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Analysis of Odor Discrimination by Multidimensional Scaling at Different Temperatures

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Abstract

Discrimination of odorants by the turtle olfactory bulb at 25° and 37°C was examined by the cross-adaptation technique and analysed by multidimensional scaling. Analysis by multidimensional scaling suggests that at 25°C odorants are grouped according to their odor qualities in the turtle olfactory system. At 37°C, the cluster formation of odorants, which have a similar odor quality, such as minty and floral alcohol odorants and molecular structure, in the plot of multidimensional scaling was poor, indicating that the ability of odor grouping according to their odor qualities was low at 37°C. Chem. Senses 20: 565–571, 1995.

Introduction

Olfactory systems have the ability to discriminate numerous odorants (Ohno *et al.*, 1984; Sicard, 1985). A high ability of odor discrimination has been observed in the receptor neuron and olfactory bulb. A recent study showed that a single olfactory cell of the turtle can discriminate odorants (Kashiwayanagi and Kurihara, 1994). Application of cAMP-dependent (or IP_3-dependent) odorants to the receptor neuron, after an inward current induced by IP_3-dependent (cAMP-dependent) odorants, induced a large inward current in the neuron. In the olfactory bulb, we observed that odor quality of optical isomers, which have similar odors and molecular structures, are discriminated (Taniguchi *et al.*, 1992). Thus, the turtle olfactory system can detect a small difference in odor qualities of most odorants.

In a previous study, we examined the effects of temperature changes on odor-discriminating ability of turtle olfactory receptors *in vivo* by applying the cross-adaptation method and found that the ability of discrimination of odorants having a similar molecular structure and odor quality decreased with an increase in temperature of the turtle olfactory epithelium (Hanada *et al.*, 1994). On the other hand, the discrimination ability of odorants, which have quite different molecular structures and odor qualities, did not decrease greatly with an increase in temperature. In general, odorants can be grouped into several classes based on their odor quality (Amoore *et al.*, 1964). In the present study, we applied the cross-adaptation technique to the turtle olfactory system at 25° and 37°C, using systematic combinations of odorants, taking account of molecular structure and odor quality, and analysed the results by using the multidimensional scaling technique (Kruskal, 1964a,b).

Materials and methods

Recording of olfactory bulbar response

Turtles, *Geoclemys reevesii*, weighing 150–300 g, were obtained from commercial suppliers and maintained at 22°C. The animals were fed porcine and bovine liver *ad libitum*. Olfactory bulbar responses were recorded essentially as

described previously (Taniguchi *et al.*, 1992). In brief, turtles were weakly anesthetized with the necessary and minimum amount of urethane to reduce pain during the operation on the animal, immobilized by an injection of *d*-tubocurarine chloride (450 μg/100 g body wt) and locally anesthetized with lidocaine at the wound and head fixation points. The stimulant-induced brain waves (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 at a 3–300 Hz frequency and integrated by 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 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. The temperature of the olfactory epithelium was changed by perfusing the epithelium with Ringer solution at different temperatures. Before application of the stimulating solution, the epithelium was irrigated with 30 ml of Ringer solution to adjust the temperature of the epithelium to 25° or 37°C. After application of the first 5 ml of stimulating solution, a second 5 ml of stimulating solution was applied immediately. Stimulating solutions of the same temperature as the irrigating solution were used. There was no time delay between the first and second applications of stimulating solutions. After each application of the stimulating solution to the epithelium, the epithelium was rinsed with Ringer solution. The interval between cross-adaptation experiments was 5–15 min.

Preparation of solutions

The Ringer solutions contained (in mM) 116 NaCl, 4 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 5 HEPES-NaOH (pH 7.4). All odorants were dissolved in ethanol to prepare stock solutions at a concentration of 0.1 M (except *n*- and *sec*-amyl acetate). Concentrations of *n*- and *sec*-amyl acetate stock solution were 1 M. These stocks were added to normal Ringer solution to give the indicated concentrations of odorants.

Statistical analysis

Non-metric multidimensional scaling is a method of representing *n* objects geometrically by *n* points, so that the interpoint distances correspond to dissimilarities between objects (8, 9). In the present study, the mean value of the degree of discrimination shown in Tables 3 and 5 was used as δ_{ij} (dissimilarity value) for the analysis. For a configuration of points x_1, ..., x_n in *t*-dimensional space, with interpoint distances d_{ij}, the stress, *S*, of the configuration is defined by:

$$S = [\Sigma(d_{ij} - D_{ij})^2 / \Sigma d_{ij}^2]^{1/2}$$

where the values of D_{ij} are those which minimize *S* to the constant that D_{ij} have the same rank order as the δ_{ij}. We calculated a three-dimensional solution among nine odorants plotted in Figures 2 and 3. The stresses of our solutions were 0.076 and 0.059, respectively, suggesting that goodness of fit was fair (Kruskal, 1964a).

Chemicals

d-Menthol and *l*-menthol were kindly supplied from Takasago International (Tokyo). *n*-Amyl acetate, *sec*-amyl acetate, nonanol, octanol and menthone were purchased from Wako Pure Chemical Industry (Osaka). β-Ionone and citral were purchased from Nakarai Tesque, Inc. (Kyoto) and Kanto Chemicals Co. (Tokyo), respectively.

Results and Discussion

In the present study, we recorded odor responses from the olfactory bulb to explore the odor discrimination ability at the olfactory bulb. In addition, because the method allows us to record reproducible and stable responses for long time, it is easy to obtain quantitative results (Taniguchi *et al.*, 1992, 1994; Hanada *et al.*, 1994; Kashiwayanagi *et al.*, 1994a, in press). Figure 1a shows a typical summated olfactory bulbar response to octanol after the epithelium was adapted to Ringer solution at 25°C. In Figure 1b the response to *d*-menthol, applied first to the epithelium and then octanol, is shown. Octanol induced a large response after *d*-menthol, indicating that the turtle olfactory system clearly discriminates octanol and *d*-menthol. Figure 1c shows the responses to *n*-amyl acetate and octanol after *n*-amyl acetate. In this case, the magnitude of response to octanol after *n*-amyl acetate was small, indicating that the turtle olfactory system poorly discriminated *n*-amyl acetate and octanol.

The degree of discrimination was quantified according to a previous paper (Kashiwayanagi *et al.*, 1994b). The magnitude of the summated responses to secondary applied odorants was measured from a level immediately before the rise of the summated value to the peak. The magnitude of a response to odorant *A* after application of the Ringer solution was defined as *y*. The magnitude of a response to odorant *A* after the response to a second odorant *B* was defined as *y'*. We defined the degree of discrimination between odorant

A and odorant *B* as *y'/y*. For example, the magnitude of the response to octanol after *d*-menthol was 73% of that of the control (Figure 1). In this case, the degree of discrimination was 0.73.

Table 1 shows the relative magnitudes of bulbar responses to various odorants at 25 and 37°C. The magnitude of the summated responses to the first applied odorant was measured from the base level to the peak. Odorant solutions used in the present study induced similar magnitudes of responses at the same temperature. Table 1 indicates that the magnitude of responses at 25°C was essentially similar to those at 37°C.

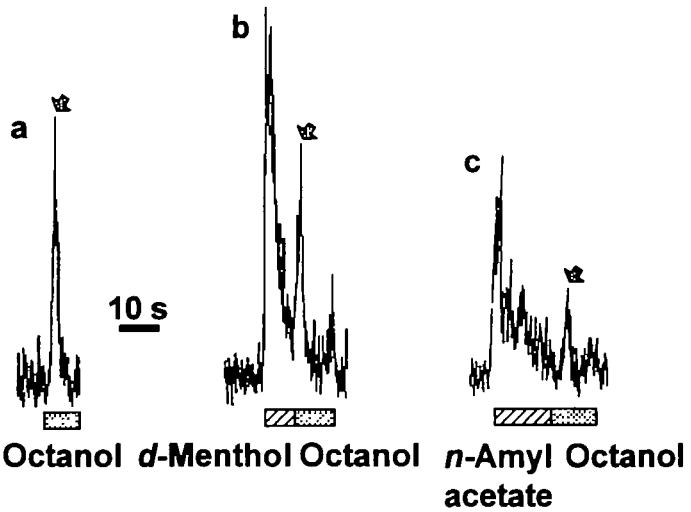

Figure 1. Summated olfactory bulbar responses to odorants at 25°C After the application of the Ringer solution to the turtle epithelium, 0.3 mM octanol solution was applied (a) After the responses to 0.1 mM *d*-menthol (b) and 1 mM *n*-amyl acetate (c) were recorded, 0.3 mM octanol solution (b) was applied. The response peaks to the odorants are indicated by arrows. These responses were recorded from the same preparation.

Table 2 shows the degree of discrimination between various odorants. The values above the oblique line represent the degree of discrimination when an odorant in a column is applied first and then an odorant. Those below the oblique line represent the degree of discrimination when an odorant in a row is applied first and then an odorant in a column. The degree of discrimination varied from 0.29 to 0.89 in the upper half and from 0.18 to 1.09 in the lower half. The degree of discrimination in the upper half was similar to that in the lower half. The mean values of the degree of discrimination were 0.56 ± 0.13 (SE) in the upper half and 0.65 ± 0.18 (SE) in the lower half.

Table 3 shows the mean values of the degrees of discrimination in the upper and lower halves as calculated from the data in Table 2. The values vary from 0.38 to 0.82. The value between octanol and nonanol is 0.38, indicating that the turtle olfactory system poorly discriminated these odorants, which have a similar odor and molecular structure. On the other hand, the value between citral and *d*-menthol is 0.82, indicating that the olfactory system discriminates effectively between these odorants.

Figure 2 shows a spatial configuration reflecting the difference in odor quality in the turtle olfactory system as analysed by multidimensional scaling. Odorants having a similar odor locate closely and odorants having different odors locate separately in the figure. For example, minty odorants (*d*-menthol, *l*-menthol and menthone) locate closely to each other and floral alcohol odorants (octanol and

Table 1. Relative magnitude of turtle olfactory responses to various odorants

	25°C			40°C		
	Mean	SE	*n*	Mean	SE	*n*
1 mM *n*-amyl acetate	1.02	0.19	7	1.08	0.24	8
1 mM *sec*-amyl acetate	1.15	0.22	11	1.18	0.21	9
0.3 mM nonanol	0.75	0.18	13	0.92	0.18	7
0.3 mM octanol	0.73	0.16	13	0.94	0.16	8
0.05 mM ionone	1.25	0.3	11	1.07	0.14	7
0.1 mM citral	0.87	0.19	9	1.17	0.21	6
0.1 mM menthone	1.16	0.26	11	1.31	0.35	6
0.1 mM *l*-menthol	1.11	0.09	11	1.25	0.36	8
0.1 mM *d*-menthol	1.23	0.24	10	1.1	0.19	7

The magnitudes of responses to odorants after Ringer solution are shown. The magnitude of response to 0.3 mM *n*-amyl acetate at 25°C is taken as unity. *n*: number of animals.

Table 2. The values of discrimination between various odorant pairs at 25°C

	NAA	SAA	NON	OCT	ION	CIT	MEN	LME	DME
1 mM *n*-amyl acetate		0.44	0.66	0.56	0.5	0.58	0.44	0.42	0.67
1 mM *sec*-amyl acetate	0.71		0.69	0.89	0.65	0.61	0.52	0.67	0.71
0.3 mM nonanol	0.23	0.18		0.29	0.57	0.39	0.38	0.44	0.44
0.3 mM octanol	0.26	0.56	0.47		0.69	0.36	0.66	0.48	0.64
0.05 mM ionone	0.69	0.59	0.74	0.66		0.56	0.63	0.61	0.72
0.1 mM citral	0.7	0.54	0.54	0.5	0.71		0.3	0.62	0.55
0.1 mM menthone	0.66	0.72	0.72	0.7	0.69	0.9		0.66	0.62
0.1 mM *l*-menthol	0.63	0.69	0.86	0.69	0.79	0.76	0.62		0.39
0.1 mM *d*-menthol	0.78	0.77	0.75	0.74	0.58	1.09	0.5	0.58	

The values were mean obtained from at least three preparations.

Table 3. The mean values of the degrees of discrimination at 25°C

	NAA	SAA	NON	OCT	ION	CIT	MEN	LME
1 mM *n*-amyl acetate								
1 mM *sec*-amyl acetate	0.58							
0.3 mM nonanol	0.45	0.41						
0.3 mM octanol	0.41	0.73	0.38					
0.05 mM ionone	0.6	0.62	0.66	0.68				
0.1 mM citral	0.64	0.58	0.47	0 43	0.64			
0.1 mM menthone	0.55	0.62	0.55	0.68	0.66	0.6		
0.1 mM *l*-menthol	0.53	0.68	0.65	0.59	0.7	0.69	0.64	
0.1 mM *d*-menthol	0.73	0.74	0.6	0.69	0.65	0.82	0.56	0.49

nonanol) also located closely, suggesting that odorants whose odors are classified as being similar by the human olfactory system, are sensed as being similar odors by the turtle olfactory system. The cluster of the minty odorants located away from that of the floral alcohol odorants, indicating that odors classified as different odor in the human olfactory system are well discriminated in the turtle olfactory system. These results suggest that odorants are grouped according to their odor quality in the turtle olfactory system.

In a previous study, we found that the odor discrimination ability of odorants having similar molecular structure and odor quality was decreased with an increase in temperature and the degree of discrimination of structurally different odorants was unchanged from 5° to 37°C (Hanada *et al.*, 1994). Since the effects of temperature change on the odorants were examined with only limited combinations, we systematically examined the effects of temperature change on odor discrimination ability, among odorants having a

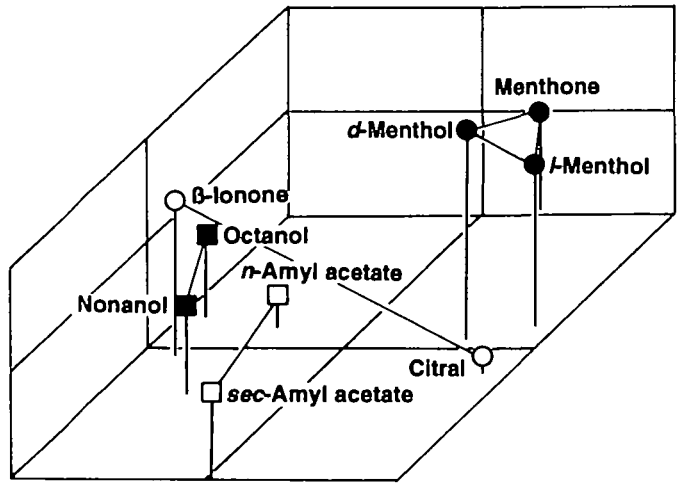

Figure 2. Three-dimensional solution of a multidimensional scaling among nine odorants at 25°C. Data were taken from Table 3. *d*-Menthol, *l*-menthol and menthone; minty odor. β-Ionone and citral; floral odor (enone) Nonanol and octanol; floral odor (alcohol). *n*-Amyl acetate and *sec*-amyl acetate; floral odor (ester).

Table 4. The values of discrimination between various odorant pairs at 37°C

	NAA	SAA	NON	OCT	ION	CIT	MEN	LME	DME
1 mM *n*-amyl acetate		0.38	0.31	0.59	0.55	0.83	0.53	0.61	0.46
1 mM *sec*-amyl acetate	0.74		0.97	0.43	0.55	0.49	0.48	0.58	0.75
0.3 mM nonanol	0.24	0.24		0.54	0.47	0.65	0.48	0.34	0.61
0.3 mM octanol	0.41	0.41	0.42		0.8	0.65	0.4	0.57	0.73
0.05 mM ionone	0.36	0.4	0.43	0.53		0.52	0.48	0.77	0.62
0.1 mM citral	0.44	0.51	0.51	0.63	0.73		0.5	0.59	0.71
0.1 mM menthone	0.54	0.5	0.68	0.49	0.49	0.67		0.65	0.61
0.1 mM *l*-menthol	0.64	0.59	0.8	0.57	0.7	0.56	0.43		0.64
0.1 mM *d*-menthol	0.62	0.29	0.9	0.57	0.77	0.47	0.51	0.47	

The values were mean obtained from at least three preparations.

Table 5. The mean values of the degrees of discrimination at 37°C

	NAA	SAA	NON	OCT	ION	CIT	MEN	LME
1 mM *n*-amyl acetate								
1 mM *sec*-amyl acetate	0.56							
0.3 mM nonanol	0.28	0.61						
0.3 mM octanol	0.5	0.42	0.48					
0.05 mM ionone	0.46	0.48	0.45	0.67				
0.1 mM citral	0.64	0.5	0.58	0.64	0.63			
0.1 mM menthone	0.54	0.49	0.58	0.45	0.49	0.59		
0.1 mM *l*-menthol	0.63	0.59	0.57	0.57	0.74	0.58	0.54	
0.1 mM *d*-menthol	0.54	0.52	0.76	0.65	0.7	0.59	0.56	0.56

different molecular structure and odor. Table 4 shows the degree of discrimination between various odorants at 37°C. The degree of discrimination varied from 0.31 to 0.97 in the upper half and from 0.24 to 0.90 in the lower half. The mean values of the degrees of discrimination were 0.58 ± 0.14 (SE) in the upper half and 0.54 ± 0.15 (SE) in the lower half. Table 5 shows the mean values of the degrees of discrimination in the upper half and the lower half, as calculated from the data in Table 4. The values varied from 0.28 to 0.76. The mean value in Table 5 (0.56 ± 0.09 ; mean ± SE) was slightly smaller than that at 25°C (Table 3, 0.60 ± 0.10; mean ± SE). This finding is consistent with that of the previous study.

However, analysis by multidimensional scaling showed a different spatial configuration at 37°C. Figure 3 shows a spatial configuration analysed by multidimensional scaling at 37°C. Odorants having a similar odor did not form a clear cluster, in contrast to that at 25°C (see Figure 2). For example, floral odorants (*sec*-amyl acetate and octanol) located near the cluster of minty odorants (*d*-menthol, *l*-menthol and menthone). These results suggest that the ability of discrimination of odorants having various molecular structures and odor quality, decreases at higher temperatures.

It is widely believed that odor responses are induced via GTP-binding protein linked cAMP-accumulation and cAMP-gated channels (Pace *et al.*, 1985; Nakamura and Gold, 1987). On the other hand, odorants induce cAMP accumulation not only in olfactory tissues, but also in non-olfactory tissues. For example, odorants activate adenyryl cyclase in renal epithelial cells and in the melanophore (Friendlander *et al.*, 1987; Lerner *et al.*, 1988). In the fetal rat, olfactory cells responded to odorants before the appearance of G-protein coupled receptors, at an early stage of development (Margalit and Lancet, 1993). A recent study showed that hydrophobic substances directly stimulated G-proteins in the chemosen-

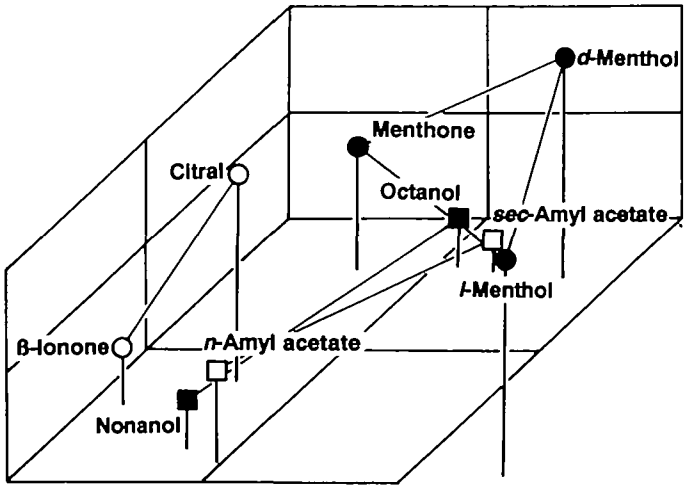

Figure 3. Three-dimensional solution of multidimensional scaling at 37°C. Data were taken from Table 5.

sory system (Naim *et al.*, 1994). These results suggest that odor responses are induced not only via G-protein coupled receptors, but also via non-specific membrane constitutes, as shown in the olfactory model systems (Kashiwayanagi and Kurihara, 1984; Nomura and Kurihara, 1987; Kashiwayanagi *et al.*, 1990).

It was shown that membrane lipids are important for odor reception; an incorporation of phosphatidylserine into the turtle olfactory epithelium enhanced odor responses to fatty acids (Taniguchi *et al.*, 1994). In a previous study, we measured membrane fluidity, using 1, 6-diphenyl-1, 3, 5-hexatriene, of a cell preparation from the turtle olfactory epithelium and a liposome made by lipid extracted from the turtle olfactory epithelium (Hanada *et al.*, 1994). The membrane fluidity of the cell preparation and the liposome decreased with an increase in temperature between 5° and 37°C. The membrane fluidity changed within a similar temperature range for a decrease in odor discrimination activity. The increase in membrane fluidity may be associated with a loss of cluster formation at 37°C. These results suggest that membrane lipids in the receptor membrane also play roles in odor reception and odor discrimination. GTP-binding protein is located on the membranes facing the inside of a cell and, hence, for the direct stimulation of GTP-binding protein, chemical stimuli applied to the cell surface must make contact with the membrane lipids. An increase in temperature increases the fluidity of the lipids in the receptor membranes, which seems to bring about a decrease of odor discrimination ability.

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