Concentration and Membrane Fluidity Dependence of Odor Discrimination in the Turtle Olfactory System

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Abstract

In the present study, we examined the concentration dependence of odor discrimination in turtle olfactory bulbar responses using the cross-adaptation technique. In the odorant pairs with diverse molecular structures, the degree of discrimination was unchanged or only slightly decreased with an increase in odorant concentrations, suggesting that odorants are well discriminated even at high concentrations. In the odorant pairs with closely related molecular structures, the degree of discrimination was decreased with an increase in odorant concentrations. An increase in the temperature of turtle olfactory epithelium also decreased the ability to discriminate these odorants. There was a good correlation between changes in the odor discriminating ability induced by an increase in odor concentrations and those induced by a temperature increase. The liposomes were made of lipids extracted from the turtle olfactory epithelia and changes of their membrane fluidity induced by adsorption of odorants were monitored with DPH. There was a good correlation between a decrease in odor discriminating ability and the membrane fluidity changes induced by odorants. We suggest that decreases in odor discriminating ability induced either by an increase in odor concentration or by a temperature increase are ultimately caused by changes in the membrane fluidity. **Chem. Senses 22: 553–563, 1997.**

Introduction

A large family of G-protein-coupled receptors (olfactory GCRs) cloned from olfactory epithelia are considered to be receptors for odorants (Buck and Axel, 1991). *In situ* hybridization experiments to identify olfactory GCRs in the olfactory epithelium have suggested that odor coding might be afforded by specific expression of only a single type of olfactory GCR in each neuron (Axel, 1995; Sullivan *et al.*, 1995). It has been also reported, however, that single olfactory neurons of various species of animals respond to various species of odorants (Gesteland *et al.*, 1982; Duchamp-Viret *et al.*, 1990; Ivanova and Caprio, 1993; Kashiwayanagi and Kurihara, 1994; Kang and Caprio, 1995; Kashiwayanagi *et al.*, 1996b). In a study on bullfrog olfactory neurons, most single olfactory neurons responded to multiple odorants, including both those that selectively accumulate cAMP content and those with unchanging cAMP content (Sklar *et al.*, 1986), suggesting that most olfactory neurons have multiple receptors (Kashiwayanagi *et al.*, 1996b).

The results indicating the presence of multiple receptors in a single cell were usually obtained by using odorants at higher than threshold concentrations. Ressler *et al.* (1994)

have pointed out the possibility that individual olfactory neurons may be highly specific for a small number of odorants only at concentrations of odorant nearer to threshold detection levels. If this is true, it follows that the olfactory system will not discriminate various odorants at high concentrations. In the present study, we examined this hypothesis by applying the cross-adaptation method to turtle olfactory bulbar responses. The results showed that various odorants with diverse molecular structures were well discriminated even at high concentrations.

In a previous study we found that the degree of discrimination between odorants with closely related molecular structures was greatly reduced by an increase in temperature of the turtle olfactory epithelium (Hanada *et al.*, 1994). The increase in temperature increased the membrane fluidity of either cell suspensions prepared from the olfactory epithelia or liposomes made of lipids extracted from the turtle olfactory epithelia. There was a good correlation between the decrease in the degree of odor discrimination and the increase in membrane fluidity. It is known that membrane fluidity is also changed by adsorption of odorants to the membranes (Kashiwayanagi and Kurihara, 1985; Kashiwayanagi *et al.*, 1990; Enomoto *et al.*, 1991; Hanada *et al.*, 1994). In the present study, we found that the ability of the turtle olfactory system to discriminate odorants with closely related structures was decreased with an increase in odorant concentrations. We suggest that this decrease in odor discriminating is due to an increase in membrane fluidity induced by adsorption of odorants.

Materials and methods

Recording of olfactory bulbar responses

Turtles, *Geoclemys reevesii*, weighing 150–300 g, were obtained from commercial suppliers and maintained at 25°C on a diet of porcine and bovine liver *ad libitum*. Olfactory bulbar responses were recorded essentially as described previously (Taniguchi *et al.*, 1992; Kashiwayanagi *et al.*, 1997b). Briefly, turtles were anesthetized with the minimum amount of urethane (usually 0.1 ml of 20% urethane) necessary to lessen pain during the operation, immobilized by injection of D-tubocurarine chloride (450 μg/100 g body wt), and locally anesthetized with lidocaine at the wounded and head-fixation points. The stimulant-induced brainwaves (bulbar responses) were recorded by attaching a pair of silver bipolar electrodes to the medial part of the anterior bulb. The responses were amplified by a DC-amplifier, filtered into 3–300 Hz, and integrated using an electric integrator (time constant 0.3 s). All experiments were carried out at 20 ± 3°C.

Stimulating procedure

The olfactory epithelium was stimulated by various odorants dissolved in Ringer's solution. The irrigating and stimulating solutions were applied to the whole area of the epithelium through a stainless steel tube at a flow rate of 27 ml/min. Before application of the stimulating solution, the epithelium was irrigated with 30 ml of Ringer's solution. After application of 5 ml of the first stimulating solution, 5 ml of the second stimulating solution was immediately applied. There was no time delay between the first and second applications of stimulating solutions. Odor responses which are sometimes generated by the offset of stimulation are called 'off-responses' (Takagi and Shibuya, 1959; Kashiwayanagi *et al.*, 1994b). We used animals in which odorants did not generate off-responses in order to exclude the effects of these components from the responses to the odorants applied secondarily. After each application of the stimulating solution to the epithelium, the epithelium was rinsed with Ringer's solution. The interval between cross-adaptation experiments was 5–15 min.

Measurement of membrane fluidity

Lipids for preparing liposomes were extracted from the fresh olfactory epithelium with a mixture of methanol and chloroform. Liposomes were prepared as described previously (Nomura and Kurihara, 1987). The lipid solution in chloroform was evaporated to dryness in a round-bottom flask using a rotary vacuum evaporator. To disperse the dried lipid film, glass beads and 200 mM mannitol solution containing 0.1 mM NaCl were added, and the flask was shaken with a vortex mixer at room temperature. The lipid suspension was sonicated at 5°C for 2 h in a bath-type sonicator (W-375; Heat Systems- Ultrasonic Inc., New York). The procedure for labeling with DPH has been described previously (Kashiwayanagi and Kurihara, 1985). After the liposomes were added to the 5 mM HEPES buffer, DPH was added to the liposome suspension and the mixture was stirred for 20 min at 30°C. The fluorescence polarization of DPH was then measured using a fluorescence spectrophotometer (650–1S; Hitachi Ltd., Tokyo, Japan) as described previously (Kashiwayanagi *et al.*, 1990).

Preparation of solutions

The Ringer's solution contained (in mM) 116 NaCl, 4 KCl, 2 $CaCl_2$, 2 $MgCl_2$ and 10 HEPES–NaOH (pH 7.4). The odorants (citralva, D-carvone, L-carvone, D-menthol, L-menthol, cineole, L-limonene, lilial, phenylethylamine and furfuryl mercaptan) were dissolved in ethanol to prepare stock solutions at a concentration of 0.1 M. Separate dilutions of *n*-Amyl acetate, *sec*-amyl acetate and isoamyl acetate were prepared by directly dissolving 10 mM concentrations of each in Ringer's solution. These stocks were added to normal Ringer's solution to give the indicated concentrations of odorants. Ethanol alone, at a concentration of 0.5%, had no effect on the integrated olfactory bulbar response.

Chemicals

D-Carvone, L-carvone, D-menthol, L-menthol, L-limonene, lilial and hedione were kindly supplied by Takasago International (Tokyo, Japan). *n*-Amyl acetate, *sec*-amyl acetate, isoamyl acetate, furfuryl mercaptan, phenylethylamine and cineole were purchased from the Wako Pure Chemical Industry (Osaka, Japan). DPH was purchased from the Tokyo Chemical Industry (Tokyo, Japan).

Results

Concentration dependence of odor discrimination

The cross-adaptation experiments were carried out using furfuryl mercaptan and phenylethylamine. The first peak in Figure 1a shows an integrated olfactory bulbar response to 0.01 mM furfuryl mercaptan. After the response to furfuryl mercaptan had returned to the basal level during continuous application, 0.03 mM phenylethylamine was applied. As shown by the second peak in Figure 1a, this application of phenylethylamine after furfuryl mercaptan induced a response. In Figure 1b, the order of application of odorants was reversed; 0.03 mM phenylethylamine was applied first, followed by 0.01 mM furfuryl mercaptan. Furfuryl mercaptan induced a response after phenylethylamine, indicating that the turtle olfactory system discriminated between these odorants. To examine the concentration dependence of this odor discrimination, the cross-adaptation experiment was repeated using the same odorants at 100 times higher concentrations (Figure 1c and d). Pre-application of the odorants at high concentration did not decrease the magnitude of responses to odorants applied secondarily, indicating that the turtle olfactory system discriminates well between odorants even at high concentrations.

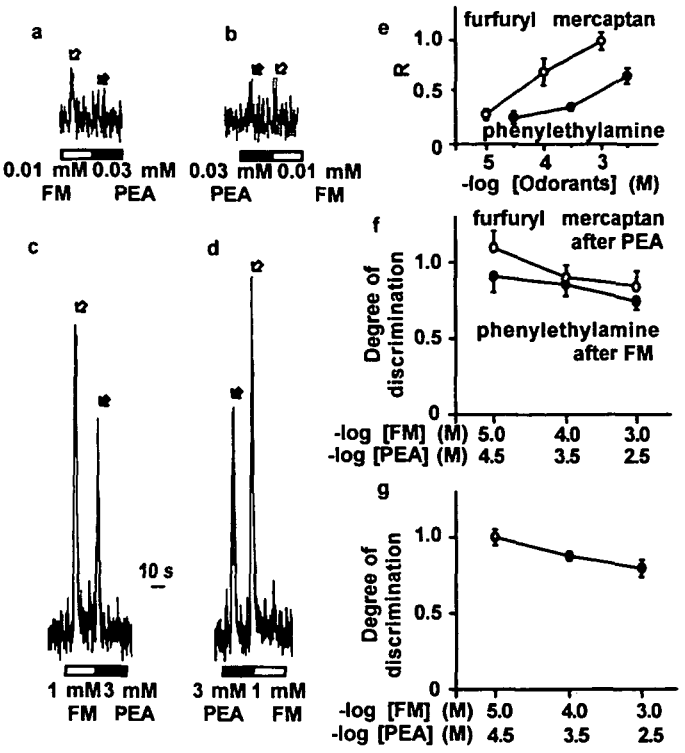

Figure 1 Integrated olfactory bulbar responses to furfuryl mercaptan (FM) and phenylethylamine (PEA) at high and low concentrations. After application of Ringer's solution to the olfactory epithelium, 0.01 mM **(a)** and 1 mM **(c)** FM or 0.03 mM **(b)** and 3 mM **(d)** PEA were applied. After the responses to the first set of odorants were adapted, 0.03 mM (a) and 3 mM (c) PEA or 0.01 mM (b) and 1 mM (d) FM were applied. All responses shown in the figure were recorded from the same preparation. **(e)** Relative magnitude of responses to FM (open circle) and PEA (closed circle) as a function of odorant concentrations. The magnitude of response to 0.1 mM citralva was taken as a unit. **(f)** The degree of discrimination obtained from FM after PEA (open circles) and from PEA after FM (closed circles) at different odorant concentrations. **(g)** The mean degree of discrimination between PEA and FM as a function of odorant concentrations. Values were calculated from the data shown in (f). Data were obtained from at least three preparations. Values were mean ± SEM.

Figure 1e plots the peak height of the response to phenylethylamine and furfuryl mercaptan against their concentrations. Figure 1f shows the degrees of discrimination between furfuryl mercaptan and phenylethylamine at various concentrations. The degree of discrimination was quantified according to a previous paper (Kashiwayanagi *et al.*, 1994c). That is, the magnitude of the integrated response to the odorant applied secondarily was measured from a level immediately before the rise of the integrated

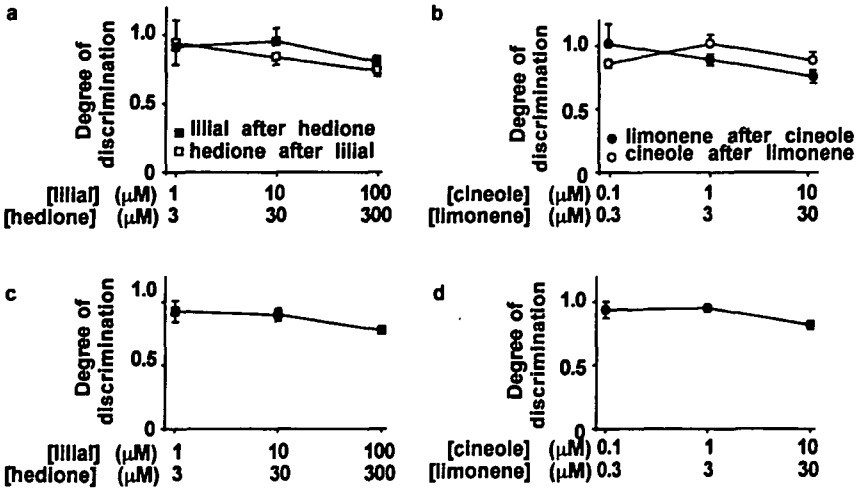

Figure 2 The degree of discrimination obtained from the magnitude of response to **(a)** hedione after lilial (open square) and lilial after hedione (closed square), and **(b)** cineole after limonene (open circles) and limonene after cineole (closed circles) at different odorant concentrations. **(c** and **d)** Mean degree of discrimination between hedione and lilial and between cineole and limonene respectively. Values were calculated from the data shown in (a and b). Data were obtained from at least three preparations. Values were mean ± SEM.

value to the peak. The magnitude of the response to odorant *A* after application of Ringer's solution was defined as *y*, and that after the response to odorant *B* was adapted was defined as *y'*. We defined the degree of discrimination between odorant *A* and odorant *B* as *y'*/*y*. The magnitude of the response to phenylethylamine after furfuryl mercaptan was 97% of that of the control (Figure 1c and d), which implies that the degree of discrimination was 0.97. The response to phenylethylamine after furfuryl mercaptan and that to furfuryl mercaptan after phenylethylamine decreased slightly but not significantly with an increase in odor concentrations (Figure 1f). The mean degree of discrimination between these odorants decreased slightly with a 1000-fold increase in odorant concentration, but again, the decrease was not statistically significant (Figure 1g). This result indicates that the ability to discriminate was practically unchanged with an increase in odor concentration.

The concentration dependence of the odor discrimination was examined using different combinations of odorants (Figure 2). The response to hedione after lilial or that to lilial after hedione was practically unchanged by an increase in odor concentrations (Figure 2a). Similar results were obtained from the combination of cineole and limonene (Figure 2b). The mean degrees of the odor discrimination ability between these odorants were practically unchanged with an increase in odorant concentrations (Figure 2c and d).

Next, we examined the concentration dependence of degree of discrimination between odorants with closely related molecular structures, such as *n*-amyl acetate and isoamyl acetate. As shown in Figure 3e, these particular odorants had similar concentration–response curves. Figure 3a–d shows the integrated responses obtained by the cross-adaptation experiment. The response to 0.01 mM isoamyl acetate was reduced by pre-application of 0.01 mM *n*-amyl acetate, though the reduction was not remarkable (Figure 3a). Reverse application of the odorants gave similar results (Figure 3b). Figure 3c and d shows that the response to 1 mM isoamyl acetate was greatly reduced by pre-application of 1 mM *n*-amyl acetate and vice versa. The degrees of discrimination calculated when isoamyl acetate was applied after *n*-amyl acetate and those calculated when *n*-amyl acetate was applied after isoamyl acetate decreased with an increase in odorant concentrations (Figure 3f). The mean degree of discrimination decreased from 0.9 to 0.4 with an increase in concentrations of odorants from 0.01 to 10 mM (Figure 3g).

The degree of discrimination between optical isomers, such as D- and L-menthol, or D- and L-carvone, was also examined at different concentrations (Figure 4). The degrees of odor discrimination between the optical isomers decreased with an increase in odor concentrations.

Table 1 shows the degree of discrimination between various combinations of odorants at low and high concentrations. The data indicate that the turtle olfactory system discriminates well between odorants at low concentration (Table 1). On the other hand, the degree of

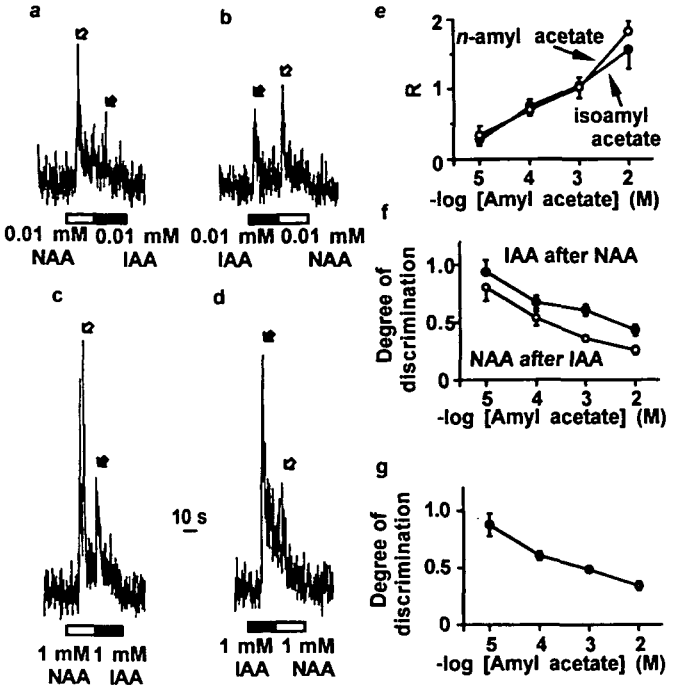

Figure 3 Integrated olfactory bulbar responses to isoamyl acetate (IAA) and *n*-amyl acetate (NAA). After application of Ringer's solution to the turtle epithelium, 0.01 mM **(a)** and 1 mM **(c)** NAA or 0.01 mM **(b)** and 1 mM **(d)** IAA were applied. After the responses to the first set of odorants had adapted, 0.01 mM (a) and 1 mM (c) IAA or 0.01 mM (b) and 1 mM (d) NAA were applied. All responses were recorded from the same preparation. **(e)** Relative magnitude of responses to IAA (closed circle) and NAA (open circle) as a function of odorant concentrations. The magnitude of response to 0.1 mM citralva was taken as a unit. **(f)** The degree of discrimination obtained from NAA after IAA (open circles) and IAA after NAA (closed circles) at different odorant concentrations. **(g)** Mean degree of discrimination between NAA and IAA as a function of odorant concentrations. Values were calculated from data shown in (f). Data were obtained from at least three preparations. Values were mean ± SEM.

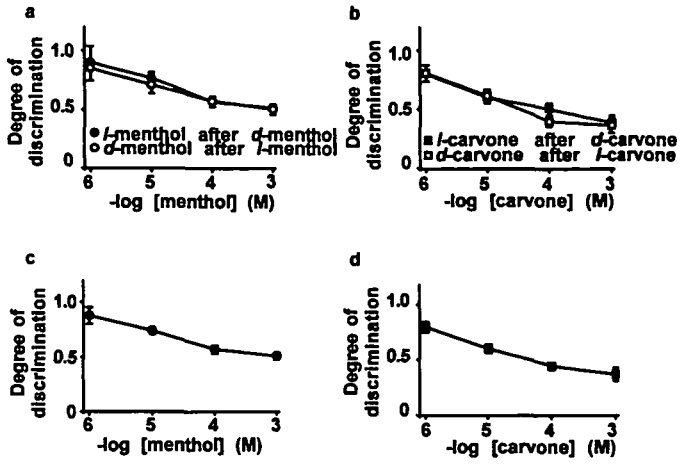

Figure 4 The degree of discrimination obtained from the magnitude of response to **(a)** D-menthol after L-menthol (open circle) and L-menthol after D-menthol (closed circle), and **(b)** D-carvone after L-carvone (open square) and L-carvone after D-carvone (closed square) at different odorant concentrations. **(c** and **d)** Mean degree of discrimination between D- and L-menthol and between D- and L-carvone respectively. Values were calculated from the data shown in (a and b). Data were obtained from at least three preparations. Values were mean ± SEM.

discrimination at high concentration varied among odorant pairs from 0.28 to 0.92. Figure 5 shows the ratios of the degree of discrimination at the low concentration to that at the high concentration. The ratios varied from 0.3 to 1.1. Ratios for the pairs with diverse molecular structures were large in general, and those for the pairs with closely related molecular structures, such as stereoisomers and structural isomers, were rather small.

Temperature dependence of odor discrimination

As reported previously, at low temperature (e.g. 5°C) the turtle olfactory system discriminates well even between pairs of odorants with closely related molecular structures, but it does not discriminate between them at high temperature (e.g. 40°C) (Hanada *et al.*, 1994). In the present study, we added data obtained with new pairs of odorants. For example, the response to 1 mM D-menthol after 1 mM L-menthol was appreciable at 5°C (Figure 6b), but absent at 40°C (Figure 6d). Figure 6e represents the ratios between the degree of odor discrimination at 5°C and that at 40°C, using data obtained in the present and previous studies. The ratios for the pairs of odorants with diverse molecular structures were much larger than those for the pairs with closely related molecular structures.

Figure 7 plots the ratios of the degree of odor discrimination at 5°C to that at 40°C against those at the low concentration to those at the high concentration. The resultant correlation coefficient was 0.75, indicating that odorant pairs showing a decrease in degree of odor discrimination at 40°C also show a decrease in degree of odor discrimination at the high concentration.

Membrane fluidity

To examine the relationship between the decrease in degree of odor discrimination at high concentration and changes in membrane fluidity, we prepared liposomes made of lipids extracted from turtle olfactory epithelia and measured changes in fluorescence polarization of DPH in the liposomes in response to application of odorants. Application of *n*-amyl acetate and isoamyl acetate to the liposomes decreased the degree of fluorescence

Table 1 Degree of discrimination between various combination of odorants at low and high concentrations

	Low concentration		High concentration	
	Mean ± SE	*n*	Mean ± SE	*n*
Hedione–eugenol	0.85 ± 0.13	4	0.92 ± 0.06	13
Citralva–eugenol	0.77 ± 0.09	6	0.78 ± 0.04	13
Limonene–cineole	0.94 ± 0.07	3	0.82 ± 0.03	20
Citralva–geraniol	0.67 ± 0.12	3	0.56 ± 0.04	13
Geraniol–nerol	0.66 ± 0.15	3	0.53 ± 0.03	13
Lilial–hedione	0.93 ± 0.08	4	0.77 ± 0.03	20
Geraniol-cineole	0.87 ± 0.01	3	0.69 ± 0.04	13
Phenylethylamine–furfuryl mercaptan	1.00 ± 0.05	5	0.80 ± 0.06	12
Eugenol–geraniol	1.10 ± 0.04	3	0.73 ± 0.07	10
Anisole–cineole	1.09 ± 0.15	5	0.80 ± 0.06	15
Citralva–hedione	0.88 ± 0.06	3	0.59 ± 0.03	13
D-Menthol–L-menthol	0.74 ± 0.03	13	0.51 ± 0.03	12
D-Carvone–L-carvone	0.61 ± 0.04	14	0.38 ± 0.06	14
n-Amyl acetate–isoamyl acetate	0.87 ± 0.10	5	0.48 ± 0.03	7
Cineole–camphor	0.99 ± 0.04	3	0.48 ± 0.05	9
n-Amyl acetate–*sec*-amyl acetate	0.75 ± 0.02	6	0.28 ± 0.05	6
trans-3-Hexenol–*cis*-3-hexenol	0.57 ± 0.09	5	0.42 ± 0.12	5

Data were obtained from *n* preparations.

polarization in a dose-dependent manner (Figure 8a), indicating that these odorants increased the membrane fluidity dose-dependently. Application of D- and L-carvone or of D- and L-menthol also increased the membrane fluidity dose-dependently (Figure 8b and c). Figure 8d plots the degree of polarization induced by various concentrations of *n*-amyl acetate against the degree of odor discrimination when isoamyl acetate was applied after *n*-amyl acetate. There is a good correlation between both values ($r = 0.981$). Similarly, the degree of polarization induced by various concentrations of isoamyl acetate (Figure 8g), D-carvone (Figure 8e), L-carvone (Figure 8h), D-menthol (Figure 8f) and L-menthol (Figure 8i) was correlated with degree of odor discrimination between respective isomers. These results indicate that there is a good correlation between the membrane fluidity and the degree of discrimination between odorants with closely related molecular structures.

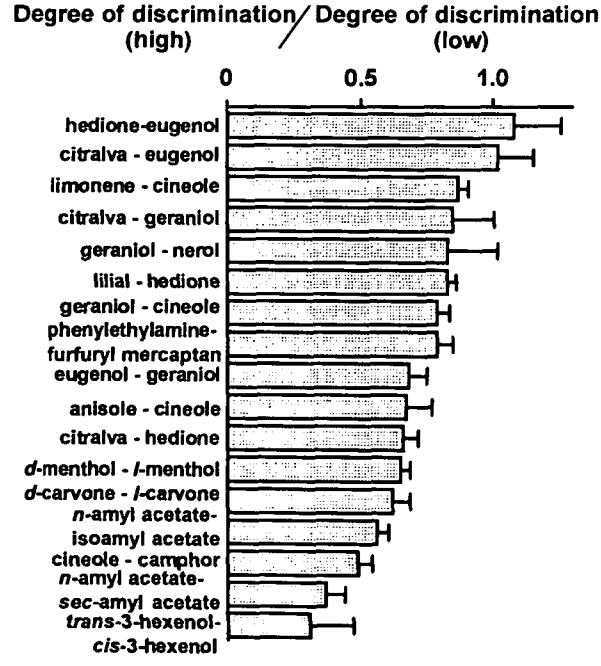

Figure 5 The ratio between the degree of odor discrimination at the high concentration and that at the low concentration. The values were calculated from Table 1. Concentrations of odorants and the mean relative magnitudes of responses to odorants were shown in Table 2. Values were mean ± SEM.

Discussion

In the present study, we examined the concentration dependence of the odor discriminating ability of the turtle olfactory system by recording olfactory bulbar responses. The recording of bulbar responses is useful for obtaining stable and quantitative responses for long periods (Taniguchi *et al.*, 1992, 1994; Hanada *et al.*, 1994; Kashiwayanagi *et al.*, 1994a,b, 1996a, 1997a,b). In addition, it has been demonstrated that there is a good correlation

Table 2 Relative magnitude of turtle olfactory responses to various odorants at low and high concentrations

	Low concentration (mM)	Relative magnitude of response		High concentration (mM)	Relative magnitude of response	
		Mean ± SEM	*n*		Mean ± SEM	*n*
Hedione	0.0005	0.33 ± 0.10	3	0.05	0.73 ± 0.06	19
Eugenol	0.001	0.23 ± 0.05	13	0.1	0.73 ± 0.05	36
Citralva	0.0005	0.27 ± 0.05	12	0.05	1	
Limonene	0.0005	0.28 ± 0.11	3	0.05	0.88 ± 0.05	20
Cineole	0.0001	0.24 ± 0.08	3	0.01	0.74 ± 0.06	20
Geraniol	0.001	0.22 ± 0.03	12	0.1	0.99 ± 0.04	53
Nerol	0.001	0.24 ± 0.05	3	0.1	1.11 ± 0.07	13
Lilial	0.001	0.34 ± 0.09	4	0.1	0.67 ± 0.05	19
Phenylethylamine	0.03	0.32 ± 0.08	5	3	0.84 ± 0.09	10
Furfuryl mercaptan	0.01	0.36 ± 0.07	5	1	1.27 ± 0.11	10
Anisole	0.01	0.28 ± 0.07	5	1	0.93 ± 0.08	15
D-Menthol	0.01	0.74 ± 0.09	12	1	1.52 ± 0.11	10
L-Menthol	0.01	0.70 ± 0.05	12	1	1.43 ± 0.16	10
D-Carvone	0.01	0.62 ± 0.09	13	1	1.77 ± 0.28	13
L-Carvone	0.01	0.61 ± 0.08	13	1	1.74 ± 0.27	13
n-Amyl acetate	0.01	0.33 ± 0.13	5	1	0.98 ± 0.13	5
Camphor	0.001	0.31 ± 0.13	3	0.1	1.49 ± 0.33	9
Isoamyl acetate	0.01	0.34 ± 0.09	5	1	1.35 ± 0.23	5
sec-Amyl acetate	0.1	0.80 ± 0.06	6	10	2.65 ± 0.39	6
trans-3-Hexenol	0.1	0.24 ± 0.12	3	10	1.16 ± 0.19	5
cis-3-Hexenol	0.1	0.22 ± 0.05	3	10	1.34 ± 0.16	5

The magnitude of response to 0.05 mM citralva was taken as a unit.

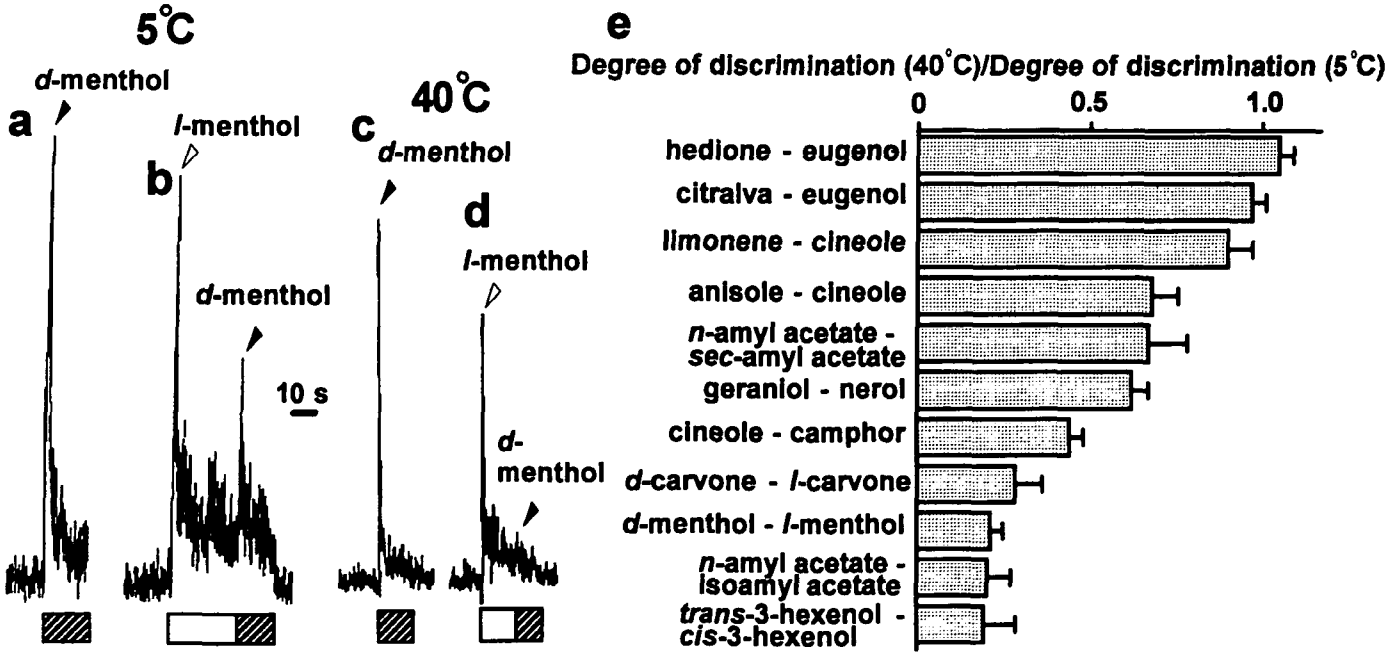

Figure 6 Integrated olfactory bulbar responses to D- and L-menthol at 5 and 40°C. After application of Ringer's solution to the turtle epithelium, 1 mM D-menthol (**a** and **c**) or 1 mM L-menthol (**b** and **d**) was applied at 5 and 40°C respectively. After the response to L-menthol was adapted, 1 mM D-menthol (b and d) was applied. All responses in the figure were recorded from the same preparation. (**e**) The ratio between the degree of discrimination at 40°C and that at 5°C of various odorant pairs. The values between limonene and cineole, *n*-amyl acetate and *sec*-amyl acetate, geraniol and nerol, cineole and camphor, D- and L-carvone, *n*-amyl acetate and isoamyl acetate, and *trans*- and *cis*-3-hexenol were taken from a previous paper (Hanada *et al.*, 1994).

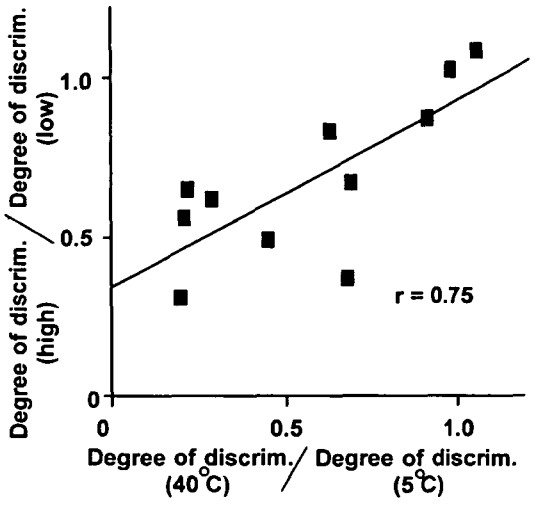

Figure 7 Correlation between ratio of the degree of discrimination at high and low concentrations of various odorant pairs and ratio of the degree of discrimination at 40 and 5°C. Values were taken from Figures 5 and 6.

between EOG, olfactory neural responses and bulbar responses (Caprio, 1977; Byrd and Caprio, 1982; Hara, 1982).

Quantitative analysis of the percentage of neurons that hybridized to olfactory GCR probes has suggested that each olfactory neuron may express only a single type of olfactory GCR gene (Axel, 1995; Sullivan *et al.*, 1995). In a number of electrophysiological studies, however, single olfactory neurons of the frog, catfish, rat, turtle and bullfrog have been shown to respond to various odorants with diverse structure and distinct odor qualities (Gesteland *et al.*, 1982; Duchamp-Viret *et al.*, 1990; Ivanova and Caprio, 1993; Kashiwayanagi and Kurihara, 1994; Kang and Caprio, 1995; Kashiwayanagi *et al.*, 1996b). It is known that various odorants induce responses not only in the olfactory system but also in non-olfactory systems, such as the turtle trigeminal nerve (Tucker, 1963), neuroblastoma cells

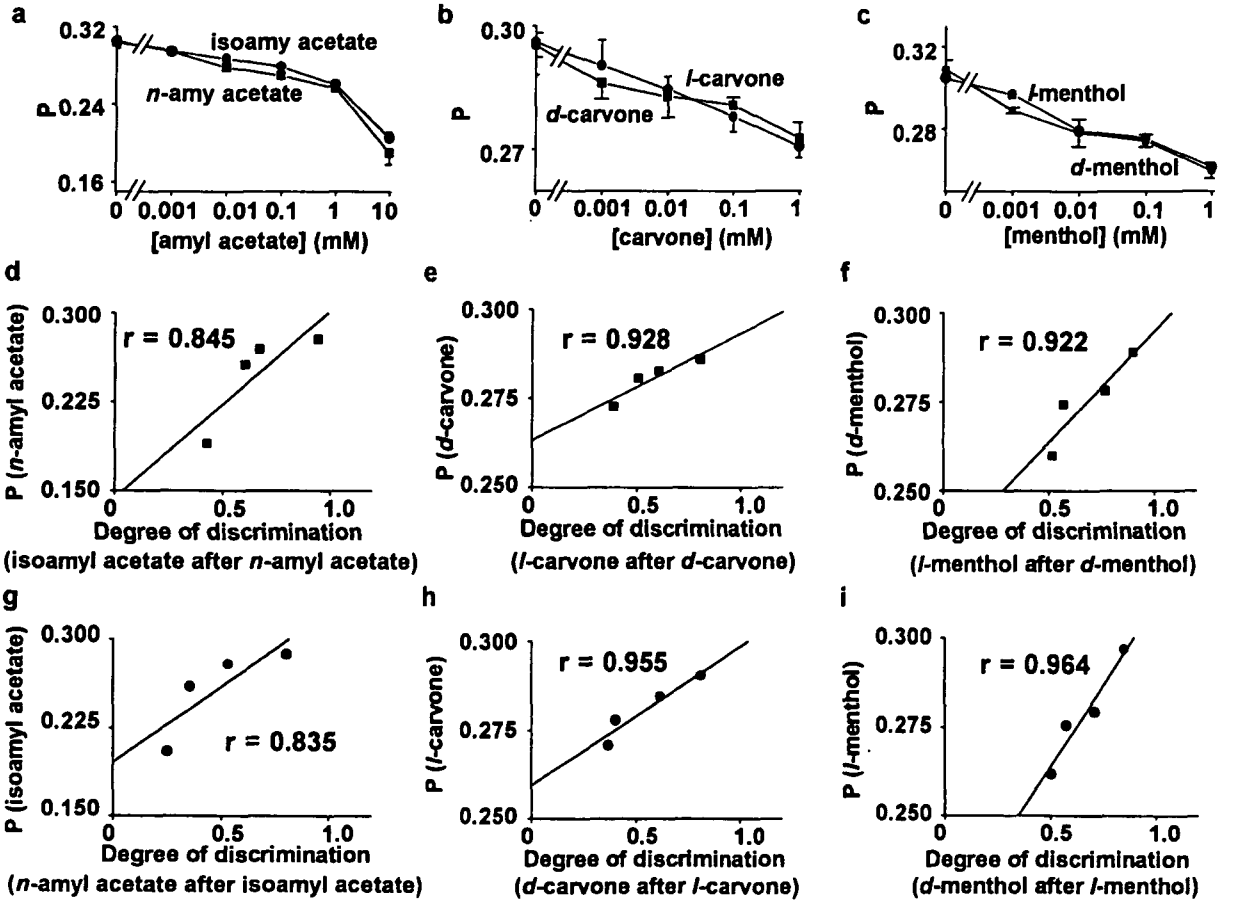

Figure 8 The degree of the fluorescence polarization of DPH in the liposomes as a function of concentrations of **(a)** isoamyl acetate (closed circle) and *n*-amyl acetate (closed square), **(b)** D- carvone (closed square) and L-carvone (closed circle), and **(c)** D-menthol (closed square) and L-menthol (closed circle). Values were mean ± SEM of data obtained from at least three preparations. **(d–i)** The ordinates in the figure show the degree of polarization induced by various concentrations of an odorant, and the abscissas show the degree of odor discrimination. The values of the degree of odor discrimination were taken from Figures 3 and 4.

(Kashiwayanagi and Kurihara, 1984, 1985) and bullfrog taste cells (Kashiwagura *et al.*, 1977; Kashiwayanagi *et al.*, 1994c). The concentrations of odorants required to induce responses in these non-olfactory systems (with the exception of the trigeminal nerve) were relatively high. Therefore, it has been suggested that the responsiveness of a single olfactory neuron to many odorants may be brought about via the non-specific receptors, as it is in the non-olfactory systems.

The present results, however, showed that the odor discriminating ability of the turtle olfactory system was independent of odor concentration provided that pairs of odorants with closely related structures were not used. These results are consistent with those of Duchamp-Viret *et al.* (1990), who found that the discriminatory ability of the frog olfactory neurons obtained by calculating the correlation coefficient of odor responses in single olfactory neurons was independent of odor concentration. The specific reception of odorants at high concentrations was also supported by the whole cell recording of turtle and bullfrog olfactory cells (Kashiwayanagi and Kurihara, 1994; Kashiwayanagi *et al.*, 1996b); the application of cAMP-increasing odorants of several hundred micromolar concentration to single turtle neurons after an inward current induced by IP_3-increasing odorants of several hundred micromolar concentration was adapted induced a large inward current and vice versa (Kashiwayanagi and Kurihara, 1994). Thus the olfactory system discriminates well between odorants even at high concentrations, and there is no reason to believe that the olfactory neuron is highly specific to odorants at low concentrations and non-specific to odorants at high concentrations.

The turtle olfactory system discriminates well between various odorants at low and room temperature, but at high temperature (e.g. 40°C) it discriminates poorly between odorants with closely related molecular structures (Hanada *et al.*, 1994). The temperature dependence of the odor discriminating ability has been closely correlated with that of the membrane fluidity of cells isolated from turtle olfactory epithelia or liposomes made of lipids extracted from the epithelia. Hence, we proposed that the increase in membrane fluidity of lipid layers of olfactory cells is attributable to a decrease of the odor discriminating ability. In the present study, we found that application of high concentrations of odorants decreased the ability to discriminate odorants with closely related molecular structures. We also showed that adsorption of odorants to liposomes brought about an increase in membrane fluidity. There was a good correlation between the concentration dependence of the odor discriminating ability and that of the membrane fluidity of the liposomes. These results suggest that a decrease in the odor discriminating ability by application of high concentrations of odorants is due to an increase in the membrane fluidity of lipid layers of olfactory receptor membranes. In addition, there was a good correlation between a decrease in odor discrimination by a temperature increase and that by an increase in odorant concentration. These results suggest that both the decrease in odor discrimination by a temperature increase and that by an increase in odorant concentration can be consistently explained in terms of the membrane fluidity changes of lipid layers of olfactory receptor membranes.

Based on the above discussion, we assume that the receptor sites for odorants are composed of proteins and lipids. At low temperature, the lipids have a rather rigid structure and the receptor sites seem to have a fine structure to discriminate the isomers. High temperature or adsorption of odorants brings about an increase in the fluidity of the lipids involved in the receptor sites and hence the sites become flexible. In the flexible state, the receptor sites will not discriminate the isomers. There is also a possibility that an increase in temperature or adsorption of odorants directly affects the protein structure. In general, the membrane fluidity changes induced by a small temperature increase such as up to 40°C come not from conformational changes of the protein but from phase transition of lipids. To our knowledge, changes in specificity of receptors to ligands by a temperature increase are not known in other receptor systems. It seems that olfactory receptor sites have unique structures which somehow involve lipids. Previously, we showed that changes in the lipid composition of olfactory model systems, such as neuroblastoma cells and liposomes, changed odor selectivity (Kashiwayanagi and Kurihara, 1985; Nomura and Kurihara, 1987; Enomoto *et al.*, 1991). Incorporation of phosphatidylserine into turtle olfactory receptor membrane selectively enhanced olfactory responses to such organic acids as valeric acid and isovaleric acid (Taniguchi *et al.*, 1994). Therefore, it is likely that lipids composing receptor membranes play a great role in odor discrimination.

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