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Receptor Neuron Discrimination of the Germacrene D Enantiomers in the Moth *Helicoverpa armigera*

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Abstract

Plants release complex mixtures of volatiles, including chiral constituents. In the search for the biologically relevant plant odorants, gas chromatography linked to electrophysiological recordings from single receptor neurons has been employed. In heliothine moths, including the females of the Eurasian cotton bollworm moth *Helicoverpa armigera*, a major type of receptor neurons is identified, showing high sensitivity and selectivity for the sesquiterpene germacrene D. In the present study, gas chromatography with a chiral column linked to single cell recordings were performed. It was found that all germacrene D neurons belonged to one type; all responded to both enantiomers, but (–)-germacrene D had ~10 times stronger effect than (+)-germacrene D. Parallel dose–response curves for the two enantiomers were obtained by direct stimulations. The enantiomeric composition of germacrene D, which differed in six plant species and in different individuals of one species, was determined on the basis of the neuron responses. The results, showing the presence of one neuron type for receiving the information about germacrene D in the various plants, suggests that the two enantiomers mediate the same kind of information to the moth, but with different intensity.

Introduction

Chiral recognition of organic molecules is considered to be one of the most important criteria of biological activity, based on the fact that about half of all active chemicals have a chiral centre (Ohloff, 1994). Since the early confirmation of the ability of humans to discriminate between enantiomers of the taste stimulant asparagine (Piutti, 1886), many examples on enantioselectivity have been shown in taste as well as in olfaction. A well known example is the different smell of (+)- and (–)-carvone, caraway and peppermint, respectively (Friedman and Miller, 1971). Interesting discoveries of enantioselectivity in the insect communication systems were made by Lanier *et al.* (Lanier *et al*., 1980), showing that geographically isolated populations of bark beetles (*Ips pini*) used different ratios of the two enantiomers, (+)- and (–)-ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol), as aggregation pheromones. In fact, the major enantiomer used by one population inhibited the attraction of the other, a phenomenon that was shown to be important for the isolation from a sympatric species (Birch *et al*., 1980). The receptor neuron responses underlying the behavioural discrimination of enantiomers were demonstrated in these species of bark beetles by the identification of two neuron types, one tuned to (+)-ipsdienol and the other to (–)-ipsdienol (Mustaparta *et al*., 1980). Other studies of various insect species have also demonstrated the use of chiral compounds in intraspecific and interspecific communication, showing synergistic as well as antagonistic effects of enantiomers on the behaviour (Tumlinson *et al*., 1977; Silverstein, 1979, 1988; Tumlinson, 1979; Hansen *et al*., 1983). Accordingly, distinct types of enantioselective receptor neurons have been shown in some of these species (Hansen *et al*., 1983; Hansen, 1984; Mustaparta, 1990; Wojtasek *et al*., 1998). Whether one species possesses one or two types of neurons for enantiomers correlates well with the specific message the signals mediate. In the case where only one enantiomer is produced by the insects, one enantioselective receptor neuron type is found, e.g. neurons with enantioselectivity exclusively for (–)-ipsenol (2-methyl-6-methylene-7-octen-4-ol) in bark beetle species (Mustparta *et al.*, 1980).

The other important group of odorants for herbivorous insects is plant-produced volatiles. In spite of the significance of these odours for host finding, knowledge is still scarce about which of the hundreds of plant volatiles are biologically relevant in the various species. Progress is being made by the use of gas chromatography linked to electrophysiology (Guerin *et al*., 1983; Baur *et al*., 1993; Blight *et al*., 1995; Wibe and Mustaparta, 1996; Wibe *et al*., 1998;

Barata *et al*., 2000; Røstelien *et al*., 2000a,b) (M. Stranden *et al*., unpublished data). In heliothine moths ~12 types of receptor neurons have been identified that responded selectively to monoterpenes and sesquiterpenes (Røstelien *et al*., 2000a,b) (M. Stranden *et al*., unpublished data). Of particular interest was the finding of a receptor neuron type with high sensitivity to and selectivity for the sesquiterpene germacrene D (7-iso-propyl-10-methyl-4-methylene-cyclodeca-5,10-diene) (Røstelien *et al*., 2000b). This neuron type was obtained in 80% of all recordings from the females of the tobacco budworm moth *Heliothis virescens*, and responses to germacrene D were shown in tests of many plant mixtures. The presence of a major neuron type with the same sensitivity and selectivity for germacrene D has also been demonstrated in females of another heliothine species, the Eurasian cotton bollworm moth, *Helicoverpa armigera* (M. Stranden *et al*., unpublished data).

Like most sesquiterpenes, germacrene D is a chiral compound synthesized as one or both enantiomers in various plants, fungi and animals. It is considered as an important intermediate in the formation of many sesquiterpenes, which are biosynthesised via cyclization of farnesyl diphosphate catalysed by synthases (Yoshihara *et al*., 1969; Cane, 1990; Bülow and König, 2000). In higher plants the (–)-configuration of germacrene D is shown to be the most common enantiomer (Beechan *et al*., 1978; Lorimer and Weavers, 1987; König *et al*., 1996; Bülow, 1998; Bülow and König, 2000). However, exceptions have been found in *Solidago* species, in which the synthesis of both enantiomers is controlled by two enantioselective synthases (Niwa *et al*., 1980; Schmidt *et al*., 1998, 1999).

In principle, two plant compounds that give different messages to the animal would be expected to activate different receptor neuron types. An interesting question is whether the two germacrene D enantiomers, produced by different enzymes, are perceived as different odour qualities. Which kind of message the heliothine moth species receives in the detection of the germacrene D enantiomers, is not yet clear. However, the large number of germacrene D neurons indicates that the compound is of particular importance in the host location by *H. armigera* females. The presence of one, possibly two, types of receptor neurons would indicate how well the moth may discriminate between (+)- and (–)-germacrene D. The objective of the present study was (i) to determine the enantioselectivity of the germacrene D receptor neurons in the female moth *Helicoverpa armigera*, and (ii) to find out whether the moth possesses one or two types of neurons tuned to each of the enantiomers.

Materials and methods

Insects

Females of *Helicoverpa armigera* originated from a laboratory culture at the Agricultural Research Organisation, The Volcani Centre, Bet Dagan, Israel. They arrived as pupae and were kept as previously described by Røstelien *et al*. (Røstelien *et al*., 2000a). When eclosed, they were placed in separate boxes marked with eclosing date and given honey water and pure water *ad libitum*. Insects aged 2–5 days old were used in the experiments.

Chemicals

As the sesquiterpene germacrene D was not available commercially as pure material, it was tested as constituent in essential oils, extracts and headspace samples of plant material as well as in isolated fractions.

Extracts and headspace samples

One hexane extract was made from materials of a dried root of ginger (*Zingiber* sp.). In addition, pentane extracts of leaf tissues of sunflower (*Helianthus annuus*), Canadian goldenrod (*Solidago canadensis*) and yarrow (*Achillea millefolium*), were included as test material. The extract of sunflower (cultivated plants) and yarrow were made of leaves from several plants, whereas the two extracts of Canadian goldenrod were each made from one individual. The plant materials were collected in the area of Stockholm, Sweden (in October, outdoor temperature 1°C). The ginger extract was made of the cut root material, which was stirred for 4.5 h with hexane. The other extracts were made of freshly cut leaves washed several times with pentane. One headspace extract of wild briar (*Rosea dumalis*) collected in the Trondheim area was also included in this study [procedure as described by Røstelien *et al*. (Røstelien *et al*., 2000a)].

Fraction containing (–)-β-caryophyllene and germacrene D

A fraction containing (–)-β-caryophyllene (42.5%) and germacrene D (46%) was isolated from a sesquiterpene fraction of cubebe pepper (*Piper cubeba*) essential oil (20 g) containing <2% of germacrene D (Schmaus, 1988). The isolation procedure is described in Røstelien *et al*. (Røstelien *et al*., 2000b), and involves several parallel series of medium pressure liquid chromatography (MPLC), first on a column loaded with silica gel and then on one with silver nitrate ($AgNO_3$) impregnated silica gel. The solvent gradient was made of *n*-hexane and methyl acetate in different proportions. The fractions were followed by thin layer chromatography and gas chromatography mass spectrometry (GC-MS). The fractions containing germacrene D were pooled and a second $AgNO_3$-MPLC run with dried *n*-hexane only was performed, which resulted in the fraction containing (–)-β-caryophyllene and germacrene D (~1 ng/µl for both compounds). Further isolation of germacrene D using MPLC was unsuccessful.

Isolated enantiomers

Reference samples of defined germacrene D enantiomers were kindly provided by Dr W.A. König (University of Hamburg, Germany). The samples were obtained by hydro distillation from two Canadian goldenrod (*Solidago*

canadensis) individuals, one containing mainly the (+)-enantiomer and the other mainly the (–)-enantiomer. The volatiles were collected in hexane and the concentrations of the samples were ~10 µg/µl and 0.1 µg/µl for (+)-germacrene D and (–)-germacrene D, respectively. The purity of the samples were 86% for the (+)-enantiomer and 75% for the (–)-enantiomer. Both samples contained ~10% of the opposite isomer, the optical purity being 79% *ee* (enantiomeric excess) for the (+)-sample and 76% *ee* for the (–)-sample. From these samples, dilution in hexane (>99%) were made in decade steps down to 1 ng/µl for (+)-germacrene D and 10 pg/µl for (–)-germacrene D. Test cartridges with the enantiomers were made by inserting in each tube a piece of filter paper on which 1 µl of a dilution was applied.

Linked gas chromatography single cell recording (GC-SCR)

The insect preparation and the electrophysiological recordings of nerve impulses from single olfactory receptor neurons on the antennae were carried out as described by Røstelien *et al*. (Røstelien *et al*., 2000a). Contact with the neuron was made with the tungsten microelectrode positioned into the base of the sensillum. The neuron was then tested for sensitivity to germacrene D by direct stimulation with the different mixtures, i.e. by blowing air through a cartridge containing a small sample of each solution on a filter paper. If a cell responded, we further tested its selectivity by injection of the mixtures into the GC-column. A split at the end of the column led half of the effluent to the GC-detector and the other half over the insect antennae, resulting in simultaneously recorded gas chromatograms and responses to the separated compounds. Each neuron was tested in sequence via two capillary columns installed in parallel in the GC, one polar DB-wax column (30 m, i.d. 0.25 mm, film thickness 0.25 µm,) and one chiral column [25 m, i.d. 0.25 mm, Heptakis (6-*O*-*t*-Butyldimethylsilyl-2, 3-di-*O*-methyl)-β-cyclodextrin (50% in OV1701)] (Schmidt *et al*., 1998; König *et al*., 1999). Separation in the polar column was performed from 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. In the chiral column, enantiomeric separation of germacrene D was found to be optimal at the isothermal temperature 125°C. The detection limit for the columns were found for (–)-β-caryophyllene (Fluka, 99%); between 0.1 and 0.01 ng/µl for DBwax and ~1 ng/µl for the chiral column. (–)-β-Caryophyllene was used as a standard for determining the concentrations of the germacrene D enantiomers.

Direct stimulation with different concentrations of (+)- and (–)-germacrene D were performed by blowing an air stream (3.3 ml/s) through the test cartridges and over the insect antenna. The stimulations were performed from low to high concentrations, alternating stimulation with the (+)- and the (–)-enantiomers. Each cartridge was tested twice in the dynamic area. Between the stimulations, purified air was blown over the antenna. The interstimulus intervals were 1 min for the low concentrations and longer for the high concentrations, depending on the response strength. Spike activity was recorded on an analogue tape recorder in parallel with the computer program Electro Antenno Detection (version 2.3, Synthec NL, Hilversum, The Netherlands), and analysed in the computer program AutoSpike-32 (Synthec NL). The response strength of the receptor neuron to the enantiomers was plotted as numbers of spikes per 0.5 s for each concentration, resulting in dose–response curves for each enantiomer.

Results

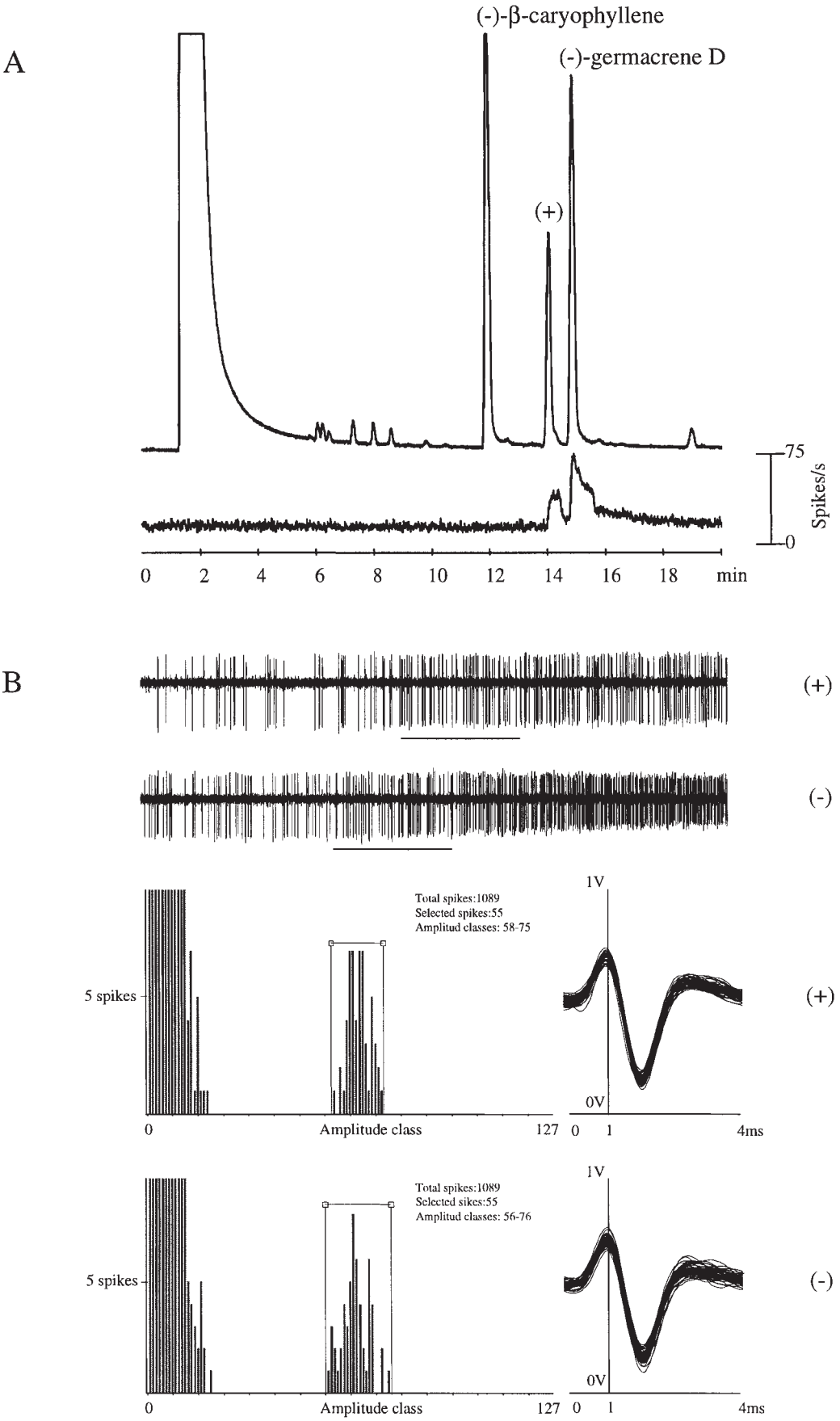
The results are based on seven receptor neurons in seven females of *Helicoverpa armigera*; all responding with high selectivity and sensitivity to germacrene D. Six neurons were tested individually up to 28 times via the polar as well as the chiral GC-column (Table 1). When tested for the same mixture, all neurons showed the same response characteristics. The seventh neuron, which was not tested via the GC, was characterized by direct stimulation with the reference samples of germacrene D enantiomers. When stimulated via the polar GC-column, each of the six neurons showed a strong excitatory response to germacrene D. Injection of the samples into the chiral column displayed chromatograms with two well-separated GC-peaks of (+)- and (–)-germacrene D, both eliciting responses in all six neurons. This is exemplified in Figure 1A showing separation of the germacrene D enantiomers in the cubebe oil fraction containing 35% of the (+)-enantiomer and 65% of the (–)-enantiomer. Both enantiomers were eluted after (–)-β-caryophyllene. The simultaneous electrophysiological recording from the germacrene D neuron showed selectively strong responses to both enantiomers. The increased spike activity of the neuron during the elution of (+)- and (–)-germacrene D is shown in Figure 1B. Histogram conversions of the spike amplitudes and selected spike classes presented as overlays

Table 1 Responses by seven receptor neurons to germacrene D stimulated via the polar GC-column, the chiral GC-column and directly via cartridges

Cell no.	Polar GC-column	Chiral GC-column		Direct stimulation	
		(+)-G*	(–)-G*	(+)-G*	(–)-G*
1	+ (1)	+ (1)	+ (1)	–	–
2	+ (2)	+ (1)	+ (1)	–	–
3	+ (9)	+ (3)	+ (8)	–	–
4	+ (3)	+ (2)	+ (7)	–	–
5	+ (2)	+ (1)	+ (1)	–	–
6	+ (12)	+ (7)	+ (16)	+ (6)	+ (10)
7	–	–	–	+ (6)	+ (5)

The numbers of stimulations are indicated in the parentheses. +, excitation; –, not performed.

G*, germacrene D.


A
(-)-β-caryophyllene
(-)-germacrene D
(+)
75
Spikes/s
0
0 2 4 6 8 10 12 14 16 18 min
B
(+)
(-)
Total spikes:1089
Selected spikes:55
Amplitud classes: 58-75
1V
0V
5 spikes
0
Amplitude class
127
0 1 4ms
(+)
Total spikes:1089
Selected sikes:55
Amplitud classes: 56-76
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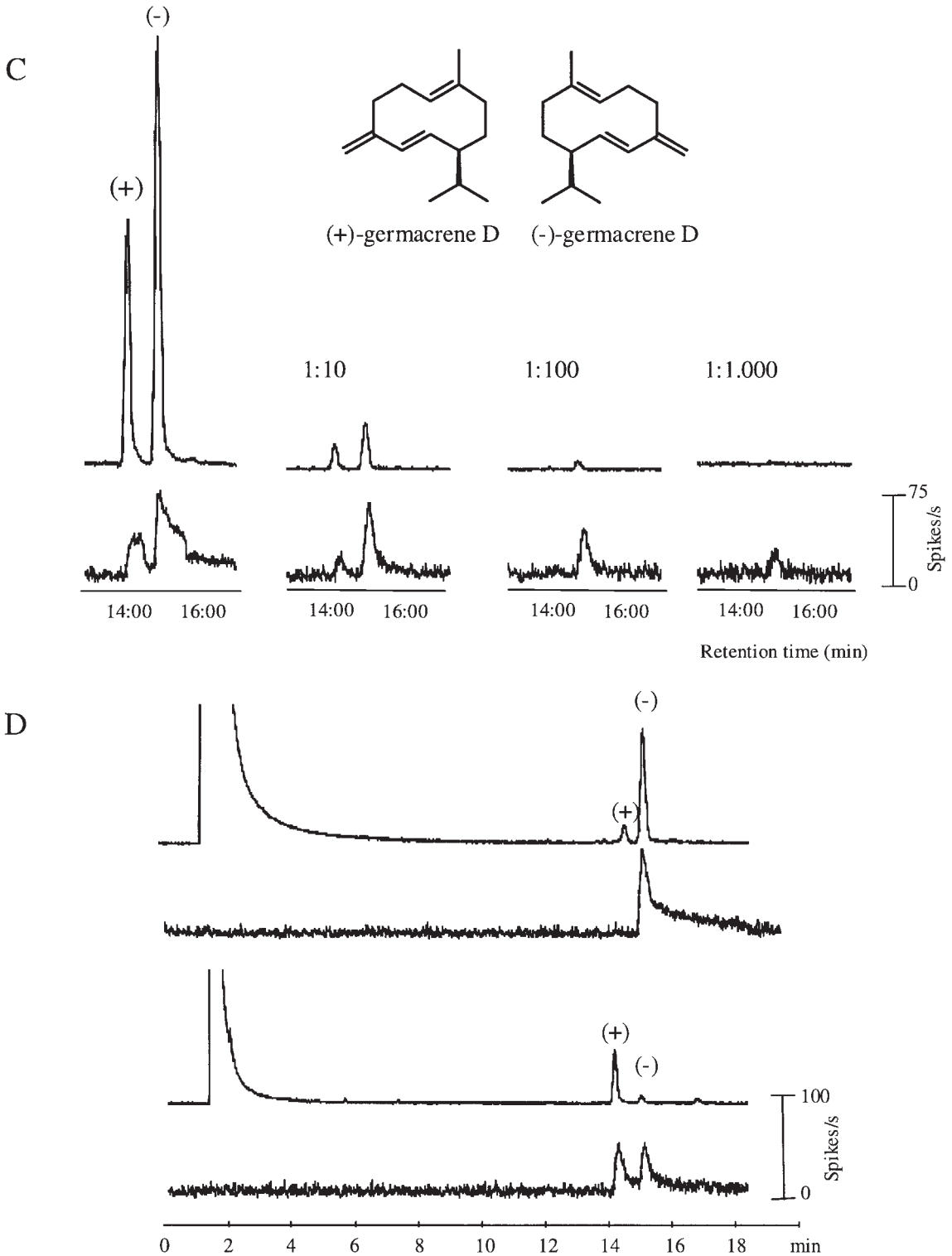

Figure 1 Gas chromatogram of plant samples (separated in a chiral column) and simultaneously recorded responses by the same germacrene D receptor neuron. **(A)** Gas chromatogram of an essential oil fraction from cubebe pepper (*Piper cubeba*) showing the separated peaks of (–)-β-caryophyllene, (+)- and (–)-germacrene D and the activity of the neuron (impulses/s). **(B)** Above: Spike activity of the receptor neuron responses during elution of the separated enantiomers shown in A. The period selected (2 s) for spike analyses are indicated by the bars. Below: Histogram conversions of the spike amplitudes (left) and the selected population of spike classes presented as overlay (×3.160 amplification) (right). **(C)** Section of the chromatogram of the cubebe oil fraction (shown in A) for different dilutions, showing the peaks of (+)- and (–)-germacrene D and the activity of the neuron. The lowest dilution is under the detection limit of the chiral GC-column (1 ng/µl determined for (–)-β-caryophyllene). Bond-line structures of the enantiomers are shown. **(D)** Gas chromatogram of the two germacrene D reference samples containing 90% of the (–)-enantiomer (above) and (+)-enantiomer (below) and the activity the neuron.

show that the responses to the enantiomers were based on one population of spikes with uniform amplitudes and waveforms.

Dose-dependent responses to both enantiomers were demonstrated for all neurons by injecting dilutions of the cubebe oil fraction in decade steps down to 1:1000. This is shown in Figure 1C (same neuron as in Figure 1A), where a decrease of responses to both enantiomers follows the decrease of germacrene D concentrations. For all dilutions, the strongest response was elicited by (–)-germacrene D. This applied to all six neurons. The different stimulatory effect of the two enantiomers were further demonstrated by testing on the same neuron two reference samples of (+)- and (–)-germacrene D, each containing ~10% of the opposite enantiomer (Figure 1D). In the (–)-germacrene D sample, 10% of the (+)-enantiomer did not elicit a detectable response. However, in the other sample 10% of (–)-germacrene D was enough to elicit about the same response as the nine times larger amount of the (+)-configuration. Thus, the stimulation via the gas chromatograph showed an ~10 times better effect of (–)-germacrene D than of the (+)-enantiomer. This was confirmed by the dose–response curves obtained with direct stimulations of two neurons with the same reference samples of (+)- and (–)-germacrene D. Figure 2A shows duplicated dose–response curves for one neuron, where the (+)-germacrene D curve is shifted ~1 logarithmic unit to the right for the curve of the (–)-configuration. As shown in Figure 2B the structures of the two enantiomers differ in the direction of the isopropyl group in relation to the 10-carbon ring.

The various plant materials, selected due to the germacrene D content, were found to contain different enantiomeric ratios of the compound. Thus, the neurons showed consistent excitatory responses to both (+)- and (–)-germacrene D when tested for the cubebe oil fraction, the extract of ginger and the different extracts of Canadian goldenrod (Figures 1A and 3). Interestingly, the different samples of Canadian goldenrod showed different enantiomeric ratios, ~90% of the (–)-enantiomer in extract 1 and ~70% of the (+)-configuration in extract 2. The ginger extract seemed to contain at least 85% of (+)-germacrene D, whereas the small amount of the (–)-enantiomer was masked by an overlapping GC-peak of another compound. Only one response to (–)-germacrene D was obtained when testing the neurons for the extracts of sunflower and yarrow and for a headspace sample of wild briar. The absence of response at the retention time for the (+)-enantiomer indicates that these samples do not contain detectable amounts of (+)-germacrene D.

Discussion

In this study we have employed (i) a chiral GC-SCR column, and (ii) stimulation directly from cartridges to study the enantioselectivity of the germacrene D receptor neurons in

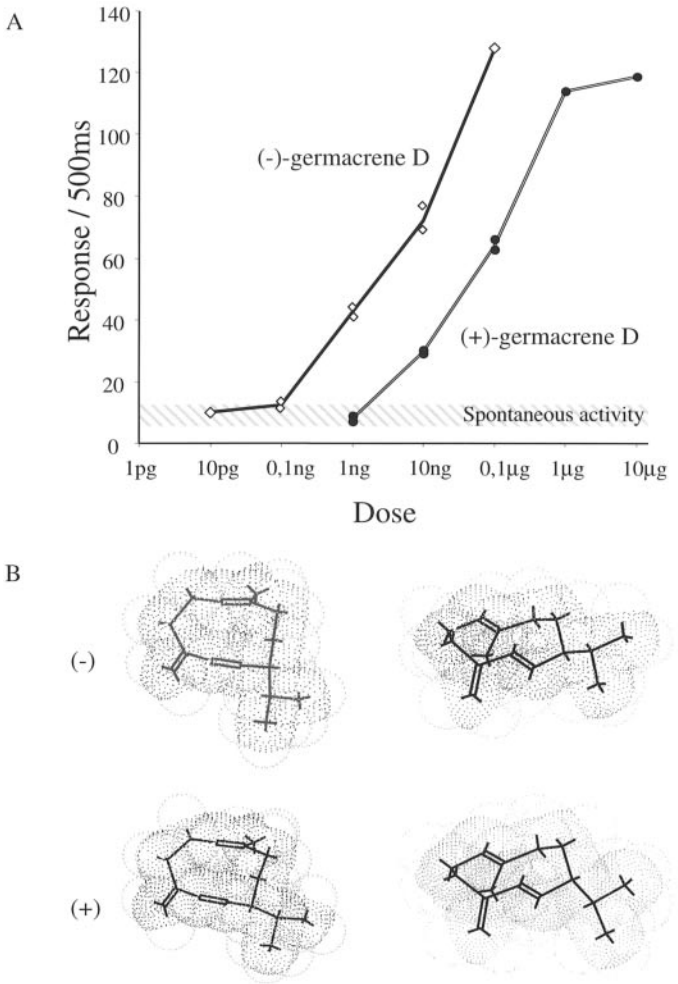

Figure 2 **(A)** Dose–response curves of a receptor neuron tuned to (+)- and (–)-germacrene D. The curves show the mean of two responses elicited by repeated stimulation with the same cartridge. The shaded area marks the low spontaneous activity. **(B)** The structures of (–)- and (+)-germacrene D, indicating van der Waals radia and the different orientation of the isopropyl group relative to the 10-carbon ring.

H. armigera. The results were consistent for all the recorded germacrene D neurons, indicating that they belonged to one functional type. In addition to the strong responses to germacrene D and weak responses to three other structurally related sesquiterpenes (Stranden *et al*., unpublished data), the present results have demonstrated that all of the neurons possessed the same enantioselectivity. The well separated germacrene D enantiomers in the chiral GC-column consistently elicited a stronger response during the elution of the (–)- than of the (+)-enantiomer. The dose–response relationships demonstrated ~10 times better stimulatory effect of (–)-germacrene D, both in the experiments with GC-SCR and/or by direct stimulation of the seven neurons. The similarity of spike amplitudes and waveforms of responses to both enantiomers indicated that they originated from the same neuron. During the selected period (beginning) of the responses, the spikes of the two

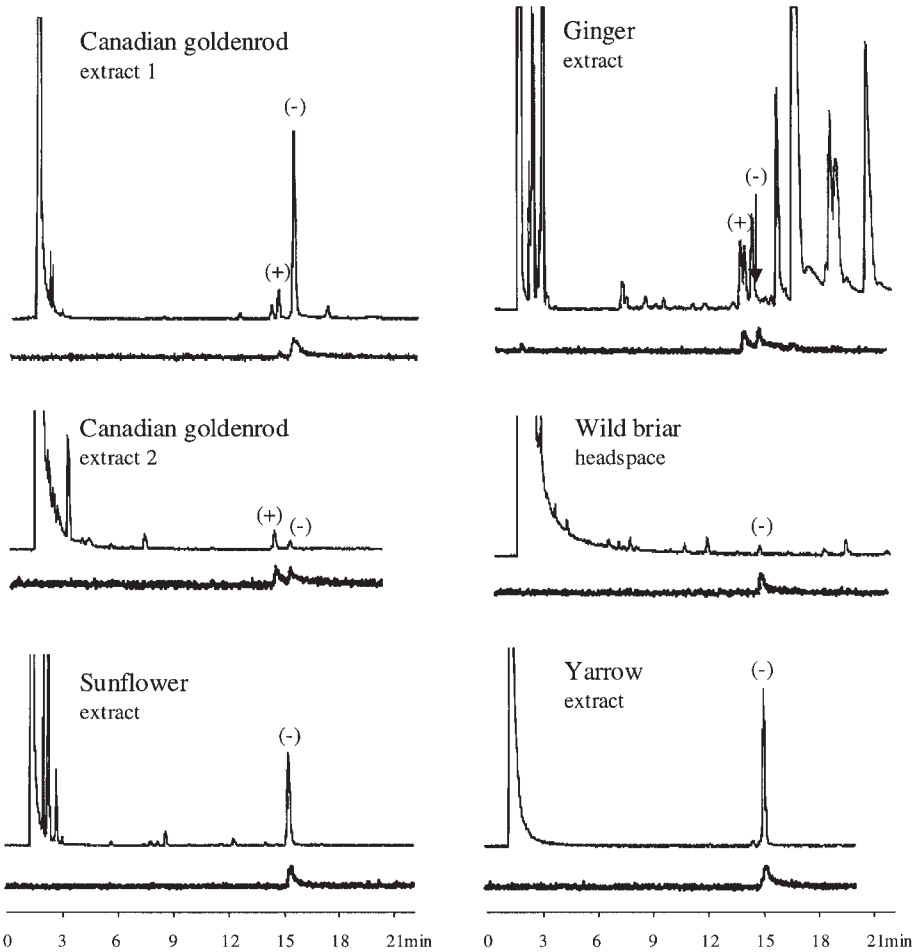

Figure 3 Gas chromatograms of pentane extracts made from leaf tissue of Canadian goldenrod (two individuals in separate samples), sunflower (several individuals) and yarrow (several individuals). The other two gas chromatograms are from wild briar (headspace from one individual) and ginger (hexane extracts). In the gas chromatogram of ginger, (–)-germacrene D (arrow) is probably masked by another component eluted at about the same retention time.

responses showed overlapping waveforms of one population of spike amplitudes, i.e. having a normal distribution around the same amplitude value. Thus, the present results have demonstrated enantioselectivity of one germacrene D receptor neuron type, which responds to both enantiomers, but with a 10 times higher affinity for the (–)-configuration. The presence of only one neuron type for receiving the information about germacrene D indicates that the two enantiomers mediate the same kind of message to the moth, i.e. the effect of the (–)-enantiomer can be simulated by a 10 times higher concentration of the (+)-configuration. It is in contrast to pheromone enantiomers that activate two different receptor neuron types, having synergistic or antagonistic effects on the behaviour. The behavioural response of *H. armigera* females to the germacrene D enantiomers is presently under investigation. Our hypothesis is that female *H. armigera* will show the same behavioural responses to both enantiomers of germacrene D. If a moth species need to distinguish between the two enantiomers for finding suitable host plants, at least two different types of receptor neurons should be needed, e.g. one tuned to (+)-germacrene D and the other to (–)-germacrene D.

It is obvious that the enantiomers of chiral odorants may be critical in the interaction with the olfactory receptor proteins, like in other interactions between chemicals and receptors. However, the question is to what extent in each case the chiral centre takes part in the interaction. As both enantiomers of germacrene D activate the same receptor neuron, it can be assumed that they are transported by the same odour binding protein and interact with the same membrane receptor protein but with different affinity. The difference between (+)- and (–)-germacrene D is the direction of the isopropyl group. Thus, it is likely that this group is an active part of the ligand in the interaction with the receptor, and that its different orientation causes the lower effect of the (+)-enantiomer than of the (–)-enantiomer. In previous studies of enantioselectivity of olfactory receptor neurons, it has been difficult to assess the difference of the stimulatory effect of enantiomers because of impurities. For instance, the (–)-ipsdienol receptor neuron has been

stimulated with (+)-ipsdienol samples containing different impurities of the (–)-enantiomer (Mustaparta *et al*., 1980) (H. Mustaparta, unpublished data). The dose–response curves obtained for the (+)-samples were shifted to the right according to the lower content of (–)-ipsdienol. Stimulation with highly pure enantiomers has shown that some receptor neurons tuned to one enantiomer may not respond to the opposite configuration. In the study of the ipsenol enantiomers, one sample with pure (+)-ipsenol (provided by Dr K. Mori, University of Tokyo, Japan) showed no stimulatory effect on the (–)-ipsenol receptor neurons (H. Mustaparta, unpublished data). In another study of three scarab beetle species (the Japanese beetle *Popilia japonica*, the Osaka beetle *Anomala osakana* and the scarab beetle *Anomala cuprea*), using highly pure enantiomers (japonilure, buibuilactone, >99% *ee*), the receptor neurons showed either no response to the opposite enantiomer or a weak response at high concentrations (Wojtasek *et al*., 1998; Larsson *et al*., 1999). For biologically relevant plant odours, enantioselectivity has been demonstrated for two receptor neuron types in the pine weevil (*Hylobius abietis*), one tuned to (+)-α-pinene and the other one to (–)-limonene, for which the opposite configuration elicited a weaker response (Wibe *et al*., 1998). However, the stimulation was only performed via non-chiral GC-columns, making it difficult to determine the contributions of the opposite enantiomers. Studies of olfactory receptor neurons in mammals by the use of the patch clamp technique have revealed neurons selective for one enantiomer as well as neurons responding to both configurations of carvone (Ma and Shepherd, 2000).

The importance of germacrene D as a cue for *H. armigera* and other heliothine moths in the interaction with plants is indicated by the large number of selective receptor neurons tuned to this constituent, shown in the present and previous studies (Røstelien *et al*., 2000b) (Stranden *et al*., unpublished data). Germacrene D seems to be widely distributed among higher plants, both in hosts and non-hosts of heliothine moths. The receptor selectivity for (–)-germacrene D might have evolved as an adaptation to the most common configuration. However, the significance of germacrene D for the behaviour of *H. armigera* is not clear, e.g. whether (–)-germacrene D is involved in location of favourable hosts (for nutrition or oviposition) or in avoidance of unsuitable hosts. In other insect species, different behavioural responses to (–)-germacrene D have been demonstrated. As a host volatile produced by healthy pines (*Pinus densiflora*) it is reported to act as an allomone, masking the attraction of the cerambycid beetle (*Monochamus alternatus*) to the oxygenated terpenes of the pines (Yamasaki *et al*., 1997). (–)-Germacrene D is also known as a mimic of the sex pheromones of the female American cockroach (*Periplaneta americana*), eliciting the sexual attraction of the males (Tahara *et al*., 1975; Kitamura *et al*., 1976). These examples indicate a wide distribution and function of (–)-germacrene D in insect–plant interactions.

The present study also allowed determining with a very sensitive method the enantiomeric ratio of germacrene D in the various plant species. Our findings of the (–)-configuration in all six plant species, and exclusively in three of them, appear to be in accordance with the hypothesis that (–)-germacrene D is the abundant enantiomer in higher plants, whereas (+)-germacrene D is rare and more likely to occur in lower plants (Beechan *et al*., 1978; Lorimer and Weavers, 1987; König *et al*., 1996; Bülow, 1998; Bülow and König, 2000). However, we also found a significant amount of the (+)-enantiomer in the extracts of Canadian goldenrod, cubebe pepper and ginger, and in two cases it was the major enantiomer. The presence of both enantiomers has also been documented in *Torilis japonica* (Itokawa *et al*., 1983), *Araucaria bidwilli* (Pietsch and König, 2000) and 11 different *Solidago* species (Niwa *et al*., 1980; Bülow, 1998; Bülow and König, 2000). Altogether it seems that the (+)-enantiomer is more widely distributed than has earlier been thought. Variation of the enantiomeric ratio in *Solidago* individuals presented here is in accordance with previous results (Bülow, 1998). In fact, Bülow found variations over the whole spectrum of enantiomeric ratios in *Solidago* individuals depending on their geographical locations, and within an individual the ratio was constant over a 3-year period. It was hypothesized that the enantiomeric ratios of germacrene D in plants of *Solidago* are genetically determined.

In conclusion, the use of gas chromatograph with a chiral column linked to recordings from single receptor neurons, have demonstrated that all germacrene D neurons belong to one functional type. They responded to both enantiomers, but had a 10 times higher affinity for the (–)-configuration. The slopes of the dose–response curves shifted 1 logarithmic unit, were parallel indicating that (+)- and (–)-germacrene D may interact with the same membrane receptor. The responses by this enantioselective neuron type showed the presence of one or both enantiomers in six plant species tested.

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