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MULTIPLE ACCEPTORS FOR PHEROMONAL ENANTIOMERS ON SINGLE OLFACTORY CELLS IN THE DOUGLAS-FIR BEETLE, *Dendroctonus pseudotsugae* HOPK. (COLEOPTERA: SCOLYTIDAE)[1,2]

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Abstract—Olfactory perception of pheromonal enantiomers by male and female Douglas-fir beetles, *Dendroctonus pseudotsugae* Hopk. (Coleoptera: Scolytidae), was investigated by electrophysiological techniques and behavioral bioassays. Electroantennograms (EAGs) and single-cell responses indicated both sexes to be more responsive to racemic frontalin and the (−)-enantiomer at lower dosages. At higher dosages, little difference was noted in responses to either enantiomer. However, response to the racemic mixture at higher dosages was slightly greater than responses to either enantiomer alone. In laboratory behavioral bioassays, responses to low concentrations of (−)-frontalin and the racemic mixture exceeded response to the (+)-enantiomer alone. At a higher concentration, responses to the racemic mixture or either enantiomer alone did not differ. The results indicate that separate enantiomer-specific acceptors may exist on the same pheromone receptor cell.

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INTRODUCTION

The chirality of semiochemicals has been found to play an important role in both communication within insect species and insect–host plant interactions (see Silverstein, 1979; Städler, 1974). Enantiomeric specificity is maintained by the insect and its host through both the chirality of pheromone precursors available from the host (Renwick et al., 1976) and enantiomeric specificity of synthetic enzymes (Renwick and Dickens, 1979). In addition, communication of chirality is further facilitated by enantiomer-specific acceptors (receptor sites) on insect olfactory cells (Kafka et al., 1973; Dickens and Payne, 1977; Mustaparta et al., 1980; Payne et al., 1982; Wadhams et al., 1982).

The aggregation pheromone of the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopk. (Coleoptera: Scolytidae), is released by females initiating host attack and attracts both sexes for mass attack of a potential host (Rudinsky, 1973; Rudinsky and Ryker, 1977). This pheromone consists of at least seven odorants (Ryker et al., 1979) including: 3,2-MCHone (3-methyl-2-cyclohexenone) (Kinzer et al., 1971); 3,2-MCHol (3-methyl-2-cyclohexenol) (Vité et al., 1972; Rudinsky et al., 1974); frontalin (1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane) (Kinzer et al., 1969; Pitman and Vité, 1970; Rudinsky et al., 1974); verbenone (Rudinsky et al., 1974) and *trans*-verbenol (Rudinsky et al., 1972). However, a mixture of 3,2-MCHone (in low concentrations), 3,2-MCHol, and frontalin was found to be most attractive in field tests (Rudinsky et al., 1974).

The olfactory receptor system of *D. pseudotsugae* for pheromones and host odors has been investigated at both the electroantennogram (EAG) (Dickens et al., 1983) and single-cell levels (Dickens et al., 1984). These investigations revealed that antennal olfactory cells could be classified into four types based on their sensitivity and specificity for the pheromones and host odors tested. Three of the cell types were primarily sensitive to one of the major aggregation pheromone components, i.e., 3,2-MCHone, 3,2-MCHol, or frontalin, while a fourth cell type responded primarily to compounds which were behavioral synergists.

Previous experiments showed racemic frontalin to be active at the antennal receptor (Dickens et al., 1983, 1984) and behavioral levels (Rudinsky et al., 1974). However, frontalin exists in two enantiomorphic forms (Mori, 1975; Stewart et al., 1977) and in other *Dendroctonus* species, specificity occurs in both neural perception (Payne et al., 1982) and behavioral response to individual enantiomers (Wood et al., 1976; Payne et al., 1982).

The purpose of this investigation was to elucidate antennal receptor and behavioral responses of *D. pseudotsugae* to the enantiomers of frontalin using electrophysiological techniques and laboratory bioassays.

METHODS AND MATERIALS

Insects. Adult *D. pseudotsugae* used in this study emerged from naturally infested bolts of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, from the McDonald Forest of Oregon State University, Corvallis, Oregon. Following emergence, beetles were sexed (Jantz and Johnsey, 1964) and maintained on moist filter paper in Petri dishes at 6°C until use within 1–3 weeks.

Electrophysiology. EAG and single-cell recording techniques were previously described in detail (Dickens, 1979) and were a modification of earlier techniques (Schneider, 1957; Boeckh, 1962). In brief, EAGs were recorded with Ag–AgCl capillary electrodes filled with 3 M KCl. The recording electrode was inserted into the distal end of the antennal club following prepuncture with a sharpened tungsten needle. The indifferent electrode was implanted in the mouth.

Single-cell recordings were made with 50.8 μm diameter tungsten wire electrolytically sharpened to a tip diameter of ca. 1–2 μm. The recording electrode was positioned under optical control (320×) with a Leitz high-power micromanipulator near the base of one of the three sensory bands encircling the antennal club. The indifferent electrode was implanted in the body of the beetle through the oral cavity.

Electrical signals were amplified 10× by a Bioelectric NF1 preamplifier prior to display on a Tektronix 561B oscilloscope. For single-cell recordings, the signal was further conditioned by a Tektronix 122 low-level preamplifier. Records of electrical activity were made on Polaroid film with a Tektronix 405 oscilloscope camera.

Chemical Stimuli and Experimental Protocol. Stimulus compounds and their source and purity are summarized in Table 1. Compounds were prepared as serial dilutions in nanograde pentane and presented as 10 μl samples placed on filter paper (20 × 7 mm) inserted into glass cartridges (75 mm; 5 mm ID) oriented toward the preparation from ca. 1 cm. Stimulus duration was 1–2 sec; air flow was ca. 2 liters/min.

Stimulus dilutions were presented from the lowest to the highest concentration. At least 3 min were allowed between each stimulus, except at higher concentrations, when 5 min were allowed between successive stimuli. These intervals were adequate for complete recovery of both EAG and single-cell activity.

To compare EAGs from different preparations, stimulation with a racemic frontalin standard at 10 μg occurred between every two stimulations with serial

TABLE 1. SOURCE AND PURITY OF STIMULUS COMPOUNDS USED IN ELECTROPHYSIOLOGICAL AND BEHAVIORAL EXPERIMENTS

Compound	Chemical purity (%)	Optical purity (%)	Source
(±)-Frontalin	99		A[a]
(+)-Frontalin	99	>98	B
(−)-Frontalin	99	>98	B
(±)-Limonene	99		A

[a] A, Chem. Samp. Co., Columbus, Ohio, B, Dr. K. Mori (Mori, 1975).

dilutions. Responses to intervening test stimuli were represented as a percent of the mean of the nearest two responses to the standard.

For each EAG stimulus, five replicates (i.e., five males and five females) were recorded from both sexes. Single-cell studies included responses of two frontalin cells (one male; one female) and four synergist cells (one male; three females) to both the racemic mixture and the individual frontalin enantiomers (for classification of cell types see Dickens et al., 1984). Each stimulus was presented only once to a particular cell.

Laboratory Bioassays. Laboratory behavioral bioassays were conducted using an olfactory walkway described in detail elsewhere (Jantz and Rudinsky, 1965; Kinzer et al., 1971). A complete stop over the test vial was used as the criterion for arrestment. Dilutions of test chemicals used in the bioassay were prepared in 95% ethanol. Each treatment was replicated four times (total of 20 males; five insects per replicate). Means were compared for significant differences using analysis of variance and F statistic (Ostle, 1963).

RESULTS

Electrophysiology. Dosage–response curves constructed from EAGs of male and female *D. pseudotsugae* to the racemate and individual enantiomers of frontalin were similar (Figure 1). For each sex, (−)-frontalin elicited EAGs greater than (+)-frontalin at the lowest dosages; response to the racemate was intermediate. At the highest dosage tested, the greatest response was elicited by the racemic mixture followed by (+)-frontalin then the (−)-enantiomer. The dosage–response curve for racemic frontalin seemed to parallel that of the (−)-enantiomer at the lower dosages. At the higher dosages, a similar parallel relationship was found between the dosage–response curves for the (+)-enantiomer and the racemate.

Results from the single-cell studies were similar to those from EAGs in that

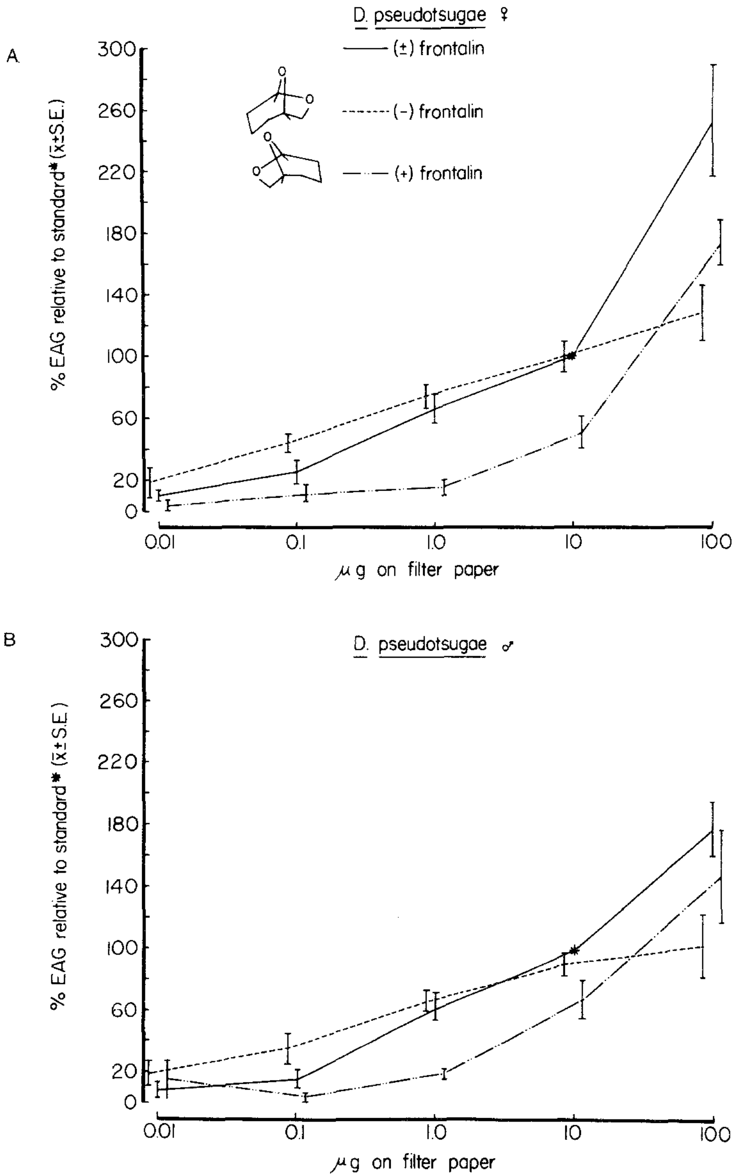

FIG. 1. Dosage–response curves constructed from EAGs of female (A) and male (B) *D. pseudotsugae* to racemic frontalin and its optical antipodes. Each point represents the mean of five replicates. Vertical bars represent standard errors.

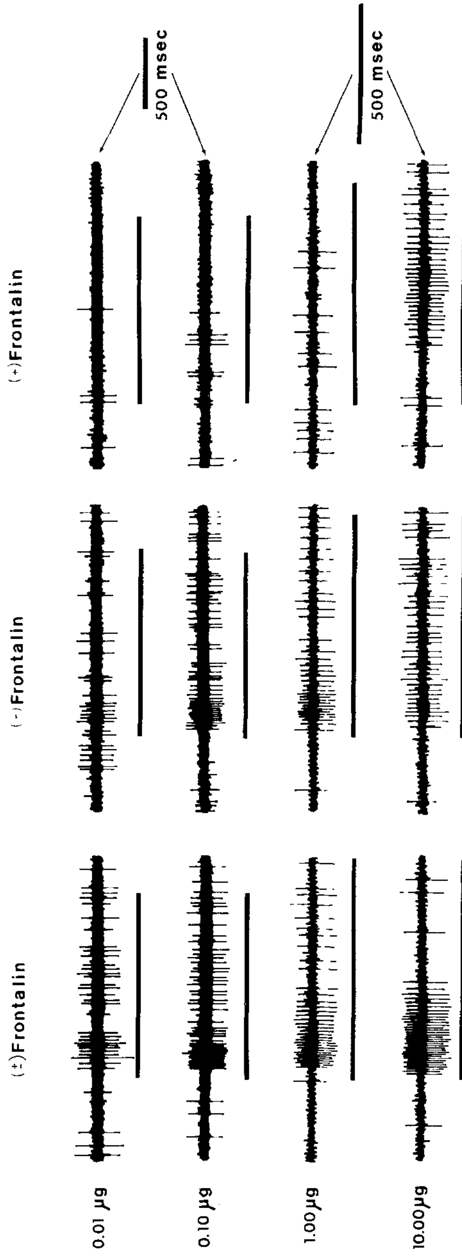

FIG. 2. Response of a frontalin cell to serial dilution of racemic frontalin and its optical antipodes.

at lower dosages racemic frontalin and the (−)-enantiomer elicited considerably more spikes than did the (+)-enantiomer (Figures 2 and 3). However, response to (+)-frontalin at the highest dosage tested was nearly equal to the number of impulses elicited by (−)-frontalin, while response to the racemate exceeded response to either enantiomer alone.

A similar response pattern was observed for synergist cells activated by relatively high dosages of frontalin (Figure 4). For example, a cell primarily sensitive to the host terpene, limonene, was most responsive to racemic frontalin at the 1.0 μg and 10 μg dosages with an intermediate response to the (−)-enantiomer relative to the least active (+)-enantiomer. However, at the highest dosage, i.e., 100 μg, little difference was noted between responses to all three compounds.

Laboratory Bioassays of Frontalin Enantiomers. Pedestrian bioassays confirmed results obtained in electrophysiological experiments (Table 2). At the lowest dosage tested, both (−)-frontalin and racemic frontalin arrested a larger number of male *D. pseudotsugae* than did the (+)-enantiomer. However, at the highest dosage tested, little difference was noted between the number of males arrested by any of the three treatments.

DISCUSSION

The fact that the enantiomers of frontalin stimulated a common single receptor cell is evidence that a single receptor cell can possess acceptors for both enantiomers. Furthermore, greater response at the EAG and single cell levels to racemic frontalin, relative to either enantiomer alone at the highest dosage tested,

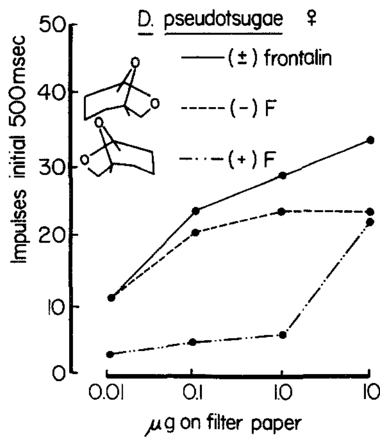

FIG. 3. Dosage–response curves constructed from response of a frontalin cell to racemic frontalin and its optical antipodes.

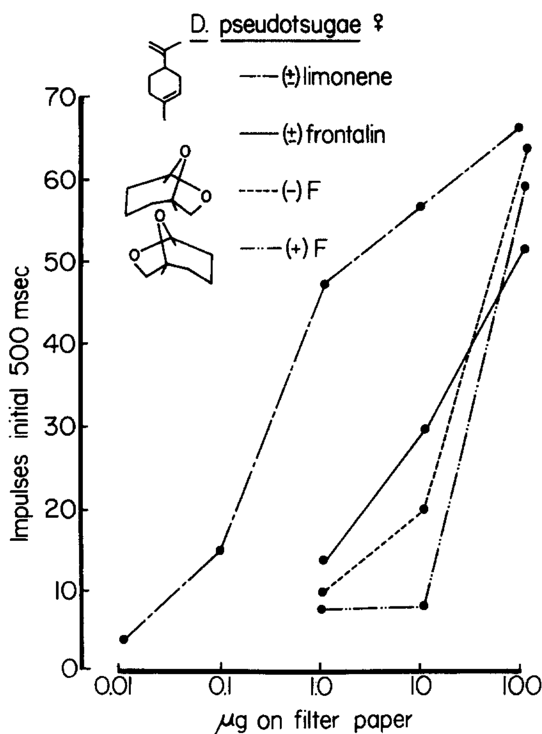

FIG. 4. Dosage–response curves constructed from response of a synergist (limonene) cell to racemic frontalin and its optical antipodes.

TABLE 2. RESPONSE OF WALKING MALE DOUGLAS-FIR BEETLES TO ETHANOL SOLUTIONS OF FRONTALIN ENANTIOMERS

Frontalin (0.01 μg/μl)[a]	Arrested[b]	Frontalin (0.1 μg/μl)	Arrested
(+)-Frontalin	0.8 ± 0.42	(+)-Frontalin	3.0 ± 0.41
(−)-Frontalin	3.8 ± 0.48**	(−)-Frontalin	4.3 ± 0.48
(±)-Frontalin	4.0 ± 1.08**	(±)-Frontalin	2.8 ± 0.71
Ethanol	0.0		

[a] All solutions in 95% ethanol.
[b] Mean ± SE of 20 beetles (four replications). Means significantly different by ANOVA and F statistic, $^{**}P < 0.10$.

suggests the existence of separate specific acceptors for each enantiomer on a single cell.

Existence of separate acceptors for individual enantiomers on the same receptor cell is further substantiated by recordings from synergist cells (Figure 4). At both the 1.0 μg and 10 μg dosages, the number of impulses elicited by frontalin exhibited the following order: (±)-frontalin > (−)-frontalin > (+)-frontalin. The greater response to the racemic mixture in this instance would also indicate the existence of both (+)- and (−)-enantiomer acceptors on this cell.

In support of the electrophysiological data, laboratory behavioral bioassays revealed a lower threshold for racemic frontalin and the (−)-enantiomer (Table 2). However, at a higher dosage, little difference was seen between the responses to either treatment.

These results indicate that separate acceptors for each enantiomer may exist on the same frontalin receptor cell. Furthermore, even in the case of less specialized synergist cells, differential responses to the individual enantiomers were preserved. Thus perception of frontalin enantiomers by *D. pseudotsugae* appears to occur via across-fiber patterning (O'Connell, 1975) elicited by the interaction of individual enantiomers with chiral acceptors on individual cells. A similar mechanism for the perception of frontalin enantiomers was hypothesized for the sourthern pine beetle, *D. frontalis* (Dickens and Payne, 1977; Payne et al., 1982). However, dosage–response data to further support the proposed hypothesis was unavailable for this species.

While an examination of the nerve impulses recorded from the sensitive sensilla suggest that the acceptors sensitive to both (+)- and (−)-frontalin are on the same neuron, a remote possibility exists that they may occur on separate neurons. It is possible, although highly unlikely, that the recording electrode was positioned in such a way between two cells, one sensitive to (+)-frontalin, the other sensitive to (−)-frontalin, so that the recorded spikes would be similar. However, neither doubling nor short interspike intervals occurred in response to the racemate. Thus we are left to conclude that multiple acceptors may occur on the same cell.

These findings for *Dendroctonus* species are similar to those observed for other insects. Kafka et al. (1973) first observed olfactory discrimination of enantiomers by the migratory locust and honeybee, both in single-cell recordings and behavioral experiments. They explained their observations by hypothesizing the presence of separate acceptors for each enantiomer on the same cell. Similarly, single cells responsive to both optical antipodes of a compound have been reported for other coleopterous species (Wadhams et al., 1982; Hansen, 1983).

These observations are an interesting contrast to the system proposed for discrimination of enantiomers by *Ips* species (Mustaparta et al., 1980). It was hypothesized for *Ips* species that olfactory cells possessed acceptors for only one enantiomer of a pheromone. But, in fact, these authors found individual receptor

cells primarily sensitive to one enantiomer did respond to its optical antipode at a higher dosage. They proposed that since the enantiomers they used were impure (i.e., 92% optically pure), response to the least active enantiomer could be attributed to the presence of its optical antipode. Furthermore, the intermediate response to the racemate was considered to be supportive of their hypothesis. In addition, a response similar to that of the most active enantiomer was obtained from stimulation with the racemate at 2× concentration (i.e., 100% of active enantiomorph). However, as they point out, their hypothesis is contradicted by different saturation levels for each enantiomer on individual cells.

Thus two slightly different mechanisms for the perception of pheromonal enantiomers appear to exist among insects. In *Dendroctonus* species, as in several other insects, individual cells specialized for the perception of an odorant may have varying numbers of acceptors for each enantiomer, with the resulting chiral message being coded across several cells (Kafka, 1973; Dickens and Payne, 1977; Wadhams et al., 1982; Hansen, 1983). As previously reported (Dickens and Payne, 1977; Dickens et al., 1984), and shown here, the occurrence of multiple acceptors on a single olfactory cell is not limited to enantiomers of a single pheromone. In fact, a single cell may possess acceptors for different pheromones and even host odors. Such a system of olfactory perception provides the insect with greater flexibility in coding sensory messages than might be possible via labeled lines. Another mechanism for deciphering pheromonal chirality proposed for *Ips* species involves "labeled lines," whereby acceptors for only one enantiomer occur on each cell (Mustaparta et al., 1980). However, this second mechanism should be considered tentative until pure enantiomers are available for testing.

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