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ELECTROANTENNOGRAM RESPONSES OF THE MALE MOTH, *ARGYROTAENIA VELUTINANA* TO MIXTURES OF SEX PHEROMONE COMPONENTS OF THE FEMALE

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Abstract—Electroantennogram (EAG) responses were recorded from male redbanded leafroller moth, *Argyrotaenia velutinana*, antennae using mixtures of the three female-produced sex pheromone components: *cis*-11-tetradecenyl acetate, *trans*-11-tetradecenyl acetate, and dodecyl acetate (12:Ac). Binary mixtures containing 3%, 8%, and 15% *trans* in *cis* elicited significantly higher amplitude responses than other isomeric mixtures as well as pure *cis* and *trans* alone. The higher responses to such mixtures were less than additive at high dosages and additive at lower dosages. Receptor adaptation studies using the two isomers support a previous single unit study demonstrating the presence of at least two functionally different receptor sites on male *A. velutinana* antennae; adaptation to an airstream containing one isomer did not eliminate response to the opposite isomer presented concurrently. An airstream containing the third component, 12:Ac, caused a significant slowing of the recovery rate during the entire recovery period of EAG responses to *cis* but not *trans*, suggesting that a possible temporal modulation of neuronal response by 12:Ac may be a means of coding for this component by antennal sensory neurons.

THE REDBANDED leafroller moth, *Argyrotaenia velutinana* (Walker), responds to a female-produced sex pheromone comprised of three components (ROELOFS *et al.*, 1975). The primary sex pheromone component of *A. velutinana* was identified as *cis*-11-tetradecenyl acetate (c11–14:Ac) (ROELOFS and ARN, 1968). Two other pheromone components, *trans*-11-tetradecenyl acetate (t11–14:Ac) and dodecyl acetate (12:Ac) were shown to be instrumental in increasing the trap catch of male *A. velutinana* in the field. The former was shown to be necessary for attractancy when present in low ratios to the *cis* isomer and gave optimum attractancy when mixed in a *trans*:*cis* ratio of approximately 7:93 (KLUN *et al.*, 1973; ROELOFS *et al.*, 1975). At ratios higher than 7:93, trap catch of males was reduced. Dodecyl acetate was found to increase trap catch of *A. velutinana* males in field tests when evaporated with the other two components (ROELOFS and COMEAU, 1968, 1971a). Further tests showed that optimum attractancy was obtained with the addition of 12:Ac at ratios greater than 3:2 to the *trans*:*cis* (8:92) blend (ROELOFS *et al.*, 1975). All three pheromone components were isolated and identified from female *A. velutinana* abdominal tip extract or calling female effluvia (ROELOFS *et al.*, 1975). The *trans*:*cis* ratio was found to be present in a ratio of about 9:91, and 12:Ac was found in airborne collections of calling female effluvia at a 5:4 ratio to Δ11–14:Ac.

Recently, BAKER *et al.* (1976) demonstrated that in laboratory olfactometers, male *A. velutinana* exhibited the highest percentage of wing fanning response to the 8:92 *trans*:*cis* blend. In addition, 12:Ac, when added at a 1:1 ratio to this blend, elicited a prolongation of the wing fanning response resulting in increased orientation to the pheromone source. Field observations (BAKER *et al.*, 1976) revealed that 12:Ac acted as a close-range mediator of behaviour for males attracted to the 8:92 mixture; simultaneous evaporation of 12:Ac resulted in an increased frequency of landing for males lured to within 0.5 m of the trap surface.

With the behavioural rôle of each pheromone component defined, it became of interest to investigate further the male antennal responses to these components. In single unit studies O'CONNELL (1972, 1975) had found two odor-sensitive sensory neurons in each antennal sensillum trichodeum and conducted extensive experiments investigating their relative sensitivities to many compounds, some over a large range of dosages. Preliminary electroantennogram (EAG) recordings from *A. velutinana* antennae had been conducted (ROELOFS and COMEAU, 1971b) with a large number of chemicals and showed differences in both amplitude and shape (recovery rate) of responses. This paper describes a more detailed study of the EAG responses to the three pheromone components.

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MATERIALS AND METHODS

Biological

A. velutinana larvae were reared on a pinto bean diet modified from SHOREY and HALE (1965) in wax-coated paper cups, (5.2 cm diameter bottom, 12.5 cm long), covered with plastic snap-on lids. Approximately 75 larvae were reared in each cup until pupation when pupae were removed and sexed. Male pupae were placed in a screened cage (38 cm × 38 cm × 47 cm) with access to a solution of 5% sucrose. Adult and immature insects were maintained at 24°C on a 16 hr:8 hr light:dark photoperiod regime with photophase at 1400 lux. Eggs for each generation of diet-reared larvae were produced by adults from a greenhouse colony maintained on fava bean plants (GLASS and HERVEY, 1962).

Chemical

Solutions of synthetic pheromone components (various ratios of c11– and t11–14:Ac) were prepared by making serial dilutions in 2 ml of Skellysolve B™ in 10-fold increments starting with neat material. Ratios of c11– and t11–14:Ac (Farchan Corp.) were prepared volumetrically and analyzed by gas–liquid chromatography (GLC). The following mixtures of the two isomers were used: thin layer chromatography (TLC) pure c11–14:Ac; 3% t11–14:Ac; 8% t11–14:Ac; 15% t11–14:Ac; 30% t11–14:Ac; 50% t11–14:Ac; 80% t11–14:Ac and TLC pure t11–14:Ac. Dodecyl acetate (Eastman Kodak) was purified by preparative GLC and used in Skellysolve B solution. All solutions were stored at −10°C in 1 dram screw-cap vials with teflon-lined lids and were used within 18 months.

Electroantennogram

The electroantennogram (EAG) setup used was described by Roelofs (1976). It included a Syracuse watch glass with its interior partially lined with wax, which was covered with saline solution. The saline solution consisted of the following (from Kaissling, personal communication): NaCl (7.5 g/l); $CaCl_2$ (0.21 g/l); KCl (0.35 g/l); and $NaHCO_3$ (0.2 g/l). A short piece of chlorodized silver wire partially submerged anywhere in the saline solution served as the ground electrode and was connected to the EAG amplifier which amplified electrical activity 100 times, had an impedance of 10^{12} ohms, and a band pass of 0 to 12 kHz. The input electrode also consisted of a short piece of chlorodized silver immersed in saline solution contained within a 1 mm (i.d.) glass tube. The tube was the drawn end of a Pasteur pipet into which the silver electrode with its shielded connecting wire was placed. Aluminum foil enveloping the pipet and grounded to the amplifier served as a shield against extraneous electrical signals. A Tektronix™ 564B storage oscilloscope was used to display the signal. Tygon™ tubing connected to an activated charcoal filter and finally to 1 cm (i.d.) glass tubing that terminated 2 to 3 cm from a male's antenna was used to deliver air. A 0.5 cm diameter hole in the glass tubing 2.5 cm from the end served as the portal for injection of samples into the airstream.

Males were captured and their heads removed and placed on the wax in the Syracuse watch glass so that the base of the head was barely enveloped by the saline solution. One antenna was teased up into a standing position and the terminal three or four segments excised. The capillary of the input electrode was then moved into position so that the saline-containing tip contacted the severed antennal end. The airstream blew continuously across the antenna at a rate of about 1 m/sec at the air-tube opening. The oscilloscope was set at 10 seconds per sweep and the vertical scale set at 1 mV/division.

Dosage–response experiments

Dosage–response curves were obtained for various ratios of c11–14:Ac and t11–14:Ac at a range of dosages spanning six orders of magnitude (10^{-4} μg to 10^{2} μg). Filter paper (2.4 × 0.5 cm) was impregnated with 20 μl of solution, the solvent allowed to evaporate, and the papers inserted into disposable pipets which were then stored at −10°C.

A particular *cis:trans* mixture (for instance c11– and t11–14:Ac's at 10 sec intervals produced no steps in dosage was tested double blind in a randomized complete block design by puffing 1 ml of room air from a glass syringe through each pipet and into the airstream (ROELOFS and COMEAU, 1971b) at 20-sec intervals. Response amplitudes were read directly from the oscilloscope screen and recorded on paper. A puff of the standard (10^{-2} μg TLC pure c11–14:Ac) preceded each test sample by 10 sec (it was found that repeated presentations of various percentages of c11–and t11–14:Ac's at 10 sec intervals produced no adaptation) and all responses were corrected for diminution with age of the preparation by the formula:

$$\frac{\text{test response (mV)} \times 10}{\text{response to standard preceding test response (mV)}} = \text{corrected response.}$$

Thus the corrected response of the standard is always 10 by the formula, and all responses greater than 10 are of greater amplitude than the standard response.

EAG receptor adaptation

Studies of EAG responses after antennal receptors had been adapted to an airstream containing one of the three *A. velutinana* pheromone components were conducted similar to receptor adaptation experiments by PAYNE (1975) on two bark beetle species. Airstream saturation was accomplished by applying 10 μg (in solution) of either TLC pure c11–14:Ac, TLC pure t11–14:Ac, or 12:Ac to a 1.3 cm diameter filter paper disc and placing it at the mouth of the glass air delivery tube. The tube was first tilted up

so that the chemically-laden air blew directly into an exhaust vent 20 cm behind the preparation. Once the antenna was connected and the trace located on the oscilloscope screen (time elapsed about 1 min), the airstream was lowered quickly to blow across the antenna. Five to 6 sec were then needed to re-adjust the deflected oscilloscope trace and puff the desired test chemical into the airstream, using a disposable pipet containing 10 μg of one of the above three components on filter paper.

Responses, including amplitude and recovery time, were recorded either by tracings or with a Polaroid CR-9™ oscilloscope camera. Antennae were used for one response and then discarded. The glass air delivery tube was removed and rinsed thoroughly with acetone after every response.

Recovery rate of the antennal receptors after maximum depolarization was calculated for three phases during recovery: 1st 1/3, 2nd 1/3, and final 1/3 return to baseline, which were estimated by dividing each maximum response amplitude into three equal segments and dividing by the measured time that elapsed during each segment (each 1/3 return to baseline). The mean recovery rates were then calculated and for each 1/3 recovery phase they appear as a straight line with the mean recovery rate as their slope (Fig. 3).

RESULTS

Dosage–response experiments

Figure 1 shows the mean corrected responses of TLC pure c11–14:Ac, TLC pure t11–14:Ac and 12:Ac. The greatest increases in response amplitude occurred between 10^{-1} μg and 10^{1} μg. It was also in this range of dosages that the greatest differences among these three treatments occurred, as evidenced by some separations of standard deviations of the means. Pure *cis*, pure *trans*, and 12:Ac responses at dosages greater than 10^{-1} μg were all significantly different from each other by the *t* test ($P < 0.01$). Responses to *cis* and *trans* were significantly different from the mean corrected response to puffs from a blank cartridge down to 10^{-4} μg.

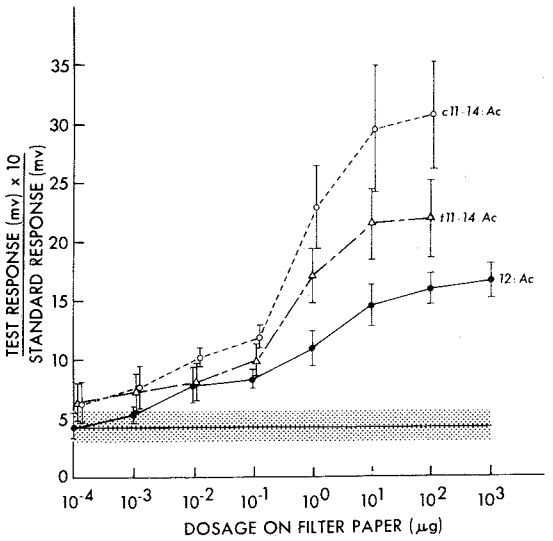

Fig. 1. Corrected EAG amplitudes of response to c11–14:Ac ($n = 12$, 7 antennae), *t*11 to 14:Ac ($n = 14$, 7 antennae), and 12:Ac ($n = 12$, 6 antennae). Brackets around the means denote standard deviations. Horizontal line and shaded region represent the mean corrected response of a blank cartridge and its standard deviation ($n = 43$, 5 antennae). Standard was 10^{-2} μg c11–14:Ac.

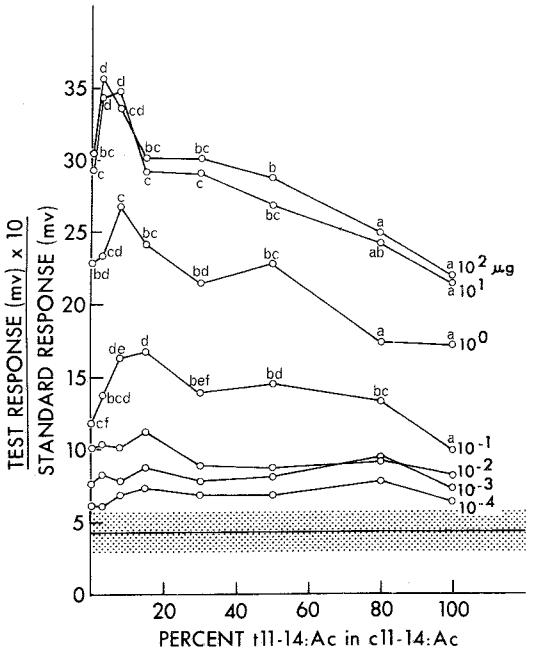

Fig. 2. Mean corrected EAG amplitudes of response to: c11–14:Ac ($n = 12$, 7 antennae); 3% t11–14:Ac ($n = 12$, 5 antennae); 8% t11–14:Ac ($n = 13$, 6 antennae); 15% t11–14:Ac ($n = 13$, 6 antennae); 30% t11–14:Ac ($n = 9$, 6 antennae); 50% t11–14:Ac ($n = 8$, 5 antennae); 80% t11–14:Ac ($n = 10$, 5 antennae); and 100% t11–14:Ac ($n = 14$, 7 antennae). Horizontal line and shaded area represent the mean corrected response of a blank cartridge $\pm$ S.D. ($n = 43$, 5 antennae). Standard was 10^{-2} μg c11–14:Ac. For a given dosage, means having no letters in common are significantly different according to the *t* test ($P < 0.05$).

The mean corrected responses to mixtures of *cis* and *trans* over six decade steps in concentration are shown in Fig. 2 along with the statistical analyses of these responses using the *t* test. For a given dosage, means having no letter on common are significantly different at the 5% level. The data reveal significant differences among the mean responses to many of the mixtures, with the highest responses occurring to 3%, 8% and 15% t11–14:Ac in c11–14:Ac. At 10^{2} μg and 10^{1} μg the 3% *trans* treatment was significantly different from all treatments except 8% *trans*, whereas 8% *trans* at 10^{1} μg differed significantly from all treat-

ments except 3% *trans*. Thus, a small amount of *trans*, when substituted for *cis* elicited a corrected antennal response that was significantly higher than that to pure *cis*, which gave the highest response of all single compounds tested. Although responses to some mixtures of the *cis* and *trans* isomers at high dosages were greater than to *cis* alone, they were less than the sum of responses to comparable amounts of either isomer alone. For example, 8% *trans* at 10^1 μg gave the second highest mean corrected response of all treatments tested, 34.7, but the sum of responses to the two isomers presented singly at equivalent dosages in this mixture was 41.8 (17.1 for .1 μg *trans* +29.0 for 9 μg *cis* minus 4.3 for one of the two blank puff values that would appear in this sum). Also, a 50:50 mixture of the *cis* and *trans* isomers at 10^1 μg gave a corrected response of 26.9, considerably lower than 43.3, the sum of the estimated responses to both isomers alone at 5 μg minus the value of one blank cartridge response.

At slightly lower dosages, though, the increased responses elicited by some isomeric mixtures were approximated more closely by the simple addition of the responses to each isomer alone. For instance, at 10^{-1} μg, the increased response to 8% *trans*, 16.3, can be approximated by adding 11.7 and 8.4 (extrapolated responses to 9×10^{-2} μg *cis* and 1×10^{-2} μg *trans* respectively) and subtracting 4.3 (one of the two blank puff values), resulting in a sum of 15.8. The sub-additive behavior of responses to mixtures at the highest dosages may have been due to physiological limitations upon response that may be encountered at such dosages; the cells were simply not capable of discharging with any greater intensity regardless of the mixture. However, at lower dosages the neurons may have been functioning at well below their maximum level, and so the additive nature of responses to some isomeric mixtures could be observed.

Receptor adaptation studies

Schematic drawings of the shapes of mean responses to all combinations of airstreams and injected samples appear in Fig. 3 and were calculated from the number of responses indicated. It is apparent that the amplitudes of all responses in chemically-laden airstreams were diminished from those to the same chemicals when injected into a clear airstream. Evidence that a given chemical airstream was saturating its receptor sites was obtained by comparing responses to chemicals injected into their own airstream (Figs. 3I and N); their amplitudes were not different from responses to blank puffs in those same airstreams (Figs. 3L and P) except for 12:Ac which gave a response slightly larger than to a blank puff (Fig. 3G).

Inspection of the mean response amplitudes sug-

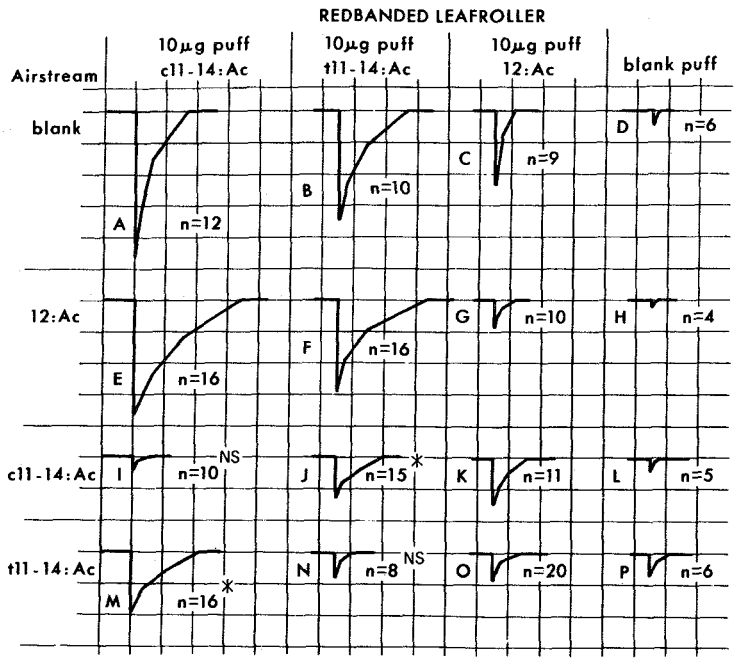

Fig. 3. Schematic drawings of EAG responses to c11– and t11–14:Ac and 12:Ac after .5 to 6 sec of antennal exposure to the airstreams listed in the left-hand column. The number of antennae used for each mean response is indicated by '*n*'. Each vertical division equals 1 mV; each horizontal division equals 1 sec. Asterisk denotes response amplitude significantly different from that to a blank puff in the same airstream (*t*-test, $P < 0.05$); NS denotes response amplitude not significantly different from a blank puff in the same airstream. Traces not labeled with an asterisk or NS were not tested statistically.

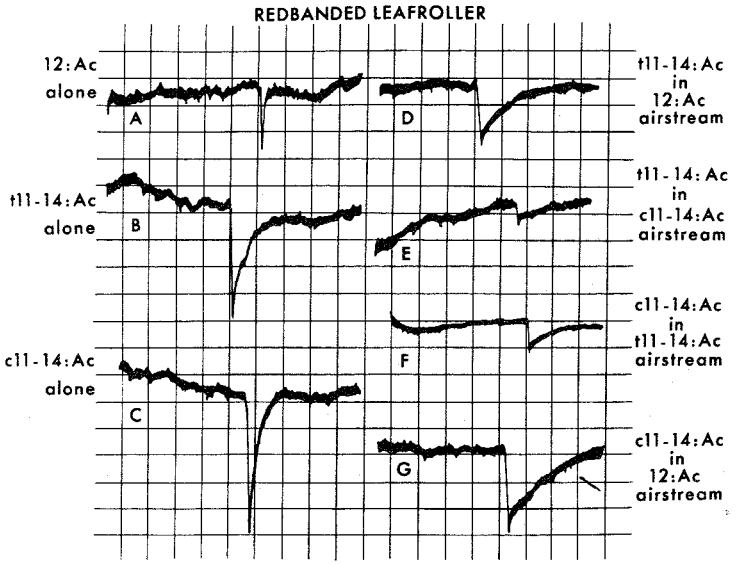

Fig. 4. Photographs of male *A. velutinana* EAG responses: in a clear airstream, A–C; and after adaptation to the indicated chemical airstream, D–G. Each vertical division equals 1 mV; each horizontal division equals 1 sec.

gests that more than one receptor site is involved in the perception of the *cis* and *trans* geometric isomers. A maximum amplitude response to the *trans* airstream did not prevent a further response to *cis* injected into this airstream, but it drastically reduced its amplitude (Figs. 3M, 4F). Response to *trans* in this airstream was little more than to a blank cartridge (Figs. 3N and P). Likewise maximum depolarization in response to the *cis* airstream reduced the response to *trans* dramatically, but did not eliminate it (Figs. 3J, 4E), whereas injected *cis* in this case elicited no more of a response than a blank cartridge (Figs. 3I and L). The amplitudes of responses to *cis* and *trans* injections into a 12:Ac airstream were not nearly so diminished from their maximum in a clear airstream (Figs. 3E and F, 4D and G) when compared to the decreases seen in *cis* and *trans* airstreams.

The striking effect on recovery rate in the case of *cis* in the 12:Ac airstream suggests that 12:Ac may alter the temporal aspects of sensory neuron response to *cis* (Figs. 3E, 4G). Although *cis* in a clear airstream gave a larger amplitude response than *trans*, its recovery rate was significantly faster than *trans* at 2nd 1/3 and final 1/3 of recovery (Table 1). In a 12:Ac airstream, however, the recovery rate of receptors to *cis* was altered dramatically so that it not only was significantly slower than that to *cis* in a blank airstream, but slower than that to *trans* as well (Table 1; Figs. 3E and 4G). The rate of recovery of receptors to *trans* in a 12:Ac airstream was not significantly different from that to *trans* in a blank airstream, except during the final 1/3 return to baseline where it had a reduced rate of return (Table 1; Figs. 3F and 4D).

DISCUSSION

EAG response amplitudes

Roelofs and Comeau (1971b) observed differences in EAG amplitude and recovery rate to the three *A. velutinana* pheromone components and demonstrated that t11–14:Ac had a slower rate of return to baseline than did c11–14:Ac. This present study also showed

Table 1. EAG mean rates of recovery (in mv/sec), plus or minus the standard deviation to c11– and t11–14:Ac in a clear airstream and in a 12:Ac airstream

	c11–14:Ac alone ($n = 12$)	t11–14:Ac alone ($n = 10$)[1,2]	c11–14:Ac in 12:Ac airstream ($n = 16$)[2]	t11–14:Ac in 12:Ac airstream ($n = 16$)[2,3]	12:Ac alone ($n = 9$)[1,2]
1st 1/3 return to baseline	2.17 ± 0.85	1.54 ± 0.86 NS	0.72 ± 0.36§[1,3]	2.06 ± 1.58 NS	2.75 ± 2.15 mV/sec NS
2nd 1/3 return to baseline	1.48 ± 0.71	0.59 ± 0.23†	0.35 ± 0.09§[1,3]	0.44 ± 0.27 NS	2.62 ± 2.07 mV/sec†
3rd 1/3 return to baseline	0.38 ± 0.12	0.25 ± 0.10*	0.18 ± 0.08§[1,3]	0.15 ± 0.07†	0.98 ± 1.15 mV/sec§

[1] Tested for significance against the c11–14:Ac response in the same row using the *t* test.
[2] $*P < 0.05$; $\dagger P < 0.01$; $\S P < 0.001$; NS $P > 0.05$.
[3] Tested for significance against the t11–14:Ac response in the same row using the *t* test.

significant differences in EAG response amplitudes and recovery rates, both of which help determine the shape of a response trace. The response amplitude to *cis* at a given dosage was greater than to *trans*, which was greater than the 12:Ac response. The recovery rates followed a different order. Dodecyl acetate had the fastest rate of return, then *cis*, and finally *trans* has the slowest recovery rate.

The present EAG study of response amplitudes to mixtures of c11– and t11–14:Ac showed that maximum response within a dosage was elicited by mixtures containing 3%, 8%, or 15% *trans* in *cis*. These optimal responses to mixtures appeared to be less than additive at higher dosages but additive at lower dosages and possibly indicate the existence of a system among or within sensory neurons providing a basis for odour quality coding at the peripheral nervous system level. MINKS *et al.* (1974) recorded increased EAG responses to mixtures of the two pheromone components of two other tortricids, *Adoxophyes orana* (Roslerstamm) and *Clepsis spectrana* (Treit.) that also were merely additive at lower dosages. Because it records from many neurons at once, the EAG cannot explain whether such responses are due to inter- or intra-unit interactions, but single unit studies for *A. velutinana* (O'CONNELL, 1972) suggest that at least part of the modulation is from within units; inhibition and synergism were observed in two sensory neuron types to binary mixtures of the three pheromone components.

O'CONNELL (1972) showed that there are two odour-sensitive neurons in each sensillum trichodeum on male *A. velutinana* antennae, and both respond to c11– and t11–14:Ac. The first cell, termed 'unit A,' produced larger amplitude spikes than the second cell, 'unit B.' The A unit typically responded with greater intensity to the *cis* isomer and the B unit with greater intensity to *trans* (O'CONNELL, 1975). In addition, the receptor sites specific for *trans* on the A unit appeared to be able to give inhibitory generator potentials as evidenced by a decrease in spike frequency by some A units when exposed to *trans*. Differences in response functions of the two cells generated by a dosage-response series of the *cis* and *trans* isomers led to the conclusion that 'at least two different functionally independent receptor sites' must exist on the dendritic processes of cells A and B (O'CONNELL, 1975). Thus cell A dendrites apparently possess more sites specific for *cis* than for *trans*, and cell B has more sites for *trans* than for *cis*. In addition, the two cells often exhibited 'clear-cut differences in response latency, duration, and interspike interval distribution for a given compound' (O'CONNELL, 1975). It was hypothesized 'these temporal characteristics of receptor neuron discharge could also code for odour quality' (O'CONNELL, 1975).

EAG recovery rates

The difference in EAG response shapes to the two isomers could be a function of the 'temporal characteristics of receptor neuron discharge' mentioned by O'Connell, such as response latency and rates of response onset and offset. If thousands of neurons having short response latencies and high rates of onset and offset were to discharge in response to a chemical stimulus, a high amplitude, fast recovery rate EAG might be recorded due to the high summation of responses early in the time interval. By contrast, if the same amount of electrical discharge were produced by different units and spaced more evenly throughout the same time interval due to longer response latencies and slower rates of response onset and offset, a lower amplitude, slower recovery rate EAG might result.

Such an hypothesis might be used to explain the relatively rapid EAG recovery rate to *cis* after maximum depolarization. Since A units' responses to a given concentration of *cis* are greater than those of B units (O'CONNELL, 1975), the A units' responses should be the dominant element in an EAG response to *cis*. As suggested by the higher EAG amplitude and more rapid recovery rate to *cis*, a population of A units should exhibit relatively short response latencies with high rates of onset and offset. By contrast, B unit responses to concentrations of *trans* are greater than those of A units (many times A units gave inhibitory or very low responses to this component) (O'CONNELL, 1975). It follows then that an EAG response to *trans* should be comprised nearly totally of the responses of B units. From the lower EAG amplitude and slower recovery rate to *trans*, one then might predict that a population of B units should exhibit relatively longer response latencies and slower rates of onset and offset.

Receptor adaptation

Results of the receptor site saturation studies with c11– and t11–14:Ac were consistent with O'CONNELL'S (1975) findings that two different, functionally independent receptor sites are present on *A. velutinana* antennae, although unlike single unit recordings, the EAG gives no evidence as to the distribution of these sites on the antenna. Either isomer puffed into the opposite isomer's airstream produced a response larger than that from a blank puff (Figs. 3J and M). When either isomer was puffed into its own airstream, however, the resulting response was no more than the response to a blank cartridge, proving that receptor site saturation had been accomplished (Figs. 3I and L, N and P). Elicitation of additional response by one isomer after the other had saturated its available sites leads to the conclusion that different sites were still available for occupation by the second isomer.

The observed ability of a 12:Ac airstream to change dramatically the shape of EAG responses to *cis* and not to *trans* by slowing the recovery rate has the following implications: (1) modulation of the temporal characteristics of response to *cis* by 12:Ac may be occurring at the receptor neuron level; (2) since

a response to *cis* is possibly comprised of predominantly unit A activity, the site of action of 12:Ac modulation might be on cell A; (3) since a response to *trans* is possibly comprised predominantly of unit B activity, and little modification of EAG recovery rate occurs with *trans* in a 12:Ac airstream, the site of action of 12:Ac does not appear to be on cell B in conjunction with *trans*.

The EAG and behavioural response

A possible behavioural correlate exists for *A. velutinana* (BAKER *et al.*, 1976) to the optimal EAG response amplitudes to mixtures approximating 8% *trans*. In laboratory olfactometers 8% *trans* elicited significantly higher levels of activation and wing fanning responses than all other *cis–trans* mixtures as well as pure *cis* or *trans* alone. The increased behavioural response to 8% *trans* occurred over a wide range of dosages, and so it was concluded that the insect must be detecting isomeric ratios and responding accordingly rather than responding to absolute quantities of either isomer.

A behavioural correlate to the temporal modulation of sensory neuron output by 12:Ac during response to *cis* also appears to exist (BAKER *et al.*, 1976). In laboratory olfactometers 12:Ac evaporated with pure *cis* and with 8% *trans* resulted in an increase in the duration of wing fanning response and orientation to the source. When evaporated with pure *trans*, however, no such increases were observed, and an involvement with *cis* rather than *trans* receptor sites by 12:Ac was hypothesised. Dodecyl acetate alone evoked no response at equivalent dosages. In the field, 12:Ac evaporated with 8% *trans* modified the behaviour of feral males close to the chemical source, resulting in an increase in the frequency of landing and close approach to the dispenser.

It can be envisioned, then, that after flying upwind as a result of optimal sensory neuron activity in response to the correct *cis–trans* ratio, a male would arrive within a short distance of and downwind to the pheromone emitter. Visual cues indicating a potential landing surface would cause a cessation of forward progress (BAKER *et al.*, 1976) with narrow oscillation casting flight to maintain contact with the odour plume. If by this stage the presence of 12:Ac had sufficiently altered the encoded *cis–trans* message the male would be more likely to land and complete the approach to the source (BAKER *et al.*, 1976). For *A. velutinana* the succession of these long and close-range responses to its species-specific component blend might be sufficient to prevent contact between *A. velutinana* males and non-conspecific females emitting a related pheromone blend. A basis for these behaviours may lie initially in the temporal aspects of odour-quality encoding by single cells (O'CONNELL, 1975) and ultimately in the complete pattern of impulse production across the many thousands of sensory neurons on the antenna (ERICKSON, 1963; O'CONNELL and MOZELL, 1969). The ability to even attempt to interpret the summated electrical activity of these neurons as recorded by the EAG is made possibly only by consideration of detailed morphological and single unit recording studies such as have been done for this species.

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