above 500 Hz were filtered out and the EMG signal was passed into the integrator for rectification (time constant 10 sec). The averaged potential indicating shivering intensity was recorded with a Rikadenki (Tohshin) potentiometer. The direct EMG signals were monitored continuously with a Tektronix 502A dual beam oscilloscope. The details of the measurement procedures used in this study have been described earlier[11,12].

Results and discussion. As shown in the table the intrahypothalamic administration of TRH induced or potentiated shivering at all ambient temperatures tested. Shivering was stimulated within a few seconds and the maximum was attained in 2–6 min. This was followed by an elevated T_b (fig. 1). The foot temperature increased in all cases (fig. 2). No correlations in the thermoregulatory responses were seen among various dosages, ambient temperatures and injection sites, although a slight dependence was recorded for the seasons (fig. 1). Injections of 1 µl of distilled water did not induce any significant changes in the measured variables.

For the body temperature, our results are in agreement with those observed in the fowl[7]. Intrahypothalamic infusion of TRH (100–500 ng) was shown to increase body temperature of the fowl maximally by 0.8 °C. In the present study the rise in the foot temperature seems paradoxical since it suggests an increased peripheral heat loss. It is, however, in contrast with the responses obtained in the fowl[7].

It is now well known that intrahypothalamic microinjections of various putative neurotransmitters and neuromodulators cause hypothermia in the pigeon in most cases[13,14]. As shown in the present study, a hyperthermic response after TRH injections was always recorded. An explanation for hyperthermia may be drawn from the evidence which shows that TRH is an analeptic agent, at least in mammals, i.e. it has the ability to counteract the depressant effects of other drugs[5]. TRH increases muscle tone and motor activity and activates heat production mechanisms i.e. shivering and tremor[7]. Accordingly, the increase in shivering activity after injection of TRH is associated with the stimulation of excitatory neuronal pathways between cold-sensors and heat production effectors. This idea is supported by the fact that the rise in body temperature is independent of ambient temperature or dosage. A similar observation has been made in rats, where intracerebrally administered TRH increases body temperature independent of the dosage[15].

It was also of interest to note that the intensified shivering was always associated with an activation of a part of the heat loss effectors (vasodilation). The maximum foot temperature was recorded about 20 min later than maximum shivering. It is suggested that the primary function of TRH is to increase shivering and overall activity, followed by a compensatory response through the vasomotor tone to lower the body temperature.

Taken together, our results suggest that TRH is an non-specific excitatory neuromodulator or neurotransmitter in heat production in the pigeon.

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Olfactory receptor systems for sex pheromone mimics in the American cockroach, *Periplaneta americana* L.

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Summary. By application of a differential saturation EAG technique, the olfactory receptor system for compounds active as sex pheromones in the American cockroach was elucidated. The interaction of sex pheromone mimics with receptors responsive to a sex pheromone (periplanone-B) was revealed. As suggested by the single cell recording studies, the presence of sex pheromone receptors responsive specifically to sex pheromones (periplanone-A and -B) was shown, as well as the presence of general odor receptors which are functionally different from the sex pheromone receptors.

The visible sexual excitement behavior observed in the male American cockroach (*Periplaneta americana* L.) following exposure to the monoterpenoid sex pheromone mimic, (+)-trans-verbenyl acetate[2], and its more active analogs such as (+)-verbanyl propionate (VaP)[3] is indistinguishable from the behavior induced by the natural sex pheromones, periplanone-A (PA)[4] and periplanone-B (PB)[5]. However, the chemical structures[2–5] and the threshold dose values[3,6] required for inducing the sexual behavior are quite different between the mimics and the pheromones. This fact stimulated us to question whether the mimics act upon the same receptors as the natural pheromones. The present study was undertaken to examine the receptor system associated with the monoterpenoid sex pheromone mimics by applying a differential saturation electroantennogram (EAG) technique to male antennae of the cockroach. This technique, developed for beetles[7,8] and moths[9], involves continuous antennal exposure to a high concentration of a compound through an airstream (primary odorous stimulation) until the insect receptors are

saturated with that compound. A successfully saturated antenna will not respond to further stimulation (secondary odorous stimulation) by the saturating compound, but will still respond by means of other antennal receptors to different active compounds.

EAG responses were recorded using a system illustrated schematically in figure 1. The scape end of an antenna, excised from an adult male of *P. americana* (a in figure 1), was immersed into saline (b) which was the medium permitting electrical connection of the antenna with an indifferent electrode (d) (chloridized silver wire). The distal end of the antenna was inserted into a recording electrode (c) (glass capillary composed of chloridized silver and saline). The EAG responses yielded were visible on an oscilloscope after amplifications with micro-electrode and biophysical amplifiers. The sex pheromone mimic, Vap, was synthetic[3,10]. The pheromones, PA and PB, isolated from females were pure[11]. Commercial camphor was used for the general odor[12].

From the results of pilot experiments, it was concluded that 500 µg of VaP and camphor, and 1×10^{-2} µg of PA and PB were suitable quantities for primary odorous stimulation in saturating the antennae, while 100 µg of VaP and camphor, and 2×10^{-3} µg of the pheromones were suitable for secondary stimulation to the saturated antennae. The quantity of a compound used for saturation was dispensed onto a filter paper (h) in a 10-ml syringe (e_1). An airstream (30 ml/min) was introduced through a 3-way stop-cock (l) into the path (m), so that the airstream passed through the filter paper (h) picking up a high concentration of the odor used for saturation. A secondary odorous compound on a filter paper (i) was applied to the antenna saturated by the primary odors by pressing the syringe plunger (f). In the case of the use of the other airstream path (n), the air became the saturating base, since the filter paper (g) contained no odorous compound. While an antenna was saturated with a compound, secondary odorous stimulations were carried out using all of the compounds. Such test

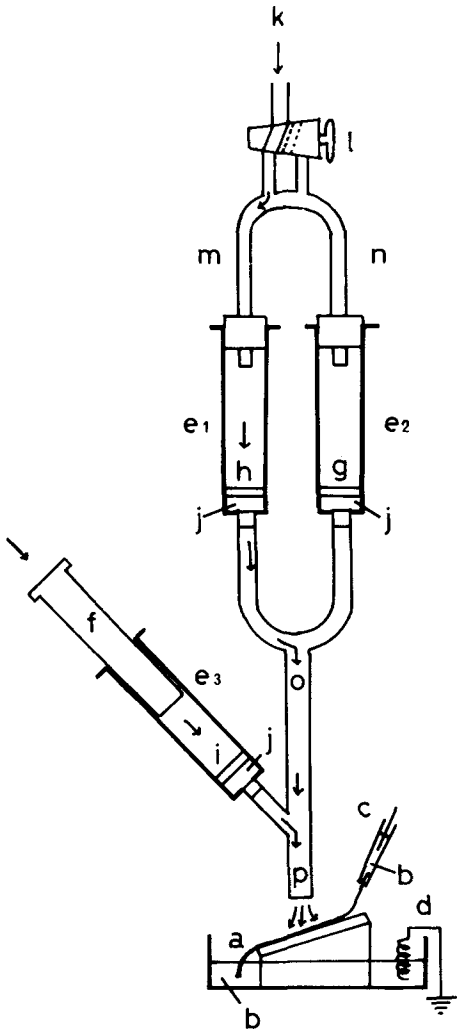

Figure 1. Schematic drawing of odor delivery system and antennal preparation for the differential saturation EAG recording. a Male antenna, b saline, c recording electrode, d indifferent electrode; e_1-e_3 10-ml suringes; f syringe plunger, g filter paper without odorous compound; h filter paper with primary odorous compound for saturation; i filter paper with secondary odorous compound; j vinyl tubing holder; k airstream; l 3-way stop-cock; m path for odor saturation; n path for control (air only) o airstream with primary odor; p airstream with both primary and secondary odors.

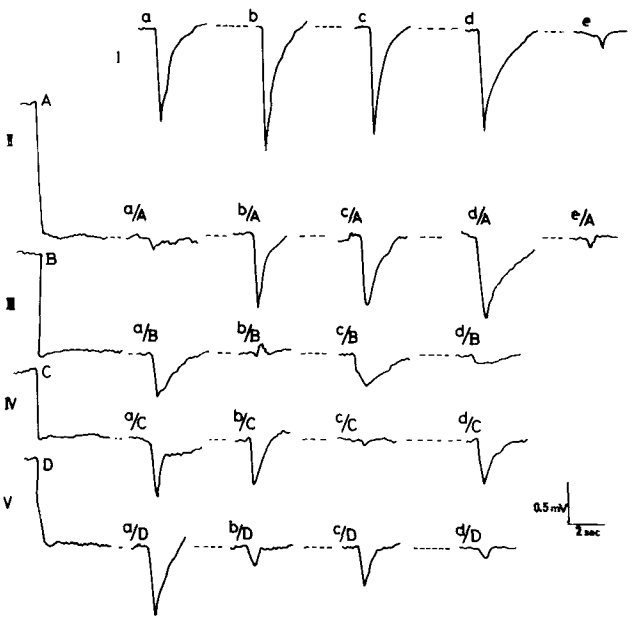

Figure 2. Typical EAG responses recorded in the differential saturation EAG experiments by stimulations with camphor (general odor), (+)-verbanyl propionate (VaP, sex pheromone mimic), periplanone-A and periplanone-B (PA and PB respectively, sex pheromones). **I**, Responses induced by 100 µg of camphor (a), 100 µg of VaP (b), 2×10^{-3} µg of PA (c), 2×10^{-3} µg of PB (d) and air (e) to the base line given by continuous stimulation with airstream without odor. **II**, Responses induced by secondary stimulations with the above quantities of the compounds to the base line saturated with 500 µg of camphor (A), in which, for example, a/A indicates a response obtained from secondary stimulation with 100 µg of camphor to the base saturated with primary odor of 500 µg of camphor. Similar stimulations were performed to the base lines produced by saturation with 500 µg of VaP (**III**-B), 1×10^{-2} µg of PA (**IV**-C) and 1×10^{-2} µg of PB (**V**-D). Good saturations are demonstrated in negligible responses elicited by secondary stimulation of a compound to the base line saturated by the same compound (**II**-a/A, **III**-b/B, **IV**-c/C and **V**-d/D). Discrimination of the general odor receptors from the sex pheromone receptors is seen in significant secondary responses of the pheromonally active compounds against camphor-saturated base (**II**-b/A, c/A and d/A) as well as secondary responses of camphor to the saturated bases by the other compounds (a/B, a/C and a/D). The strong interaction of the sex pheromone mimic, VaP, with the sex pheromone receptors responsive to PB is evident from very small responses seen in **III**-d/B and **V**-b/D. Specific receptors responsive individually to PA and PB are apparent from the responses, **IV**-d/C and **V**-c/D. Calibration: 0.5 mV and 2 sec.

EAG amplitudes (mV) of the adult male American cockroach in differential saturation experiments

		Primary odor for saturation			
		Camphor (500 µg)	VaP (500 µg)	PA (1×10^{-2} µg)	PB (1×10^{-2} µg)
Saturated base line		1.18 ± 0.28	1.06 ± 0.27	1.50 ± 0.18	0.86 ± 0.13
Odor for secondary stimulation (amplitude from saturated base line)	Camphor (100 µg)	0.25 ± 0.13[a]	0.54 ± 0.25[b]	0.50 ± 0.09[a]	0.75 ± 0.17[b]
	VaP (100 µg)	0.83 ± 0.18[b]	0.08 ± 0.04[a]	0.36 ± 0.22[c]	0.16 ± 0.06[c]
	PA (2×10^{-3} µg)	0.88 ± 0.25[b]	0.38 ± 0.22[c]	0.01 ± 0.02[a,d]	0.32 ± 0.07[d]
	PB (2×10^{-3} µg)	0.95 ± 0.25[b]	0.27 ± 0.10[c]	0.31 ± 0.04[d]	0.10 ± 0.06[a,d]

Each amplitude is given by average value ± SD of 3 tests. Values in the line 'saturated base line' means the amplitudes after saturated by the compounds and it became base lines for secondary odorous stimulations (see **II**-A, **III**-B, **IV**-C and **V**-D in fig. 2). The other amplitudes were measured for the responses from the saturated base lines whose shapes are typically shown in a/A, b/A, ..., c/D and d/D in figure 2. Amplitudes with mark [a] (secondary stimulation with a compound to the antennae saturated by the identical compound) demonstrate the smallest values in each column, suggesting good saturations. Discrimination between the general odor receptors and the sex pheromone receptors was based on the data with mark [b]. Amplitudes with mark [c] were major grounds for the participation of Vap strongly with the sex pheromone receptors responsive to PB, but very weakly with those to PA. The occupation of PA and PB to their own specific receptors was indicated by the data with mark [d].

was repeated 3 times. The average amplitudes obtained from the 3 tests are listed in the table together with SD.

Against the saturated bases (**II**-A, **III**-B, **IV**-C and **V**-D in fig. 2), additional stimulation with a compound identical with the saturating compound gave very small or negligible responses (**II**-a/A, **III**-b/B, **IV**-c/C and **V**-d/D) which are comparable to the response evoked by the air control (without odor) to the base saturated by primary odor (**II**-e/A), although the quantities for secondary stimulation were sufficient to elicit strong EAG responses in each compound (**I**-a–d), when only air was used as the saturating base (cf. **I**-e: air to air-saturation). This suggests that receptor saturation by the compounds was complete. All of the sex pheromonally active compounds used as the secondary stimulation elicited large responses from the antennae saturated by the general odor, comphor (**II**-b/A, c/A and d/A). Amplitudes of these responses corresponded to 0.83–0.95 mV which were much larger than the 0.25 mV recorded in the experiment, camphor to camphor-saturation (**II**-a/A). This implies that camphor stimulates general odor receptors[12] which are functionally different from the receptors for the sex pheromonal compounds. This was supported by the following results. Secondary stimulation with camphor to the antennae saturated by the sex pheromonal compounds produced large responses (**III**-a/B, **IV**-a/C and **V**-a/D) with 0.50–0.75 mV amplitudes. In these cases, secondary stimulation with the pheromonal compounds caused no response larger than 0.10 mV to the base when saturated by their own odor (**III**-b/B, **IV**-c/C and **V**-d/D).

In saturation experiments with VaP (see **III**), PA evoked a significantly larger response (0.38 mV, **III**-c/B) in comparison with PB (0.27 mV, **III**-d/B) suggesting 2 important facts: 1. that VaP is strongly associated with sex pheromone receptors responsive to PB, but acts weakly on receptors to PA, and 2. that there are specific receptors individually responsive to PA and PB. Therefore, male antennae were saturated by PA, and then additional stimulations with VaP and PB were performed. In both stimulations, significant responses with equal amplitudes (0.36 mV for VaP and 0.31 mV for PB) were recorded (**IV**-b/C and **IV**-d/C), while only 0.01 mV was recorded by stimulation with PA itself (**IV**-c/C). In the subsequent saturation experiment with PB, further stimulation with VaP produced a small response (0.16 mV, **V**-b/D) similar to that with PB (0.10 mV, **V**-d/D). In contrast, secondary stimulation with PA induced a significantly larger response (0.32 mV, **V**-c/D) against the base saturated by PB. From these pheromone saturation experiments, the above suggestions (1) and (2) were confirmed. Thus, the application of the differential saturation EAG technique to differentiate between olfactory receptor systems on the male antennae of *P. americana* led clearly to the following conclusions. 1. The strong interaction of monoterpenoid sex pheromone mimics with the sex pheromone receptors specific for PB. 2. The presence of individual sex pheromone receptors specific for PA and PB. 3. The presence of general odor receptors which are functionally different from the sex pheromone receptors.

It has been reported from single cell recording studies on the antennal olfactory sensilla of *P. americana* that 2 receptor cells are present, one of which is selectively responsible to PA and the other to PB[13,14], and that there are also food (general) odor receptors[14,15], i.e., (2) and (3) of our conclusions.

The present new discovery, the interaction of monoterpenoid sex pheromone mimics with the receptors for PB, suggests that the structural key moieties for active sites of the sex pheromone receptor show overlapping between the mimics and PB. The investigation of this aspect is in progress, using a molecular model.

The application of the convenient electrophysiological techniques employed here may be generally useful in the elucidation of the interactions of secondary odors in animal olfactory sensory systems adapted to primary odors.

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