Olfactory Discrimination: Electrophysiological Spatiotemporal Basis

Abstract. *Simultaneous recordings were taken from two widely separated branches of the olfactory nerve of the frog. Odors from a variety of chemical substances were blown into the nares, and each elicited a different response magnitude ratio between the two branches. In addition, the time lapse between the two nerve responses differed for different chemicals. This suggests a time-space encoding of the mucosal response to odors.*

One of the major problems in olfaction is to understand the physiological basis for the discrimination between vast numbers of different odorants. At least three possibilities exist. (i) The receptors themselves may be more or less selectively sensitive to different groups of chemicals. (ii) The receptor sheet as a whole might elicit differential time-space discharges in response to separation of the chemical vapors in a manner analogous to gas chromatography columns. (iii) There may be both types of receptor analysis.

Recordings from single units in the olfactory bulb and olfactory mucosa have given considerable support to the concept of selectively sensitive receptors with, however, the important qualifications that each unit responds to a rather large group of different chemicals and that these groups show a considerable amount of overlap (*1*). The second hypothesis, that the mucosal sheet may differentially respond to different chemicals in the time-space characteristics of its discharge, is suggested by the findings that different parts of the olfactory bulb respond differentially to different chemicals and that certain temporal aspects of the bulbar discharge also differ for different chemicals (*2*). Furthermore, there is histological evidence of some isomorphism between the mucosal and bulbar areas (*3*). However, no direct evidence that different parts of the mucosa respond differentially to different chemicals has been reported. This report presents such data.

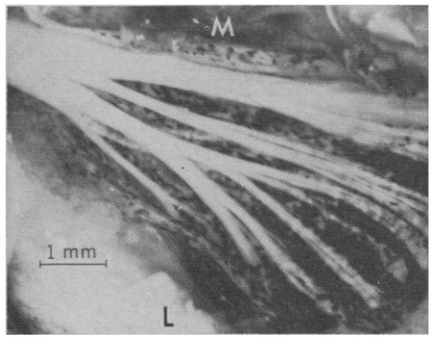

Fig. 1. Photograph of the branching olfactory nerve as it courses through the lamina propria of the olfactory mucosa on the dorsal aspect of the olfactory sac. *L*, Lateral aspect; *M*, medial aspect.

Figure 1 shows that the olfactory nerve of *Rana catesbeiana* branches considerably as it courses through the lamina propria of the olfactory mucosa. Under a 40-power dissecting microscope the preterminal ramifications of these branches were followed peripherally. Their separate and divergent courses continued into different areas of the lamina propria. It was therefore assumed that the branches subserved different regions of the mucosa and that the activity from these different regions could be sampled by recording from the appropriate branches. In order to keep the possibility of overlap of receptor fields to a minimum, it was thought best to record from the branches that came from the most divergent areas of the lamina propria. Thus, the electrodes were placed on the most lateral and the most medial nerve branches. The electrodes were stainless-steel, enameled wires, 63 μ in diameter (*4*).

A Harvard infusion pump forced deodorized air to bubble through the odorant liquid and then to puff into the anterior nares of the frog. The olfactory sac itself remained intact. The volumes puffed into the nares varied between 1 and 0.15 cm^3 for different animals but were held constant in any given animal; 0.5 cm^3 was most often used. In two of the animals the flow rate was set at 40 cm^3/min. In all the rest the flow rate was 20 cm^3/min. Only one concentration of each odorant was used; this was saturation in purified air at the ambient room temperature. The absolute magnitudes of the responses varied with the flow rates and volumes. However, the measure upon which the conclusions of this paper are based (that is, the ratio of the discharge magnitudes recorded simultaneously from different nerve branches in response to the same odorant) showed little dependence upon these two parameters. The discharges from these small branches look like the discharges recorded previously from more posterior locations on the olfactory nerve (*4*).

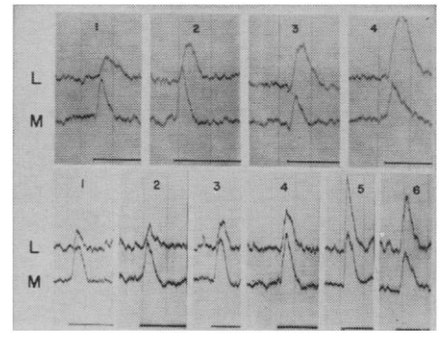

Fig. 2. Typical frequency meter responses (upper two traces) and summated responses (lower two traces) of the lateral nerve (*L*) and the medial nerve (*M*). The stimulus marker traces (retouched on upper traces) signify only the start of stimulation. The distance between the vertical lines on the chart signifies 10 seconds. Upper traces: 1, geraniol; 2, citral; 3, *d*-limonene; 4, octane. Lower traces: 1, phenyl ether; 2, napthalene; 3, geraniol; 4, citral; 5, *d*-limonene; 6, octane.

To compare these discharges quantitatively, they were passed through either an electronic frequency meter (1.2 second time constant) or an electronic summator (0.25 second time constant). The frequency meter determined the average number of occurrences per unit time of the amplified neural events which surpassed a given peak-to-peak voltage change. The total area in the neural discharge per unit time was summed electronically by the summator (*5*). The frequency meter or summator outputs were used to drive the galvanometers of a Honeywell Visicorder. Thus, depending upon whether the frequency meters or summators were used, the amplitudes of the resulting traces were proportional to either the aver-

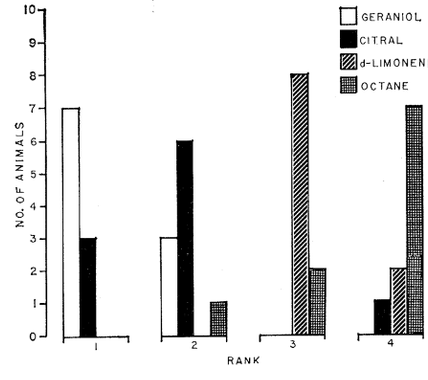

Fig. 3. The number of animals in which the ratio between the lateral nerve response and medial nerve response for a given chemical fall into a given rank. Only the data from the frequency meter are included. There were ten such animals in all.

age frequency or total area of the neural activity.

Typical frequency meter and summator responses are presented in Fig. 2. The frequency meter traces show the average frequency response on the lateral nerve (*L*) and the medial nerve (*M*) for four different chemicals. The ratio of the maximum response of the lateral nerve to the maximum response of the medial nerve increases in the following order: geraniol, citral, *d*-limonene, octane.

Each frog was presented with the series of chemicals four times, and the average lateral-nerve-to-medial-nerve ratio was computed for each chemical. These ratios for the four chemicals were ranked in order of magnitude with "1" being the lowest and "4" being the highest. This procedure was followed for ten animals, and the ranks for each chemical were compared across animals to determine whether there was any consistency in the order into which the ratios fell. Figure 3 gives a graphical representation of these data. Although no chemical gave ratios confined solely to a single rank, there is a definite trend for orderliness. Statistical testing for this orderliness across the ten animals showed it to be significant ($p < .01$) (*6*).

Further statistical testing of differences between each chemical and every other chemical revealed that the differences in rank were all significant with the exception of that between octane and *d*-limonene (*7*).

Tests were conducted with four additional animals, the summators rather than the frequency meters being used (Fig. 2). During these tests two more chemicals were added, and the data were analyzed in the same manner as before. The lateral-to-medial nerve discharge ratios showed the same order noted: geraniol, citral, *d*-limonene, and octane. In addition, phenyl ether had the smallest ratio, whereas the naphthalene ratio was much like that of geraniol. The order across animals was again statistically significant ($p < .01$) (*6*).

For both the frequency meter and summator records a temporal measure was also taken, namely, the average time that elapsed between the onset of the stimulus and the point when the response reached 70 percent of the maximum. This measure of latency showed that the lateral nerve responses for all chemicals occurred later than the medial nerve responses a statistically significant number of times ($p < .01$) (*8*). The average difference between the lateral nerve response and the medial nerve response for each chemical was computed and ranked. The ranks were then compared across animals. They increased in the following order: octane, *d*-limonene, citral, and geraniol. Again, statistical testing of order across animals yielded significance ($p < .01$) (*6*). Likewise, all the average temporal differences between chemicals were significantly different from each other except that of geraniol and citral (*7*).

These data strongly suggest that the input to the central nervous system from the olfactory peripheral system differs in temporal and spatial discharge pattern for different chemicals. It is possible to explain these different response levels of the two nerve branches either by unequal distribution of selectively sensitive receptors or by a mechanism such as the separation of the vapors by adsorption. It is more difficult to ascribe the temporal differences to the unequal distribution of selectively sensitive receptors. It should be noted again that the effect of concentration on the results described here is yet to be determined. At any rate there appears to be a time-space encoding of the mucosal analysis of incoming vapors.

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References and Notes

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6. As tested by the Kendall coefficient of concordance described in S. Siegal, *Nonparametric Statistics* (McGraw-Hill, New York, 1956), pp. 229–239.
7. As tested by the Wilcoxon matched-pairs signed ranks test described in S. Siegal, *ibid.*, pp. 75–83.
8. As tested by the sign test described in S. Siegal, *ibid.*, pp. 68–75.
9. Supported by NIH grant NB 03904–02.

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Variation in the Monoterpenes of Pinus Ponderosa Laws

Abstract. *Wide differences from tree to tree were found in the monoterpene composition of the wood oleoresin of ponderosa pines located in the same geographic area in California. Differences attributable to variation within the tree, to the season of sampling, to the year of sampling, and to methods of analysis by gas chromatography were only slight. There may be an inverse relation between the amounts of* Δ_3*-carene and* β*-pinene in a given tree.*

Few data are available on the extent to which the terpene composition of the wood oleoresin of pines varies within the species. Hagen-Smit *et al.* (*1*) analyzed the resin of 19 samples of *Pinus washoensis* and found variation in the density, index of refraction, and specific rotation of their turpentines. Specific rotation varied from plus 10 to minus 9 degrees, and the difference was attributed to varying quantities of Δ_3-carene and β-pinene. Bannister *et al.* (*2*) observed only slight differences between trees of *P. radiata* and *P. attenuata.* In ten trees of *P. maritima* and three trees of *P. pinaster* Sandermann (*3*) found differences in the percentage of α-pinene and β-pinene. Mirov's (*4*) comprehensive review of pine turpentines contains much data on the interspecies differences in terpene composition, but only limited data on intra-

Table 1. Percentage of monoterpenes in the volatile oils of selected ponderosa pines.

α-Pinene	β-Pinene	Δ_3-Carene	Myrcene	Limonene	β-Phellandrene	Unknown
7.7	8.9	65.9	8.5*	5.0	0.9	3.1
4.5	17.2	29.6	15.2	30.7*	1.8	0.8
11.5	55.3*	0.4	15.5	15.5	1.8	0.0
8.7	21.4	28.8	14.4	23.2	2.4	1.1
4.0	14.4	27.3	24.9*	26.5	1.8	1.1
4.0	16.5	52.6	10.3	12.3	1.9	2.4
6.2	16.7	59.2	15.5	†	0.3	2.1
1.5*	0.1*	82.5	11.1	1.5	1.4	1.9
13.3*	57.5*	†	8.2	12.0	1.8	1.7

* Extremes indicated. † Trace.