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Modulation by Octopamine of Olfactory Responses to Nonpheromone Odorants in the Cockroach, *Periplaneta americana* L.

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Abstract

Olfactory receptor cells in insects are modulated by neurohormones. Recordings from cockroach olfactory sensilla showed that a subset of sensory neurons increase their responses to selected nonpheromone odorants after octopamine application. With octopamine application, recordings demonstrated increased firing rates by the short but not the long alcohol-sensitive sensilla to the nonpheromone volatile, hexan-1-ol. Within the same sensillum, individual receptor cells are shown to be modulated independently from each other, indicating that the octopamine receptors reside in the receptor not in the accessory cells. A uniform decrease in the amplitude of electroantennogram, which is odorant independent, is suggested to reflect the rise in octopamine concentration in the antennal hemolymph. Perception of general odorants measured as behavioral responses changed qualitatively under octopamine treatment: namely, repulsive hexan-1-ol became neutral, whereas neutral eucalyptol became attractive. Octopamine induced a change in male behavioral responses to general odors that were essentially the same as in the state of sexual arousal. Our findings suggest that sensitivity to odors having different biological significances is modulated selectively at the peripheral as well as other levels of olfactory processing.

Key words: insect, octopamine, odorant selectivity, olfaction

Introduction

The physiological state of an animal strongly influences its behavior, including sensory perception. Behavioral modifications found in diverse taxa are, in part, due to modulation of circuits by neuroactive substances. Examples include primary olfactory centers (Wilson and Leon 1988; Gadenne et al. 2001; Greiner et al. 2002; Inokuma et al. 2002; Wilson et al. 2004; Anton et al. 2007; Sachse et al. 2007), centers for sensory association (Wilson et al. 2004; Murakami et al. 2005; Nisimura et al. 2005), and motor circuits (Hooper et al. 1999; Belanger 2005; Marder et al. 2005). Some examples of aperipheral role for neuroactive modulators have been reported for olfactory receptor neurons (ORNs). Such reports derive from studies of vertebrates (Kawai et al. 1999; Eisthen et al. 2000; Park et al. 2003; Czesnik et al. 2007; Pírez and Wachowiak 2008; Savigner et al. 2009), as well as insects (Pophof 2000, 2002; Grosmaitre et al. 2001; Meola and Sittertz-Bhatkar 2002; Zhukovskaya and Kapitsky 2006; Flecke and Stengl 2009; Ignell et al. 2009; Martel et al. 2009; Vergoz et al. 2009).

Behavioral responses of insects to sex pheromones are enhanced by octopamine pretreatment (Linn and Roelofs 1986; Zhukovskaya 2008), whereas those to general odorants can be both suppressed (Zhukovskaya 2008) or enhanced (Vander Meer et al. 2008), depending on the species and the odorant. Octopamine was shown to increase the firing rate of receptor cells to pheromones in different insect species (Pophof 2000, Grosmaitre et al. 2001; Kapitsky and Zhukovskaya 2001, Zhukovskaya and Kapitsky 2006; Flecke and Stengl 2009; Vergoz et al. 2009). In males of the moth *Bombyx mori*, octopamine receptors have been identified at the base of their sensilla trichoidea (Nickisch-Rosenegk et al. 1996). Octopamine/tyramine receptors (MbraOAR/TAR, Brigaud et al. 2009) have also been shown expressed in ORNs of *Mamestra brassicae*, although their precise location remains unknown.

Evidence for octopamine modulation of the sensitivity to nonpheromonal odorants has until now been ambiguous. The amplitude of electroantennogram (EAG) recorded in response to plant-derived volatiles was reported either to decrease (Zhukovskaya 2007, 2008) or increase (Stelinski et al.

2003; Spivak et al. 2003) under octopamine treatment. The only available data on single receptor cell activity did not show any marked effect (Pophof 2002). But because both EAGs sensilla recordings and behavioral responses were all measured using different insect species, it is not clear how sensitivity to odorants representing biological significant cues are modulated for any given species.

The American cockroach, *Periplaneta americana* L., is a well-studied laboratory insect easily reared under controlled conditions. Its sex pheromone-sensitive sensilla are visually distinguished from other morphological types and can therefore be selectively targeted for electrophysiological recording. In addition to pheromone-sensitive cells, the sensilla contain units that respond to eucalyptol (Fujimura et al. 1991), a general odorant found to be repellent (Scriven and Meloan 1984). Aliphatic alcohols, including hexan-1-ol (hexanol), were shown to activate short basiconic sensilla of the "single wall A (swA) type" as defined by Altner et al. (1977) and Schaller (1978). Although hexanol has frequently been used in cockroach olfaction studies (Fujimura et al. 1991; Getz and Akers 1997; Lemon and Getz 1997; Sakura et al. 2002), the behavioral responses to this odor have not been analyzed. Our recent studies have determined hexanol as a repellent, but the repulsion of adult male cockroaches vanished under sexual arousal as well after octopamine ingestion (Zhukovskaya 2008).

The present study describes the responses of cockroach sensilla and the ensuing behavioral responses to general odorants and their observed modulation by octopamine. The purpose of this study was to determine whether sensilla of different morphological and physiological types are selectively affected by octopamine and whether differently tuned receptor cells inside the same sensillum might be modulated independently of each other.

Materials and methods

Animals

Freshly molted imago males of *P. americana* were transferred from the stock colony to the experimental cages at least 2 weeks before experiments. Insects were kept under 12:12 LD regime at 28 ± 1 °C. Water and food were provided ad libitum. The experiments started in the first half of a dark phase, a period of maximum locomotor activity (Lipton and Sutherland 1970) and pheromone sensitivity (Zhukovskaya 1995).

Chemicals and solutions

The odorants Hexanol (Hexan-1-ol, synthesis grade; Merck) and eucalyptol (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane; Fluka) were dissolved in mineral oil (*oleum vaselini*, P 71.273.2. Tver Pharmaceutical Factory). Odorant concentrations were chosen to elicit intermediate EAG responses. Hemolymph saline (NaCl 170 mM, KCl 10 mM, $CaCl_2$ 2 mM, $MgCl_2$ 2 mM, HEPES/NaOH 10 mM, pH 7.4) was used for octopamine (OA) application and EAG recordings. Sensillum lymph saline (Kaissling 1987) was used to fill glass microelectrodes for single sensillum recordings (SSRs). Octopamine (D,L-octopamine hydrochloride, Sigma–Aldrich Co.) was dissolved in the hemolymph saline to a final concentration of 100 mg/mL for topical applications during electrophysiological recordings or in water and sugar syrup (10 mg/mL) for ingestion in behavioral experiments.

Electrophysiology

In each experiment, a male cockroach was restrained with one antenna fixed onto a stable platform. An indifferent electrode (Ag/AgCl) established contact with the surface of the proximal part of antennae through hemolymph saline (Figure 1). Outdoor air was warmed up to the room temperature, charcoal filtered, and humidified before feeding into the setup. Airflow (40 cm/s) was constantly maintained across the middle part of flagellum. An outlet with 5 mm of inner diameter was placed at a distance of 10 mm from the preparation. During the stimulation period, air was first routed through the dispenser loaded with control solvent or the stimulus odor. Valves were controlled by an electrostimulator ESL-2 (Biofizpribor). Suction from the special outlet (Figure 1, insert) accounted for 10% of the initial flow and prevented the preparation from receiving odorant stimuli between experiments. The recording electrode for EAGs was identical to the indifferent one (Ag/AgCl) and was placed distally on the antenna (Figure 1, for more details, see Kapitskii and Gribakin 1992; Zhukovskaya and Kapitsky 2006).

For SSRs, an Ag/AgCl-coupled glass microelectrode with an initial tip resistance of 20–40 MOhm was brought into contact with the cuticle around a sensillum base. The

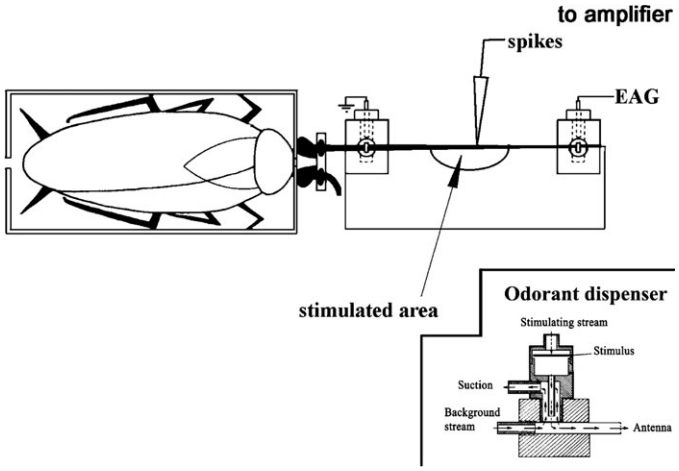

Figure 1 Stimulation and recording scheme. The same ground electrode was used both for single sensillum activity and EAG recordings. Insert—odorant dispenser; antennal outlet is directed to the "stimulated area" of the main scheme. Suction (10% of initial airstream) prevented odor leakage during interstimulus intervals.

morphological identity of the sensillum type was identified under the binocular microscope at a magnification of ×250. Responses by the ORN were preamplified 10-fold by a custom-built 10 GOhm input resistance headstage, amplified by an ISO-DAM amplifier (WPI, Gain 100), high-pass filtered at 300 Hz, and finally digitized with ADC MD88 (Molodtsov V.O., 12 bit, 10 V input range, 20 kHz rate). EAGs were recorded simultaneously with single sensillum activity using a separate custom-built amplifier with signals fed to an ADC MD88 without filtering. Stimulation, storing, viewing, and primary data processing were performed as described elsewhere (Zhukovskaya and Kapitsky 2006).

Four microliters of the test stimulus (10% v/v hexanol, 1% eucalyptol in mineral oil) was loaded onto an 8-mm circle of filter paper disc which was placed into a stimulus dispenser (Figure 1, insert). Stimulations lasted 1 s and were spaced at 300-s interval. Recordings lasted 2 s including 50 ms before and 950 ms after stimulation. The number of spikes was counted for the whole recording. Each animal was used for one experiment only.

Behavioral test

A binary-choice bioassay was used for behavioral tests. The setup consisted of 3 components: a transparent plastic cage (30 × 45 × 30 cm) containing food and water, a constantly dark shelter (17.0 × 17.0 × 5.5 cm), and an exchangeable test chamber (20 × 20 × 8 cm); the latter 2 were separated from the cage with plastic doors (Zhukovskaya 2008). Cockroaches were individually marked with a number written on a piece of medical tape attached to the pronotum. Insects were deprived of water for 20 h before being assayed. Twenty animals were living in the experimental setup. At the beginning of testing, the door between the shelter and the cage was shut, and the light was turned on. In this configuration, the test chamber, which was covered with red transparent films (thus allowing observation by the experimenter), was the only "dark" place available for the insect. One cockroach at a time was allowed to enter the test chamber, which was divided into a common zone (200 × 100 mm) and 2 test compartments (100 × 100 each), each equipped with a single test tube (6 mm inner diameter, volume 1.5 mL) containing either an odorant dissolved in mineral oil or pure mineral oil as a control stimulus (Figure 1B in Zhukovskaya 2008). Eucalyptol (0.1%) and hexanol (0.01%) v/v solutions were used for the tests. The volume of samples was 0.5 mL, filling up a lower third of the tube, which prevented cockroaches from direct contact with a solution. Simple plexiglass holders prevented the tubes from overturning. The positions of the control and test compartments were randomized across the experimental series. After the cockroach visited at least one test compartment, it was allowed to exit the test chamber and enter the shelter. The procedure was repeated until all individuals from the cage were tested. Because some of animals were hiding in the nest, their number slightly varied from day to day.

OA and control treatments

In electrophysiological experiments, the part of the antenna proximal to the indifferent electrode was mounted on a separate platform (Figure 1). A 4 mm diameter plasticine bath was built on the platform around the antenna. Ten microliters of octopamine solution or plain saline was pumped into the bath through a channel in the platform by a pneumatic system driven from outside of the experimental chamber to avoid an uncontrolled rise of the endogenous octopamine level due to stress (Mobius and Penzlin 1993). In behavioral experiments, octopamine dissolved in water and sugar syrup or control samples were placed into the cage 1 h before the tests. The overall volume of solutions was 1 mL. Oral administration of octopamine used in the present study has been successfully used previously (Schulz and Robinson 2001; Spivak et al. 2003; Barron et al. 2007) and does not require handling, anesthesia, or long recovery periods after the treatment.

Experimental protocols

Firing responses to hexanol were recorded from swA and single wall C (swC) sensilla according to Schaller's classification (Schaller 1978). Application of either octopamine dissolved in saline or control saline was performed after 5 successive recordings of hexanol stimulation. The overall duration of each experiment was 100 min. Twenty-two type swC sensilla (11 experiments with octopamine and 11 controls) and 18 type swA sensilla (9 with octopamine treatments and 9 controls) were recorded.

Firing responses to eucalyptol were recorded from single wall B (swB) sensilla (12 replicates with octopamine and 12 controls). Due to the high variation in firing rate and EAG amplitude across individuals, data were normalized by dividing the spike count obtained for each stimulation into the mean value of 3 stimulations preceding application for the same animal.

Firing responses to eucalyptol and Periplanone B (PB) were recorded from swB sensilla (12 replicates with octopamine and 12 controls). Three responses to eucalyptol and 3 responses to PB were recorded before application of OA or control saline. Following 30 min after application, the same sensillum was stimulated with eucalyptol and pheromone again (3 times each odorant). Data were normalized as described above. EAG was not recorded.

Behavioral responsiveness of male cockroaches to hexanol was tested against the solvent as described above. Three series of experiments were conducted: 1) no treatment—10 replicates, 2) control treatment, when insects were deprived from water for 20 h and were exposed to water and sugar syrup 1 h before testing—13 replicates, and 3) octopamine treatment, when water-deprived insects were allowed to consume octopamine dissolved in water and sugar syrup to the final concentration of 10 mg/mL for 1 h before testing—12 replicates.

Behavioral responsiveness of male cockroaches to eucalyptol (10 replicates, 119 choices). A 0.01% v/v eucalyptol

solution was used. Control as well as octopamine treatment experiments were done in 10 replicates.

All measurements are shown as mean ± standard error. The data were statistically evaluated with Student's *t*-test, χ^2 test and two-way analysis of variance (ANOVA).

Results

Responses of single sensilla and EAGs

Recordings of firing activity during stimulation with hexanol revealed that 2 morphological types of sensilla, the short swA and long swC, respond to the odorant. Firing activity of both sensillar types in response to the same hexanol load started from similar values: 49.3 ± 5.3 spikes per recording for swA and 54.6 ± 4.6 spikes for swC. Spiking of swC sensilla did not change over time in control experiments (one-way ANOVA $F_{19/166} = 0.87$, $P > 0.05$), whereas spiking of swA type decreased (one-way ANOVA $F_{19/144} = 2.5$, $P < 0.01$) (Figure 2). Because the exact position of recording microelectrode relative to the ORNs slightly differed between experiments, the spike amplitudes and shapes varied significantly making sorting unavailable.

Compared with controls, octopamine treatment resulted in an increased firing in swA sensilla (two-way ANOVA $F_{1/120} = 51.3$, $P < 0.001$) but not swC sensilla (two-way ANOVA $F_{1/144} = 0.76$, $P > 0.05$) (Figures 2 and 4). EAG responses to hexanol significantly decreased after octopamine application (two-way ANOVA $F_{1/330} = 25.4$, $P < 0.001$, Figure 2) consistent with recent data (Zhukovskaya 2008).

The pheromone-sensitive sensilla of swB type responded to eucalyptol stimulation with an average firing rate of 29.7 ± 4.1 spikes per recording. A slight drop in spiking with time was statistically insignificant (two-way ANOVA $F_{10/261} = 0.47$, $P > 0.05$, Figure 3, sample trace Figure 4). The firing rate of eucalyptol responses was not affected by octopamine treatment (Figure 3; two-way ANOVA $F_{1/261} = 0.66$, $P > 0.05$). EAG recorded simultaneously with single sensilla activity decreased after octopamine application (Figure 3; two-way ANOVA $F_{1/282} = 159.2$, $P < 0.001$) as well as with time (two-way ANOVA $F_{10/282} = 2.9$, $P < 0.01$).

When the same swB sensillum was stimulated with both eucalyptol and PB, the results were substantially the same, namely, octopamine application caused increased firing in response to PB but did not affect responses to eucalyptol (Figure 5, Student's *t*-test, $t = 5.94$ $P < 0.001$).

Behavioral responses

Behavioral responses to hexanol vapors were consistent with previously reported data (Zhukovskaya 2008); in particular, the repellence to male cockroaches of hexanol odor was confirmed (Table 1).

Behavioral responses in control tests (Table 1, "syrup") were practically the same as those in the untreated group. Thus, 20-h long water deprivation followed by access to water and syrup did not affect odorant preference. Choices of octopamine-treated insects were random (Table 1). The difference between the control and the octopamine-treated groups was also statistically significant ($\chi^2 = 6.97$; $P < 0.01$).

Eucalyptol neither repelled nor attracted male cockroaches in control experiments. Octopamine-treated males visited the chamber with odor sample more frequently than the other one with pure solvent (Table 2). The difference between the groups was significant ($\chi^2 = 4.7$; $P < 0.05$).

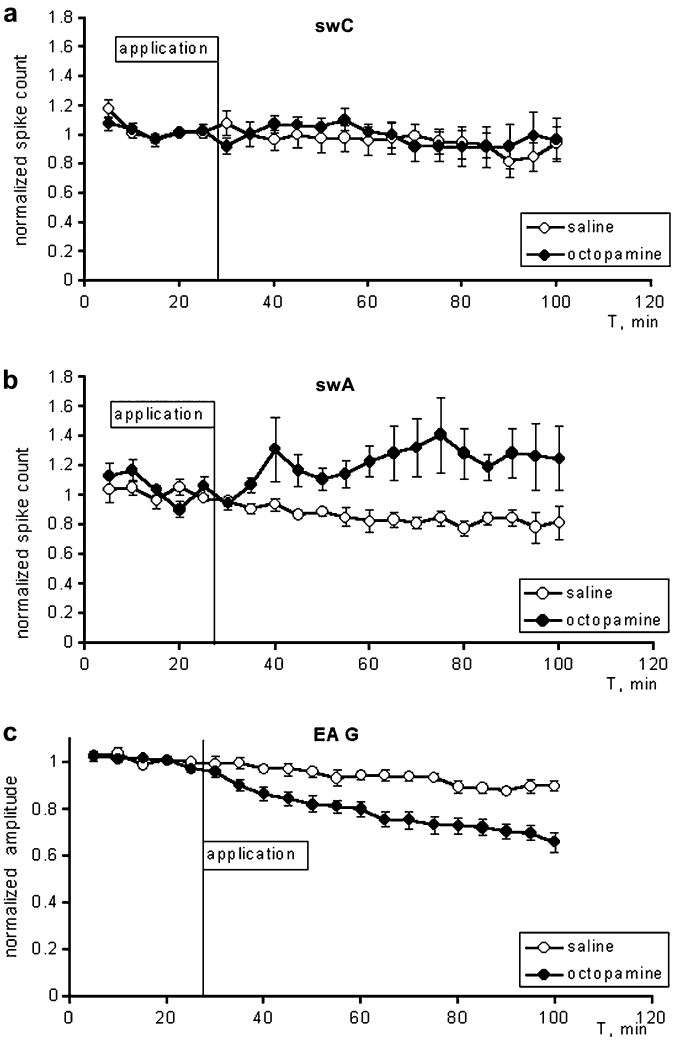

Figure 2 Electrophysiological responses to hexane-1-ol. **a**—firing activity of swC sensilla, **b**—firing activity of swA sensilla, **c**—EAG. All data are mean ± standard error.

Discussion

Although the antennal sensory system of the American cockroach has been studied for more than half a century (Roys 1954), many aspects of its physiology and behavioral roles remain obscure. Although it is not surprising that the present study showed swA sensilla responding to hexanol (Altner et al. 1977; Getz and Akers 1997; Lemon and Getz 1997),

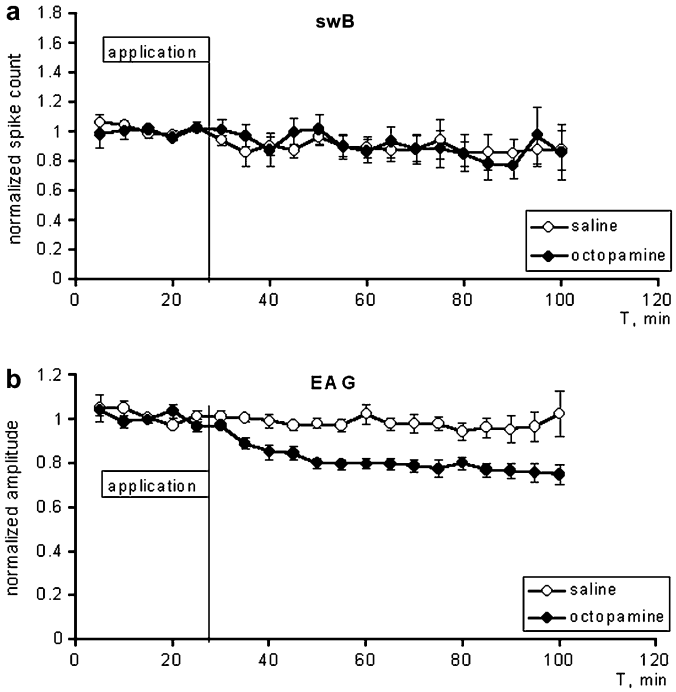

Figure 3 Electrophysiological responses to eucalyptol (1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane). **a**—firing activity of swB sensilla, **b**—EAG. All data are mean ± standard error.

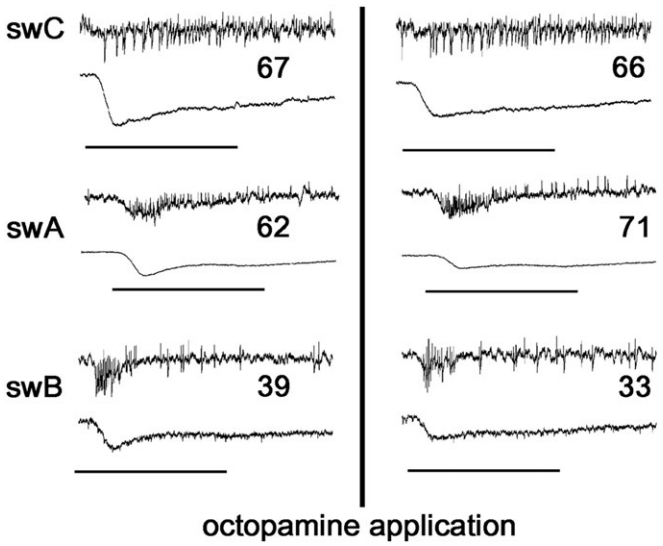

Figure 4 Sample records. Upper trace—SSR; lower trace—EAG, bar—odor stimulus (1000 ms). Numbers are the spikes counts measured for the whole recording (2000 ms) including 50 ms before and 950 ms after stimulation. swC: Responses of swC sensillum to hexan-1-ol, (left) before octopamine application; (right) 70 min after application. swA: Responses of swA sensillum to hexan-1-ol, (left) before octopamine application, (right) 80 min after application. swB: Responses of swB sensillum to eucalyptol (left) before octopamine application, (right) 75 min after application.

a response also was clearly recorded from swC sensilla (type "T," using the classification of Fujimura et al. 1991). Because only few odorants were tested for this sensillum type, its responsiveness to hexanol was probably overlooked, although its responses to the complex odor of lemon oil were studied thoroughly (Hinterwirth et al. 2004). Responses of swC sensilla to hexanol were excitatory and stable over time (Figures 2 and 4). Sometimes it was even possible to distinguish spikes generated by different receptor neurons (Figure 4 swB), but more commonly, it was not possible to discriminate between firing patterns of individual cells.

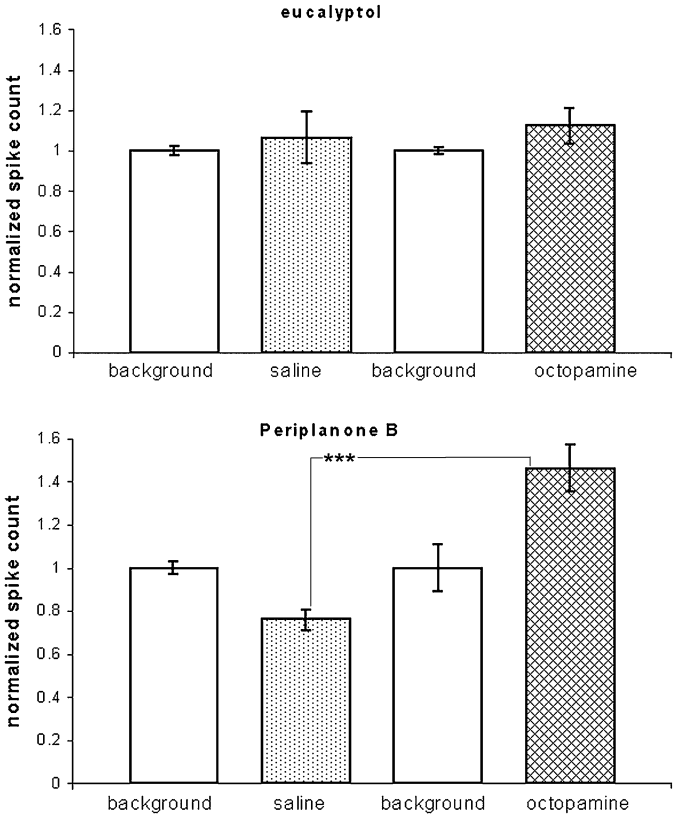

Figure 5 Firing activity of swB sensilla in response to eucalyptol and Periplanone B before (background) and after saline or octopamine application. All data are mean ± standard error. ***—the difference is statistically significant, $P < 0.001$.

A decline in firing rates of short swA sensilla with time could possibly be explained by the adaptation of hexanol sensitive ORN(s) to repeated stimulation. The presence of adapting and nonadapting cells responding to the same odorant might provide the central nervous system with precise information about the intensity of the stimulus, along with the advantage of a broader dynamic range for adapting cells. However, although unlikely, it cannot be entirely ruled out that the smaller swA sensilla are more susceptible to damage by the recording electrode than the larger swC ones.

During the course of the experiment, the antenna became partially wetted by liquid exuded by the antennae itself. However, in the fixed condition, the animal is unable to groom its antenna, so pores on swA sensilla could become blocked. However, this would not apply to the swC sensilla, as these have no pores on the basal parts of their shafts

Table 1 Behavioral responses of cockroach males to hexan-1-ol

Treatment	*N*	Sample		χ^2 (H_0—no preference)
		Hexan-1-ol 0.01%	Solvent	
Without treatment	116	41 (35%)	75	9.97, $P < 0.01$
Syrup	197	68 (35%)	129	18.9, $P < 0.001$
Syrup + octopamine	150	88 (48%)	96	0.34, $P > 0.05$

Table 2 Behavioral responses of cockroach males to eucalyptol

Treatment	*N*	Sample		χ^2 (H_0—no preference)
		Eucalyptol 0.01%	Solvent	
Syrup	118	63 (53%)	55	0.54, $P > 0.05$
Syrup + octopamine	95	64 (67%)	30	12.2, $P < 0.001$

(Schaller 1978). Firing of swB sensilla in response to eucalyptol decreases only slightly (statistically insignificant), and the dimensions of swB sensilla fall between those of swA and swC sensilla (18–28 μm for swB against 8–12 μm for swA, and 30–40 μm for swC, according to Schaller 1978), thus supporting the assumption of partial blocking of sensillar pores. In freely behaving insects, liquid exudation presumably occurs naturally on the antennae and may have adaptive function in removing particles or residual ligands. Increase in surface liquid level after prolonged odor stimulation and excessive antennal grooming of unrestrained cockroaches has been described elsewhere (Zhukovskaya 2010). Thus, the observed differences in the time course of responses of sensilla belonging to different morphological types are more likely due to the proportion of blocked pores rather than different adaptation properties.

Our previous experiments with simultaneous recordings of EAGs from both antennae showed fast and even distribution of OA (Zhukovskaya and Kapitsky 2006). Simplicity and reliability of the topical application method make it an attractive alternative to the commonly used methods of injection and oral administration (Barron et al. 2007). Indirect estimation of the concentration of octopamine gives us values about 10^{-7} to 10^{-6} M (Zhukovskaya and Kapitsky 2006), which are within the physiological range (Adamo et al. 1995).

Octopamine application to the cockroach selectively affects firing responses of its antennal receptor cells to pheromone, as well as to nonpheromone odorants. This contrasts with Pophof's (2002) observations on the silkmoth *B. mori*, showing that OA influences response to pheromone but not to general odorants. It should be cautioned that all but one of the previous studies on the effects of OA on insect ORNs dealt with pheromone stimuli, but not with general odorants (Pophof 2000; Dolzer et al. 2001; Grosmaitre et al. 2001; Flecke and Stengl 2009. Pophof (2002) showed that plant-responsive ORNs are not modulated by octopamine. However, because those experiments were done with females lacking pheromone-sensitive ORNs, it is not clear whether females modulate their antennal receptors at all or whether any of the nonpheromone-sensitive ORNs (including those of males) are under OA control. The present study demonstrates that responses to some general odorants are indeed modulated by octopamine, but responses by the same insect to some other odorants are not. In the present study, responses of swB sensilla to eucalyptol do not change after OA application, whereas responses of swA sensilla to hexanol do change after OA application. Thus, there is a selective regulation of sensitivity to a general odor in the specific condition of sexual arousal by the male.

Shorter swA sensilla increase their firing rate during octopamine treatment similar to the responses of swB sensilla to pheromones, reported earlier (Kapitsky and Zhukovskaya 2001; Zhukovskaya and Kapitsky 2006). Our data are consistent with the recent description of the putative octopamine/tyramine receptor (MbraOAR/TAR) expression patterns in the antenna of the moth *M. brassicae* (Brigaud et al. 2009), which demonstrated specific OAR/TAR labeling of specific types of olfactory sensilla. The data of Brigaud et al. (2009), however, do not disprove that OARs can be expressed in accessory cells, because the method just show labeling at the sensillum level and antennal nerve. Although mRNA expression in sensillar cell(s) does not necessarily prove the presence of the protein inside a sensillum, it does indicate that signal detection can be upregulated at the level of the sensory cell. For example, the sex peptide in *Drosophila* is bound to its receptors at the base of the antennal nerve, that is, the axons of ORNs (Ottiger et al. 2000). Similarly, γ-aminobutyric acid (Root et al. 2008) and tachykinin (Ignell et al. 2009) receptors were found in the terminals of sensory neurons in the antennal lobes. The alterations in the firing rates of ORNs shown in the present study are indicative of modulation at the level of cell bodies because only sex pheromone-sensitive ORNs inside the swB sensilla changed their firing during octopamine treatment, whereas the eucalyptol-sensitive cells remain unaffected. Until now, it could not be ruled out that octopamine modulates accessory cells through the receptors on their basal membranes, as suggested by Dolzer et al. (2001), whereas ORNs are affected presynaptically at a central location. The unchanged overall spike frequency after octopamine treatment in responses of swC sensilla to hexanol may also suggest the excitatory effect of the neurohormone on one ORN and an inhibitory effect on another ORN within the same sensillum.

It is generally believed that a voltage signal recorded from the primary olfactory organ in response to odorants—the EAG—reflects the summed receptor potentials (Kapitskii and Gribakin 1992; Mousley et al. 2006). However, because an antenna is a complex organ, the electrical properties of its components jointly contribute to the shape and magnitude of the recorded EAG. A more or less uniform decrease of EAG amplitude in response to all odorants tested so far in the cockroach does not match the changes in firing activity

or behavioral effects of octopamine. Nevertheless, such a robust drop in EAG is a very useful indicator of the rise in octopamine level in an antenna (Zhukovskaya and Kapitsky 2006; Zhukovskaya 2008).

Although an enhanced behavioral responsiveness to pheromone components directly corresponds to an increased firing in pheromone-sensitive sensilla, general odorants alter their biological significance: namely, the repulsive odor of hexanol becomes neutral and neutral eucalyptol becomes attractive. However, distinguishing alterations of sensitivity as opposed to odor quality is challenging because the same nonpheromone substances have been shown to be perceived as different odors when presented in different concentrations (Wright et al. 2005). Despite such ambiguities, octopaminergic neuromodulation in the brain relating to odor processing (Boeckh and Ernst 1987) is the most plausible explanation for qualitative alterations of behaviors described here. Octopamine treatment caused practically the same changes in behavioral responses of males to general odorants, such as hexanol and eucalyptol, as sexual arousal (Zhukovskaya 2008, 2009). These results are in agreement with the observed in male crickets rise of octopamine level during courtship (Adamo et al. 1995). Thus, the proposed scheme of odor sensitivity modulation is arousal-dependent release of octopamine into an insect hemolymph, which in turn causes changes in the sensitivity of a subset of ORNs and the modification of odor processing circuits.

The behavior of an insect in response to an odorant is the integral output of sensory information processing in the context of its physiological state. That repellency of eucalyptol to *P. americana* adult males has not been previously reported is not surprising because males have distinct morphological, physiological, and behavioral peculiarities compared with females and juveniles. Examples are eucalyptol-sensitive cells in numerous male-specific sensilla and the "perching" behavior of males (Seelinger 1984). Different sensitivities to the same nonpheromone odors between sexes are common across insect species and serve specific functions (for example, see Hern and Dorn 1999; Larsson et al. 2003; Faucher et al. 2006). One suggestion is that eucalyptol, an abundant plant volatile, is an attractant for male cockroaches to seek out places suitable for perching.

Our data support the distinction between pheromone and general odors processing (Boeckh et al. 1984; Hildebrand 1996; Sandoz et al. 2007; Galizia and Rössler 2010). At least for the cockroach, sexual state and octopamine treatment are responsible for not only quantitative adjustment to sex pheromone sensitivity, namely, a rise in the sensitivity at ORNs and behavioral levels, but also qualitative alterations in the perception of plant-derived odorants, when the biological significance of the signal is changed.

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