The Nature and Development of Sex Attractant Specificity in Cockroaches of the Genus *Periplaneta*

IV. ELECTROPHYSIOLOGICAL STUDY OF ATTRACTANT SPECIFICITY AND ITS DETERMINATION BY JUVENILE HORMONE

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ABSTRACT The antennae of male *Periplaneta americana* acquire a large number of olfactory receptors at the adult stage. Electrophysiological methods (single unit and electroantennogram recording) show that a portion of the receptors added at the adult ecdysis are sex attractant receptors. Sex attractant receptors are not present in large numbers on larval and adult female antennae. The differentiation of pheromone receptors is inhibited during normal larval development by juvenile hormone. Topical application of juvenile hormone-mimic to male antennae during the terminal larval instar inhibits their development. Comparative electrophysiological studies indicate a high degree of cross-reactivity of the *P. americana* sex attractant among four other species within the genus *Periplaneta*.

Electrophysiological recording from cockroach antennae is a line of research with a troubled history. For example, Chapman and Craig ('53) recorded muscle potentials associated with olfactory and mechanical stimulation of the antennae of *Periplaneta americana*, but incorrectly attributed the potentials to nervous elements in the antennae and central nervous system. Roys ('54) was the first to record genuine nervous activity in *Periplaneta* antennae, but did not pursue antennal recording in subsequent work on the cockroach (Smyth and Roys, '55). Loftus ('66, '68, '69) reported the first detailed electrophysiological work on cockroach antennae. He limited his investigations to single unit recording with tungsten microelectrodes from a morphologically identifiable thermoreceptor sensillum ("cold receptor") on the antennae of *P. americana*.

Boeckh et al. ('63) recorded electroantennogram (EAG) responses from *P. americana* antennae exposed to a sex attractant extract. Adult male, female, and larval antennae all responded strongly to the extract. This seemed unusual, since larval cockroaches do not show sexual behavior, and females would seem to have little need for receptors for their own sex attractant. The reported isolation and identification of the active principle of this extract (Jacobson et al., '63) was disputed in the literature (Wharton et al., '63; Jacobson and Beroza, '63). Following the synthesis of the reported structure (Day and Whiting, '64), behavioral tests demonstrated that the compound was behaviorally inactive (Jacobson and Beroza, '65).

The concluding paper in this series (Boeckh et al., '70) reported that a new attractant extract produced large EAG responses in male antennae, but not in females. They stated that, "Because there is not very much difference in the number of sensilla (olfactory hairs) on the antennae of the two sexes, the differences in response to the attractant extract is presumably due to specificities of the receptor cells. . . ." But the first part of this statement is incorrect, since male *P. americana* have nearly twice as many antennal olfactory organs as females (Schafer and Sanchez, '73; Lambin, '73). The mistake is understandable

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because there is no obvious morphological difference between individual male and female olfactory sensilla. The difference can be clearly demonstrated only by laboriously counting olfactory sense organs on specially-treated antennae (Schafer and Sanchez, '76a). Most recently, the Boeckh group has explored the problem of olfactory coding in receptors which respond to odorants other than pheromones (Sass, '73; Boeckh, '74; Boeckh et al., '75; Waldow, '75), and has not pursued the problem of specialization for pheromone reception.

Yamada ('68, '70, '71) and Yamada et al. ('70) used microelectrodes to record from the olfactory lobe of the brain of *P. americana* while stimulating the antennae with an odorous airstream. At least two aspects of these studies deserve critical examination. First, the *on, off,* and *on-off* responses attributed solely to olfactory neurons and given considerable attention in these papers (e.g., Yamada, '68: fig. 1) could easily be mechanoreceptor responses from the numerous thick-walled hairs on the antenna (Lambin, '73) or responses of central multimodal neurons receiving both mechano- and chemoreceptive input (Waldow, '75). Second, Yamada et al. ('70) claimed that sex attractant-specific receptor cells are present in both male and female antennae of *P. americana.* This claim is at variance with the results of Boeckh et al. ('70) and Washio and Nishino ('76) who recorded strong EAG responses to sex attractant extracts in male antennae, but little or no response in female antennae. On the other hand, the utility of EAG's (presumably massed receptor potentials recorded as a negative-going tip-to-base potential) has been questioned (e.g., Adler, '71; Birch, '71). Also, all of these studies employed pheromone extracts—not chemically identified compounds.

Based primarily on work with silk-moth antennal receptors, it has been generally believed that two entirely distinct classes of receptors exist on insect antennae: "generalist" receptors which respond to a variety of common odors such as those of foods, and "specialist" receptors which respond only to certain odorants such as pheromones (e.g., Schneider, '69). This cogent principle has lost some of its force with the finding that so-called "specialist" receptors in some insects have quite variable response spectra and that some individual receptors may respond both to behaviorally excitatory compounds and behavioral inhibitors (e.g., O'Connell, '75). The concept of highly specialized sugar and salt receptors in blowflies has also suffered retrenchment (Dethier, '74). It appears now that the classification of some receptors as "specialists" may be as much a heuristic convenience as a precise description of real receptors. I will employ the term "sex-attractant receptor" to designate olfactory receptors whose reaction spectra are limited primarily to responses to the appropriate pheromone. The reader should also bear in mind during subsequent discussions which simplistically refer to *the* sex attractant of *P. americana* that more than one attractant compound may be involved, as has been demonstrated in some species of moths (e.g., Jacobson et al., '70; Roelofs et al., '75) and possibly in *P. americana* (Persoons et al., '74).

The present study examined sex attractant reception in the antennae of unaltered *P. americana* and in insects whose development had been affected by experimental intervention in the endocrine system. The endocrine experiments were undertaken because the development of the antennal olfactory receptors is influenced by juvenile hormone (JH) (Schafer and Sanchez, '76b). Sexual dimorphism develops in the antennae only at the adult stage, because JH, which is secreted during the larval period but not prior to the adult ecdysis, inhibits the differentiation of olfactory sensilla in males. Adult males normally have twice as many olfactory sensilla as adult females. The adult male pattern can be induced precociously by removing the corpora allata, glands which produce JH. Or, the male pattern can be inhibited by dipping the antennae in a solution containing juvenile hormone-mimic (JH-M) early in

the terminal larval instar (Schafer and Sanchez, '76b). Electrophysiological recording was undertaken to determine whether JH might control the appearance of sex attractant receptors by inhibiting their differentiation in larvae and females.

The chemical identity of the *P. americana* sex attractant is as yet unknown, and it was therefore necessary to use crude fecal extracts containing attractant, or purified (but chemically unidentified) extracts. The extracts used were shown to be highly potent through behavioral testing (Schafer, '77), but the experiments are still inadequate to demonstrate absolute specificity.

MATERIALS AND METHODS

Experimental animals

Single Unit and EAG recordings from adult and larval *P. americana* L. were made from insects donated by J. G. Sternburg at the University of Illinois, Urbana-Champaign (strain *P.a.*/UR of Schafer and Sanchez, '76a). The sources of other strains of *P. americana* and other species within the genus *Periplaneta (P. australasiae, P. brunnea, P. fuliginosa,* and *P. japonica)* have been previously reported (Schafer and Sanchez, '76a). The *P. americana* used in most of the hormonal experiments were raised in this laboratory (*P.a.*/MR of Schafer and Sanchez, '76a). Recordings were made on adults one to four weeks after emergence from the terminal ecdysis, and on larvae about two weeks after the last larval ecdysis. Recordings on hormonally-altered insects were made two to four weeks after the last ecdysis. No age-dependency of receptor responses was noted within the period of one to four weeks post emergence (cf. Rees, '70). All recordings, except those from adult females, were made from insects which had been isolated from adult females (or other pheromone sources) for one week.

Pheromone extraction and preparation of stimulation cartridges

Crude extracts of larval, adult male, and adult female *P. americana* (*P.a.*/UR or *P.a.*/BE) feces were prepared as previously described (Schafer, '77). Highly purified *P. americana* sex attractant was obtained from K. Nakanishi and J. Tabak of the Department of Chemistry, Columbia University. One hundred microliters of each extract (in purified n-hexane) or the purified pheromone (in purified n-pentane) were applied to a 2 × 2 cm square of Whatman No. 1 filter paper contained in the barrel of a glass stimulation cartridge. After application, the ethereal solvent was allowed to evaporate to dryness as determined visually. Stimulation cartridges were prepared 15 minutes prior to each experiment. Cartridges containing purified pheromone were reused once (eight stimulations, total). All other cartridges were used for only one series of stimulations (four, total), although a pilot study showed little or no loss in stimulating ability with as many as ten stimulations, or after storage for up to two months. A control (blank) cartridge contained untreated filter paper. Two microliters of purified amyl acetate were used as a standard, highly stimulatory odorant.

Stimulation apparatus and geometry

Stimulation was programmed using a Grass S48 electronic stimulator driving a low current relay (Potter Brumfield No. JR-1051) which in turn operated a 3-way electric valve (Skinner Valve Co., New Britain, CT) in the stimulating air line. An air bottle (Airco Zero Gas) supplied dry stimulating air at 500 ml per minute. The air was passed through activated charcoal, adjusted to ambient room temperature (23-25°C), and humidified to 93 ± 3% R.H. before reaching the stimulating cartridge. Stimuli were of 0.25-second duration, given in a set order (see below) at 100-second intervals.

The stimulating cartridge consisted of a glass tube 0.75 cm I.D. × 3.8 cm long, with a 7/15 ground glass fitting for attachment to the stimulating air line. The 2 × 2 cm filter paper containing the stimulatory odor was rolled and inserted to abut the constriction

next to the ground glass joint. The orifice was situated 5.0 cm lateral to the antenna. An evacuation system continuously removed stimulatory odors from the stimulation site by slowly drawing air downward and away from the site and exhausting it to the outside of the building. A Faraday cage with solid walls shielded the preparation from electrical noise and room air currents, and prevented stimulatory odors from escaping into the laboratory.

Preparation and mounting of antennae for EAG recording

Antennae were severed 1.2 cm distal to the pedicel. A capillary electrode (15-25 μm tip diameter) filled with Ringer's solution was inserted 0.2 cm into the cut end of the antenna and the antenna fixed to the electrode with paraffin. The distal end of the antenna was then severed and a larger, Ringer-filled capillary slipped over the distal end and sealed with paraffin, leaving 1.8 cm of the antenna exposed to the stimulating air stream. The number of olfactory sensilla (thin-walled hairs) on this region of the antenna was calculated at 16,250 sensilla in adult males, 9,600 sensilla in adult females, and 8,210 sensilla in eleventh instar larvae of both sexes. Actual counts on antennae used in EAG recording showed a variation of no more than ± 10%. A better method would have been to select pieces of larval, adult male, and adult female antennae of identical length *and* total number of olfactory sensilla. However, calculations incorporating the differences in the density and distribution of sensilla along the antennae showed that this refinement was physically impossible The method used here eliminated a variable number of distal sensilla, but produced the most repeatable results in pilot tests using alternative methods, including whole-antenna recording.

Electronics and recording

Electrical signals were amplified by a Grass P16 microelectrode preamplifier and read out on a Tektronix storage oscilloscope. For EAG recording, band pass filters were set at the following points: DC or −3dB at 0.1 Hz (lower cutoff) and −3dB at 100 Hz (upper cutoff). Typically, recordings were not made in full DC mode to avoid continual readjustment to compensate for slow baseline shifts. Limiting the lower frequency to −3dB at 0.1 Hz had no effect on EAG amplitude in comparison with the amplitude achieved in full DC mode. For single unit recording the band pass was set at −3dB at 1 Hz (lower cutoff) and −3dB at 3 kHz (upper cutoff). A second and third oscilloscope trace were used to display a stimulus/amplitude marker trace and a time base.

The electrophysiological responses of the various types of antennal sensilla were surveyed prior to examination of olfactory responses. It was necessary to know the breadth of the unit response spectrum to adequately interpret the EAG results. Mechanoreceptor responses were obtained from thick-walled hairs by inserting a tungsten microelectrode at the base of the sensillum, or by slipping a Ringer-filled capillary over the tip of the sensillum. The mechanoreceptor was stimulated when recording with a tungsten electrode by moving the hair with a separate microprobe. The recording electrode moved the hair shaft when a capillary electrode was used. Tungsten microelectrodes were used to record from hair plate sensilla.

In the EAG experiments, the following stimuli were applied for 0.25 seconds at intervals of 100 seconds: (1) control (blank) cartridge; (2) larval extract; (3) male extract; (4) female extract; (5) purified pheromone; and (6) amyl acetate. This sequence was repeated four times for each antenna examined. The purified pheromone was used in fewer than half of the experiments due to its limited availability. The EAG waveform did not vary from antenna to antenna. Strict attention was given to the constancy of stimulus geometry and other stimulatory parameters.

For single unit recording from olfactory sensilla, a tungsten microelectrode (tip diameter <1 μm) was inserted near the base of the sensillum on the side toward

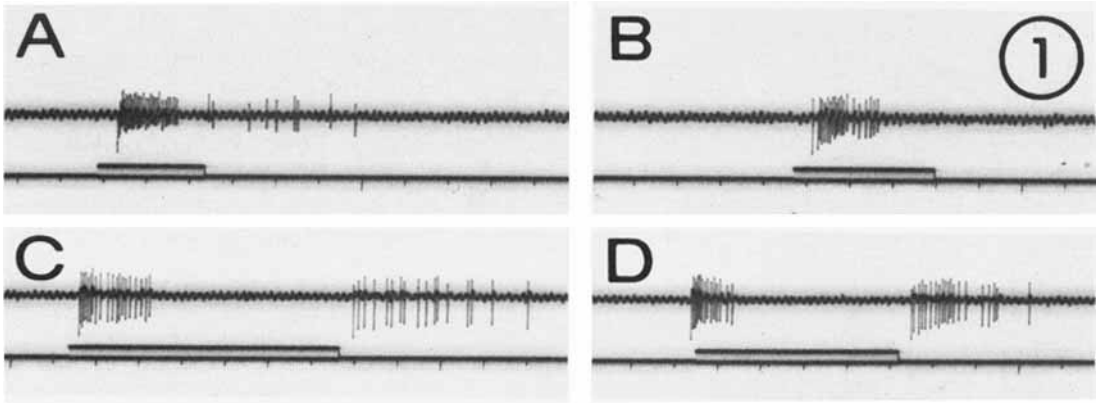

Fig. 1 Responses of mechanoreceptive neurons in thick-walled antennal sensilla of *Periplaneta americana* recorded by a capillary electrode slipped over the tip of the hair shaft.

A,B Typical response pattern. The mechanoreceptor (top trace) responds to a deflection of the hair shaft with a phasic burst of action potentials. The mechanoreceptor does not respond when the hair shaft is returned to the resting position (end of stimulus marker trace). More than 80% of the mechanoreceptors of thick-walled antennal sensilla produce this characteristic response: an "on-response" to the onset of the deflection, but no "off-response." The bottom trace contains time marks at 0.1-second intervals. Records A and B are from the same mechanoreceptor.

C,D The mechanoreceptors of a few thick-walled sensilla respond to deflection of the hair shaft both at the onset of the deflection (an "on-response") and at return of the hair to the resting position (an "off-response"). Most sensilla will not respond in this way even with repeated trials using different angles of deflection. Records C and D are from the same mechanoreceptor.

the base of the antenna. Stimulus geometry and odorants were identical to those used in EAG recording, with the addition of several other odorants. Cartridges with 2.0 μl of purified amyl acetate, hexanol, citronellal, triethylamine, and butyric acid were used. Mixed food odors of possible biological significance were also used: Swiss cheese, apple, banana, and hamburger (flesh). A fresh 0.5 cm^3 piece of each substance was smeared on a 2 × 2 cm filter paper and placed in a cartridge. For single unit analyses, 1.5-second stimuli were applied at 100-second intervals in the following order: blank control, larval extract, female extract, amyl acetate, Swiss cheese, apple, banana, hamburger, hexanol, citronellal, triethylamine, and phenyl ethyl sulfide. Stimuli of longer, 1.5-second duration (vs. 0.25 second in EAG studies), were used to increase the probability of identifying slowly-responding or less sensitive single units. Each set of stimuli was run three times on each unit. Purified pheromone was not available at the time of single unit recording.

RESULTS

Single unit response types

Mechanoreceptors of thick-walled chemoreceptors

An adult male *P. americana* antenna contains 6,000 to 14,000 thick-walled hairs, depending on the strain (Schafer and Sanchez, '76a). Mechanoreceptors responses were consistently obtained from thick-walled sensilla using either tungsten or capillary electrodes (fig. 1). Of 70 thick-walled sensilla examined from all parts of the antennal flagellum 83% produced mechanoreceptive responses, and the remainder no response. Nearly every thick-walled hair, therefore, possesses a mechanoreceptor among its usual complement of three to five receptor cells. The typical response was a phasic burst of 5-25 action potentials from a single receptor as the hair was deflected toward the surface of the antenna from its initial resting position (fig. 1). An occasional hair responded both to the onset of the deflection and the return to the resting position, but more than 80%

responded *only* to the initial deflection. The direction of the initial deflection (toward the antennal surface) corresponds to the direction the hair would be moved when the antenna contacts a solid object under natural conditions. In such a contact, hundreds of hairs would be stimulated.

The tungsten electrode recording technique was used to examine the response of thick-walled hairs to puffs of air from the olfactory stimulation apparatus. Twenty-four of 58 thick-walled hairs examined (41%) produced responses to the stimulating air stream (a 0.25 second pulse at 500 ml/minute).

Electroantennogram responses will include a mechanoreceptive component contributed by thick-walled hairs. The sections of *P. americana* antenna used for EAG recordings have an average of 3,110 thick-

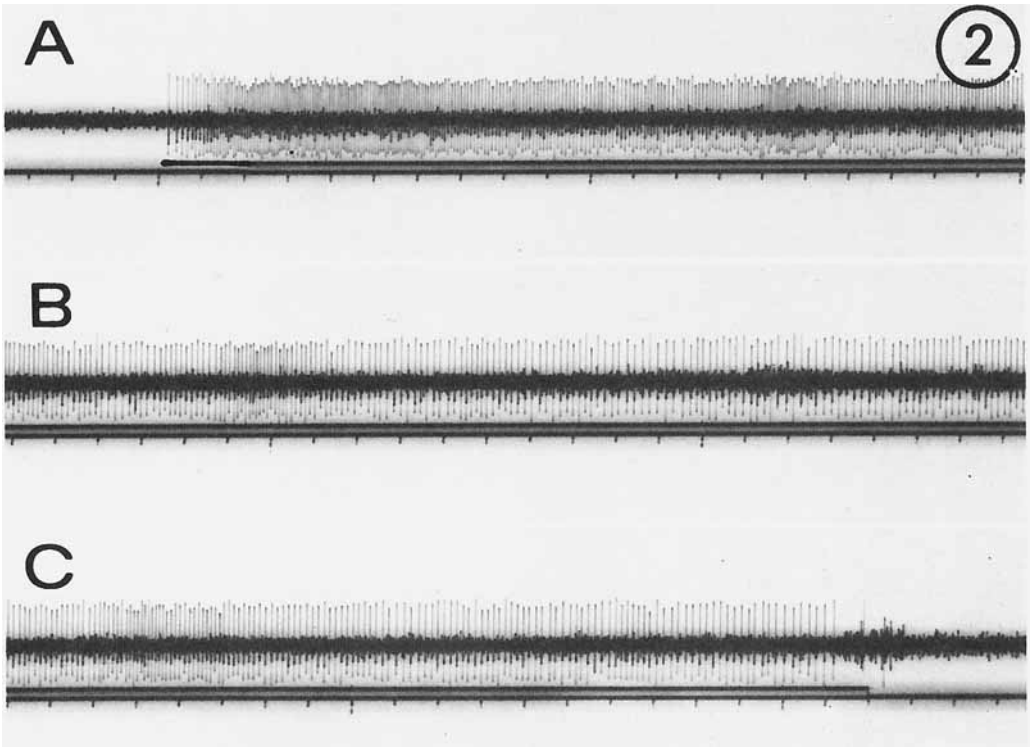

Fig. 2 Response of a hair-plate mechanoreceptor at the scape-pedical articulation in *Periplaneta americana* recorded with a tungsten microelectrode inserted near the base of the hair. The hair-plates occur at the scape-pedicel articulation and at the joint between the scape and head at the base of the antenna. Flexion of the joint causes the tips of the hairs to contact the cuticle of the adjacent segment which deflects the hair shaft (Schafer and Sanchez, '73).

A Onset of joint flexion. The mechanoreceptor responds to joint flexion with a phasic-tonic train of action potentials. The onset of the stimulus marker trace indicates the point at which the joint was flexed.

B Flexion is maintained at the new position and the receptor reaches a constant frequency of firing, indicating maintained flexion. The joint was flexed 14° from its resting position and resulted in a 30-35° displacement of the hair shaft. Tests employing different rates and angles of flexion showed that a single hair-plate sensillum is capable of registering both velocity and angle of joint flexion.

C After remaining in the flexed position for 7.2 seconds, the joint is re-extended to its original position (total record not shown). The receptor ceases responding at the instant the joint is extended. The receptor mechanism is thus highly directional.

walled hairs in eleventh instar larvae of both sexes, 2,550 in adult males, and 2,650 in adult females. Thus, the mechanoreceptor component of the EAG should be approximately equal in male and female adults, and slightly higher in larvae. Mechanoreceptors are presumably the major source of the responses to control (blank) stimuli containing no odorant (tables 1-4), with temperature and humidity effects probable minor contributors. The magnitude of the control response increased linearly with increasing air velocity within the range of 250-1,500 ml/minute. Responses to control stimuli vanished when antennae were killed with heat or cyanide. Assuming each thick-walled hair has a single mechanoreceptor, and that there are two olfactory receptors per thin-walled sensillum, the mechanoreceptors represent a population of receptors equal to 7.8% of the total number of olfactory receptors in the section of male antennae used for EAG recording. The corresponding figure for adult females is 13.8% and is 37.9% in male and female larvae.

Momentary stimulation of the tips of the thick-walled sensilla using micropipettes filled with 0.1 M NaCl or 0.1 M D-fructose produced action potentials from cells other than the mechanoreceptor. Although no systematic study was made of the chemoreceptive responses of the thick-walled sensilla, generally two, and sometimes three, cells could be discerned on the basis of spike height. Electrophysiological responses therefore indicate an innervation of at least three to four receptor cells: one mechanoreceptor and two to three contact chemoreceptors. Possible olfactory responses of the thick-walled sensilla were not tested.

Mechanoreceptors at the joints

The basal segments of the cockroach antenna have hair plate sensilla associated with the movable articulations (Schafer and Sanchez, '73). Flexion of the scape-pedicel articulation produced phasic-tonic excitatory responses from the hair-plate sensilla (fig. 2). No comprehensive study was performed, but these sensilla were highly sensitive to joint movement, thus confirming their suggested role as proprioceptors (Schafer and Sanchez, '73). These sensilla are directionally-sensitive (fig. 2), and signal both rate of joint movement and the angle of maintained flexion. No attempts were made to record from campaniform sensilla or chordotonal organs.

Responses of olfactory receptors

Olfactory receptors generally produced phasic or phasic-tonic excitatory responses to odorous stimulation (fig. 3). Inhibitory effects on tonically-active cells were rare and were not scored in the study of specificity (table 4).

EAG responses of untreated antennae

Responses to control stimuli (blank cartridges) were consistent within a series of runs on a single antenna, but the general

TABLE 1

Electroantennogram (EAG) responses of normal adult males of the species Periplaneta americana *(N = 44)*

Stimulus	Average response of each of four runs				Four runs averaged
	Run 1	Run 2	Run 3	Run 4	
Blank	0.32mV	0.29mV	0.21mV	0.29mV	0.28mV
Larval extract	0.90	0.77	0.74	0.72	0.78
Male extract	0.88	0.67	0.62	0.62	0.70
Female extract	2.09	1.55	1.34	1.27	1.56
Purified pheromone [1]	1.99	1.94	1.81	1.46	1.80
Amyl acetate	2.47	1.45	0.99	0.82	1.43

[1] N = 11.

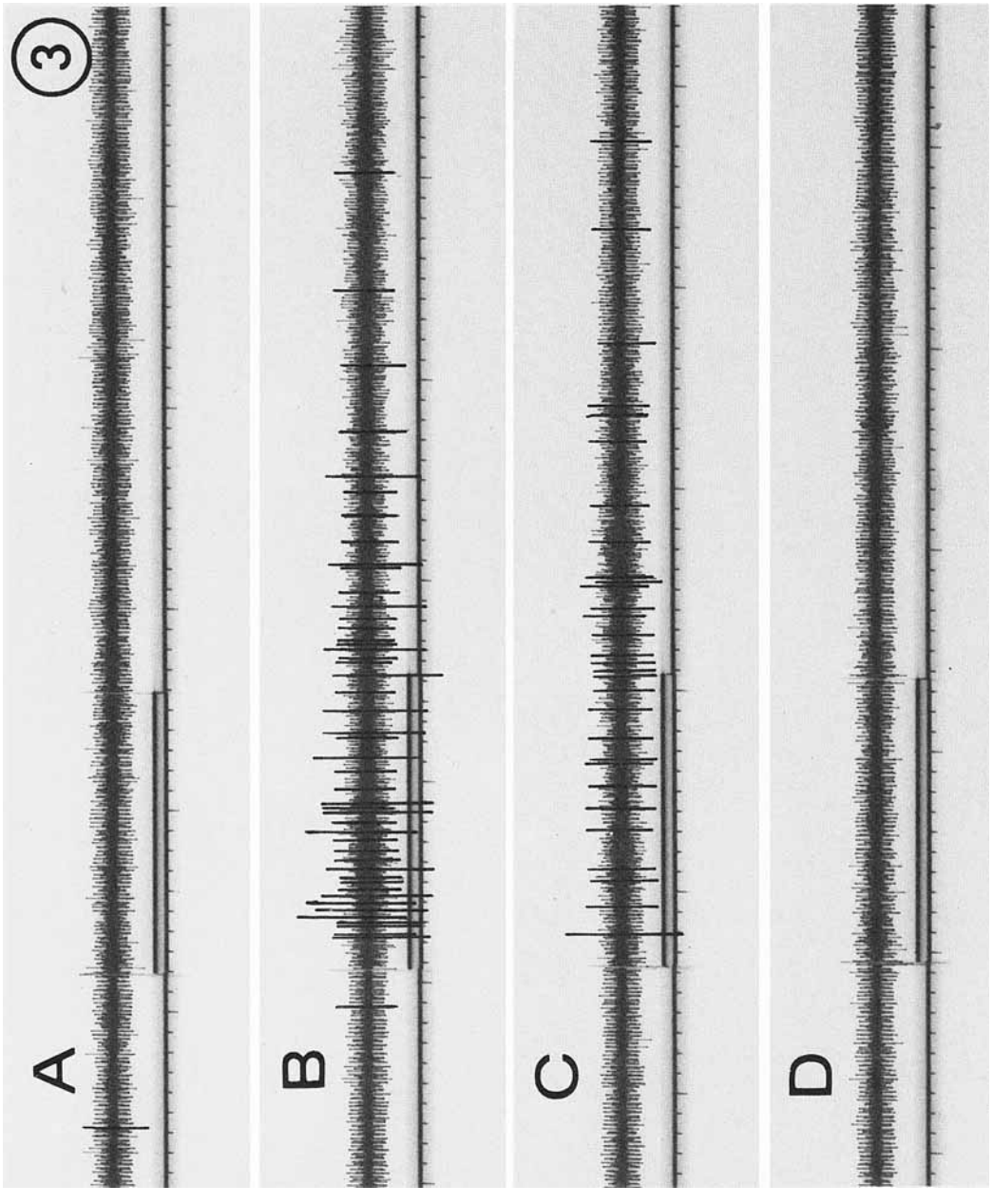

Figure 3

responsiveness among different antennae varied. Generally, those antennae which produced larger control responses were more responsive to odorous stimuli.

Table 1 shows the average responses for adult male antennae throughout a series of four stimulation runs with six stimuli given at 100-second intervals. Approximately 40 minutes elapsed between the first stimulus in the first run and the last stimulus of the fourth run (6 stimuli/run × 4 runs/experiment = 24 stimuli/experiment; 100 seconds/stimulus × 24 stimuli/experiment = 40 minutes/experiment). Figure 4 depicts a single experiment on a male adult carried out to five runs. The magnitude of the responses to each type of stimulus invariably declined during an experiment, except for the control (mechanoreceptive component). This pattern was also observed in female and larval antennae, although these data are not shown. The largest decline always appeared in the responses to amyl acetate. In males, 62% of the total decline took place between the first and second stimulus with amyl acetate. Since ten minutes elapsed between these stimuli, metabolic decline may be a more likely explanation for decreasing responsiveness than adaptation.

Fig. 3 Responses of olfactory receptors in a thin-walled antennal sensillum. Recordings A-D are from the same sensillum with stimuli applied at 100-second intervals.

A Blank (control) stimulus. A single, tonically-active receptor is firing, but is not excited or inhibited by the control stimulus (air only). The second trace is a stimulus marker trace indicating the 1.5-second duration of the stimulus. The third trace contains time marks at 0.1-second intervals.

B Amyl acetate stimulus. Two receptors, which can be differentiated by two different spike heights, are excited by the amyl acetate. The tonic cell is unaffected. The action potentials of the two responding cells have been retouched for reproduction.

C Banana stimulus. One of the two cells which responded in B is excited by banana odor about as much as it was excited by amyl acetate. The other cell responds with a single action potential. Note that the tonic cell responds to none of the stimuli.

D Female extract stimulus. None of the cells respond to the extract of female feces which contain sex attractant. By contrast with the receptors illustrated here, nine out of 52 receptors tested on adult male antennae responded only to female extract.

Responsiveness declined much less rapidly or not at all when stimulating with attractant extracts or purified pheromone (table 1, fig. 4). In fact in five of eleven male antennae, the responses to the purified pheromone increased, rather than decreased. The differences in response decline may indicate a qualitative difference between the antennal response to amyl acetate (and similar esters) and the response to other odorants, particularly the sex attractant. Amyl, ethyl, and butyl acetates are more highly stimulatory in EAG recording than other compounds with a functional group containing oxygen, i.e., alcohols, aldehydes, ketones, phenols, and ethers (Schafer, unpublished data). Amyl acetate is also a notoriously effective stimulant of vertebrate electroolfactogram responses.

The data of table 1 demonstrate that adult male antennae are highly responsive to female extract and the purified pheromone. In a pilot study to determine the possible influence of adaptation, recordings were made from adult males taken directly out of cultures containing adult females. Isolation did not significantly enhance the electrophysiological response within the range of sensitivity of the EAG method. On the other hand, behavioral responses to pheromone extract are substantially increased by isolation from females (Schafer, '76).

Table 2 and figure 5 compare the EAG responses of unaltered male and female adults, and larvae. In general, larval antennae respond less strongly to fecal extracts than adult antennae. The fact that larval responses to larval and male extracts are substantially less than that of males and females is more important than it might seem at first glance. Larval and male extracts were used as controls for (1) fecal odors which would also be present in female extract, and (2) the possible presence of an aggregation pheromone which is produced by larvae and adults (Bell et al., '72; Bell et al., '73; Brousse-Gaury, '75). Low EAG responses by larval antennae to larval and male extract indicate that either the aggregation pheromone is not present in feces,

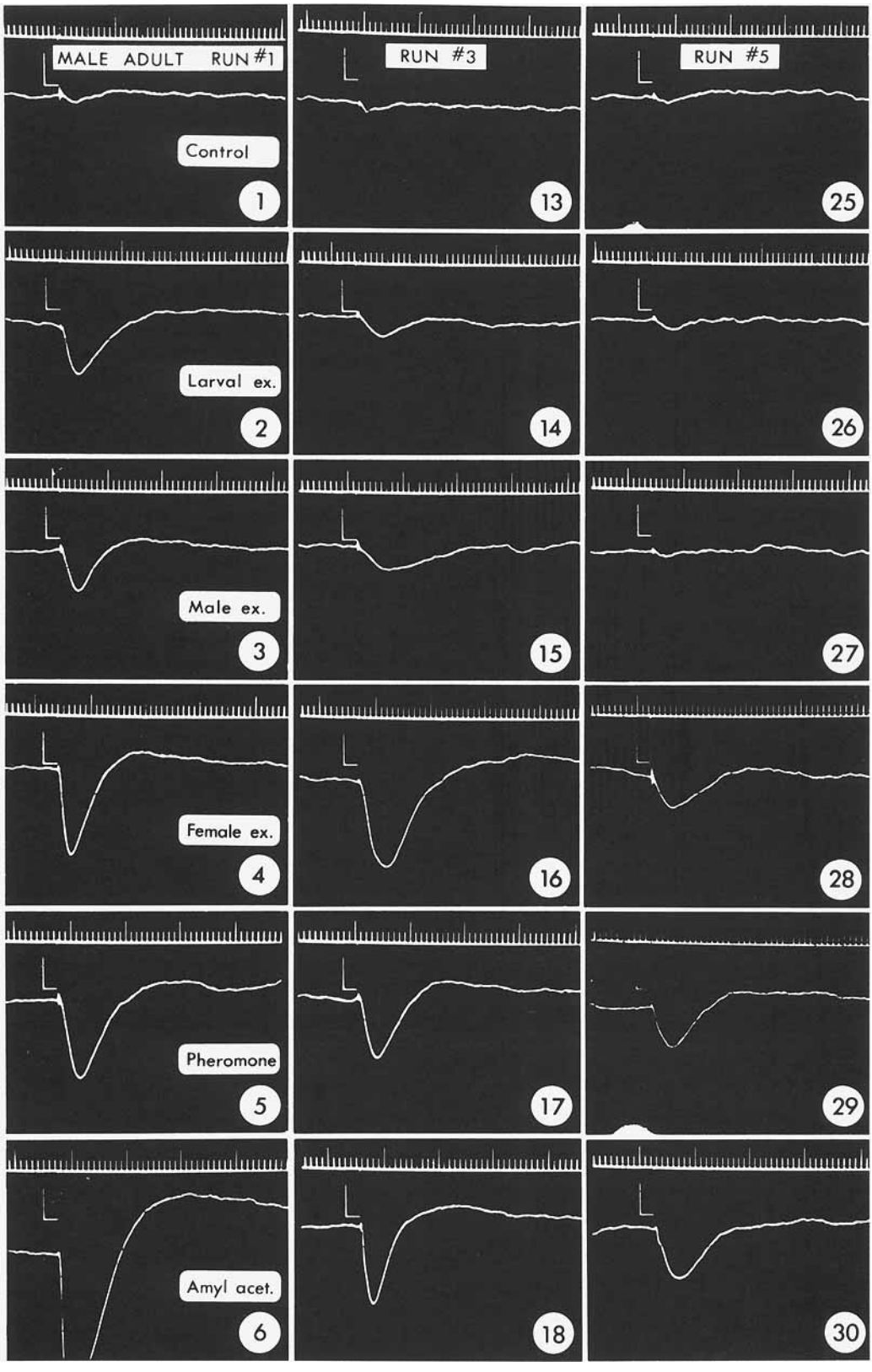
MALE ADULT RUN #1
RUN #3
RUN #5
Control
1
13
25
Larval ex.
2
14
26
Male ex.
3
15
27
Female ex.
4
16
28
Pheromone
5
17
29
Amyl acet.
6
18
30

Figure 4

TABLE 2

Electroantennogram (EAG) responses of normal adults and larvae of the species Periplaneta americana [1]

Stimulus	Male adults (N=44)	Female adults (N=29)	Male larvae (N=15)	Female larvae (N=15)
Blank	0.28mV	0.31mV	0.32mV	0.30mV
Larval extract	0.78	0.53	0.40	0.43
Male extract	0.70	0.57	0.49	0.64
Female extract	1.56	0.53	0.97	0.62
Purified pheromone	1.80 [2]	0.47 [3]	0.61 [4]	0.31 [4]
Amyl acetate	1.43	1.02	0.99	1.06

[1] Each entry represents the average EAG magnitude of N antennae with four stimulations per antenna.
[2] N = 11; [3] N = 8; [4] N = 3.

or if present, it does not stimulate antennal receptors strongly. Larval antennae respond as strongly as female antennae to amyl acetate, a result expected because there are nearly as many olfactory sensilla on larval antennal sections as on female adult sections (8,210 versus 9,600 in the region from which EAG recordings were made).

Electroantennogram responses to female extract and purified pheromone are far greater in the male adult than in the female adult. These results would be expected if there are many sex attractant receptors on male antennae, but few or none in females. The average difference between adult male and female responses to female extract and purified pheromone is statistically significant at the 2% level (Student's t-test, two-tailed). Interestingly, the response of female antennae to the purified pheromone was consistently above the control level (table 2, fig. 5).

The response of terminal instar male larvae to female extract and purified pheromone was measurably greater than the response of female terminal instar larvae and statistically significant at the 10% level (t-test). The differential response of male and female larvae to the sex attractant suggests that sensitivity to the attractant has begun to appear in the male before the adult stage is reached.

Fig. 4 Electroantennogram (EAG) responses of a single male antenna to six different stimuli applied at 100-second intervals. The top trace in each record contains time marks at 0.1- and 1.0-second intervals. The L-shaped mark indicates a stimulus duration of 0.25 seconds (the horizontal bar) and an amplitude of 0.4mV (vertical bar). The latency between the application of the odorous airstream and the EAG response is about 0.25 seconds, making it appear that the response starts with the end of the stimulus. Each vertical column is a single series of stimulations applied at 100-second intervals. The antenna was stimulated with five series of stimulations (runs 1-5). The second and fourth runs are not shown. The number on each record indicates its position in the total

Intra- and interspecific EAG responses within the genus Periplaneta

Electroantennogram tests were also run on three other strains within the genus *Periplaneta* (table 3): *P.a.*/MR, *P.a.*/BE, and *P.a.*/WE. Other species tested were *P. australasiae, P. brunnea, P. fuliginosa,* and *P. japonica.* All antennae were stimulated with the same sequence of *P. americana* (*P.a.*/BE) extracts reported previously. Purified pheromone was not available in sufficient quantity for interspecific testing. Fecal extracts of other species were also prepared, but they proved so variable in behavioral testing (Schafer, '77) that they were not used in the EAG experiments.

The average responses of ten male antennae from each strain, *P.a.*/MR, *P.a.*/BE, and *P.a.*/WE, were all within one standard deviation of the responses of *P.a.*/UR males reported previously. Thus, no strain differences in antennal responsiveness to the sex attractant could be detected with the EAG method. The four other species of *Periplaneta* tested against *P.a.*/BE extracts all gave strong responses to the *P. americana* attractant (table 3). The response of *P. brunnea* antennae were strongest and those of *P. japonica* weakest. Differences in the responses presented in table 3 should not be over emphasized, because all re-

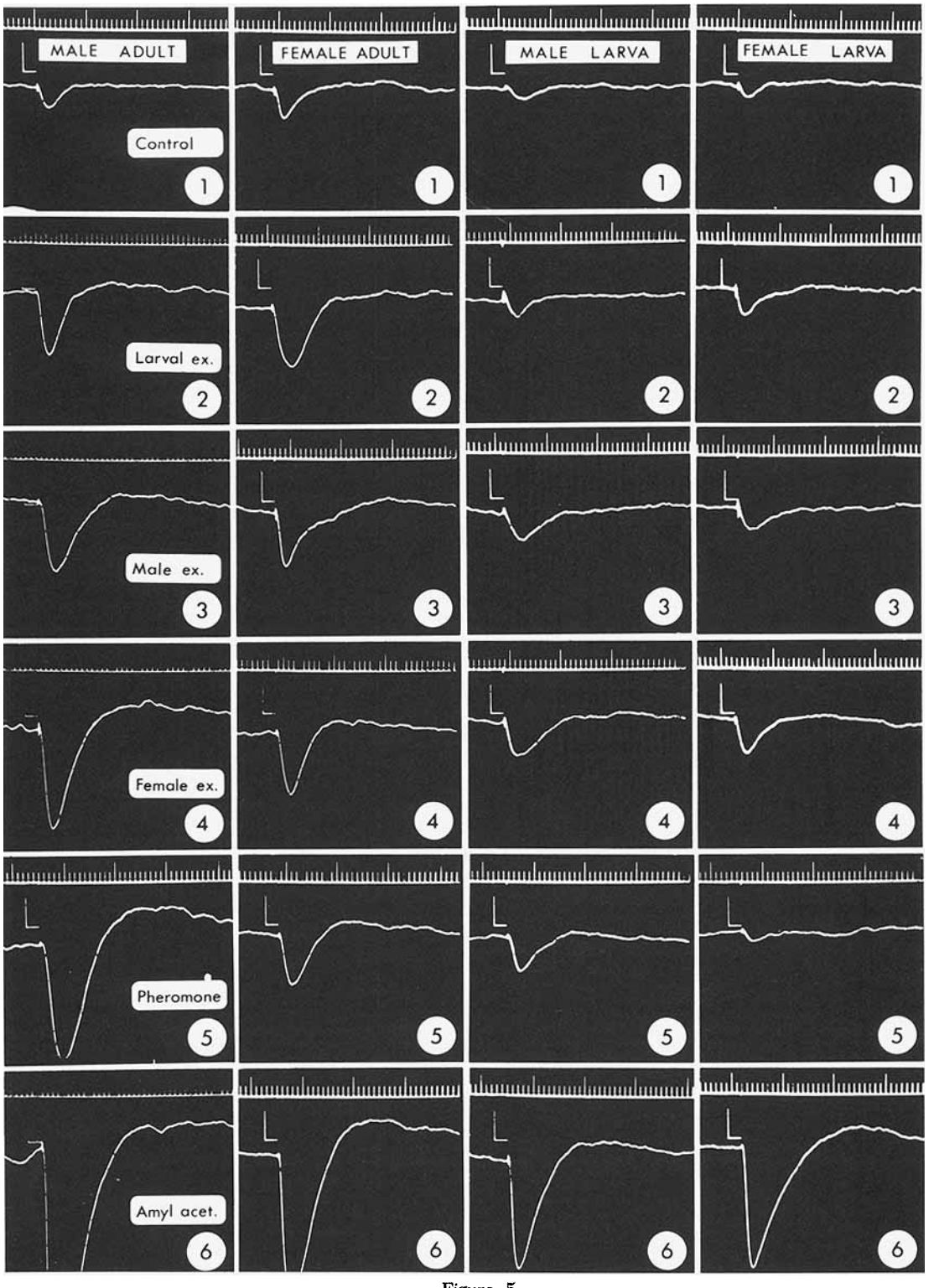
MALE ADULT
FEMALE ADULT
MALE LARVA
FEMALE LARVA
Control
Larval ex.
Male ex.
Female ex.
Pheromone
Amyl acet.
1
2
3
4
5
6

Figure 5

TABLE 3

Electroantennogram (EAG) responses of male antennae of five species in the genus Periplaneta *to* P. americana *sex attractant* [1]

Stimulus	*P. americana* (N=44)	*P. australasiae* (N=6)	*P. brunnea* (N=6)	*P. fuliginosa* (N=10)	*P. japonica* (N=6)
Blank	0.28mV	0.33mV	0.40mV	0.24mV	0.21mV
Larval extract	0.78	0.81	1.08	0.90	0.66
Male extract	0.70	0.83	0.90	0.53	0.53
Female extract	1.56	1.94	2.19	1.23	0.98
Purified pheromone	1.80 [2]	—	1.61 [3]	1.20 [3]	—
Amyl acetate	1.43	1.99	3.05	1.90	1.06

[1] Each entry represents the average EAG magnitude of N antennae with four stimulations per antenna.
[2] N = 11; [3] N = 2.

EAG responses of JH-M-treated males and male adultoids

Electroantennograms were made on three categories of insects whose development had been experimentally altered by treatment with juvenile hormone-mimic (JH-M). This treatment is fully described by Schafer and Sanchez ('76b).

Bilaterally-treated males

Adult male antennae which had been dipped in JH-M solution in the terminal larval instar did not respond well to the sex attractant (table 4). The difference between treated and untreated antennae is statistically significant at the 10% level (t-test). Control responses to extracts of larval and male feces were nearly identical (within 6%) to the responses of untreated male adult antennae. The magnitude of the response to female extract was nearly identical (within 3%) to the response level of the terminal larval instar, indicating that the increased responsiveness which usually develops at the terminal ecdysis did not develop in the antennae of bilaterally-treated animals. However, bilaterally-treated antennae were just as responsive as normal antennae to *other* odorants, as indicated by the responses to control extracts and amyl acetate. The somewhat greater response of treated antennae to amyl acetate is not statistically significant (t-test).

Unilaterally-treated males

A second method of experimentally altering male antennae was to dip *one* antenna in a 70% ethanol solution containing JH-M during the early part of the terminal larval instar. The other antenna was dipped in 70% ethanol only, as a control. Treated and control antennae examined at the adult stage responded identically to control extracts and amyl acetate (table 4). However, treated antennae did not respond well to the female extract containing sex attractant, while the control antennae responded at the normal adult level. This difference in response to female extract is significant at the 10% level (t-test). Response levels varied from animal to animal, but comparisons of right and left antennae of individual animals and statistical treatment of the averaged results provide substantial evidence of the efficacy of JH-M treatment in specifically inhibiting the development of sex attractant sensitivity.

Fig. 5 Representative electroantennogram (EAG) responses of adult male and female antennae and terminal instar larvae. Each column represents the first series of responses of an antenna to six stimuli, numbered 1-6. The male antenna responds strongly to the female extract and the purified pheromone, while the female antenna responds no more to the female extract than to the larval and male extracts. Surprisingly, the female shows a response to the purified pheromone which is above the level of the control response. Larval antennae show smaller responses to all stimuli, but the male larva responds above the control level when stimulated with the purified pheromone.

TABLE 4

Electroantennogram (EAG) responses of male Periplaneta americana *antennae treated with juvenile hormone-mimic and responses of male adultoids* [1]

Stimulus	Normal adults	Normal larvae	Bilateral treatment	Unilateral treatment		Adultoids
				JH-treated side	Untreated side	
	(N=44)	(N=15)	(N=21)	(N=15)	(N=15)	(N=6)
Blank	0.28mV	0.32mV	0.24mV	0.29mV	0.29mV	0.33mV
Larval extract	0.78	0.40	0.76	0.71	0.67	0.56
Male extract	0.70	0.49	0.66	0.69	0.61	0.42
Female extract	1.56	0.97	0.94	0.81	1.37	0.93
Purified pheromone	1.80 [2]	0.61 [3]	—	—	—	—
Amyl acetate	1.43	0.99	1.84	1.56	1.91	1.92

[1] Each entry represents the average EAG magnitude of N antennae with four stimulations per antenna.
[2] N = 11; [3] N = 3.

Male adultoids

The third method of altering antennal development was to remove the corpora allata of tenth instar males to produce adultoids with generally adult-like morphology, including the antennae (Schafer and Sanchez, '76b). Recordings from adultoid antennae (table 4) indicate that the level of responses to control extracts was less than that of adult males, but was within the range of responses of terminal instar larvae. The response to female extract was approximately twice the response to control extracts, but was well below the level of response seen in unaltered adults. This difference is just barely significant at the 10% level (t-test). The level of response of adultoids is similar in outline to that of terminal instar male larvae, except for a substantially larger response to amyl acetate in the adultoids. This similarity is not surprising in view of the fact that the adultoids and the eleventh instar larvae have passed through the same number of ecdyses. However, adultoids are unlike larvae in that they respond behaviorally to the sex attractant (Schafer, '76).

Single unit study of olfactory receptor specificity

Fifty-two olfactory single units in male *P. americana* antennae were tested with four categories of odorants: (1) pure chemicals, (2) mixed food odors, (3) larval extract, and (4) female extract. If a unit responded to a single odorant in two out of three presentations, it was scored as positive for that odorant. A positive response to one or more odorants within a category resulted in a positive score for that category. Thus, the data are reported with responses to pure chemicals combined as one category and responses to foods combined in a second category. Figure 6 gives the numerical distribution of cells falling into the 14 possible patterns of response in the four categories of stimuli.

The responses to pure chemicals and foods are combined to avoid unwarranted attention to the possible existence of response classes among receptors responding to odorants other than the sex attractant. Other investigators have examined this problem in greater detail, recording from more receptors, and using a wider variety of odorants (e.g., Kafka, '70; Boeckh, '74; Boeckh et al., '75; Waldow, '75). It seems likely that receptor specialization and across-fiber patterning (Pfaffman, '55; Erickson, '68) both contribute to olfactory discrimination and behavior in insects. The question examined in this paper concerns the possible existence of sex attractant receptors whose reaction spectra are strongly biased toward the sex attractant.

Nine of 52 receptors responded only to the female extract containing the sex attractant (column A: fig. 6). Seven out of these nine cells were excited strongly by

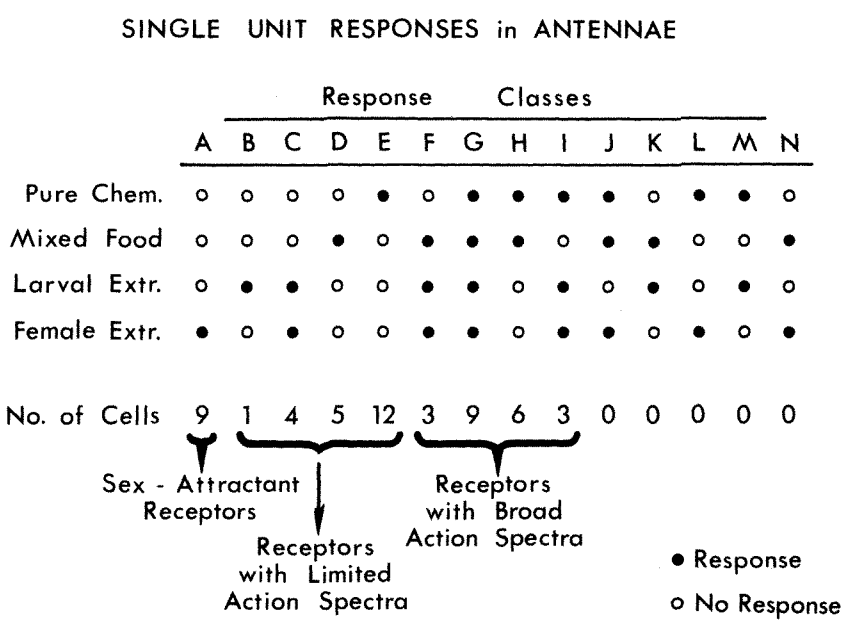

Fig. 6 Single unit responses of adult male antennal olfactory receptors (*Periplaneta americana*). Fifty-two units were tested with four classes of stimuli: (1) purified chemicals (amyl acetate, hexanol, citronellal, triethylamine, and butyric acid), (2) mixed food odors (Swiss cheese, apple, banana, and hamburger), (3) larval extract, and (4) female extract. A response to one or more of the pure chemicals or mixed foods was scored as a positive response in the appropriate category (black dots). A response to larval extract or female extract is similarly noted with a black dot. The number of receptors out of the total of 52 which responded in a given pattern is noted at the bottom of each column. Nine of the 52 cells responded only to the female extract containing the sex attractant. Experiments were not scored when none of the stimuli elicited a response. Likewise, experiments in which the blank stimulus (no odorant) elicited a response were not scored, because such responses probably originated from the mechanoreceptive neuron of a thick-walled sensillum.

the female extract; none showed any response to the larval extract which presumably contained all odorants present in the female extract, save the sex attractant. These data indicate that sex attractant receptors exist on male *P. americana* antennae. The extent of specialization is not known, because a relatively limited number of chemicals and food odors were used for comparison. It is entirely possible that these attractant receptors would have responded to other odors, had the right chemical been chosen (cf. Yamada, '71). No single unit recordings were made from larval or adult female antennae; hence no single unit data is available to reinforce the EAG data which suggest that strong sensitivity to the attractant is not found in larval or female antennae. Nor is unit data available to corroborate either the claim that female adults possess sex attractant receptors or the counter claim that they do not (Boeckh et al., '70; Washio and Nishino, '76).

Responses to odorants other than the sex attractant fell into two groups. Some receptors (columns B-E: fig. 6) had relatively limited action spectra. For example, some cells responded only to amyl acetate or citronellal (column E: fig. 6). Receptors in the second group had broader reaction spectra, with some units responding to stimuli within each stimulus class. These data are consistent with the findings of other workers who have found a wide variation in the breadth of response spectra among single olfactory receptors on insect antennae (e.g., Boeckh et al., '75).

DISCUSSION

The single unit data indicate that sex attractant receptors are present on male

Periplaneta americana antennae. The use of the term, "sex attractant receptor," should be taken to mean that the response spectra of certain receptors are strongly biased toward responding to the female-produced attractant. However, the extent of the bias or specialization is not defined beyond the limits of the stimuli used in these tests. The sex attractant receptors defined here might respond to other chemicals, perhaps to analogs of the attractant. For example, Washio and Nishino ('76) demonstrated strong EAG responses to bornyl acetate in *P. americana*—responses similar in waveform and amplitude to pheromone responses. However, all concerned agree that bornyl acetate is not the *P. americana* sex attractant (Bowers and Bodenstein, '71; Persoons et al., '74; Washio and Nishino, '76). I feel that use of the term sex attractant receptor is justified in the case of *P. americana,* but have not applied the label "specialist" since this term is identified with the high degree of specialization present in attractant receptors of the male silkworm moth, *Bombyx* (Schneider, '69).

The EAG data indicate that attractant receptors develop at the adult stage in male antennae, and are not present (in large numbers at least) on larval or female antennae. This provides some justification for the appearance of male sexual behavior only at the adult stage; larvae cannot respond to the sex attractant if their antennae do not detect it. It is tempting to suggest that differentiation of attractant receptors at the adult stage results in the appearance of "labeled lines" which make connection with or actuate central circuits which program male sexual behavior. This hypothesis could be explored by transplanting antennae. For example, adult male antennae containing functional attractant receptors could be grafted onto larvae, and, after healing and the ingrowth and connection of new receptor axons, the larvae could be tested for behavioral responses to the sex attractant.

The EAG experiments establish that juvenile hormone (JH) inhibits the differentiation of sex attractant receptors in male antennae during larval development. The morphological data (Schafer and Sanchez, '76b), behavioral data (Schafer, '77), and electrophysiological data all implicate JH as an inhibitor of sex attractant receptor differentiation. Juvenile hormone experiments were performed exclusively on *P. americana,* but it is likely that sensillar differentiation operates in the same way in other *Periplaneta* species, since all develop sexual dimorphism of the antennal olfactory receptors at the adult stage (Schafer and Sanchez, '76a) and rely on reception of a female-produced attractant (Schafer, '77). The same may be true of cockroaches and other hemimetabolous insects which depend on airborne sex attractants for mating (e.g., Schafer and Sanchez, '74).

The *P. americana* attractant was effective in exciting EAG's in all species within the genus. The uniformity of this response contrasts strongly with the variable effects of *P. americana* attractant on male behavior in the five species. The pheromone elicits an explosive behavioral response in *P. americana* and *P. brunnea,* but is a much less effective excitor of *P. australasaie, P. fuliginosa,* and *P. japonica* males (Schafer, '77). These same differences are also seen when each species is tested with its own pheromone (Frazier, '70; Simon, '71; Schafer, '77). Since the antennal receptors are excited to an equal degree, the sources of differences in behavioral responses must lie at the central level and/or in other routes of sensory input such as sensitivity of the palps and antennae to contact pheromones. Cross-reactivity in behavioral and electrophysiological responses suggests that the attractants of the five species are chemically similar (cf. Frazier, '70).

A question which remains unanswered by the present study is the possible existence of sex attractant receptors in female adults. In his single unit study of the *Periplaneta* deutocerebrum, Yamada ('71) found units in the female brain which responded to the female-produced sex attractant, but not other odorants. I made no

single unit recordings from female adult antennae, not wishing to look for sex attractant receptors where I believed none would be found. However, the EAG recordings from female adult antennae held a surprise: there were consistent and measurable responses to the purified pheromone above the level of both the blank (mechanoreceptor) EAG's and the control responses to the solvent used as a carrier for the purified pheromone. Some sensitivity to the attractant seems to develop in females at the terminal ecdysis, but whether or not specialized receptors have differentiated is unknown. Females do not show any consistent behavioral response to the attractant (Schafer, '77), so it is not clear if the odor is recognized as a pheromone.

Another important question left unanswered in this series of papers is why attractant receptors appear in profusion on male antennae at the adult stage but not on female antennae. An inhibitory role of JH effectively explains the lack of attractant receptors on larval antennae. But, if JH drops to a low titer in the terminal larval instar leading to the adult ecdysis, why don't attractant receptors differentiate on *both* male and female antennae? At least three hypotheses could be advanced to account for this discrepancy: (1) the JH titer in male and female larvae may decline at different rates, dropping to a low enough value at the critical period for antennal differentiation in males, but not in females. Or, perhaps the critical period comes at different times in male and female larvae; (2) juvenile hormone titers are intrinsically lower in males than females in the terminal larval stages; and (3) the male genome is a prerequisite to the development of attractant receptors.

The present study produced no data to allow choosing among these hypotheses, but several findings are pertinent. Apparently, males begin to develop attractant receptors in the terminal larval instar, even though they do not respond to the pheromone behaviorally (fig. 5, table 2). This, plus the fact that adultoid antennae respond to the pheromone (table 4), indicates that a critical period is not limited to the terminal larval instar, but occurs during each molting cycle. The incipient sensitivity of male larvae to the attractant in the terminal instar may be a reflection of the beginning of the decline in JH concentration which leads to the adult ecdysis. Antennal attractant receptors begin to differentiate because the antennae are the least susceptible to JH inhibition among the constellation of adult characters which emerge at the adult ecdysis (Schafer and Sanchez, '76b).

Questions concerning the absolute specificity of the attractant receptors and their possible occurrence on female and larval antennae will have to await the chemical identification of the *P. americana* attractant(s). At least five laboratories in the United States, Europe, and Japan are working on this problem, but it would be prudent not to expect a rapid solution, considering past difficulties in isolating and characterizing the pheromone(s). An area of research which can proceed using crude extracts is that of the role of the male genome in the development of attractant receptors and the problem of peripheral versus central activation of sexual behavior.

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LITERATURE CITED

Adler, V. E. 1971 Physical conditions important to the reproducibility of electroantennograms. Ann. Ent. Soc. Am., *64:* 300-302.

Barth, R. H., and L. J. Lester 1973 Neuro-hormonal control of sexual behavior in insects. Ann. Rev. Entomol., *18:* 445-472.

Bell, W. J., T. Burk and G. R. Sams 1973 Cockroach aggregation pheromone: Directional orientation. Behavioral Biol., *9:* 251-255.

Bell, W. J., C. Parsons and E. A. Martinko 1972 Cockroach aggregation pheromones: Analysis of aggregation tendency and species specificity (Orthop-

tera: Blattidae). J. Kans. Entomol. Soc., *45:* 414-421.

Birch, M. C. 1971 Intrinsic limitation in the use of electroantennograms to bioassay male pheromones in Lepidoptera. Nature, *233:* 57-58.

Boeckh, J. 1974 Die Reaktionen olfaktorischer Neurone im Deutocerebrum von Insekten im Vergleich zu den Antwortmustern der Geruschssinneszellen. J. Comp. Physiol., *90:* 183-205.

Boeckh, J., K. Ernst, H. Sass and U. Waldow 1975 Coding of odor quality in the insect olfactory pathway. In: Olfaction and Taste. Vol. V. D. A. Denton and J. P. Coghlan, eds. Academic Press, New York, pp. 239-245.

Boeckh, J., E. Priesner, D. Schneider and M. Jacobson 1963 Olfactory receptor response to the cockroach sexual attractant. Science, *141:* 716-717.

Boeckh, J., H. Sass and D. R. A. Wharton 1970 Antennal receptors: Reactions to female sex attractant in *Periplaneta americana.* Science, *168:* 589.

Brousse-Gaury, P. 1975 A trail pheromone in *Periplaneta americana.* C. R. Acad. Sci. (Paris), *280:* 319.

Chapman, J. A., and R. Craig 1953 An electrophysiological approach to the study of chemical sensory reception in certain insects. Can. Entomol., *85:* 182-189.

Day, A. C., and M. C. Whiting 1964 The structure of the sex attractant of the American cockroach. Proc. Chem. Soc., p. 368.

Dethier, V. G. 1974 The specificity of the labellar chemo-receptors of the blowfly and the response to natural foods. J. Insect Physiol., *20:* 1859-1869.

Erickson, R. 1968 Stimulus coding in topographic and nontopographic afferent modalities: On the significance of the activity of individual sensory neurons. Psychol. Rev., *75:* 447-465.

Frazier, J. L. 1970 Interspecific responses to sex pheromones among cockroaches of the genus *Periplaneta.* Ph.D. thesis, The Ohio State University, Columbus, Ohio.

Jacobson, M., and M. Beroza 1963 Sex attractant of the American cockroach. Science, *142:* 1258-1259 (letter).

——— 1965 American cockroach sex attractant. Science, *147:* 748.

Jacobson, M., M. Beroza and R. T. Yamamoto 1963 Isolation and identification of the sex attractant of the American cockroach. Science, *139:* 48.

Jacobson, M., R. E. Redfern, W. A. Jones and M. H. Aldridge 1970 Sex pheromones of the southern armyworm moth: Isolation, identification, and synthesis. Science, *170:* 542.

Kafka, W. A. 1970 Molekulare Wechselwirkungen bei der Erregung einzelner Riechzellen. Z. vergl. Physiol., *70:* 105-143.

Lambin, M. 1973 Les sensilles de l'antenne chez quelques blattes et en particulier chez *Blaberus craniifer* (Burm.). Z. Zellforsch., *143:* 183-206.

Loftus, R. 1966 Cold receptor on the antenna of *Periplaneta americana.* Z. vergl. Physiol., *52:* 380-385.

——— 1968 The response of the antennal cold receptor of *Periplaneta americana* to rapid temperature changes and to steady temperature. Z. vergl. Physiol., *59:* 413-455.

——— 1969 Differential thermal components in the response of the antennal cold receptor of *Periplaneta americana* to slowly changing temperature. Z. vergl. Physiol., *63:* 415-433.

O'Connell, R. J. 1975 Olfactory receptor responses to sex pheromone components in the redbanded leafroller moth. J. Gen. Physiol., *65:* 179-205.

Persoons, C. J., F. J. Ritter and W. H. Lichtendonk 1974 Sex pheromones of the American cockroach, *Periplaneta americana.* Isolation and partial identification of two excitants. Pr. Koninkl. Nederl. Academie van Wetenshcappen (C), *77:* 201-204.

Pfaffman, C. 1955 Physiological and behavioral processes in the sense of taste. In: Taste and Smell in Vertebrates. G. E. W. Wolstenholme and J. Knight, eds. J. A. Churchill, London, pp. 31-50.

Rees, C. J. C. 1970 Age dependency of response in an insect chemoreceptor sensillum. Nature, *227:* 740-742.

Roelofs, W., A. Hill and R. Cardé 1975 Sex pheromone components of the redbanded leafroller moth, *Argyrotaenia velutinana* (Lepidoptera: Tortricidae). J. Chem. Ecol., *1:* 83-89.

Roys, C. 1954 Olfactory nerve potentials, a direct measure of chemoreception in insects. Ann. N. Y. Acad. Sci., *58:* 250-255.

Schafer, R. 1977 The nature and development of sex attractant specificity in cockroaches of the genus *Periplaneta.* III. Normal intra- and interspecific behavioral responses and responses of insects with juvenile hormone-altered antennae. J. Exp. Zool., *199:* 73-84.

Schafer, R., and T. V. Sanchez 1973 Antennal sensory system of the cockroach, *Periplaneta americana:* Postembryonic development and morphology of the sense organs. J. Comp. Neur., *149:* 335-354.

——— 1974 Juvenile hormone inhibits the differentiation of olfactory sense organs during the postembryonic development of cockroaches. J. Insect. Physiol., *20:* 965-974.

——— 1976a The nature and development of sex attractant specificity in cockroaches of the genus *Periplaneta.* I. Sexual dimorphism in the distribution of antennal sense organs in five species. J. Morph., *149:* 139-157.

——— 1976b The nature and development of sex attractant specificity in cockroaches of the genus *Periplaneta.* II. Juvenile hormone regulates sexual dimorphism in the distribution of antennal olfactory receptors. J. Exp. Zool., *198:* 323-336.

Schneider, D. 1957 Elecktrophysiologische von Chem-und Mechanorezeptoren der Antenne des Seidenspinners *Bombyx mori* L. Z. vergl. Physiol., *40:* 8-41.

——— 1969 Insect olfaction: Deciphering system for chemical messages. Science, *163:* 1031-1037.

Simon, D. 1971 A comparative study of sexual behavior in the genera, *Periplaneta* and *Blatta.* Ph.D. thesis, University of Texas, Austin.

Smyth, T. Jr., and C. C. Roys 1955 Chemoreception in insects and the action of DDT. Biol. Bull., *108:* 66-76.

Waldow, U. 1975 Multimodale Neurone im Deutocerebrum von *Periplaneta americana.* J. Compar. Physiol. A., *101:* 329-341.

Washio, H., and C. Nishino 1976 Electroantennogram responses to the sex pheromone and other odours in the American cockroach. J. Insect Physiol., *22:* 735-741.

Wharton, D. R. A., E. D. Black and C. Merritt, Jr. 1963 Sex attractant of the American cockroach. Science, *142:* 1257-1258 (letter).

Yamada, M. 1968 Extracellular recording from single neurones in the olfactory centre of the cockroach. Nature, *217:* 778-779.

——— 1970 Electrophysiological investigation of insect olfaction. In: Control of Insect Behavior by Natural Products. D. L. Wood, R. M. Silverstein, and M. Nakajima, eds. Academic Press, New York, pp. 317-330.

——— 1971 A search for odour encoding in the olfactory lobe. J. Physiol. (London), *214:* 127-143.

Yamada, M., S. Ishii and Y. Kuwahara 1970 Odour discrimination: "Sex pheromone specialists" in the olfactory lobe of the cockroach. Nature, *227:* 855.

Note added in proof: Three research reports which deal with the electrophysiology of the antenna of *Periplaneta americana* have appeared since this paper was submitted for publication. They are:

Ruth E. 1976 Electrophysiology of sensilla chaetica on antennae of *Periplaneta americana.* J. Comp. Physiol., A. *105:* 55.

Sass, H. 1976 Zur nervösen Codierung von Geruchsreizen bei *Periplaneta americana.* J. Comp. Physiol., A. *107:* 49-65.

Yokohari, F., and H. Tadeta 1976 Moist and dry hygroreceptors for relative humidity of the cockroach, *Periplaneta americana* L. J. Comp. Physiol., A. *106:* 137-152.