.E.M.; N=6). To investigate the influence of drug injections, the resistance was measured twice within 2–10 min before the injection, twice within a period of 2–10 min after the injection, and once more 10 min later. Since no significant difference in the group of pre-injection measurements and in the group of post-injection measurements was found, each group was averaged to yield the resistances before (R_{before}) and after (R_{after}) drug injection. The normalized resistance R_{after}/R_{before} was then computed.

Results

In extracellular tip recordings from long trichoid sensilla, we investigated the transepithelial potential (TEP), spontaneous action potential activity and the effects of octopamine and serotonin injections on both these variables. So far, no report has been published on the general properties of spontaneously active ORNs (such as action potential distribution, thresholds and amplitude distributions). Thus, we first characterized these general properties before testing for any effects of biogenic amines on these variables. Because action potential patterns are known to determine neuronal gene expression (Brosenitsch and Katz, 2001), we place particular emphasis on the analysis of burst behaviour.

The recordings could be maintained for up to 100 h when no drugs were injected, but were usually terminated earlier. No steady trend in action potential frequency could be detected during the course of long-term recordings (Fig. 1), and the action potentials of both amplitude classes appeared to undergo random fluctuations. If a sensillum appeared to be damaged, as judged from rapid TEP breakdown or the disappearance of one

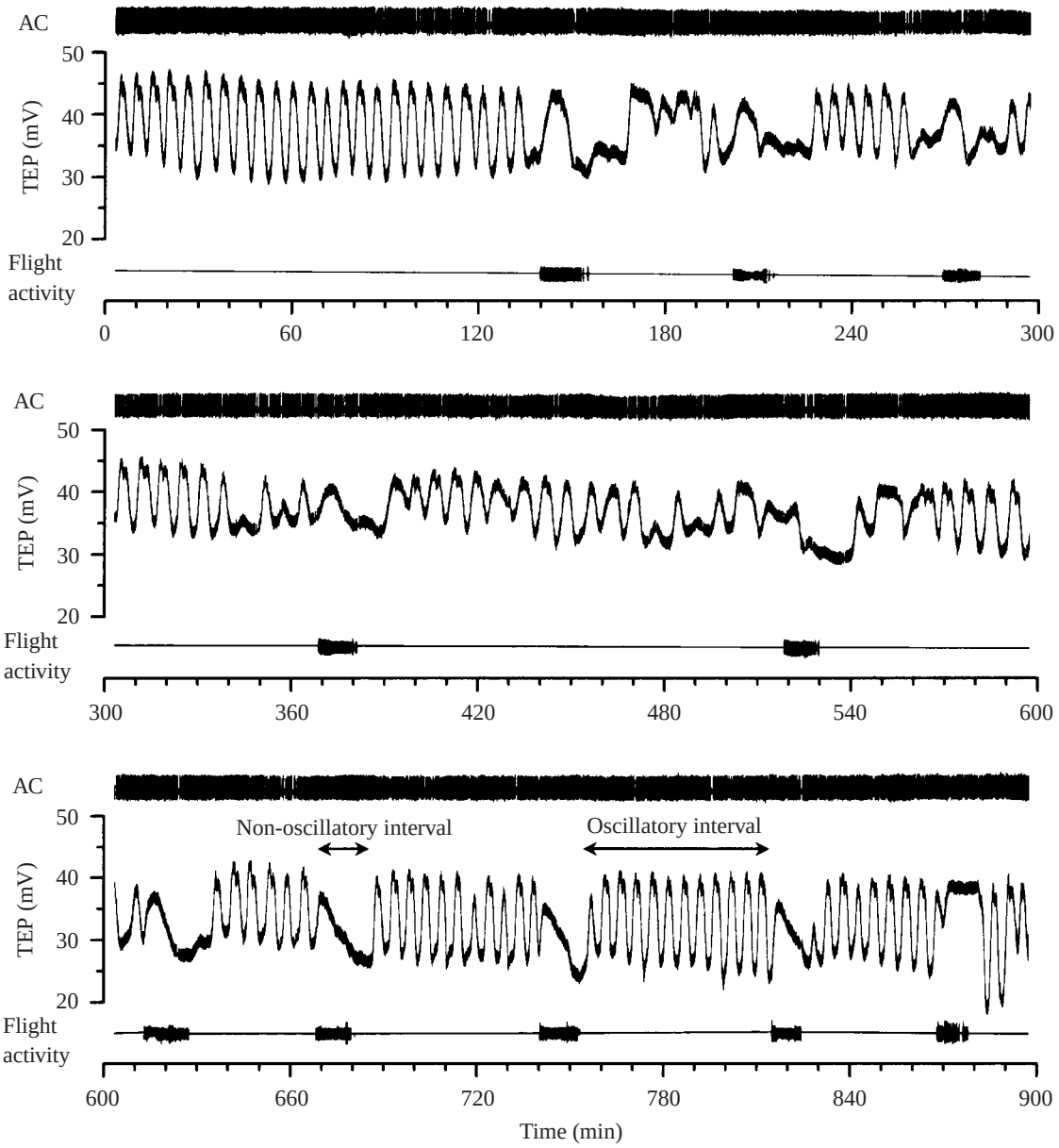

Fig. 1. Fifteen hours of a recording of action potentials and the transepithelial potential (TEP) of a trichoid sensillum with respect to the flight activity of the moth. The recording starts approximately 50 h after the beginning of the tip recording. The highpass-filtered signal (AC) reveals the action potentials superimposed on the slow fluctuations of the TEP, illustrating that there was no trend in the action potential activity. The time course of the TEP exhibited regular oscillations for periods of up to several hours, termed 'oscillatory intervals'. These were interrupted by shorter periods, 'non-oscillatory intervals', during which the TEP fluctuated less regularly or remained constant. Flight activity, recorded by a piezo-electric sensor at the thorax, was restricted to non-oscillatory intervals.

Table 1. *Transepithelial potential (TEP) oscillations and flight activity in long-term recordings*

Number of recordings	TEP oscillations	Oscillatory and non-oscillatory intervals*	Flight activity	
			In the absence of TEP oscillations	In the presence of TEP oscillations
58	57	47	58	6

The table only contains observations from recordings that lasted more than 15 h.
*A sequence of at least two oscillatory intervals interrupted by a non-oscillatory interval.

action potential class, the recording was discarded. In total, 177 sensilla from 138 animals were recorded, but most measurements were only performed on a subset.

Oscillations of the transepithelial potential

In 57 of 58 recordings that lasted more than 15 h (Table 1), the TEP exhibited a periodically oscillating time course with a peak-to-peak amplitude between 1–2 and >25 mV and a period of 2–8 min. The waveform was typically asymmetrical, with a shoulder on the decay phase of the positive peak (Fig. 1, see Fig. 3B), but sinusoidal signals were also observed. The oscillations were occasionally present from the beginning of a recording, but typically started between 2 and 15 h after the recording had been established. In most recordings, the oscillations developed gradually, with slowly increasing amplitude, thus preventing a quantitative analysis of the onset time. During the periods of oscillating TEP, referred to here as oscillatory intervals, the animals typically exhibited no flight activity (Table 1). The oscillatory intervals were interrupted by periods during which the time course of the TEP fluctuated less periodically or remained constant (Fig. 1). These periods are termed non-oscillatory intervals. Flight activity typically was observed only during non-oscillatory intervals, occurring in bursts of approximately 5–15 s duration separated by periods without flight activity of 15 to >100 s.

Oscillatory intervals typically lasted 1–5 h, but their duration was highly variable (up to >20 h). Non-oscillatory intervals, in contrast, had a shorter and more constant duration of approximately 20–50 min. Because the spontaneous occurrence and the duration of non-oscillatory intervals were very variable and appeared random, we did not attempt any further analysis. The sequence of oscillatory intervals without flight activity and non-oscillatory intervals associated with flight activity was observed in 47 of the 58 recordings of more than 15 h duration. Flight activity occasionally decreased over the course of long recordings and could be completely absent by the end. Flight activity during oscillatory intervals, however, was only observed in six experiments (Table 1).

The injection of 1–2500 nmol of octopamine into the head capsule suppressed the TEP oscillations after a delay of one to

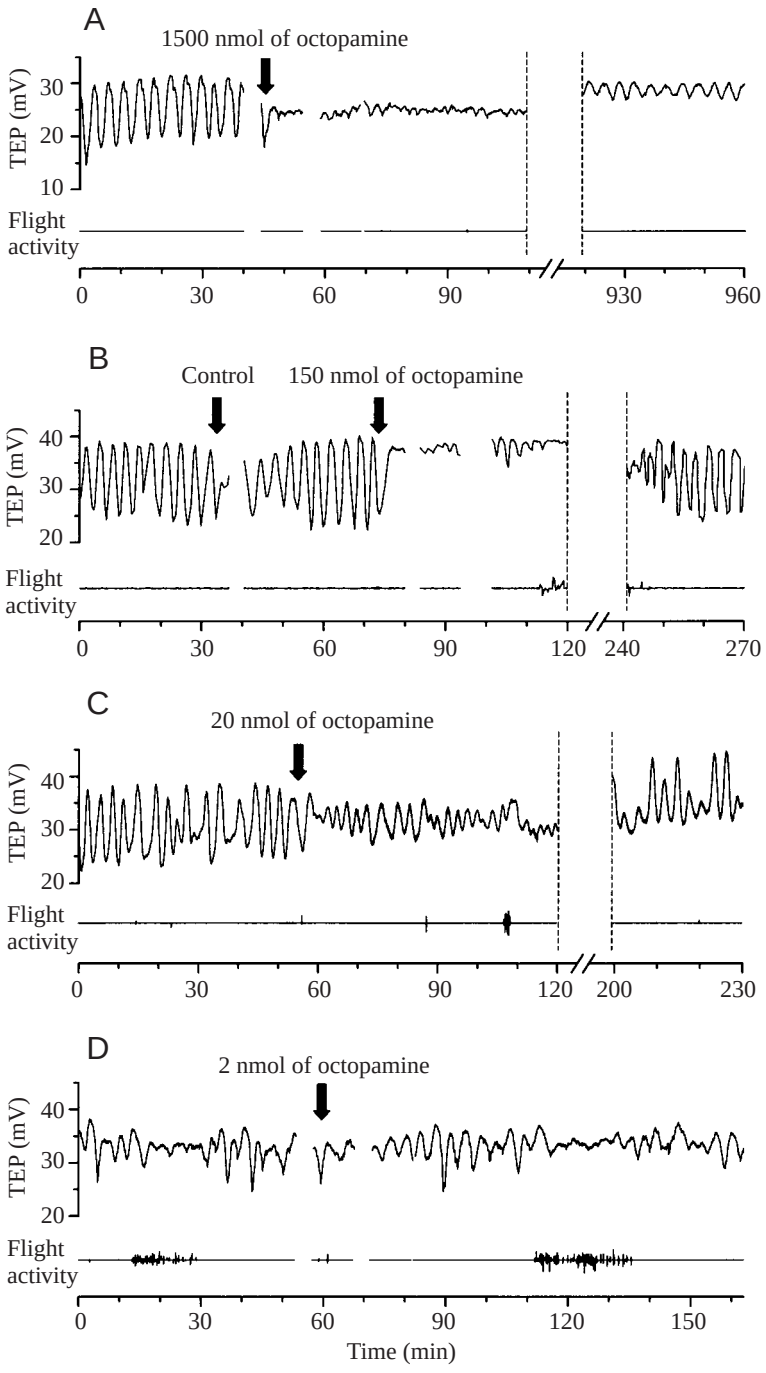

Fig. 2. (A–D) Recordings from four different animals showing the transepithelial potential (TEP) and flight activity during injections of octopamine. (A) After a delay of 1–2 min, the TEP oscillation was abolished by an injection of 1500 nmol of octopamine. Even after 15 h, the peak-to-peak amplitude had not recovered (after the axis break). During the gaps, current step protocols and resistance measurements (not shown) were performed. (B) While a control injection of haemolymph Ringer only slightly and transiently reduced the amplitude of the TEP oscillations, 150 nmol of octopamine almost completely suppressed it. At 113 min, the animal exhibited flight activity, marking the beginning of a non-oscillatory interval of approximately 2 h (not shown), after which the oscillation reappeared. (C) After an injection of 20 nmol of octopamine, the amplitude of the oscillation was reduced, but the time course of the TEP remained periodic. Over the course of several hours after the injection, the oscillation gradually regained its original amplitude and regularity. (D) The effect of an injection of 2 nmol of octopamine into an oscillation with a complex waveform could not reliably be distinguished from the effects of control injections. Note the two non-oscillatory intervals without TEP oscillations (12–30 min, 112–138 min) during which flight activity was recorded.

Table 2. *The effects of drug injections during oscillatory intervals*

	Dose (nmol)	*N*	TEP oscillation suppressed*	No effect‡
Control		21	2	19
Serotonin	10–50	6	5	1
	100–750	9	7	2
Octopamine	1–5	6	4	2
	10–50	9	8	1
	100–500	14	12	2
	1000–2500	9	8	1

TEP, transepithelial potential.

*Complete suppression of the oscillation for ⩾20 min or clear reduction in the oscillation amplitude for ⩾30 min.

‡No suppression or suppression for <20 min and no amplitude reduction or reduction for <30 min.

Table 3. *Spontaneous action potential frequency and burst behaviour*

Variable	Small APs	Large APs
Mean AP frequency (Hz)	0.542±0.129	0.390±0.057
% APs in bursts	45.7±2.1	47.4±2.7
Mean number of APs per burst	3.31±0.12	3.36±0.09
Coefficient of variation	1.95±0.09	1.78±0.06

AP, action potential.

Data from 66 recordings acquired before drug injection were analyzed in 438 segments of 10 min duration. The segments of individual recordings were averaged, and the mean and S.E.M. of these averages were then computed.

several minutes (Fig. 2; Table 2). The TEP stabilized at variable values between the maximum and minimum potential during the previous oscillations. Both the degreee of suppression and the duration of the effect were dose-dependent. At doses above 1000 nmol, the oscillations were completely absent after 89 % of the injections during an oscillatory interval. At these doses, the amplitude of the oscillation did not fully recover for the rest of the recording (Fig. 2A). At lower doses, the suppression was usually incomplete and more transient (Fig. 2B–D). After some control injections with haemolymph Ringer, we observed a transient suppression of the TEP oscillations or a phase shift (Fig. 2B, see Fig. 7A; Table 2) reminiscent of the effect of a low octopamine dose. To account for these effects, for the quantitative analysis (Table 2), we scored the complete absence of oscillations for at least 20 min or a clear reduction in the oscillation amplitude (to two-thirds or less) for at least 30 min as suppression. More transient changes or the absence of any obvious influence were scored as no effect, which applied to 90 % of the control injections during oscillatory intervals.

Serotonin injections (*N*=15) led to a less pronounced reduction in the oscillation amplitude. The effect of serotonin typically resembled that of a 10-fold lower octopamine dose. However, the waveform of the oscillation was more regular after 13 of 14 serotonin injections that were associated with a suppression of the oscillation that was sufficiently transient to allow an analysis. In addition, after five of these injections, the shoulder after the positive peaks was less prominent than before. After five injections, the shoulder was completely absent, as shown in Fig. 3. A similar change in the oscillation waveform was never observed after octopamine injections. When injected during non-oscillatory intervals (*N*=10), none of the doses of octopamine or serotonin tested had any detectable effects. Because of their variable duration, it was not possible to analyze whether the non-oscillatory intervals were prolonged by drug injections. In none of the experiments did oscillations occur earlier than 20 min after the injection.

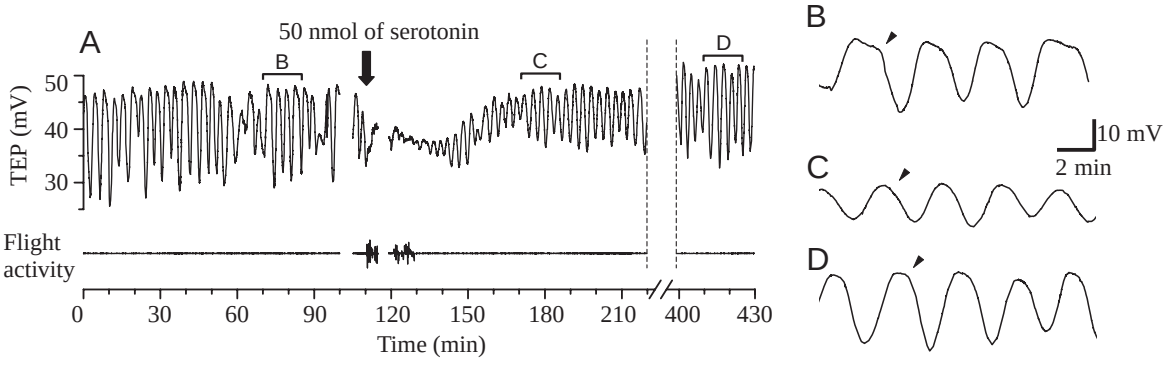

Fig. 3. (A–D) The time course of the transepithelial potential (TEP) during injection of 50 nmol of serotonin. The sections indicated in A are shown on an enlarged time scale in B–D. The TEP oscillation before drug injection was asymmetrical, exhibiting a shoulder (arrowhead) after the positive peak (B). After the injection, the oscillation was transiently suppressed and then gradually recovered to approximately two-thirds of the original peak-to-peak amplitude. After recovery, the oscillation was more regular than before, and the shoulder was absent (C). Five hours after the injection, the peak-to-peak amplitude had fully recovered, but the shoulder was not as prominent as before (D). The animal exhibited flight activity only transiently immediately after the drug injection.

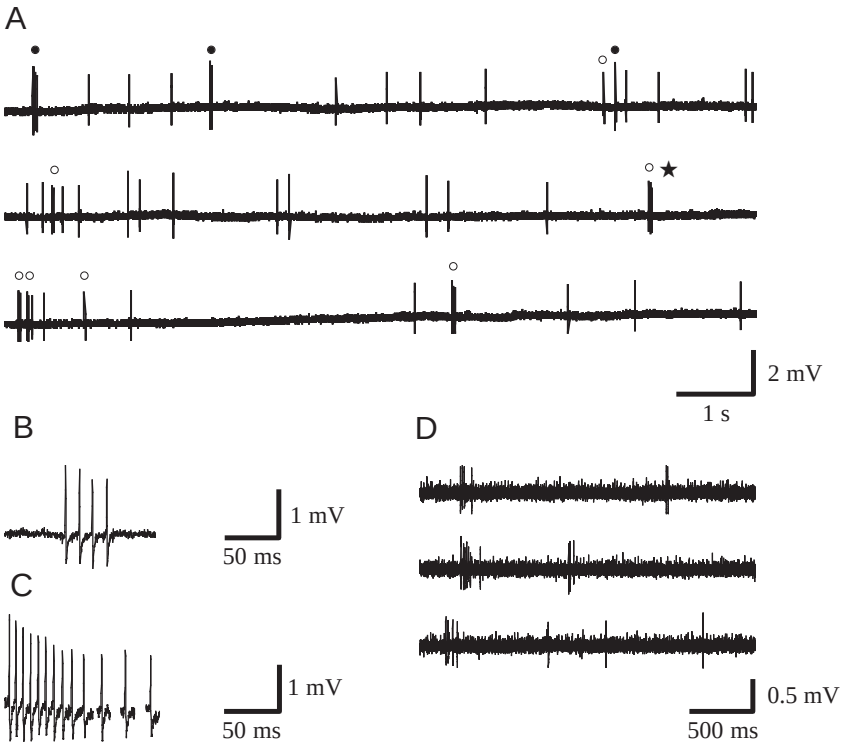

Fig. 4. (A,B) Spontaneous action potentials in the two olfactory receptor neurons (ORNs) in a trichoid sensillum, distinguishable by their amplitudes. The three parts in A are one continuous sequence. The action potentials of neither class were randomly distributed, but (both) exhibited bursting activity. Bursts of large action potentials are marked with filled circles, bursts of small action potentials with open circles. The indicated burst (star) is shown on an enlarged time scale in B. (C) A burst of large action potentials from a different recording, illustrating the reduction in amplitude of the action potentials. (D) Action potential bursts were also recorded with tungsten electrodes placed near the hair base. The three parts of D are one continuous trace, highpass-filtered at 5 Hz.

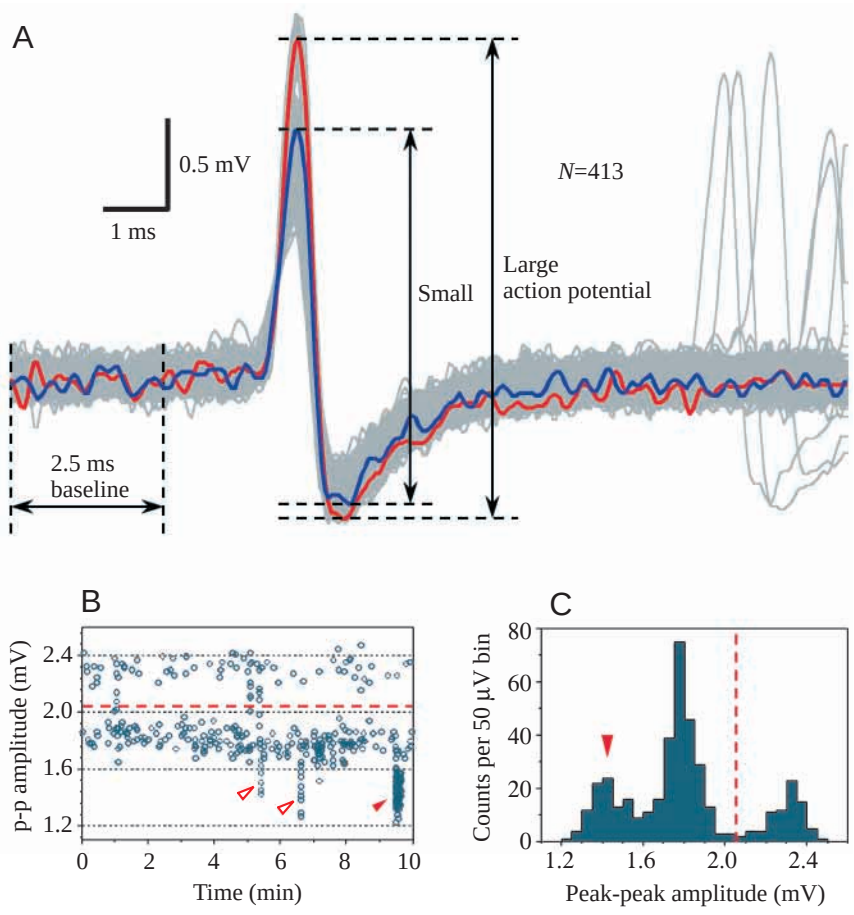

Fig. 5. (A) Action potentials recorded in sweeps of 12.75 ms duration. The initial 2.5 ms of each sweep were defined as baseline and used to measure the transepithelial potential. The baseline was then adjusted to 0 mV. One large (red line) and one small (blue line) action potential are highlighted. The peak-to-peak (p-p) amplitudes of each action potential occurring over a 10 min period were measured and plotted *versus* time (B). The amplitude reduction during bursts then became obvious (arrowheads). The massive bursting activity of the cell firing the small action potentials (solid arrowhead) gives rise to a third peak when the data are presented as an amplitude histogram (C), generating a false third action potential class (arrowhead). Both types of plot were analyzed to determine the threshold for action potential sorting (dashed lines).

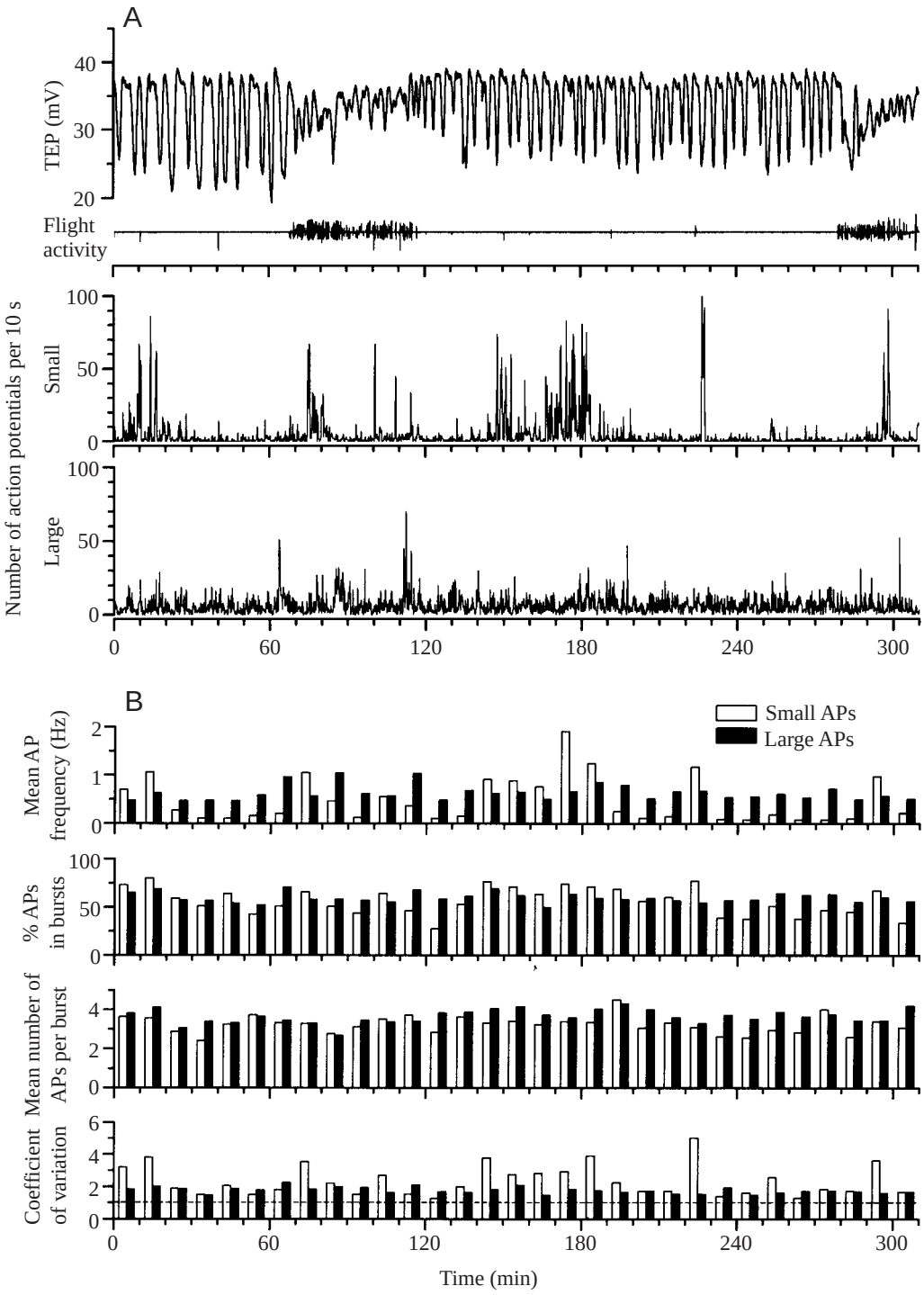

Fig. 6. (A,B) Action potential variables during oscillatory and non-oscillatory intervals of the transepithelial potential (TEP). (A) The upper panel shows the time course of the TEP and flight activity, while the lower panels illustrate the action potential activity of the small and large action potentials (APs), evaluated in bins of 10 s. Periods of elevated action potential activity and quiescent periods occurred independently of the TEP and of flight activity, during oscillatory as well as during non-oscillatory intervals. (B) No correlation between the phase of the TEP oscillations and any evaluated variable of the action potential activity was found. All variables were analyzed in bins of 10 min and plotted on the same time axis as in A. The frequency of the small action potentials was more variable than that of the large ones (Table 3). The percentage of action potentials that were members of bursts fluctuated randomly between <25 and >75 %. Similarly, the mean number of action potentials in each burst and the coefficient of variation (CV) did not exhibit any correlation with the time course of the TEP. The dashed line marks a CV of 1, which would indicate a random distribution of the action potentials.

Spontaneous action potentials

The distribution of the spontaneous action potentials was not random (Table 3). On average, 45.7±2.1 % of the small action potentials occurred in bursts made up of 3.31±0.12 action potentials and 47.4±2.7 % of the large action potentials occurred in bursts made up of 3.36±0.09 action potentials (means ± S.E.M.; Fig. 4, Fig. 5; Table 3). The action potential amplitude decreased during bursts, depending on the burst duration. Bursts were also observed in each of five recordings made using tungsten electrodes (Fig. 4D). Occasionally, a single large action potential occurred within a burst of small ones or both ORNs fired bursts simultaneously. Unless the frequency of both action potential classes was rather high (>2 Hz), these cases were very infrequent (≪1 % of the total action potentials), indicating that bursts were independent in the two ORNs. The coefficient of variation computed for the interspike intervals (ISIs) of both action potential classes was clearly higher than 1 (Table 3). The action potential frequency

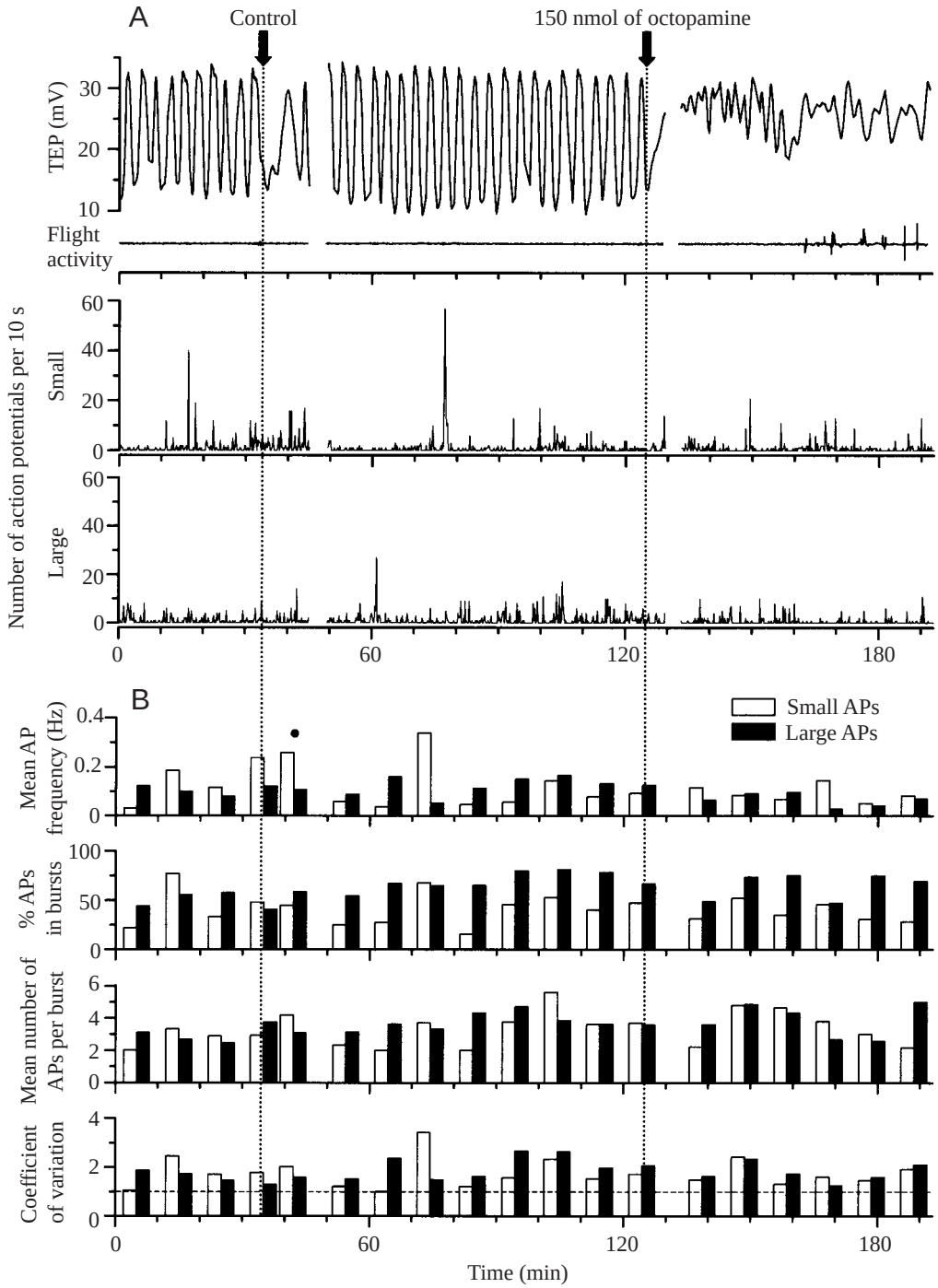

Fig. 7. No variable of the action potential (AP) activity correlated with octopamine-dependent modulation of the transepithelial potential (TEP). (A) Haemolymph Ringer (control) injection only slightly reduced and phase-shifted the TEP oscillation for less than 20 min, while injection of 150 nmol of octopamine caused a long-term depression of TEP oscillations. Flight activity marked the beginning of a non-oscillatory interval. The frequency of both action potential classes was unaffected by control or octopamine injections. (B) None of the evaluated variables of the action potential activity was affected by the injections. The first bin after the control injection (filled circle) is only 5.2 min long.

of both ORNs was highly variable between preparations and even between different sensilla of the same animal. In the complete absence of pheromone, the highest measured frequencies were 6.7 Hz for the small and 5.8 Hz for the large action potentials when averaged over 10 min. Both ORNs, however, also had transient periods when less than one action potential occurred in 10 min. The mean action potential frequencies evaluated for 438 data segments without drug injection from 66 recordings were 0.542±0.129 Hz (small action potential) and 0.390±0.057 Hz (large action potentials) (means ± S.E.M.). The frequency of each action potential class changed during the recordings, with periods of increased activity and quiescent periods in apparently random sequence (Fig. 6). However, when examined over the duration of a recording, the more active ORNs were consistently more active, while the more silent ones maintained a low average activity.

Neither with subjective judgement nor with cross-correlation tests on three sample recordings (not shown) did we find any significant correlation between the time course of the TEP oscillation and the action potential frequency.

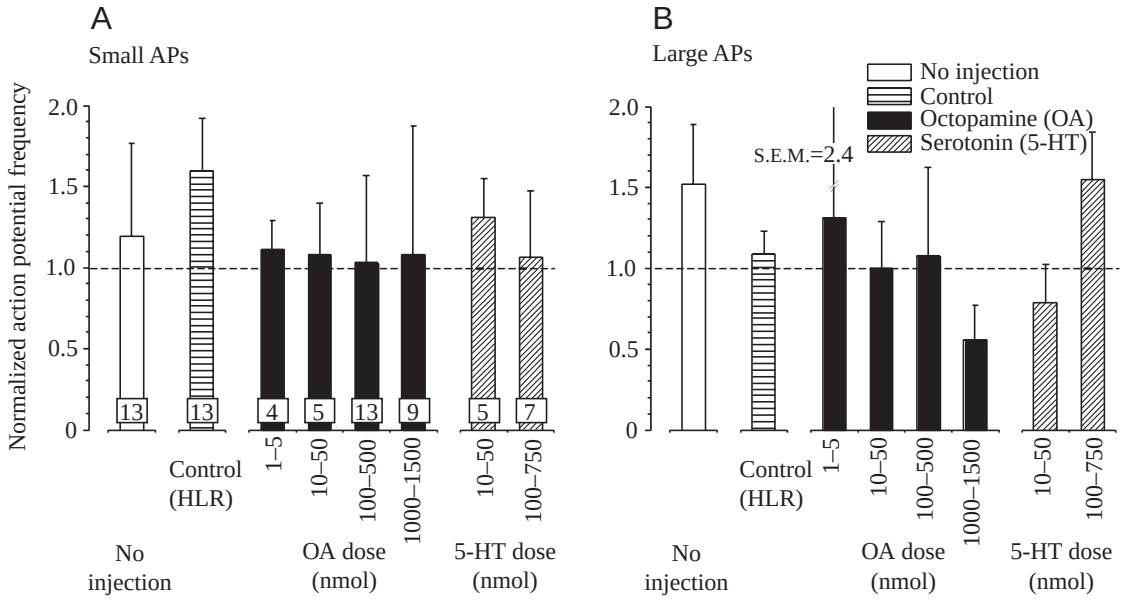

Fig. 8. (A,B) Neither of the two action potential (AP) classes was significantly influenced by amine injections. The frequencies of small and large action potentials were evaluated for segments 10 min in duration within 30 min before (ν_{before}) and after (ν_{after}) drug injections, and the normalized frequency ν_{after}/ν_{before} was computed. For comparison, data segments of 10 min, separated by 40–50 min, but without any injection, were analyzed the same way. One-way analysis of variance (ANOVA) did not reveal significant differences between any dose of the injected drugs, the control (injected with haemolymph Ringer) and the non-injected state. Values are medians + S.E.M. Values of *N* given in A also apply to B. 5-HT, serotonin; OA, octopamine; HLR, haemolymph Ringer.

Furthermore, there was no detectable difference between oscillatory and non-oscillatory intervals with respect to the action potential frequency or any of the variables evaluated that describe the burst behaviour (Fig. 6). Consequently, injections of octopamine or serotonin did not significantly influence the action potential activity either (Fig. 7, Fig. 8).

Electrically elicited action potentials

During the application of current step pulses (Fig. 9), large action potentials were elicited by a significantly smaller step amplitude (208.3±7.3 pA) than small ones (243.7±8.0 pA; means ± S.E.M., N=123, P<0.01, Student's t-test). No significant changes were found after the injection of any dose of the tested drugs or of haemolymph Ringer (not shown).

Resistance and transepithelial potential

At the beginning of each recording, when TEP oscillations were not usually present, the resistance of the preparation and the TEP were measured. The mean resistance of 159 sensilla was 68.3±2.3 MΩ (mean ± S.E.M.). Octopamine injections

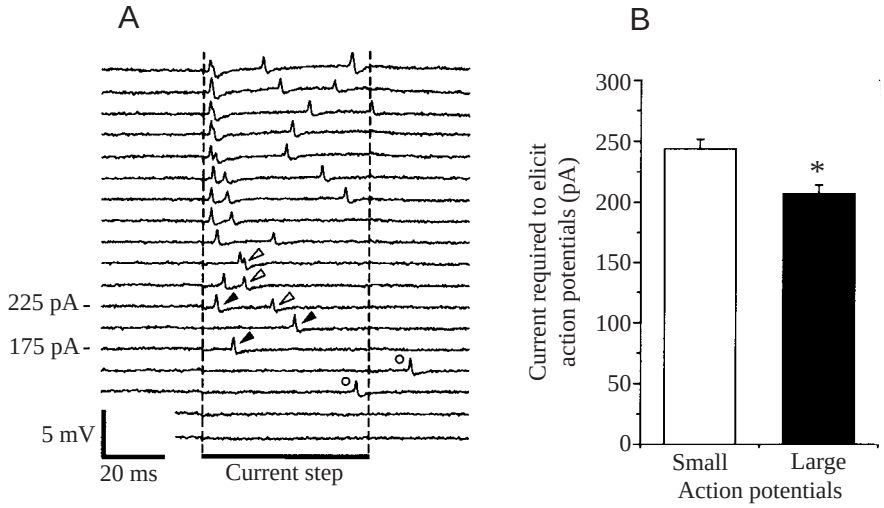

Fig. 9. Significantly more current was necessary to elicit small action potentials than large action potentials. (A) Action potentials elicited by a series of 50 ms current steps, increasing by +25 pA from bottom to top. Passive electrical responses were compensated by adding the responses of two pre-pulses of opposite polarity and half the amplitude to each sweep. The large action potentials (filled arrowheads) required a current of 175 pA, while the small action potentials (open arrowheads) were first elicited by a 225 pA step. The action potentials marked with open circles were considered to be spontaneous, since either no action potential was present in the next sweep or the action potential occurred outside the current step. (B) The small action potentials required significantly more current (244 pA) than the large ones (208 pA). Values are means + S.E.M., N=123 sets of five step protocols without drug injection. The asterisk indicates a significant difference from the control (P<0.01, Student's t-test). For both classes of action potential, no significant changes were detected after the injection of any dose of octopamine or serotonin (not shown).

reduced the resistance of the preparation by up to 20 % in a dose-dependent manner (Fig. 10). Serotonin, in contrast, did not significantly alter the resistance.

The TEP, as measured at the beginning of the recordings, was +33.8±0.8 mV (mean ± S.E.M.; N=177) irrespective of whether or not a drift or oscillation was present at the time of the measurement. During non-oscillatory intervals, the TEP fluctuated around variable values between the maximum and minimum potential in preceding and subsequent oscillatory intervals (Fig. 1). Similarly, after amine-dependent suppression of the oscillations, no consistent change in the absolute value of the TEP was found. Instead, the TEP stabilized around variable potentials between the upper and lower bounds of the previous oscillations, apparently dependent on the value of the TEP at the moment that drug action occurred (Fig. 2A–C, Fig. 3A, Fig. 7A).

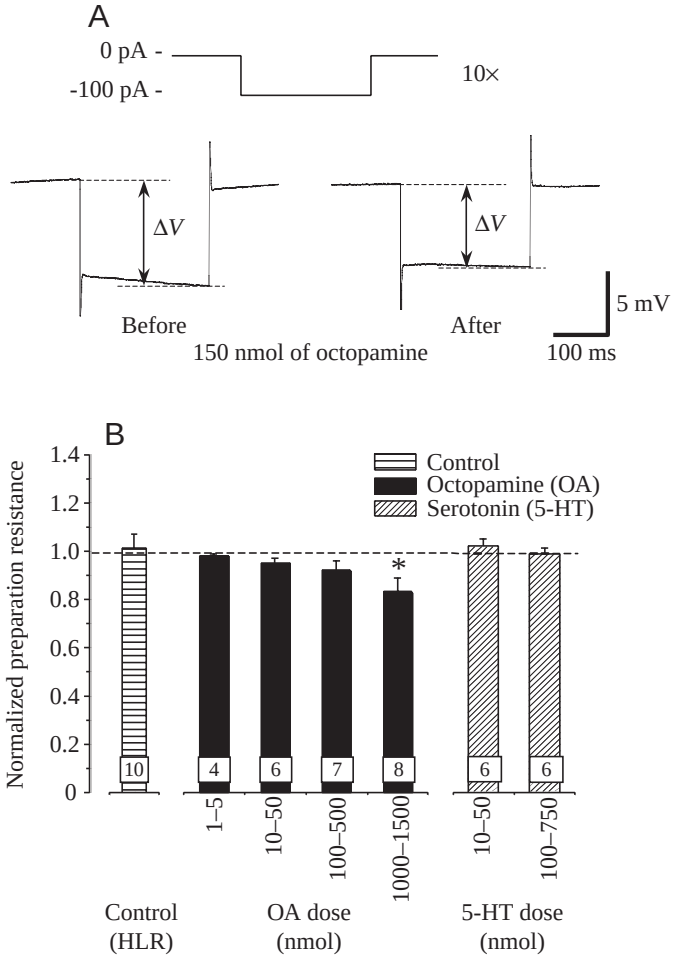

Fig. 10. Octopamine reduced the resistance of the preparation in a dose-dependent manner. (A) For resistance measurements, 10 subsequent pulses of negative current were applied. When compared with the averaged voltage response (Δ*V*) before drug injection (left), an injection of 150 nmol of octopamine decreased the response (right). (B) The normalized resistance R_{after}/R_{before} was computed. While control injections of haemolymph Ringer (HLR) did not alter the resistance of the preparation, octopamine (OA) reduced it in a dose-dependent manner. Serotonin (5-HT), however, had no effect. Values are means + S.E.M. The asterisk indicates a significant difference from the control (P<0.02, Student's t-test).

Discussion

Using extracellular tip recordings from pheromone-sensitive sensilla, we investigated whether the biogenic amines octopamine and serotonin affect the kinetics of the transepithelial potential (TEP) and the occurrence of action potentials in the absence of adequate stimuli. We wanted to know whether the octopamine-dependent increase in the peak nerve impulse frequency in response to pheromone (Pophof, 2000; Grosmaitre et al., 2001) is mediated by accessory cells that generate the TEP of long trichoid sensilla. In addition, we wanted to test whether octopamine exerts its effects *via* modulation of the spontaneous action potentials in ORNs. For the first time, we were able to distinguish the two ORNs in one sensillum and to record for several days from the same intact olfactory sensillum before and after amine injection. Thus, we could analyze slow temporal changes in the kinetics of the TEP and the properties of the action potential. While the biogenic amines did not influence spontaneous action potentials, we found that both amines affected the kinetics of the TEP, presumably acting on different targets.

Amine-dependent effects on the transepithelial potential

Interestingly, we found that octopamine suppressed the oscillations of the TEP in a dose-dependent manner, while serotonin changed the waveform of the oscillation. In accordance with our findings, octopamine did not alter the mean value of the TEP in trichoid sensilla of *Antheraea polyphemus* (Pophof, 2000). In contrast to studies in moths, Küppers and Thurm (Küppers and Thurm, 1975) found an increase in the amplitude of the TEP in whole antennae of the cockroach *Blaptica dubia* after the application of serotonin. In both these studies, the recordings lasted only a few minutes, so slow TEP oscillations were not observed. Octopamine-dependent suppression of TEP oscillations in *M. sexta* could stabilize the TEP at variable values, suggesting that an additional mechanism determines the mean value of the TEP.

Zack (Zack, 1979) concluded from her studies that accessory cells generate the TEP in insect sensilla. It is generally assumed that the TEP is generated by active K^+ transport through the apical membranes of the tormogen/trichogen cells into the sensillum lymph cavity (Thurm, 1972; Thurm, 1974). The high K^+ concentration of approximately 200 mmol l^{-1} in the sensillum lymph causes a potential difference of up to 50 mV between the sensillum lymph and the haemolymph. This potential difference is assumed to add to the transmembrane potential of the outer dendrites. The localization of the *M. sexta* midgut V-ATPase in accessory cells of antennal moth sensilla (Klein, 1992; Klein and Zimmermann, 1991) suggests the presence of the same two-step mechanism for generation of the TEP in the antennal epithelium, as has been described for the midgut and for a number of other epithelia in insects (for

reviews, see Wieczorek, 1992; Wieczorek et al., 2000). Therefore, it is likely that the slow TEP oscillations reflect feedback-coupled regulatory mechanisms both in H^+ transport by the V-ATPase and in the subsequent K^+/H^+ antiport. Because serotonin rather selectively suppressed the shoulder of the TEP waveform, it is assumed that two distinct, but coupled, mechanisms are involved in the generation of the TEP. Octopamine injection, in contrast, reduced the amplitude of the oscillation, suggesting that the octopamine-sensitive mechanism is the initial process, acting on the V-ATPase. The serotonin-sensitive process, however, is delayed, and might therefore reflect a mechanism modulating K^+/H^+ antiport. We will test this hypothesis in future experiments by targeting the V-ATPase and K^+/H^+ antiport. TEP oscillations also occur in pheromone sensilla of the moth *A. polyphemus* (Zack, 1979) and in Malpighian tubules of mosquitoes (Beyenbach et al., 2000), which also contain V-ATPases (Wieczorek et al., 2000). The question of whether the oscillations have any biological significance, however, or are just a by-product of feedback-regulated processes, remains to be determined in pheromone-stimulated sensilla. Interestingly, Zack (Zack, 1979) mentioned that TEP oscillations measured in different sensilla are not necessarily in phase with each other. Possibly, a stable phase relationship between TEP oscillations in different sensilla on the antenna could affect the temporal resolution of pheromone responses. This hypothesis needs to be examined in pheromone stimulation experiments.

It is not clear how octopamine reaches the accessory cells and what the effective drug concentration at the sensilla is. The maximal dose used in these experiments was within the range reported to influence pheromone responses in other moths (Pophof, 2000; Grosmaitre et al., 2001). We then reduced the dose by three orders of magnitude until we reached a concentration that ceased to be effective. An octopamine concentration of between 2 and 17 nmol l^{-1} has been reported in the haemolymph of *M. sexta* (Lehman, 1990). However, it is not known whether active transport can cause octopamine to accumulate in specific tissues, such as the antenna. In addition, it is not known how much octopamine is released by the 1–2 octopaminergic neurons that project into the antenna (U. Homberg, personal communication). Thus, effective doses are difficult to determine. But the findings of amine-dependent effects on the TEP in *M. sexta* reported here and on pheromone responses in other moths (Pophof, 2000; Grosmaitre et al., 2001) indicate that the doses of haemolymph-carried octopamine employed have a functional significance in the antenna. This assumption is further supported by the localization of a neuronal type 3 octopamine receptor, which inhibits cyclic AMP synthesis, at the base of olfactory sensilla on the antennae of the moths *Bombyx mori* and *Heliothis virescens* (von Nickisch-Rosenegk et al., 1996). In addition, the weak and transient octopamine-like effects observed after several of the control injections (Fig. 2B, Fig. 7A) suggest that the TEP oscillations are equally affected by endogenous octopamine. The manipulations during an injection aroused the animals and occasionally caused flight activity (Fig. 3). Flight activity is known to be elicited and maintained by octopamine (Kinnamon et al., 1984; Orchard et al., 1993). Thus, we assume that, during some control injections, a stress-dependent increase in endogenous octopamine levels caused the observed transient suppression of the TEP oscillations together with the initiation of flight activity.

Action potentials

The absence of effects of octopamine and serotonin on action potential activity in the absence of adequate stimuli indicates that these amines alone do not directly influence the action potential generator. It appears that a synergistic factor is required to increase the background activity after octopamine injections in the presence of low pheromone doses (Pophof, 2000; Grosmaitre et al., 2001). It has yet to be tested whether rises in intracellular Ca^{2+} concentration or increases in cyclic GMP or cyclic AMP levels mimic the presence of a low pheromone dose for these octopamine effects. In addition, no correlation between the time course of the TEP and any aspect of the spontaneous action potential activity of the ORNs was found, despite the fact that the TEP adds to the driving force for the generator potential (Thurm, 1972). Thus, the ionic conductivity of the plasma membrane in the outer dendritic segment, the location of the chemo-electrical transduction machinery, is obviously very low in the absence of adequate stimuli. If this were not the case, changes in the driving force up to 20 mV, such as were present during oscillatory intervals, would very likely be reflected in the action potential frequency. This assumption is further supported by the fact that no ion channel openings occurred in unstimulated pheromone-dependent ORNs either *in situ* or *in vitro* (Zufall and Hatt, 1991; Stengl et al., 1992).

Action potentials were elicited by the injection of positive, but not negative, current (see de Kramer, 1985; de Kramer et al., 1984). Positive currents hyperpolarize the outer dendritic segments of the ORNs, but depolarize membranes that are situated basal to the zones of septate junctions located at the transitional region between the inner and outer dendritic segment (Keil, 1989). Together with the polarity of the recorded action potentials (positive phase first), this further confirms the hypothesis that the action potential generator is located in the soma or axon hillock region. Action potentials with the opposite polarity, as would be expected for dendritic action potentials, were never observed. Unexpectedly large currents of more than 200 pA were necessary to elicit action potentials. In current-clamp recordings of vertebrate (Lynch and Barry, 1989; Iida and Kashiwayanagi, 1999; Ma et al., 1999), lobster (Schmiedel-Jakob et al., 1989) and cultured insect (M. Stengl, unpublished observations; I. Jakob, personal communication) ORNs, currents as small as 1–10 pA were sufficient to elicit action potentials. Thus, it appears likely that most of the injected current does not flow through the ORNs, but through a shunt pathway, i.e. through the accessory cells and the surrounding epithelium. Since the measurement of preparation resistance is also based on current injections, the measured resistance is presumably governed by the resistance

of the shunt pathway. This was also assumed by Zack (Zack, 1979) in *A. polyphemus*. The reduction in the resistance of the preparation found after octopamine injection therefore further supports the hypothesis that the accessory cells are targets for the biogenic amines. It has yet to be resolved whether and how this decrease in the resistance of accessory cells can influence TEP oscillations. Zack (Zack, 1979) also showed that the preparation resistance oscillates. These resistance oscillations, however, cannot be directly responsible for the TEP oscillations because the two types of oscillation differed in their kinetics and showed a stable phase difference. If resistance oscillations are also present in *M. sexta*, the actual changes in the preparation resistance after octopamine injection might be even larger than observed.

Physiological implications

What is the physiological relevance for a biogenic-amine-controlled olfactory sensillum? Because biogenic amines can act as hormones, they could adjust the general sensitivity (or state of adaptation) of various targets, at the periphery as well as centrally, at the same time. Such a sensitivity adjustment could possibly be triggered by a biologically relevant signal, such as sex pheromones. The octopamine-dependent increase in the action potential activity of ORNs only in the presence of a low pheromone dose (*Antheraea polyphemus*, Pophof, 2000; *Mamestra brassicae*, Grosmaitre et al., 2001) is consistent with this hypothesis of a sensitivity adjustment in response to a relevant stimulus. In addition, an amine-dependent sensitivity adjustment could be governed by the circadian clock of a nocturnal moth because increased sensitivity to pheromone is strongly dependent on photoperiod cues (Linn, 1997; Linn and Roelofs, 1986; Linn and Roelofs, 1992; Linn et al., 1992; Linn et al., 1996). It is known that octopamine levels in the haemolymph show a circadian rhythm and are high at night, during the activity phase of the moths (Lehman, 1990), when the females release pheromone (Itagaki and Conner, 1988). In our experiments, we tried to keep endogenous octopamine levels constant by disturbing the circadian rhythm of endogenous octopamine release using constant light application (which stops the circadian clock). Thus, we do not know whether the TEP oscillations show a circadian rhythm in undisturbed moths. In future experiments, we will test whether there is a circadian rhythm in the pheromone response of *M. sexta*, whether it is dependent on the presence of octopamine and whether it correlates with circadian changes in the kinetics or the amplitude of the TEP.

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References

Bell, R. A. and Joachim, F. A. (1976). Techniques for rearing laboratory colonies of tobacco hornworms and pink bollworms. *Ann. Ent. Soc. Am.* **69**, 365–373.

Beyenbach, K. W., Aneshansley, D. J., Pannabecker, T. L., Masia, R., Gray, D. and Yu, M.-J. (2000). Oscillations of voltage and resistance in Malpighian tubules of *Aedes aegypti*. *J. Insect Physiol.* **46**, 321–333.

Brosenitsch, T. A. and Katz, D. M. (2001). Physiological patterns of electrical stimulation can induce neuronal gene expression by activating N-type calcium channels. *J. Neurosci.* **21**, 2571–2579.

de Kramer, J. J. (1985). The electrical circuitry of an olfactory sensillum in *Antheraea polyphemus*. *J. Neurosci.* **5**, 2484–2493.

de Kramer, J. J., Kaissling, K.-E. and Keil, T. A. (1984). Passive electrical properties of insect olfactory sensilla may produce the biphasic shape of spikes. *Chem. Senses* **8**, 289–295.

Grosmaitre, X., Marion-Poll, F. and Renou, M. (2001). Biogenic amines modulate olfactory receptor neurons firing activity in *Mamestra brassicae*. *Chem. Senses* (in press).

Iida, A. and Kashiwayanagi, M. (1999). Responses of *Xenopus laevis* water nose to water-soluble and volatile odorants. *J. Gen. Physiol.* **114**, 85–92.

Itagaki, H. and Conner, W. E. (1988). Calling behavior of *Manduca sexta* (L.) (Lepidoptera: Sphingidae) with notes on the morphology of the female sex pheromone gland. *Ann. Ent. Soc. Am.* **81**, 798–807.

Kaissling, K.-E. (1995). Single unit and electroantennogram recordings in insect olfactory organs. In *Experimental Cell Biology of Taste and Olfaction* (ed. A. I. Spielman and J. G. Brand), pp. 361–377. Boca Raton, New York: CRC Press.

Kaissling, K.-E., Hildebrand, J. G. and Tumlinson, J. H. (1989). Pheromone receptor cells in the male moth *Manduca sexta*. *Arch. Insect Biochem. Physiol.* **10**, 273–279.

Keil, T. A. (1989). Fine structure of the pheromone-sensitive sensilla on the antenna of the hawkmoth, *Manduca sexta*. *Tissue & Cell* **21**, 139–151.

Kinnamon, S. C., Klaassen, L. W., Kammer, A. E. and Claassen, D. (1984). Octopamine and chlordimeform enhance sensory responsiveness and production of the flight motor pattern in developing and adult moths. *J. Neurobiol.* **15**, 283–293.

Klein, U. (1992). The insect V-ATPase, a plasma membrane proton pump energizing secondary active transport: immunological evidence for the occurrence of a V-ATPase in insect ion-transporting epithelia. *J. Exp. Biol.* **172**, 345–354.

Klein, U. and Zimmermann, B. (1991). The vacuolar-type ATPase from insect plasma membrane: immunocytochemical localization in insect sensilla. *Cell Tissue Res.* **266**, 265–273.

Küppers, J. and Thurm, U. (1975). Humorale Steuerung eines Ionentransportes an epithelialen Rezeptoren von Insekten. *Verh. Dt. Zool. Ges.* **67**, 46–50.

Lehman, H. K. (1990). Circadian control of *Manduca sexta* flight. *Soc. Neurosci. Abstr.* **16**, 1334.

Linn, C. E. (1997). Neuroendocrine factors in the photoperiodic control of male moth responsiveness to sex pheromone. In *Insect Pheromone Research. New Directions* (ed. R. T. Cardé and A. K. Minks), pp. 194–209. New York: Chapman & Hall.

Linn, C. E., Campbell, M. G., Poole, K. R., Wu, W.-Q. and Roelofs, W. L. (1996). Effects of photoperiod on the circadian timing of pheromone response in male *Trichoplusia ni*. *J. Insect Physiol.* **42**, 881–891.

Linn, C. E., Campbell, M. G. and Roelofs, W. L. (1992). Photoperiod cues and the modulatory action of octopamine and 5-hydroxytryptamine on locomotor and pheromone response in male gypsy moths, *Lymantria dispar*. *Arch. Insect Biochem. Physiol.* **20**, 265–284.

Linn, C. E. and Roelofs, W. L. (1986). Modulatory effects of octopamine and serotonin on male sensitivity and periodicity of response to sex pheromone in the cabbage looper moth *Trichoplusia ni*. *Arch. Insect Biochem. Physiol.* **3**, 161–172.

Linn, C. E. and Roelofs, W. L. (1992). Role of photoperiodic cues in regulating the modulatory action of octopamine on pheromone-response threshold in the cabbage looper moth. *Arch. Insect Biochem. Physiol.* **20**, 285–302.

Lynch, J. W. and Barry, P. H. (1989). Action potentials initiated by single

channels opening in a small neuron (rat olfactory receptor). *Biophys. J.* **55**, 755–768.

Ma, M., Chen, W. R. and Shepherd, G. M. (1999). Electrophysiological characterization of rat and mouse olfactory receptor neurons from an intact epithelial preparation. *J. Neurosci. Meth.* **92**, 31–40.

Nicolson, S. W. and Isaacson, L. C. (1987). Transepithelial and intracellular potentials in isolated Malpighian tubules of tenebrionid beetle. *Am. J. Physiol.* **252**, 645–653.

Orchard, I., Ramirez, J.-M. and Lange, A. B. (1993). A multifunctional role of octopamine in locust flight. *Annu. Rev. Ent.* **38**, 227–249.

Pass, G., Sperk, G., Agricola, H., Baumann, E. and Penzlin, H. (1988). Octopamine in a neurohaemal area within the antennal heart of the American cockroach. *J. Exp. Biol.* **135**, 495–498.

Pophof, B. (2000). Octopamine modulates the sensitivity of silkmoth pheromone receptor neurons. *J. Comp. Physiol. A* **186**, 307–313.

Redkozubov, A. (2000). Guanosine 3′,5′-cyclic monophosphate reduces the response of the moth's olfactory receptor neuron to pheromone. *Chem. Senses* **25**, 381–385.

Roeder, T. (1999). Octopamine in invertebrates. *Prog. Neurobiol.* **59**, 533–561.

Rospars, J.-P., Lánský, P., Vaillant, J., Duchamp-Viret, P. and Duchamp, A. (1994). Spontaneous activity of first- and second-order neurons in the frog olfactory system. *Brain Res.* **662**, 31–44.

Schmiedel-Jakob, I., Anderson, P. A. and Ache, B. W. (1989). Whole cell recording from lobster olfactory receptor cells: responses to current and odor stimulation. *J. Neurophysiol.* **61**, 994–1000.

Stengl, M., Zufall, F., Hatt, H. and Hildebrand, J. G. (1992). Olfactory receptor neurons from antennae of developing male *Manduca sexta* respond to components of the species-specific sex pheromone *in vitro*. *J. Neurosci.* **12**, 2523–2531.

Thurm, U. (1972). The generation of receptor potentials in epithelial receptors. In *Olfaction and Taste IV* (ed. D. Schneider), pp. 95–101. Stuttgart: Wissenschaftliche Verlagsgesellschaft.

Thurm, U. (1974). Mechanisms of electrical membrane responses in sensory receptors, illustrated by mechanoreceptors. In *Biochemistry of Sensory Functions* (ed. L. Jaenicke), pp. 367–390. Berlin, Heidelberg, New York: Springer.

Thurm, U. and Wessel, G. (1979). Metabolism-dependent transepithelial potential differences at epidermal receptors of arthropods. I. Comparative data. *J. Comp. Physiol. A* **134**, 119–130.

von Nickisch-Rosenegk, E., Krieger, J., Kubick, S., Laage, R., Strobel, J., Strotmann, J. and Breer, H. (1996). Cloning of biogenic amine receptors from moths (*Bombyx mori* and *Heliothis virescens*). *Insect Biochem. Mol. Biol.* **26**, 817–827.

Wieczorek, H. (1992). The insect V-ATPase, a plasma membrane proton pump energizing secondary active transport: molecular analysis of electrogenic potassium transport in the tobacco hornworm midgut. *J. Exp. Biol.* **172**, 335–343.

Wieczorek, H., Grüber, G., Harvey, W. R., Huss, M., Merzendorfer, H. and Zeiske, W. (2000). Structure and regulation of insect plasma membrane H^+ V-ATPase. *J. Exp. Biol.* **203**, 127–135.

Williams, J. C., Jr and Beyenbach, K. W. (1984). Differential effects of secretagogues on the electrophysiology of the Malpighian tubules of the yellow fever mosquito. *J. Comp. Physiol. B* **154**, 301–309.

Zack, C. (1979). Sensory adaptation in the sex pheromone receptor cells of saturniid moths. PhD dissertation, Ludwig-Maximilians-Universität, München.

Zufall, F. and Hatt, H. (1991). Dual activation of a sex pheromone-dependent ion channel from insect olfactory dendrites by protein kinase C activators and cyclic GMP. *Proc. Natl. Acad. Sci. USA* **88**, 8520–8524.