Electroantennogram Responses from Parts of Antennae of the American Cockroach

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Electroantennograms (EAG) were recorded from the distal, middle and basal parts of the antennae of adult male and female American cockroach. Stimulating chemicals employed were three sex pheromone mimics [(–)-bornyl acetate, (+)-*trans*-verbenyl acetate and germacrene-D] and a general odor (nerol). EAG responses produced from the male antennae were greater than those from females at any part of the antenna in all compounds. Among the antennal regions, the middle region was stimulated with the strongest EAG response in the male, whereas the distal region was most strongly stimulated in the female with any stimulus. From the above EAG results, the distribution of olfactory sensilla on the antennae is discussed along with the observations of morphologists.

INTRODUCTION

The male American cockroach, *Periplaneta americana* L. (Orthoptera : Blattidae), has been found to respond sexually to several sex pheromone mimics other than the female sex pheromone called periplanone-B (V in Fig. 1) (PERSOONS et al., 1976). The first discovery of the mimics was reported by BOWERS and BODENSTEIN (1971). They found that (+)-bornyl acetate [(+)-BA] (I) and three sesquiterpenoids induce the typical sexual behavior in the males. Recently, NISHINO et al. (1977 b) found other sex pheromone mimics, (–)-bornyl acetate [(–)-BA] (II) and (+)-*trans*-verbenyl acetate (VA) (III) among bicyclic monoterpenoids. TAHARA et al. (1975) isolated germacrene-D (GD) (IV) from several plants as a sex pheromone mimic of the cockroach. This plant-derived compound is of interest, since its structure was the basic skeleton of the natural sex pheromone, periplanone-B.

The electroantennogram (EAG) recordings of these behaviorally active compounds have been examined by the authors and other groups. A crude (WASHIO and NISHINO, 1976) and purified sex pheromone (KAWASAKI et al., 1977; SCHAFER, 1977) produced much greater EAG responses from male antennae than those from females of the American cockroach. GD was also observed to produce basically the same EAG patterns to the pheromone from both of the sexes antennae (WASHIO et al., 1976). The great EAG responses of these sexually active compounds in the male suggest that sex pheromone receptors being on only the male antenna are stimulated.

For the compound which gives no significant behavioral response to either sex, but evokes great EAG responses from both sexes of the insect, we proposed the term “general odor” as opposed to “special odor” which causes some behavioral reaction against both or either sex(es) (NISHINO et al., 1977a). Compounds having hydroxyl or carbonyl groups were found to be good general odors to the American cockroach (WASHIO and NISHINO, 1976; NISHINO and WASHIO, 1976). Nerol (N) used in the present

Fig. 1. Structures of behaviorally active compounds against *P. americana*. Sex pheromone mimics; (+)- and (−)-bornyl acetate (I and II), (+)-*trans*-verbenyl acetate (III) and germacrene-D (IV). Sex pheromone: periplanone-B (V).

study is one of such general odors. These general odors seemed to stimulate general odor receptors on the antennae of both sexes.

Both (+)- and (−)-BA were demonstrated to stimulate strongly both adult male and female, and male nymphal antennae of the American cockroach in our EAG experiment (WASHIO et al., 1976; and unpublished data), although the compounds induced behavioral response in the male only. Accordingly, the acetates are considered to interact not only with the sex pheromone receptor but also with the general odor receptor on the antennae.

We have proposed the significance of the male to female EAG ratio (M/F ratio) (NISHINO et al., 1977a). The ratio reflects the patterns of perception of stimuli to the olfactory receptors mentioned above. In fact, GD gave the largest M/F ratio value, whereas the general odors afforded the smallest values. The ratio of (+)-BA was between those of GD and general odors (NISHINO et al., 1977a). Thus, the M/F ratio seems to be a convenient and useful guide to simply characterize stimuli, in particular, between sexually active compounds and general odors.

Several morphological works have been done on the antennae of the American cockroach (SCHAFER and SANCHEZ, 1973, 1976a, b; NORRIS and CHU, 1974). Most of these focused their investigations on the classification and population of various kinds of sensilla, especially, olfactory sensilla. Quite recently, SCHALLER (1978) morphologically investigated the frequency and distribution of various sensilla on antennal segments of mainly male and nymph of the cockroach. In order to follow SCHALLER's morphological work, we applied the EAG technique to record responses from restricted areas of the cockroach antenna using sex pheromone mimics and a general odor, since the EAG responses from such areas were presumed to reflect the distribution of the olfactory sensilla responsive to the chemicals.

MATERIALS AND METHOD

Stimulating chemicals. Commercial (−)-BA and N were used after distillation. VA was obtained after distillation and column chromatography of a 97% pure VA from a commercial (+)-*trans*-verbenol. GD was isolated from *Solidago juncea* (NISHINO

Fig. 2. Schematic drawings of the antennal preparations in the present experiment. Using silicon grease, 2/3 parts of antenna were covered, and the remaining 1/3 part was exposed to stimulating chemical to record response.

et al., 1977a). The purity of all chemicals were established as almost 100% pure by gas chromatography, IR and PMR spectra. The sex pheromone mimics were behaviorally active at the concentrations reported in (NISHINO et al., 1977a, b).

Antennal preparation for EAG recording. We sectioned first a whole antenna excised from the adult male or female cockroach into three sections, distal, middle and basal parts, and attempted to record EAG from each section. No section, however, gave EAG response. In this case, small electrical resistance due to too short antennal length may cause the undetected response voltage. Therefore, the whole antenna served for the antennal preparation. After 1 mm of the tip was cut off, the antenna was placed on a glass slide with small pieces of adhesive tape, and then the 2/3 parts of the antenna were covered with silicon grease (Toray). After test chemicals were puffed onto the 2/3 antennal parts covered with the silicon grease to confirm no EAG response, the remaining 1/3 parts (distal, middle and basal) were examined (see Fig. 2).

EAG recording. A stainless steel electrode (indifferent) and a silver-halide coated wire in a glass capillary filled with Ringer's solution (recording electrode) were inserted into the scape and distal part of the antenna, respectively. Both electrodes were connected to an amplifier (MZ-4, Nihon Kohden). The amplified signal on an oscilloscope (Tektronix 5115, storage type) was photographed with a Polaroid camera. A filter paper disk (1.3 cm in diameter) impregnated with a test chemical [300 μ g of (—)-BA, VA or N, or 150 μ g of GD] was placed in a 10 ml syringe. After 10 ml of air had been drawn into the syringe, the odor was mixed into the air stream (30 ml/sec) by pushing the plunger, so that the air including odor was puffed onto the antennal preparation through 0.5 cm-diameter glass tubing. The glass tubing was terminated 1.5 cm from the middle part of the antennal preparation in the recording from whole antenna. The distance between the tubing and the preparation was shortened to 1.0 cm in the experiment with the parts of antenna. An interval of 30 sec between tests was used routinely. As a control the same procedure was performed without a test chemical. Each sample was repeated twice for each antenna.

RESULTS AND DISCUSSION

EAGs from whole antenna

The EAG magnitudes and M/F ratios from the whole antennae of male and female are listed in Table 1.

All chemicals stimulated the male antennae more strongly than the female, supporting the morphological observations in SCHAFER and SANCHEZ (1973), and SCHALLER (1978) that the number of sensilla containing olfactory cells is greater in the male antenna of the American cockroach than in the female.

Furthermore, SCHAFER and SANCHEZ (1973) morphologically found that more than half of olfactory trichoid sensilla on the adult male antenna appear in the adult stage in the animal. They also stated that the sexual dimorphism apparent in adult antennae is limited solely to an extra component of thin walled sensilla trichodea which are almost certainly connected with pheromone receptors. SCHALLER (1978) pointed out from his morphological study of the cockroach that in the sensilla of adult male antenna, long sensilla responsible for reception of sex pheromone are most frequent and that approximately half of these sensilla are newly-generated during the final molting. In the present data, GD evoked strong EAG responses from the male, but weak responses from the female, as observed previously (WASHIO et al., 1976). Consequently, the M/F ratio became very high value (7.9). The tremendously high ratio value indicates that this compound interacts with the sex pheromone receptors on the sex specific sensilla suggested by SCHAFER and SANCHEZ (1973) and SCHALLER (1978).

N elicited prominent EAGs from the antennae of both sexes with the smallest M/F ratio value (1.8) given to the compounds stimulating the general odor receptors on the male and female antennae. The M/F ratio of (–)-BA (2.0) and VA (2.4) were present between those of GD and N. VA which has been found to possess the same EAG pattern as that of (+)-BA (WASHIO et al., 1976) in addition to the similarities of the chemical structure and behavioral activity (NISHINO et al., 1977b) as those of (+)-BA, suggest that VA also has both the sex pheromonal and general odorous functions.

EAGs from parts of antenna

Table 2 shows the EAG magnitudes obtained from three parts of the antennae of male and female, and Table 3 represents the distribution (%) of EAG responses of

Table 1. EAG Responses (mV)^a FROM THE WHOLE ANTENNA OF *P. americana*
TO (–)-BORNYL ACETATE [(–)-BA], (+)-*trans*-VERBENYL ACETATE
(VA), GERMACRENE-D (GD) AND NEROL (N)

Stimulus	Male	n ^b	Female	n	M/F ratio ^c
(–)-BA (300 µg)	0.83±0.04	40	0.41±0.02	38	2.0
VA (300 µg)	0.93±0.05	40	0.38±0.02	39	2.4
GD (150 µg)	1.10±0.08	40	0.14±0.01	43	7.9
N (300 µg)	0.83±0.05	40	0.46±0.02	38	1.8

^a Mean response±standard error.

^b Number of repetitions.

^c Male mean response divided by female mean response.

Table 2. EAG RESPONSES IN PARTS OF ANTENNA OF *P. americana*

Stimulus		Antennal part		
		Distal	Middle	Basal
(–)-BA (300 µg)	Male	0.34±0.03 ^a	0.41±0.04	0.20±0.01
	Female	0.21±0.03 ^b	0.14±0.01	0.11±0.01
Va (300 µg)	Male	0.36±0.04	0.43±0.05	0.25±0.02
	Female	0.23±0.02	0.15±0.01	0.13±0.01
GD (150 µg)	Male	0.39±0.04	0.54±0.07	0.28±0.04
	Female	0.08±0.02	0.04±0.00	0.01±0.01
N (300 µg)	Male	0.35±0.05	0.52±0.04	0.22±0.02
	Female	0.26±0.02	0.18±0.02	0.18±0.02

^a Mean response±standard error of 18 tests in mV.^b Mean response±standard error of 16 tests in mV.Table 3. DISTRIBUTION OF EAG RESPONSES ON MALE AND FEMALE ANTENNAE OF *P. americana*

Stimulus		Antennal part						Total (A) + (B) + (C)	M/F
		Distal (A)	(%) ^a	Middle (B)	(%)	Basal (C)	(%)		
(–)-BA	Male	0.34 ^b	(36)	0.41	(43)	0.20	(21)	0.95	2.1
	Female	0.21	(46)	0.14	(30)	0.11	(24)	0.46	
VA	Male	0.36	(35)	0.43	(41)	0.25	(24)	1.04	2.0
	Female	0.23	(45)	0.15	(29)	0.13	(26)	0.51	
GD	Male	0.39	(32)	0.54	(45)	0.28	(23)	1.21	9.3
	Female	0.08	(61)	0.04	(31)	0.01	(8)	0.13	
N	Male	0.35	(32)	0.52	(48)	0.22	(20)	1.09	1.8
	Female	0.26	(42)	0.18	(29)	0.18	(29)	0.62	

^a $\frac{(A), (B) \text{ or } (C)}{(A) + (B) + (C)} \times 100$.^b Values are mean responses in Table 2.

the partial antennal parts, which were calculated on the basis of the average magnitudes in Table 2.

The male antenna always showed greater EAG magnitude than the female at any part and with any stimulus. The distribution of EAG response among the three parts of the male antenna decreased in the following order, middle (41–48%), distal (32–36%) and basal part (20–24%) in all stimuli. This means that the olfactory sensilla possessing the general odor and/or sex pheromone receptor are most abundantly scattered on the middle portion of male antenna.

SCHALLER (1978) classified sensilla of the American cockroach antenna into three categories, *Wp* (wall pore), *Np* (no-pore) and *Tp* (terminal pore) sensilla on the basis of his TEM and SEM observations. The *Wp* sensilla are composed of five types of olfactory sensilla, *sw A*, *sw B*, *sw C*, *dw* (double walled) *A* and *dw B*, in which *sw B* sensillum is considered to be concerned with the sex pheromone reception. The data of Figs. 4 and 5 in SCHALLER's report (1978) briefly demonstrate the frequency and distribution of the sensilla on the male and nymphal antennae, and are suitable for discussion of our EAG distribution results. The figures include the sensilla, *sw B*, short *wp* (combined *sw A* and *dw A*), *sw C* and *Tp*. In these, the short *wp* and *sw*

C sensilla seemed to have a function of reception of our general odors from his description. The distribution of *wp* and *sw C* in the figures indicates that these are scattered most numerous on the middle part of male antenna, and decrease toward the distal and basal parts. The trend of our EAG distribution in (—)-BA, VA and N on the male antenna matches the above observation by SCHALLER (1978).

The population of the sex pheromone sensillum, *sw B*, is displayed as middle, basal and distal portion in order of abundance on the male antenna (Figs. 4 and 5 in SCHALLER, 1978). In GD, which stimulates the sex pheromone receptor, the EAG distribution appeared most abundantly in the middle region (45%) on the male antenna. The following order, distal (32%) and basal (23%), was, however, inconsistent with the result of SCHALLER (1978). To resolve the contradiction, further investigations will be required from both morphological and electrophysiological fields.

In the EAG experiments with the parts of female antenna, the distribution of EAG response decreased in the following order, distal (42–46%), middle (29–30%) and basal portion (24–29%) in (—)-BA, VA and N. Accordingly, the olfactory sensilla responsive to the general odor seemed to be most abundant on the distal region in the female antenna unlike the male. For GD, although the EAG magnitude was very weak in the distal and middle, and was negligible in the basal region of female antenna (Table 2), the trend of EAG distribution was similar to that for the other compounds (Table 3).

The interpretation of the significant (notwithstanding very weak) EAG responses of the female antenna to the sex pheromone and GD has not been performed yet. SCHALLER (1978) found that among the sensilla of the adult male antenna of the American cockroach, long type *sw B* with sex pheromone receptor is most frequent. He also mentioned that about half of the long *sw B* are newly-formed with at the time of the imerginal ecdysis, whereas the other half are derived from the short type *sw B* which are present in all larval stages of both sexes and in adult female. This short *sw B* on adult female antenna might respond to GD and the sex pheromone in EAG.

No study has been done morphologically on the population and distribution of sensilla in antennal parts of the female, although the total number of various kinds of sensilla on the female antenna of the American cockroach was investigated (SCHAFER and SANCHEZ, 1973). Therefore, our EAG distribution results on the female antenna can not be compared with morphological aspects at present.

We have felt uneasy about whether or not the penetration of stimulating chemical to olfactory sensilla on whole antenna is adequate in EAG. The summated EAG magnitudes of the three parts (Total in Table 3) always appeared to be 10–30% larger than the magnitudes with the whole antennae (Table 1) in all compounds in both sexes. Although this indicates that the penetration of stimulus to olfactory cells is insufficient, it is practically not so important because the M/F ratios in Table 1 and 3 are similar.

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