13 Wilson, J. E., Trends Biochem. Sci. *3* (1978) 124.
14 Wilson, J. E., Biophys. J. *16* (1982) 997.
15 Fiek, C., Benz, R., Ross, N., and Brdiczka, D., Biochim. biophys. Acta *688* (1982) 429.
16 Kliman, H. J., and Steck, T. L., J. biol. Chem. *255* (1980) 6314.
17 Lluis, C., Int. J. Biochem. *16* (1984) 1005.
18 Lluis, C., J. Prot. Chem. *5* (1986) 423.
19 Ehmann, J. D., and Hultin, H. O., Archs Biochem. Biophys. *154* (1973) 471.
20 Lluis, C., Int. J. Biochem. *16* (1984) 997.
21 Ratner, J. H., Nitisewojo, P., Hirway, S., and Hultin, H. O., Int. J. Biochem. *5* (1974) 522.
22 Ross, R. E., and Hultin, H. O., J. Cell Physiol. *105* (1980) 409.
23 Lluis, C., Int. J. Biochem. *17* (1985) 1219.
24 Hultin, H. O., Ehmann, J. D., and Melnick, R. L., J. Food Sci. *37* (1972) 269.
25 Nitisewojo, P., and Hultin, H. O., Eur. J. Biochem. *67* (1976) 87.
26 Masters, C. J., Reid, S., and Don, M., Molec. cell. Biochem. *76* (1987) 3.
27 Brdiczka, D., Krebs, W., and Kloock, P., Biochim. biophys. Acta *297* (1973) 203.
28 Kline, E. S., Brandt, R. B., Laux, J. E., Spainhour, S. E., Higgins, E. S., Roger, K. S., Tinsley, S. B., and Water, M. G., Archs Biochem. Biophys. *246* (1986) 673.
29 Mattison, A. G. M., Johansson, R. G., and Bostrom, S. L., Comp. Biochem. Physiol. *41 B* (1972) 475.
30 Skilleter, D. N., and Kun, E., Archs Biochem. Biophys. *152* (1972) 92.
31 Wilson, J. E., J. Neurochem. *19* (1972) 223.
32 Cercek, B., and Houslay, M. D., Biochem. J. *207* (1982) 123.
33 Selwyn, M. J., Biochim. biophys. Acta *105* (1965) 193.
34 Bergmeyer, H. U., Gawehn, K., and Grassl, M., in: Methods of Enzymatic Analysis, vol. 1, p. 425. Ed. H. U. Bergmeyer. Academic Press, New York 1974.
35 Houslay, M. D., and Palmer, R. W., Biochem. J. *174* (1978) 909.
36 Schmidt, E., in: Methods of Enzymatic Analysis, vol. 2, p. 650. Ed. H. U. Bergmeyer. Academic Press, New York 1974.
37 Canela, E. I., and Nin, C. M., J. Prot. Chem. *4* (1985) 305.
38 Arnol, H., Henning, R., and Pette, D., Eur. J. Biochem. *22* (1971) 121.
39 Sagrista, L., and Bozal, J., Biochimie *69* (1987) 205.
40 Desmoulin, F., Cozzone, P. J., and Canioni, P., Eur. J. Biochem. *162* (1987) 151.
41 Spriet, L. L., Soderlund, K., Bergstrom, M., and Hultman, E., J. appl. Physiol. *62* (1987) 616.
42 Clarke, F. M., Shaw, F. D., and Morton, D. J., Biochem. J. *186* (1980) 105.
43 Wals, T. P., Masters, C. J., Morton, D. J., and Clarke, F. M., Biochim. biophys. Acta *675* (1981) 29.
44 Carpenter, D. O., Hovey, M. M., and Bak, A. F., Ann. N.Y. Acad. Sci. *204* (1973) 502.
45 Cope, F. W., and Damadian, R., Physiol. Chem. Phys. *6* (1974) 17.
46 Melnick, R. L., and Hultin, H. O., J. Food Sci. *35* (1970) 67.

0014-4754/88/030203-06$1.50 + 0.20/0

Spatial variation in response to odorants on the rat olfactory epithelium

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Received 29 September 1987; accepted 8 December 1987

Summary. We have measured the electro-olfactogram produced by four odorants, nicotine, i-pentyl acetate, i-pentanoic acid and cineole from twelve positions on an in vitro preparation of rat olfactory tissue. Each odorant shows a different pattern of response over the twelve positions which can be explained by differences in olfactory receptor populations between regions of the rat olfactory epithelium.
The result for nicotine is further evidence that there are olfactory receptors which are stimulated by nicotine when it is presented as a vapour.
Key words. Electro-olfactogram; olfaction; spatial patterning; nicotine.

Introduction

The olfactory epithelium in the rat is located on bony turbinate structures and on the septum which separates the two halves of the nasal cavity. This sensory epithelium is the site of a complex series of events following odorant stimulation, culminating in the generation of an action potential in the primary olfactory neurons. These primary neurons synapse in the olfactory bulb where subsequent processing of the information from the epithelium occurs [2, 3].
The mechanisms by which the olfactory system can distinguish between the very large number of 'smells' found in the environment using a finite number of receptors is still not fully understood. One level at which determination of odour quality can occur is at the initial interaction of odorant with the olfactory epithelium. The layer of mucus which covers the olfactory epithelium will affect odorants which dissolve in it in different ways [4, 5] and will therefore affect the rate and concentration at which odorants reach the olfactory receptors. This mode of discrimination between odorants is an example of 'imposed' patterning of the stimulus-olfactory epithelium interaction. A second type of discrimination is more specific, in that the stimulus can be identified through differences in the stimulated receptor populations, in the transduction pathways activated and in the arrangement of neuronal connections to the olfactory bulb.
This specific 'patterning' of the response to odorants has been demonstrated in several ways at different levels in the transduction process. Electrophysiological studies have shown that an odorant stimulates more than one receptor

type[6,7] and that the olfactory neurons differ in the range of odorants to which they respond[8]. In vitro experiments using the olfactory odorant-modulated adenylate cyclase also suggest that odorants stimulate a heterogeneous population of receptors and that some odorants are poor stimulants for this particular transduction mechanism[9,10]. The electro-olfactogram (EOG), a summated receptor potential from many olfactory neurons, has been used to demonstrate regional differences in response to odorants in the frog[11,12] and the salamander[13], and histological studies have shown that the membraneous particles of vertebrate olfactory cilia, supposedly the olfactory receptor sites, are unevenly distributed within the epithelium[14]. Recent studies have demonstrated that specific regions of the olfactory bulb are connected with specific regions within the olfactory epithelium[15–17]. These results support the hypothesis that the olfactory neurons are arranged non-randomly within the epithelium and that different odorants may stimulate receptors at different regions of the olfactory epithelium.

The spatial patterning of the response to odorants by the rat olfactory epithelium is suggested from EOG recordings taken from the olfactory neurons on the dorsal side of the cribriform plate[18]. Here we have used the EOG as a measure of the response from twelve positions on the epithelium itself to four odorants with markedly different structures and odours; i-pentyl acetate, i-pentanoic acid, cineole and nicotine.

Materials and methods

The in vitro preparation, odorants and methods used in this study are described in detail elsewhere[19]. The exposed epithelium of each of the four olfactory turbinates was allocated three positions from which it would be possible to record EOGs. These positions were essentially the same in each rat studied, though some variation in topography of the turbinates of different preparations was observed during the study. Thus, there were twelve positions from which EOGs could be recorded (fig. 1). The right side of the head was routinely used in this study.

Initially, EOG recordings were taken from an arbitrary position (position 8) on every rat studied. In this laboratory recordings are frequently made from position 8 because this location gives a large EOG amplitude to many odorants. Following this, the electrode was raised and repositioned by movement of the head stage holding the preparation. An identical sequence of the odorants (each presented in duplicate as a 1-s pulse of vapour followed by a 1-min recovery period) was then presented to the new position. By moving the preparation in this manner, the distance between the electrode tip and odorant delivery nozzle was fixed throughout each experiment. The same vapour concentration of each odorant was applied to each position studied (nicotine 65 nM, cineole 309 nM, i-pentanoic acid 383 nM and i-pentyl acetate 981 nM). The preparation of odorants for the experiments and calculation of odorant concentrations was as described elsewhere[19].

Recordings were made from position 8 in each rat to provide a common reference position for the experiment, since time did not permit us to record EOGs from all twelve positions on each rat studied. The same electrode was used to record from any one preparation but was replaced as required during the study; this will not significantly affect the EOGs recorded. The order of positions from which recordings were taken was as random as possible after the following criteria were obeyed. Neither adjacent positions were used on the same turbinate nor opposite areas on neighbouring turbinates, where the first presentation of odorants may have reduced the sensitivity of the second or subsequent areas to be studied. In a single preparation it was possible to record from four or five positions before there was a risk of the tissue drying out. The olfactory epithelium was not superfused with Ringer solution between recordings from each position.

Discriminant analysis on the results from the study was performed on an IBM 4381 computer utilising the Statistical Package for the Social Sciences (SPSSx) programmes.

Results

An example of a typical experiment is shown in figure 2. The EOG traces were recorded consecutively from five positions (positions 8, 12, 4, 6 and 1 shown in fig. 1) on the same rat olfactory preparation and show that there are differences in response of the rat olfactory epithelium to the four odorants at the five positions. In all, 19 rats were studied.

The mean EOG amplitude to the odorants at each position is shown in figure 3. The data were collected from all presentations of odorant made to the olfactory epithelium during the study, typically three presentations of i-pentyl acetate

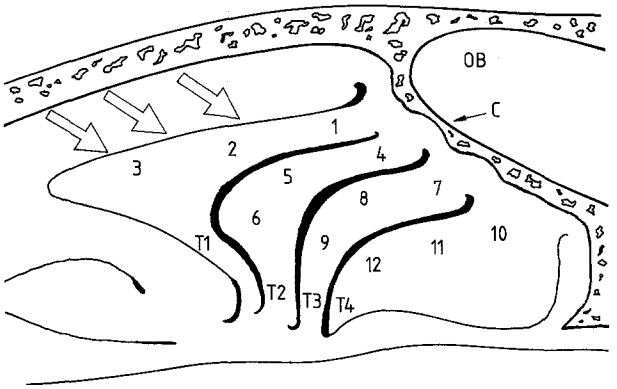

Figure 1. Schematic diagram of the olfactory turbinates following sagittal sectioning of a rat head and removal of the septum. The 12 positions from which EOGs were recorded are marked 1 to 12, three on each of the four turbinate structures, T1–T4. C = Cribriform plate, OB = Olfactory Bulb. The arrows show the direction from which the odorants were presented to the epithelium.

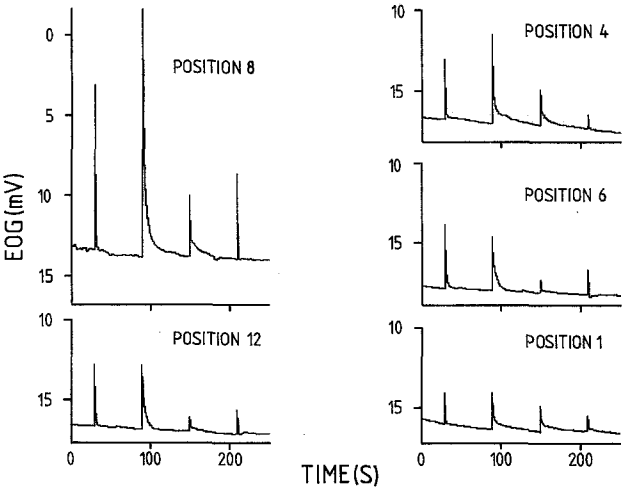

Figure 2. Patterning of the EOG response to the four odorants from a single rat half-head preparation. The presentation order (and the vapour phase concentration) of the odorants is from left to right, i-pentyl acetate (981 nM), cineole (309 nM), nicotine (65 nM) and i-pentanoic acid (383 nM). The order of recordings is position 8 first, then 12, 4, 6 and 1.

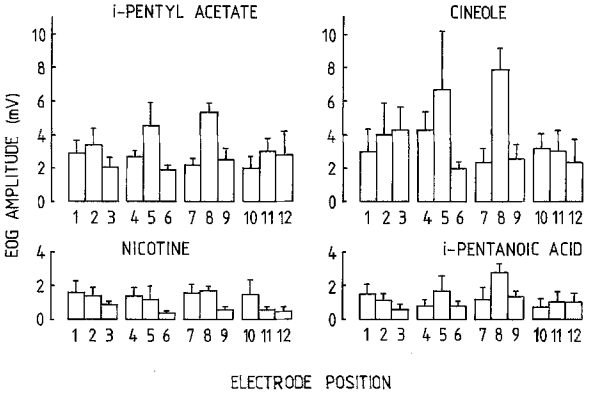

Figure 3. Mean EOG peak amplitude + 95% confidence interval versus recording position. The minimum number of presentations to any one position is 10 (nicotine, cineole and i-pentanoic acid to positions 5 and 11) and the maximum is 69 (i-pentyl acetate to position 8).

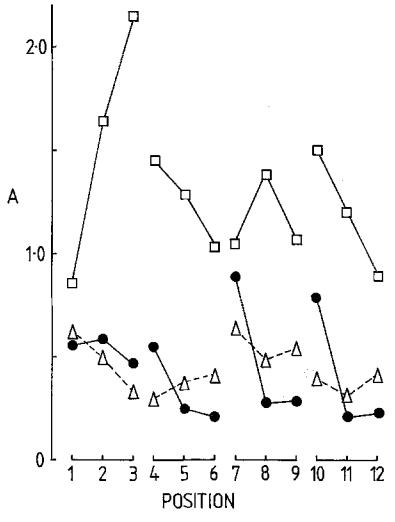

Figure 4. Mean A value versus recording position for cineole □, nicotine • and i-pentanoic acid △. The 95% confidence intervals of the mean A values for positions 1 to 12 are as follows;
cineole ± 0.24, 0.37, 0.54, 0.29, 0.29, 0.20, 0.46, 0.10, 0.18, 0.37, 0.53, 0.23.
nicotine ± 0.28, 0.23, 0.18, 0.20, 0.14, 0.04, 0.49, 0.03, 0.05, 0.41, 0.13, 0.08.
i-pentanoic acid ± 0.42, 0.15, 0.25, 0.10, 0.26, 0.14, 0.41, 0.05, 0.18, 0.19, 0.27, 0.17.

and two each of nicotine, cineole and i-pentanoic acid at each position, remembering that it was not possible to record EOGs from all twelve positions on the same preparation and that position 8 was studied on every preparation. The results shown in figure 3 suggest that there are spatial differences in the amplitude of response to the odorants tested. However, accurate interpretation of these results is difficult when it is realised that in addition to experimental effects (see discussion) the data do not account for variation in response between animals. In previous studies we have used the A value to account for this variation [6, 7, 19], and we have applied this analysis here also. The value A was calculated for each odorant by normalising the EOG peak amplitude recorded from nicotine, cineole or i-pentanoic acid to the EOG peak amplitude recorded from i-pentyl acetate at the same position. Thus, we are describing the EOG for nicotine, cineole and i-pentanoic acid by a value that is unrelated to EOG amplitude and is consistent for each odorant between animals.
The mean A value for each odorant at each position is shown in figure 4 and gives an estimate of changes in A value versus

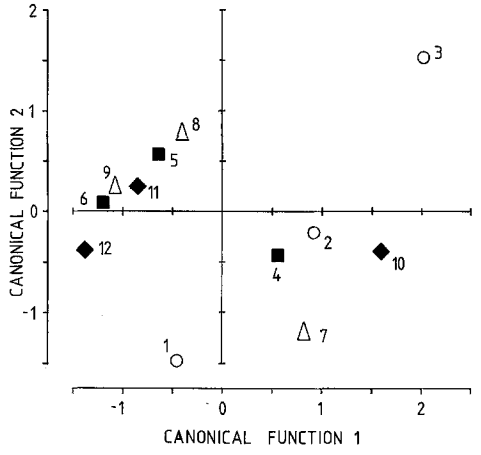

Figure 5. Plot of the centroid value for each recording position (mean canonical value for canonical functions 1, 2, and 3) determined using discriminant analysis of the A values for cineole, nicotine and i-pentanoic acid. The shaded symbols represent a negative value for canonical function 3. All points start at the origin before the analysis. Numbers 1 to 12 represent the centroid value for the recording positions on ○ T1, □ T2, △ T3 and ◇ T4 of figure 1.

recording position. The A value can change due to a relative increase or decrease in EOG amplitude measured from one or both of the 'test' odorants (cineole, nicotine or i-pentanoic acid) or 'reference' odorant (i-pentyl acetate). As can be seen from figure 4, the change in A value for the 'test' odorants is not identical across the 12 positions, again suggesting that there are spatial differences in response to the test and/or reference odorant on the rat olfactory epithelium.
The variation in A value across the 12 positions for each test odorant may not be identical for each animal tested, thus evidence for spatial patterning may be obscured by simply calculating the mean A value. We therefore analysed the A value data from each preparation using discriminant analysis, in which each position studied on each animal was described mathematically by canonical values relating the normalised response (A value) of the olfactory epithelium to the three test odorants. The equations used to determine these values calculated the maximum discrimination possible between the A values. A plot of the two most discriminating functions on x–y axes represents the movement of the value from the origin during the analysis. The canonical values for each position are then averaged, giving a mean value, the centroid, for each position studied (fig. 5). Using this analysis we represent the original EOG data from four odorants in a two-dimensional plot. Figure 5 shows that the centroid values for the positions do not fall into the same quadrant of the plot, again suggesting that there are regional differences in response to the odorants tested which can be identified by the normalised responses to cineole, nicotine and i-pentanoic acid. The data points used in the discriminant analysis were related to the actual recording position by a number from 1 to 12 (as in fig. 1). The analysis classified the data from 33 out of 92 recordings as coming from the expected recording position. The probability that this result has been generated by chance is a function of the Poisson distribution and gives $p \ll 0.001$. This indicates that there is a similar pattern of response from the 19 animals used.

Discussion

The results obtained in this study show that there is spatial patterning of response to the four odorants tested in both EOG amplitude (fig. 3) and variation in the normalised response (fig. 4). These results must be interpreted with cau-

tion in order to distinguish imposed patterning (including experimental effects) from the patterning which should be observed if the odorants stimulate different regions of the olfactory epithelium.

The EOGs measured at the twelve positions will be affected by differences in mucus thickness and composition between each recording position, the proportion of non-responsive respiratory epithelium in the regions tested and the position of the recording electrode relative to the earth electrode. Smaller EOGs are likely to be recorded near to the edges of the turbinates due to current leakage being greater in these regions. This should affect the EOG to all four odorants equally. The same is true of the amount of respiratory epithelium at each position (likely to be greatest at positions 3, 6, 9 and 12). The mucus layer covering the epithelium may impose patterning on our preparation in the following way. The response to odorants that have a large water/air partition coefficient (nicotine and i-pentanoic acid) will be affected to a larger extent by changes in mucus thickness than the less soluble odorants (i-pentyl acetate and cineole). As the mucus thickness increases, the decrease in EOGs to nicotine and to i-pentanoic acid will be greater than any change in the EOG to cineole and to i-pentyl acetate. Evidence suggests that in the rat the mucus layer is of uniform thickness (about 5 μm [20]). Thus imposed patterning via mucus effects is unlikely to explain large differences in the response measured from the twelve positions, particularly between nicotine and i-pentanoic acid.

The design of our electrode and stimulus source [21], although reducing imposed variation in the response, may influence the EOGs in a different manner. Diffusion of the odorants (or even transport [22]) through the mucus may allow adaptation to occur in an adjacent position on the same turbinate. The experimental protocol we used avoided recording from two such positions without leaving time for the odorants to disperse (by recording the EOGs from a position on a different turbinate). If such adaptation had occurred, then the response of an odorant would be reduced at the subsequent recording position. Observations made during the experiment suggested that this was not the case.

The differences between recording position determined by discriminant analysis have no experimental parameter, but are a useful pointer to positions which show large differences in response to others. By referencing to the EOG data shown in figure 3, it is possible to discover why these differences have been identified by the discriminant analysis. For example, position 3 is distinguishable from all others because of a relatively larger response to cineole than is expected for a uniform distribution of receptors for all four odorants. Another example is the response to nicotine vapour at position 10 which is larger than expected, whereas the response to i-pentanoic acid vapour at the same position is not, suggesting that imposed patterning in this case is unlikely. In a previous study we have shown that the lectin concanavalin A reduces the EOG to nicotine, cineole and i-pentanoic acid to different extents [19], suggesting that the three odorants stimulate different combinations of olfactory receptor. Thus, the unrelated variation in response over the twelve positions to each of the three odorants seen here is not unexpected.

The relative arrangement of the centroids for each turbinate is also interesting (fig. 5). The first turbinate (positions 1, 2 and 3) is easily distinguished from the others on the basis of the response to the odorants tested. The other three turbinates are not so easily separated by canonical functions 1 and 2, though all have differences in response between the anterior and posterior of each turbinate. Such positional differences have been observed previously in the salamander [13]. Values for canonical function 3 for each centroid do appear to separate the responses of turbinates 1 and 3 from those of turbinates 2 and 4. Such patterns may be expected from a non-random arrangement of receptors within the olfactory epithelium.

These observations suggest that specific patterning of response to the odorants is seen on rat olfactory epithelium. This patterning is most likely explained by differences in receptor populations between the positions studied and is consistent with other studies suggesting this type of arrangement of olfactory receptors in the rat and other species [12, 13, 15–18].

In addition, the result for nicotine confirms our previous finding [19] that nicotine stimulates olfactory receptors of rat olfactory epithelium in a manner similar to other odorants.

Acknowledgments. D.A.E. and R.A.M. were supported by a grant from the Science and Engineering Research Council. We would also like to thank Dr S. G. Shirley for useful discussion during this study.

1 To whom reprint requests should be made.
2 Getchell, T. V., Physiol. Rev. *66* (1986) 772.
3 Lancet, D., A. Rev. Neurosci. *9* (1986) 329.
4 Mozell, M. M., and Jagodowicz, M., Science (Washington) *181* (1973) 1247.
5 Getchell, T. V., Margolis, F. L., and Getchell, M. L., Prog. Neurobiol. *23* (1984) 317.
6 Shirley, S. G., Polak, E. H., Edwards, D. A., Wood, M. A., and Dodd, G. H., Biochem. J. *245* (1987) 185.
7 Shirley, S. G., Polak, E. H., Mather, R. A., and Dodd, G. H., Biochem. J. *245* (1987) 175.
8 Revial, M. F., Sicard, G., Duchamp, A., and Holley, A., Chem. Senses *7* (1982) 175.
9 Shirley, S. G., Robinson, C. J., Dickinson, K., Aujla, R., and Dodd, G. H., Biochem. J. *240* (1986) 605.
10 Sklar, P. B., Anholt, R. R. H., and Snyder, S. H., J. biol. Chem. *261* (1986) 15538.
11 Mustaparta, H., Acta. physiol. scand. *82* (1971) 154.
12 Daval, G., Leveteau, J., and MacLeod, P., J. Physiol. Paris *76* (1980) 559.
13 Mackay-Sim, A., and Kubie, J. L., Chem. Senses *6* (1981) 249.
14 Menco, B. Ph. M., in: Nasal Tumors in Animals and Man, vol. 1, p. 45. Eds G. Reznik and S. F. Stinson. CRC Press, Florida 1983.
15 Pederson, P. E., Jastreboff, P. J., Stewart, W. B., and Shepherd, G. M., J. comp. Neurobiol. *250* (1986) 93.
16 Astic, L., and Saucier, D., Brain Res. Bull. *16* (1986) 445.
17 Saucier, D., and Astic, L., Brain Res. Bull. *16* (1986) 455.
18 Thommesen, G., and Doving, K. B., Acta. physiol. scand. *99* (1977) 270.
19 Edwards, D. A., Mather, R. A., Shirley, S. G., and Dodd, G. H., Experientia *43* (1987) 868.
20 Menco, B. Ph. M., Cell Tissue Res. *207* (1980) 183.
21 Shirley, S. G., Chem. Senses (1987) in press.
22 Pevsner, J., Sklar, P. B., and Snyder, S. H., Proc. natl Acad. Sci. USA *83* (1986) 4942.

0014-4754/88/030208-04$1.50 + 0.20/0