

Discrimination between Enantiomers of Linalool by Olfactory Receptor Neurons in the Cabbage Moth *Mamestra brassicae* (L.)

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Abstract

Plants emit complex blends of volatiles, including chiral compounds that might be detected by vertebrates and invertebrates. Insects are ideal model organisms for studying the underlying receptor neuron mechanisms involved in olfactory discrimination of enantiomers. In the present study, we have employed two-column gas chromatography linked to recordings from single olfactory receptor neurons of *Mamestra brassicae*, in which separation of volatiles in a polar and a chiral column was performed. We here present the response properties of olfactory receptor neurons tuned to linalool. The narrow tuning of these receptor neurons was demonstrated by their strong responses to (*R*)-(–)-linalool, the weaker responses to the (+)-enantiomer as well as a few structurally related compounds, and no responses to the other numerous plant released volatiles. The enantioselectivity was verified by parallel dose-response curves, that of (*R*)-(–)-linalool shifted 1 log unit to the left of the (*S*)-(+)-linalool curve. A complete overlap of the temporal response pattern was found when comparing the responses of the same strength. Analysis of the spike amplitude and waveform indicated that the responses to the two enantiomers originated from the same neuron.

Key words: enantiomeric discrimination, GC–SCR, *Mamestra brassicae*, olfactory receptor neurons, (*R*)-(–)-linalool

Introduction

Chiral recognition by receptors and enzymes is well demonstrated in biochemical, pharmaceutical, and chemosensory research. Many chiral odorants are perceived by humans as different qualities and/or having different intensities (Friedman and Miller, 1971; Leitereg *et al.*, 1971; Pike *et al.*, 1988; Ohloff, 1994; Brenna *et al.*, 2003), like (*R*)-(–)-linalool which smells slightly different from (*S*)-(+)-linalool, and has a much stronger intensity (Christoph and Drawert, 1985). In contrast, the odor of other enantiomers may not be distinguished by humans (Ohloff, 1994). The importance of enantiomers in olfaction is well demonstrated in insects where optical configurations of pheromone components may be critical and contribute to population differences within species as well as to isolation between species (Tumlinson *et al.*, 1977; Birch *et al.*, 1980; Lanier *et al.*, 1980; Hagaman and Cardè, 1984; Wallner *et al.*, 1984). The presence of different olfactory receptor neurons (RNs) for these enantiomers have been demonstrated by extracellular recordings in several insect species (Mustaparta *et al.*, 1980; Hansen *et al.*, 1983; Wojtasek *et al.*, 1998; Plettner *et al.*, 2000; Nikonov and Leal, 2002).

Studies of herbivorous insects have also provided interesting data on RN detection of chiral compounds emitted by plants. Most interesting are the results obtained by the use of gas chromatography (GC), employing chiral columns linked to electrophysiological recordings from single olfactory RNs [gas chromatography–single cell recording (GC–SCR)] (Stranden *et al.*, 2002, 2003a,b; Bichão *et al.*, 2005b). This is because chiral GC is a means of providing optically pure compounds, which is a challenge when testing the effects of enantiomers. In three related heliothine moths, the sesquiterpene germacrene D, consisting of two enantiomers, activates a major group of RNs (Røstelién *et al.*, 2000a; Stranden *et al.*, 2003a). Stimulation with compounds eluting from a chiral column have shown that all the RNs responding to this compound in heliothine moths were tuned to (–)-germacrene D that has a 10-fold stronger effect than the (+)-enantiomer. A few other compounds of related structures had a weak effect. In the strawberry blossom weevil *Anthonomus rubi*, RNs tuned to germacrene D were found to have a similar enantioselectivity but differed in respect to other secondary odorants (Bichão *et al.*, 2005a). RNs tuned to linalool have been

found or indicated in several insect species, where linalool may act as a host plant attractant or repellent (Hori, 1998) or as a pheromone, or synergist to the pheromone (Ochieng *et al.*, 2002; Borg-Karlson *et al.*, 2003). In addition, other RN types tuned to plant odorants with a chiral center have been reported in various insect species. However, these RNs have not been tested with 100% pure enantiomers separated via chiral GC columns. We here present results obtained using GC-SCR with two different columns installed in parallel to stimulate enantioselective RNs tuned to linalool in the herbivorous moth species, *Mamestra brassicae*.

Materials and methods

Insects

Mamestra brassicae pupae were supplied by The Norwegian Crop Research Institute, Ås, Norway. The sexed pupae were stored in separate containers placed in climate chambers (22°C, 14:10h light:dark photoperiod, onset of dark cycle at 10:00 AM.). After eclosion, the adult insects were kept in cylindrical containers with access to water containing sucrose (5%). The age of adult insects used in the experiments ranged from 2 to 14 days. Both sexes were used in the experiments.

Chemicals and headspace samples

Volatiles were collected from several plant species by using a headspace technique (Byrne *et al.*, 1975; Pham-Delegue *et al.*, 1989; Wibe and Mustaparta, 1996; Røsteliën *et al.*, 2000b). The plants were placed in a closed oven bag through which purified air was sucked and led into glass tubes containing the adsorbents (Tenax TA and Porapak Q, 1:1). The air was purified by a filter of activated charcoal before the intake to the bag, and the collection was carried out for 24 or 48 h. The trapped volatiles were eluted with hexane and ethyl acetate (1:1) and stored in vials kept in a freezer. The plant materials used for collecting volatiles were *Brassica oleracea*, *Brassica napus*, and four ecotypes of *Arabidopsis thaliana*. Table 1 gives an overview of the headspace samples, essential oils, standard mixtures, and single compounds used to stimulate the RNs via the GC.

Direct stimulation via cartridges

Direct stimulation via glass cartridges was used for screening of RN sensitivity to the various samples of headspace and other mixtures. Five microliters of each test sample was applied to a filter paper placed inside the cartridge letting the solvent evaporate before use. The RN was exposed for the test sample by puffing air (8 ml/s) through the cartridge and over the antenna. Direct stimulation via glass cartridges was also used for determining dose-response curves. In these tests, 100 µl of each dilution of linalool enantiomers (decadic steps) was applied to a filter paper, and the solvent was evaporated by N₂ flow before inserting the filter paper into the glass cartridge. This resulted in test tubes containing the

following amounts of linalool: 0.01 ng, 0.1 ng, 1 ng, 0.01 µg, 0.1 µg, and 1 µg. In dose-response experiments, using direct stimulation, the tests started with concentrations below the detection limit of the RN and continued toward higher concentrations. Between the stimulations, the antenna was exposed to a continuous flow (500 ml/min) of purified air. The interstimulus interval varied from 30 s at low concentrations to 3 min at high concentrations.

GC linked to single-cell recordings, GC-SCR

The insects were mounted in a Plexiglas holder, and the head and antennae were stabilized with tape and wax as described by Røsteliën *et al.* (2000b). Electrophysiological recordings from single RNs were made by the use of electrolytically sharpened tungsten microelectrodes; the recording electrode placed into the base of a sensillum and the reference electrode into the base of the antenna. The neurons were initially screened for responses to mixtures of plant odors and single compounds via cartridges. When responding, 0.5–1 µl of the solution was injected into the column of the GC. The column was equipped with a splitter at the end, leading half of the effluent to the flame ionization detector (FID) and the other half into a constant airflow (500 ml/min) blowing over the insect antenna (Røsteliën *et al.*, 2000b). This made it possible, together with the simultaneous single-cell recording, to determine which compounds in the mixture elicited the responses. The spike rate and the gas chromatogram were recorded using EAD software (Syntech, Netherlands), while the spikes were recorded and stored using Spike2 software (Cambridge Electronic Design Limited, Cambridge, Great Britain). The GC was equipped with two columns of different separation properties, installed in parallel (Stranden *et al.*, 2002). In the present experiments, we used a DBwax [25 m, inner diameter (i.d.) 0.25 mm, film thickness 0.25 µm, J&W Scientific, Agilent Technologies, Palo Alto, CA] and a chiral column [25 m, i.d. 0.25 mm, octakis (6-O-methyl-2,3-di-O-pentyl)- γ -cyclodextrin (80% in OV 1701), König *et al.*, 1990]. Separation in the polar column was performed with two different programs, the first and most frequently used program starting at the initial temperature 80°C with an increase rate of 6°C/min to 180°C and a further increase rate of 15°C/min to 220°C. The second program was used to achieve better separation of the compounds in some of the headspace samples: performed from the initial temperature 50°C isothermal for 2 min followed by a 3°C/min increase to 180°C and a final increase of 15°C/min to 220°C. In the chiral column, enantiomeric separation of linalool, dihydrolinalool, and tetrahydrolinalool was optimal at the isothermal temperature 80°C. The FID temperature was set to 230°C for all programs. The GC was equipped with a cold on-column injector.

Spike analysis and cell classification

The spikes from RNs were analyzed using the Spike2 software. Separation of the cell types in one recording was based on differences in spike amplitudes and waveforms. The RNs

Table 1 Overview of the samples tested on each linalool RN via the two-GC columns

	RN no. I	RN no. II	RN no. III	RN no. IV	RN no. V	RN no. VI	RN no. VII	RN no. VIII	RN no. IX	RN no. X	RN no. XI	RN no. XII
Test samples												
Racemic linalool ^a	12	2	1	1	—	1	—	—	2	1	—	—
(S)-(+)-linalool ^b	7	1	—	—	—	—	—	1	1	—	1	1
Headspace/essential oils												
<i>Arabidopsis thaliana</i> Cvi ^c	2	—	—	—	—	—	—	1	—	—	—	—
<i>Arabidopsis thaliana</i> Col ^c	2	—	—	—	—	—	—	1	—	—	—	—
<i>Arabidopsis thaliana</i> L ^c	3	—	—	—	—	—	—	—	—	—	—	—
<i>Arabidopsis thaliana</i> Was ^c	—	—	—	—	—	—	—	1	—	—	—	—
<i>Brassica napus</i> ^c	2	—	—	—	—	—	—	—	—	—	—	—
<i>Brassica oleracea</i> ^c	2	—	—	—	—	—	—	—	—	—	—	—
Cinnamon essential oil ^d	—	—	—	—	—	—	—	—	—	—	1	—
Lemon essential oil ^d	1	—	—	—	—	—	—	1	—	—	—	—
Ylang ylang essential oil ^e	—	—	—	1	1	1	1	—	1	1	1	—
Synthetic compounds												
Camphor ^b	—	1	—	—	—	—	—	—	—	—	—	—
Racemic citronellol	1	—	—	—	—	—	—	—	—	—	—	—
Racemic dihydrolinalool ^b	6	—	—	—	—	—	—	—	—	—	—	1
Linalyl acetate ^e	1	—	—	—	—	—	—	—	—	—	—	—
3-Octanone ^b	1	—	—	—	—	—	—	—	2	—	1	—
Racemic 1-octen-3-ol ^e	1	—	—	—	—	—	—	—	2	—	1	—
<i>p</i> -, <i>m</i> -, <i>o</i> -Methylanisol ^f	—	—	—	1	—	—	—	—	—	—	—	—
Racemic tetrahydrolinalool ^e	5	—	—	—	—	—	—	—	—	—	—	1
Standard 1	2	—	1	—	—	—	—	2	—	—	1	1
3,7-Dimethyl-1-Octanol												
3,7-Dimethyl-1,6-Octanol												
2,6-Dimethyl-2,7-Octadiene												
Racemic citronellol												
Dihydromyrcene												
Myrcenol												
Dihydromyrcenol												
Geraniol												
Myrcene												
(<i>Z</i>)- and (<i>E</i>)- β -ocimene (1:1)												
Standard 2	—	2	—	—	—	—	—	1	—	—	—	—
(<i>Z</i>)- and (<i>E</i>)- β -ocimene (70 %)/												
Limonene (25%) ^a												
Camphor												
Racemic linalool ^a												

Table 1 Continued

	RN no. I	RN no. II	RN no. III	RN no. IV	RN no. V	RN no. VI	RN no. VII	RN no. VIII	RN no. IX	RN no. X	RN no. XI	RN no. XII
Methyl benzoate ^a												
Isoborneol ^b												
(+)-(E)-Verbenol ^b												
Methyl salicylate ^a												
Standard 3	1	—	—	—	—	—	—	—	—	—	—	—
(+)-3-Carene												
Citral [Geranial and Neral (1:1)]												
Benzylalcohol												
Benzylcyanide												
Indole												

Numbers indicate how many times the stimulation was performed on the individual RNs.

^aFluka, Buchs, Switzerland.

^bBorg-Karlson, Royal Institute of Technology RIT, Stockholm, Sweden.

^cUlland, Norwegian University of Science and Technology, Trondheim, Norway.

^dNorsk Medicinal Depot (NMD), Oslo, Norway.

^eAldrich, Steinheim, Germany.

^fLancaster, Lancashire, UK.

^gMerck, Darmstadt, Germany.

were classified according to which odorant elicited the strongest response (primary odorant) as well as those having weaker effects (secondary odorants).

Results

General response characteristics

Excitation and temporal response pattern

Out of 43 olfactory RNs recorded in *M. brassicae* females and males, 12 neurons responded best to the plant volatile linalool. The 12 RNs were obtained in six females and six males. Altogether, the RNs were tested 89 times via the GC (GC = 89), out of which 49 tests were performed on the same neuron (Table 1, RN no. I). In 30 out of the 49 tests, this neuron responded strongly to linalool present in the various headspace samples of host and nonhost plants as well as to synthetic linalool. In addition, the other RNs tested repeatedly with samples containing linalool demonstrated reproducibility of the responses, which appeared as increased firing rate upon stimulation with linalool (Figure 1A). Three other compounds elicited weaker responses in the most sensitive neurons. No inhibition was observed. When stimulating via the GC, the firing rate of the RNs followed the concentration profile of the active GC peak, regaining the spontaneous activity within half a minute or a few minutes. Responses to high concentrations elicited responses that far outlasted the GC peak. Most of the 12 RNs showed responses also to the solvents hexane and ethyl acetate.

Stimulation directly with linalool via cartridges, giving a fast onset and more constant concentration profile, showed a phasic-tonic response pattern. After stimulation with high concentrations of linalool, the RNs showed adaptation which might last for several minutes. Most recordings presented from the GC-SCR indicated activity of two or three RNs responding to different compounds. The responses of the RNs were distinguished based on spike analysis in the Spike2 software. Similar amplitudes and waveforms of the spikes were ascribed to the same RN.

Concentration dependency

Ten out of the twelve RNs responded to linalool at low concentrations. The threshold concentration for the most sensitive neurons was approximately 0.5 ng of *racemic* linalool when tested via the polar GC column. Dose-dependent excitation was shown by stimulation with decadic increase of concentrations over 5 log units. As shown in Figure 1B, the most sensitive linalool RN displayed an approximately linear increase of the firing frequency over the three highest concentrations (5 ng–0.5 µg), reaching a maximum firing rate of more than 200 spikes/s. The temporal response pattern of the same RN when stimulated with the three highest concentrations is shown in Figure 1C. The firing rate of the neuron increased from the onset of the stimulus, that is, when the GC peak appeared, reaching a maximum after 300–400 ms at the peak of the concentration profile. During the high firing frequency of the strongest response, the spike amplitude decreased below the noise level making spike counting

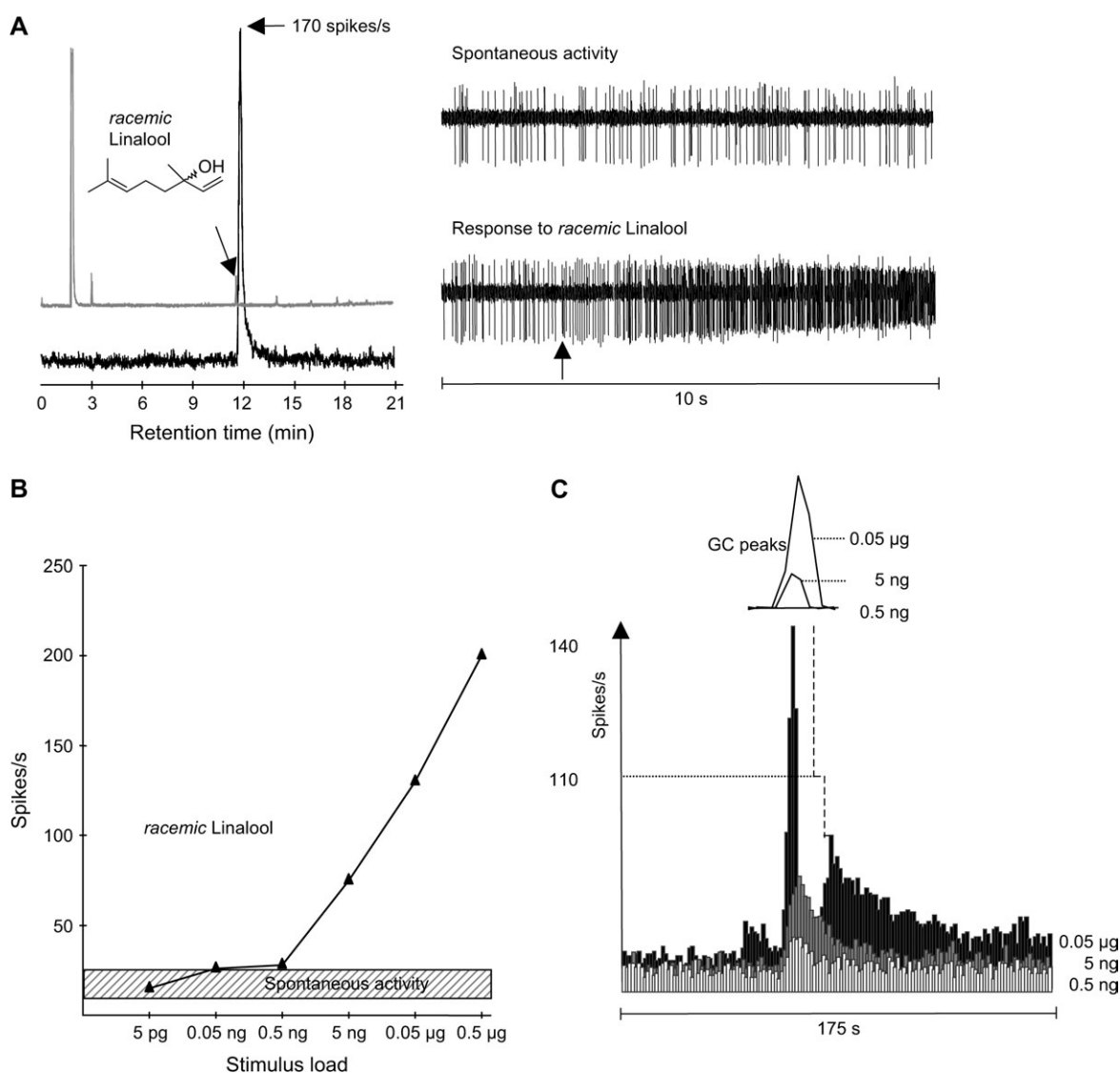


Figure 1 Response characteristics of a single RN to stimulations with *racemic* linalool via the polar GC column. **(A)** Gas chromatogram of standard 1 (GC trace above) and simultaneously recorded activity of the RN (trace below), showing response to linalool eluted at the retention time 11.29 min (left). Traces (10 s) of the spontaneous activity and the response (right). Arrow indicates the start of the stimulation. **(B)** Dose-response curve indicating threshold concentration at 0.5 ng and an approximately linear increase up to 0.5 μg. **(C)** Temporal patterns of the responses to decadic increase of the concentration (0.5 ng–0.05 μg). The stimulus period and relative concentrations of the GC peak is indicated above. Maximum firing rate appeared at about the same time after onset of the stimulus. The decay of the strongest response was particularly prolonged. The sudden drop appearing at the maximum firing rate was caused by the decrease of spike amplitudes, disabling spike counting in the Spike2 software. The dashed line indicates the real decay of the response.

difficult. This was the cause of the apparently rapid drop in spike frequency in Figure 1C. Nevertheless, recovery of spike amplitude showed that the actual spike frequency recovered fairly quickly toward baseline levels, that is, to about 50% of maximum within 5 s.

The molecular receptive range and enantioselectivity

Stimulation with compounds eluting from the polar column

The molecular receptive range was described by stimulating the neurons with headspace samples, essential oils, and

synthetic compounds via the polar GC column. Figure 2A shows the gas chromatogram of the headspace sample of the host plant *B. oleracea* var. *italica* and the simultaneously recorded activity of a single RN. The response appeared at the retention time of linalool (11.29 min), present in trace amount below the detection limit of the FID. In addition, weak responses to two other compounds appeared at the retention times 12.47 and 14.07 min. The trace amount did not allow identification of these two compounds by GC–mass spectrometry. Responses during the elution of the large amounts of the solvents (hexane and ethyl acetate) were

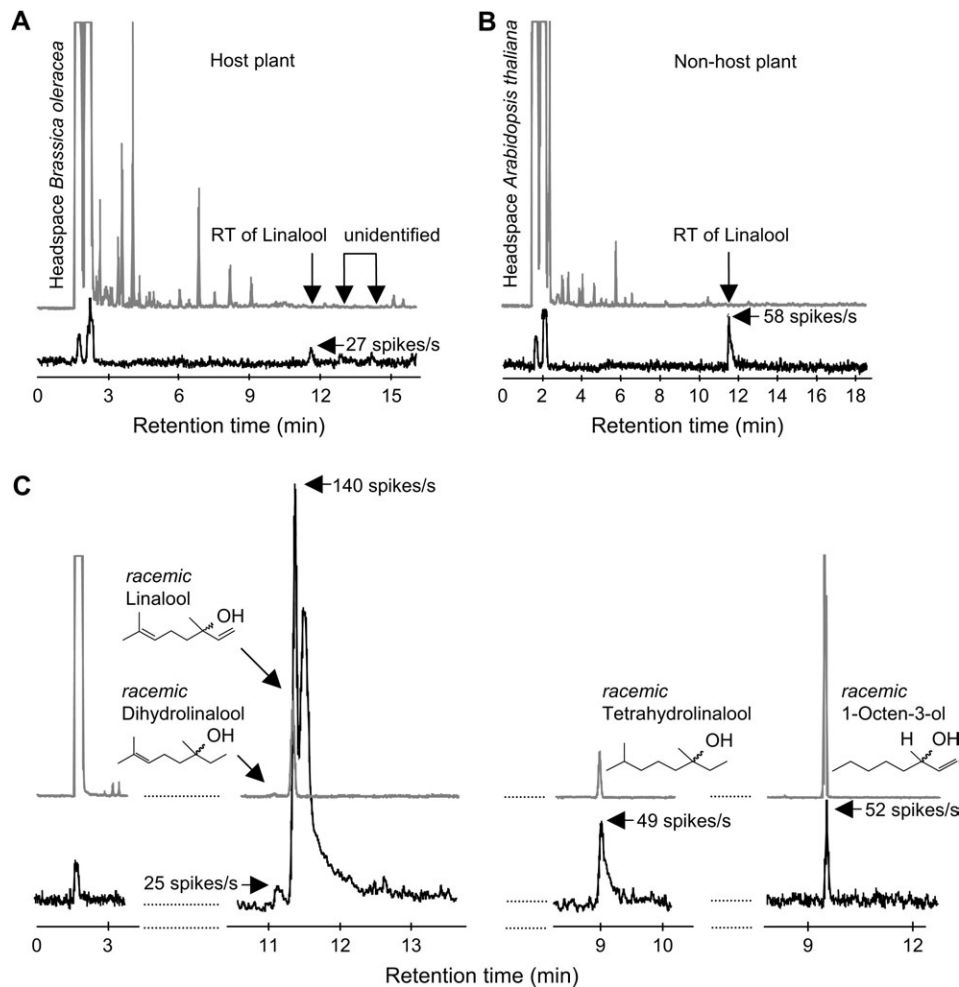


Figure 2 Gas chromatograms of headspace samples of the host *Brassica oleracea* (A) and the nonhost *Arabidopsis thaliana* Cvi (B) separated in the polar GC column (GC trace above). Below, simultaneously recorded activity of a single RN stimulated with the separated components, showing responses at the retention time (RT) 11.29 min of linalool (trace amount). Two unidentified compounds present in *B. oleracea* elicited weak responses (retention times 12.47 and 14.07 min). Responses to the high concentrations of the solvents (hexane and ethyl acetate) also appeared. (C) Responses of the same RN (traces below) to stimulation via the polar GC column (GC trace above) with synthetic material of *racemic* linalool, *racemic* tetrahydrolinalool, and *racemic* 1-octen-3-ol.

also obtained in this type of neuron of high sensitivity. When stimulating with the headspace sample of *A. thaliana*, the most sensitive neurons responded during the elution of the two solvents and at the retention time (11.29 min) of linalool (Figure 2B). Also in this sample, the amount of linalool was below the detection limit of the FID. Altogether, six headspace samples of *Brassica* spp. and *A. thaliana* and two essential oils were tested on this neuron, all eliciting responses at the retention time of linalool. *Racemic* linalool injected in the polar column elicited one strong response to the linalool peak. Also, a weaker response to dihydrolinalool appeared before the elution of linalool. In addition, weak responses were obtained to two structurally related compounds, tetrahydrolinalool and 1-octen-3-ol (Figure 2C). The selectivity for linalool was further demonstrated by no responses to the numerous other compounds present in the standards and headspace samples tested.

Stimulation with compounds eluting from the chiral column

Eight of the twelve RNs (RN no. I, II, V, VIII–XII) were tested with compounds eluted from the chiral column. All of them showed enantioselectivity by responding stronger to (*R*)-(–)-linalool than to (*S*)-(+)-linalool. Approximately a 10-fold stronger effect of the (–)-enantiomer than of (*S*)-(+)-linalool was found for all eight neurons. This is illustrated for RN no. I in Figure 3A where the concentrations 0.05 μg of (*R*)-(–)-linalool and 0.5 μg of (*S*)-(+)-linalool elicited the same firing frequencies. Analysis of the spikes of the two responses showed similar amplitudes and waveforms, indicating that they originated from the same neuron. The 10-fold difference in stimulatory effect was also shown by the dose-response curves obtained by direct stimulation, the (*R*)-(–)-linalool curve shifted 1 log unit to the left of the (*S*)-(+)-linalool curve (Figure 3B). Enantioselectivity was also found for the responses to dihydrolinalool and

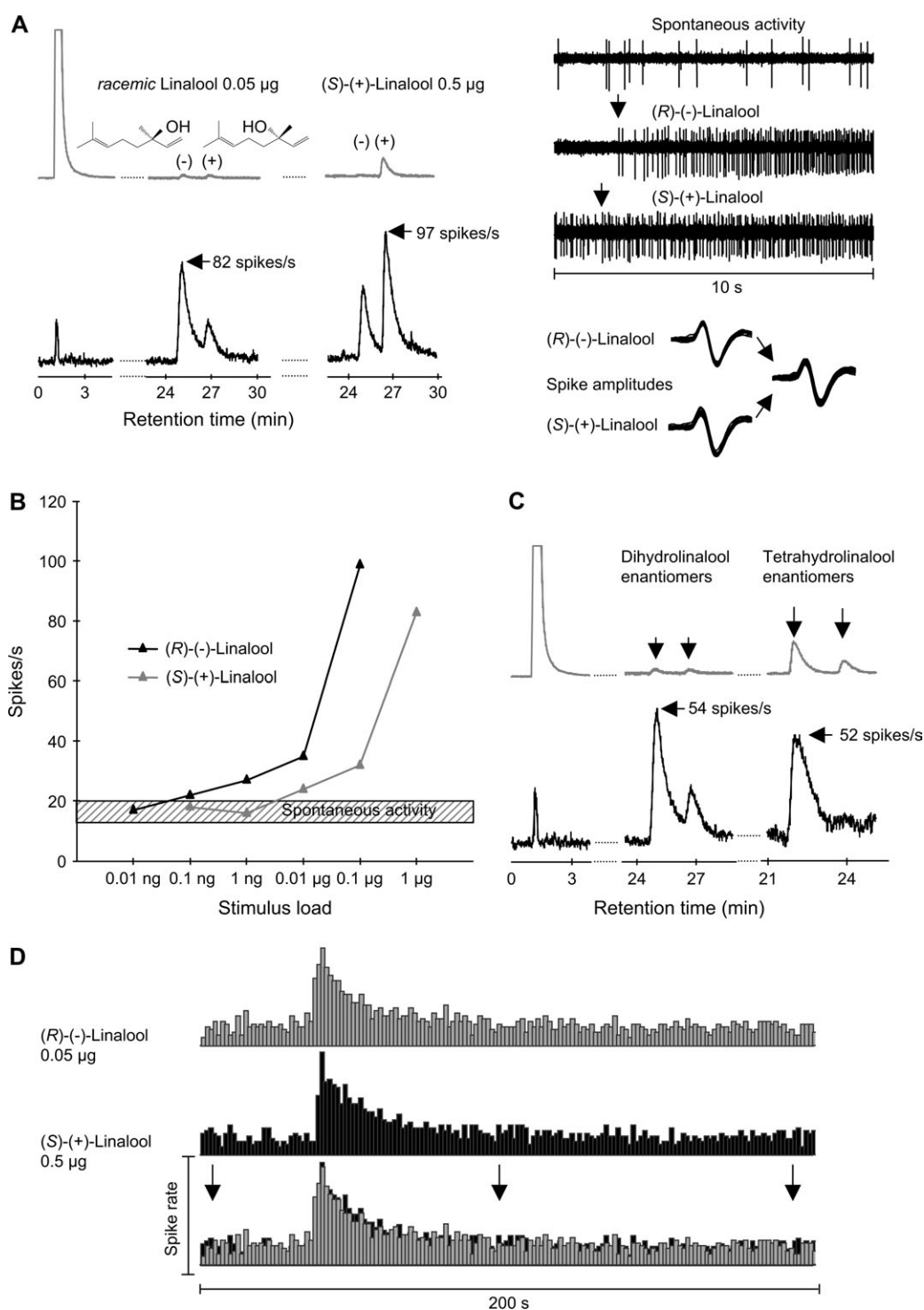


Figure 3 (A) Responses of a single RN (traces below) to enantiomers of linalool during elution from a chiral GC column (GC traces above) (left). The responses elicited by solutions with different enantiomeric ratios show about 10 times higher effect of (R)-(-)-linalool than (S)-(+)-linalool. Traces of the spontaneous activity and the responses to the enantiomers (right). Arrows indicates the start of the stimulations. Overlapping spike waveforms and amplitudes indicated that the responses originated from the same RN. (B) Dose-response curves for linalool enantiomers obtained by direct stimulation with two samples, one containing 96% (S)-(+)-linalool and the other 97% (R)-(-)-linalool, verified a 10-fold stronger effect of (R)-(-)-linalool. (C) Responses of the RN (traces below) to synthetic *racemic* dihydrolinalool and *racemic* tetrahydrolinalool eluted from the chiral GC column (GC trace above) shows a marked stronger response to one of the enantiomers (the sequences of the enantiomers are unknown). (D) A complete overlap of the temporal patterns of the responses to 0.05 μg (R)-(-)-linalool and to 0.5 μg (S)-(+)-linalool is shown.

tetrahydrolinalool by two RNs (RN no. I and XII) stimulated with the compounds eluted from the chiral GC column (Figure 3C). However, the elution sequence of these enantiomers in the chiral column is not known. Comparison of the responses to 0.05 μg (*R*)-(–)-linalool and to 0.5 μg (*S*)-(+)-linalool showed complete overlap of the temporal response pattern (Figure 3D).

Discussion

Discrimination between odors is based on the presence of a large number of olfactory receptor protein types, each expressed in subsets of sensory neurons, as shown in various vertebrates and insects (Buck and Axel, 1991; Clyne *et al.*, 1999; Krieger and Breer, 1999; Mombaerts, 1999; Vosshall, 2001). The convergence of axons belonging to one subtype in one or two specific glomeruli in the primary olfactory center, the olfactory bulb in vertebrates, and antennal lobe in insects further elucidate the principle called “the molecular logic of smell” (Axel, 1995). Depending on the tuning of the receptors, the information about one compound may be mediated by one or several types of RNs resulting in activation of one or more glomeruli. Among the olfactory RN types recorded in *M. brassicae*, the neurons tuned to linalool constituted the largest group. They all showed similar functional characteristics, indicating that *M. brassicae* detect linalool by one type of RNs that has a narrow tuning to (*R*)-(–)-linalool. This RN type is 10 times more sensitive to (*R*)-(–)-linalool than to (*S*)-(+)-linalool and show the same temporal response pattern to the two enantiomers. It suggests that *M. brassicae* perceive the odors of these enantiomers as similar quality but with different intensity. Although RNs tuned to (*S*)-(+)-linalool have not been found in this species, we can not exclude the possibility that they might be present among the large number of olfactory RNs responding to plant odors, for example, indicated by the numerous ordinary glomeruli (67 ± 1) in the antennal lobe of this species (Rospars, 1983). It is also possible that the two enantiomers of linalool have differential effects on RNs tuned to other compounds, resulting in different across-glomerular stimulation patterns and behavioral discrimination. In another insect species, the strawberry blossom weevil *A. rubi*, two types of linalool RNs have been described, one tuned to the (*S*)-(+)-linalool and the other to the (*R*)-(–)-linalool, both showing considerably lower sensitivity to the opposite enantiomer (Bichão *et al.*, 2005b). So far, the results obtained in these two species indicate that the strawberry blossom weevil may easily discriminate between the two enantiomers, whereas *M. brassicae* may perceive these odors as being of the same or similar quality. How the information about the two enantiomers is represented by specific glomerular activation is not known in these species. However, in another species of moths *Manduca sexta*, two specific glomeruli have been identified that receive enantioselective information about linalool (Reisenman *et al.*, 2004). According to these results, *M. sexta*

certainly possesses at least two populations of RNs that discriminate linalool enantiomers. In fact, another study of linalool responding RNs of *M. sexta* showed individual variations of the molecular receptive ranges when directly stimulated with numerous selected compounds (Shields and Hildebrand, 2001). It would be interesting to test these neurons by GC-SCR and with the same protocol as in *M. brassicae*, for comparison of specificity and enantioselectivity.

Linalool is a typical floral constituent produced in a wide range of plants. It is synthesized via a condensation of dimethyl allyl pyrophosphate and isopenthyl pyrophosphate to geranyl diphosphate (GPP) and converted to linalool in a reaction catalyzed by linalool synthase, which is enantioselective (Dudareva *et al.*, 1996; Cseke *et al.*, 1998). Both enantiomers of linalool are present in many plant species, but in a few species only one of them is present (Borg-Karlson *et al.*, 1996; Casabianca *et al.*, 1998). Each inflorescence can produce its own specific composition of the enantiomers (A.-K. Borg-Karlson, unpublished data). It is not known whether *M. brassicae* moths uses a narrow or a broad range of flowering plants for nectar feeding, but their larvae are particularly associated with plants of the genus *Brassica* (Skou, 1991). The enantiomeric ratio of linalool released by these host plants has previously not been reported and was not investigated in the present study by separating headspace samples in the chiral column. However, in *A. thaliana* (Columbia ecotype) of the same family, Brassicaceae, a larger amount of (*R*)-(–)-linalool than (*S*)-(+)-linalool is emitted (ratio 2:1) (Chen *et al.*, 2003). Furthermore, a gene coding for a protein involved in catalyzing GPP to pure (*S*)-(+)-linalool has been identified, which implies that there is also a particular gene coding for the (–)-enantiomer. Emission of linalool in *A. thaliana* (Columbia ecotype) is shown exclusively from the flowering parts, and the release is continuous with no indications of induction by stress (J. Gershenzon, personal communication). If Brassicaceae plants have a similar distribution of linalool enantiomers, we may assume that *M. brassicae* uses (*R*)-(–)-linalool as a cue in the nectar feeding and not in the oviposition. In fact, in wind tunnel experiments, mated *M. brassicae* females did not show upwind flight to stimulation with linalool (Rojas, 1999).

Important in demonstrating enantioselectivity of RNs is to obtain an optimal separation in the chiral GC column. This is in order to make the sensory neurone able to recover enough from adaptation to the first eluted enantiomer before stimulated with the second one, which was the case in the present study. The RN responded to (*S*)-(+)-linalool although it eluted after the stronger response to (*R*)-(–)-linalool. The 10-fold difference of the stimulatory effect of the (–)- and (+)-enantiomers also demonstrated by direct stimulation corresponds to what is found in other enantioselective plant odor RNs in insects when stimulated via the chiral GC column (Stranden *et al.*, 2002, 2003a; Bichão *et al.*,

2005a). The molecular properties of enantiomers are interesting in considering odor-receptor interactions. The present results show that the chiral center at carbon 3 in the linalool molecule is important in the interaction with the receptor of *M. brassicae*. The molecule with hydroxyl group in *R* configuration at carbon 3 in combination with the allylic double bond elicits the highest response, followed by the *S* configuration. A similar ratio of the stimulatory effect can be assumed for the corresponding configurations of the weaker stimulants dihydrolinalool and tetrahydrolinalool, for which the sequence of elution is not known. When stimulating with the smaller molecule, 1-octen-3-ol, lacking both of the methyl groups at carbon 3 and 7, the response is even more reduced. Speculations about the binding between the receptor protein and the linalool enantiomers are difficult without knowing the amino acid sequence of the binding area of the receptor protein.

Because the linalool enantiomers are common in many plant species, it would be interesting to know the enantioselectivity of the receptors in insects of different genera and families as well as in vertebrates. Based on this, it might be possible to figure out why the receptors for detecting these enantiomers have evolved similar or different specificities across species and why some species have one and others have two types of enantioselective linalool receptors.

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