# The Spatiotemporal Analysis of Odorants at the Level of the Olfactory Receptor Sheet

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ABSTRACT Activity in two separate regions of the frog olfactory mucosa was sampled by simultaneously recording the summated neural discharges from the olfactory nerve branches originating from them. The difference in the activity from these two regions in response to a stimulus was measured by: (*a*) the ratio of the response amplitude recorded from the lateral nerve branch to that recorded from the medial nerve branch (LB/MB ratio), (*b*) the latency difference (or time interval) between these two responses. Equal concentrations of four different odorants were drawn into the nose by an artificially produced sniff of known dimensions. At each concentration in every animal the four chemicals were ranked in order of the magnitudes of their LB/MB ratios and again in order of their latency differences. Regardless of their concentration, the same chemicals fell into the same ranks in different animals. In addition, for each chemical the magnitudes of the ratios and latency differences showed only minimal changes with concentration. Thus, spatiotemporal patterns of relative response magnitudes and latency differences across the mucosa differentially represented the odorants. Such a spatiotemporal code, together with physicochemical considerations, suggested that the nose separates vapors in a manner similar to a gas chromatograph. This is further supported by the previously observed reversal of the ratio patterns with reversal of air flow direction through the olfactory sac.

## INTRODUCTION

Recent evidence appears to show that one of the mechanisms basic to olfactory quality discrimination at the level of the olfactory mucosa is the selective sensitivity of individual receptors for different groups of chemicals (6). Other mechanisms, which may act in concert with the above, have been proposed. One hypothesis states that different chemicals are not equally effective stimuli for the same regions of the mucosa, so that each chemical would yield a unique pattern of regional activity across the receptor sheet (1, 4, 11, 12). Another proposed mechanism is a unique time course in the growth and decay of the mucosal activity elicited by different chemicals. The credibility of

both these latter proposed mechanisms is supported by extrapolating to the olfactory mucosa the spatiotemporal code observed by recording from the secondary olfactory neurons of the olfactory bulb (1, 8, 9). However, until now there has been no direct evidence that different chemicals do indeed yield differential activity both temporally and spatially across the receptor sheet. It is the purpose of this paper to present such direct evidence.

In order to determine whether a given pattern of activity across the mucosa is dependent upon the particular vapor itself rather than upon its concentration, it is necessary to present different chemicals at the same concentration and the same chemical at different concentrations. This present study, unlike the preliminary reports (11, 12), distinguishes between the effects of molecular species and molecular concentration by incorporating a suitable olfactometer to control concentration.

As previously shown, different branches of the primary olfactory nerve subserve different areas of the olfactory mucosa (12, 14). This makes it possible to sample the activity occurring in different regions of the frog's receptor sheet in response to a stimulus by recording from different olfactory nerve branches simultaneously. Since there are no synaptic junctions between the receptor endings and the primary fibers, the representation of the receptor sheet activity on the primary nerve is not confounded by synaptic modifications.

## APPARATUS AND PROCEDURES

### A. *Stimulus Control*

The required concentration of odorant is achieved by a flow dilution olfactometer (Fig. 1) in which an air stream is first saturated with an odorant and then is diluted the necessary amount with an independent nonodorized air stream. The air for both streams is initially dried and deodorized by passing it through calcium chloride, activated charcoal, and silica gel. The air for the odorized stream is drawn into the syringe of an infusion pump. Reversal of this pump bubbles the air through undiluted liquid odorant. To insure saturation the bubbles passing through the liquid are made quite fine by forcing the air through Pyrex wool as it leaves the submerged inlet tube. The room temperature is held at 23 $\pm$ 1° C, and the temperature of the liquid odorants is found to remain within this range even during bubbling. Thus, knowing the temperature, the partial pressure of the odorant which saturates the air can be specified. The flow rate at which this odorized air enters the mixing chamber can be regulated by adjusting the above-mentioned infusion pump. The tube carrying the odorized air extends halfway into the mixing chamber, and it is supported there by a Teflon piece which also acts as a baffle.

Rotary vane pumps supply a nonodorized air stream with a much greater flow rate than the odorized stream because of the considerable dilution needed to reach the lower limits of the frog's response range. This nonodorous stream is bubbled through deodorized water to prevent dehydration of the mucosa. The flow rate at

which this dilution stream enters the mixing chamber is read on the inlet rotameter and is varied by a stopcock at the rotary vane pump.

The two air streams are combined in different proportions by regulating their respective flow rates. Thus the odorant is diluted to the desired partial pressure, and it is allowed to flow through the mixing chamber and into the room exhaust system. A short tube, which fits snugly into one of the frog's nares, is inserted halfway into the mixing chamber via a small side arm.

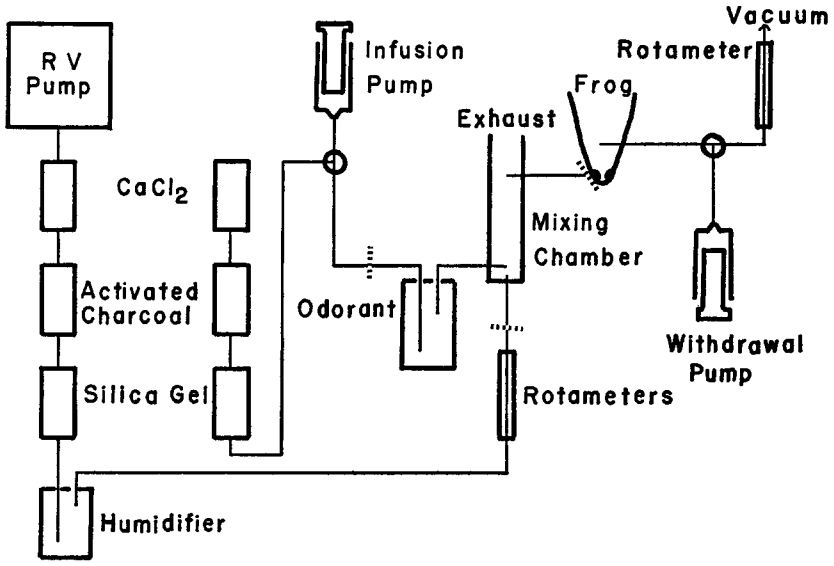


FIGURE 1. Diagram of the stimulation apparatus. See text for explanation. The section bounded by the dotted line is replaced when shifting from one chemical to the next or when desiring a reduction in the concentration of any given chemical. This protects against residual odors. Two rotary vane pumps (RV pump) with a combined maximum output of 47 liters per min are used to supply nonodorized air. The distilled water in the humidifier is deodorized by boiling with activated charcoal. It is considered deodorized when air bubbled through it no longer produces a neural response from the frog.

A cannula is introduced into the buccal cavity through a tight fitting hole drilled in the maxillary bone. The other end of this cannula is connected by a three-way stopcock either to a constant vacuum, via a rotometer, or to a withdrawal pump. When the syringe of this withdrawal pump is pulled back, a suction is transferred through the animal to the mixing chamber thus drawing into the olfactory sac a sample of the air flowing in the mixing chamber. The integrity of this flow path through the stimulated naris is maintained by closing off all other entrances to the buccal cavity (i.e., other naris, trachea, esophagus, jaws). The flow rate and volume of this artificially produced sniff are controlled by varying these parameters on the withdrawal pump. The volumes ranged between 0.2 and 0.6 cc for different animals but were held constant in any given animal. The flow rate for all animals was 8.24 cc/min. Thus, the duration of stimulation ranged between 1.46 and 4.38 sec.

The initial movement of the withdrawal syringe, which closed an electrical circuit, was defined as the onset of the stimulation.

During the interstimulus interval (2.5 to 3.5 min) humidified nonodorized air was flushed through the olfactory sac at 8.00 cc/min by allowing only the nonodorized air stream to enter the mixing chamber and by turning the three-way stopcock to the constant vacuum.

From the deodorizing agents to the frog's naris the olfactometer is made entirely of glass and Teflon. Those parts of the olfactometer that must be contaminated by contact with the odorant (Fig. 1) could be replaced after the stimulation. The residual odors in these parts were removed by long term boiling and oven heating.

### B. *Stimulus Presentation*

The odorants used as stimuli were geraniol, citral, *d*-limonene and octane.[1] Each of the four chemical vapors was presented at each of the following partial pressures: 0.25, 0.56, 1.20, and 2.50 $\times$ $10^{-2}$ mm Hg. Octane and *d*-limonene were presented at two additional partial pressures, 12.0 and 56.0 $\times$ $10^{-2}$ mm Hg. These latter partial pressures could not be reached at room temperature with geraniol and citral because of their lower vapor pressures.

The order of stimulus presentation was dictated in part by the attempt to minimize the number of olfactometer replacements necessitated by residual odors. Consequently, for most animals, each of the concentrations of a given chemical was presented once in ascending order and then a different chemical was begun. However, the order of presentation of the four chemicals was randomized beforehand. To determine whether there was an effect due to the sequence of presentation, this procedure was changed in two animals so that stimulation with all chemicals at the same partial pressure was completed before going on to the next concentration. These results did not differ from those obtained with the more usual order of presentation.

Deodorized, humidified air was presented as the first stimulus of each concentration series. If a response occurred, the presence of residual odors was suspected and the olfactometer component replacement procedures described earlier were followed.

A complete experiment was defined as one in which all four chemicals were presented to an animal at all the above partial pressures at least three times.[2] Thus, each of the ten animals yielded three response arrays like the single array seen in Fig. 2. The definition also required that the electrode positions not be changed and the amplification (see below) not be altered. As decided beforehand, the experimental group for this study was composed of the first ten animals yielding such complete sets of data.

### C. *Recording*

After each frog (*Rana catesbeiana*) was anesthetized with urethan, it was placed in a head holder and the dorsal aspect of the olfactory sac was exposed revealing the several branches of the olfactory nerve (11). The most lateral branch (LB) and the most

[1] Eastman Kodak Distillation Products Catalog Nos. are respectively: T378, P932, 1980, P1107. The same batch of each chemical was used throughout the work reported here.

[2] Occasionally, a given concentration of a given chemical was presented more than three times. All these additional presentations are included in the data analysis which follows.

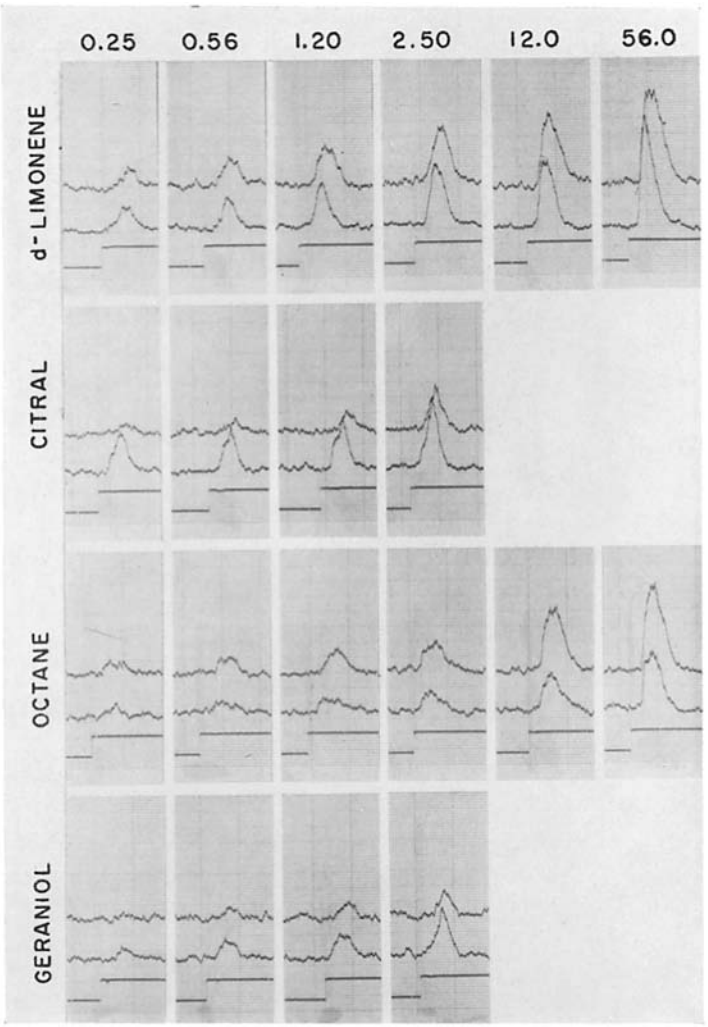


FIGURE 2. Visicorder records of summated neural discharges showing the responses of one frog to a single presentation of every stimulus. Thirty such complete response arrays (i.e., three from each of the ten animals) were included in the analysis. Partial pressures are given along the top in terms of $\times 10^{-2}$ mm Hg. At room temperature (23°C) the partial pressures of $12 \times 10^{-2}$ and $56 \times 10^{-2}$ mm Hg cannot be reached for geraniol and citral. The upper response of each pair is recorded from the lateral nerve branch and the lower is from the medial nerve branch. The stimulus marker shows only the onset of the stimulus. Vertical time lines occur once every 10 sec. In this animal the stimulus duration was 3 sec. The stimulus volume was 0.4 cc.

medial branch (MB) were desheathed. The mucosal area subserved by LB is more distant from the external naris than is the area subserved by MB. These two nerve branches diverge as they are followed peripherally (11, 12). Depending on the animal's size, the branches are 6 to 9 mm apart at the most distal mucosal area and 1.5

to 2.5 mm apart at the most proximal mucosal area. The olfactory sac itself was left intact, thus protecting the integrity of the receptor sheet and the air flow path.

The electrodes were stainless steel wires, 63 $\mu$ in diameter and quadruply enamelled to the tips (10). The neural activity from each nerve branch was recorded differentially with the active electrodes pressed lightly against the nerve branches. The in-

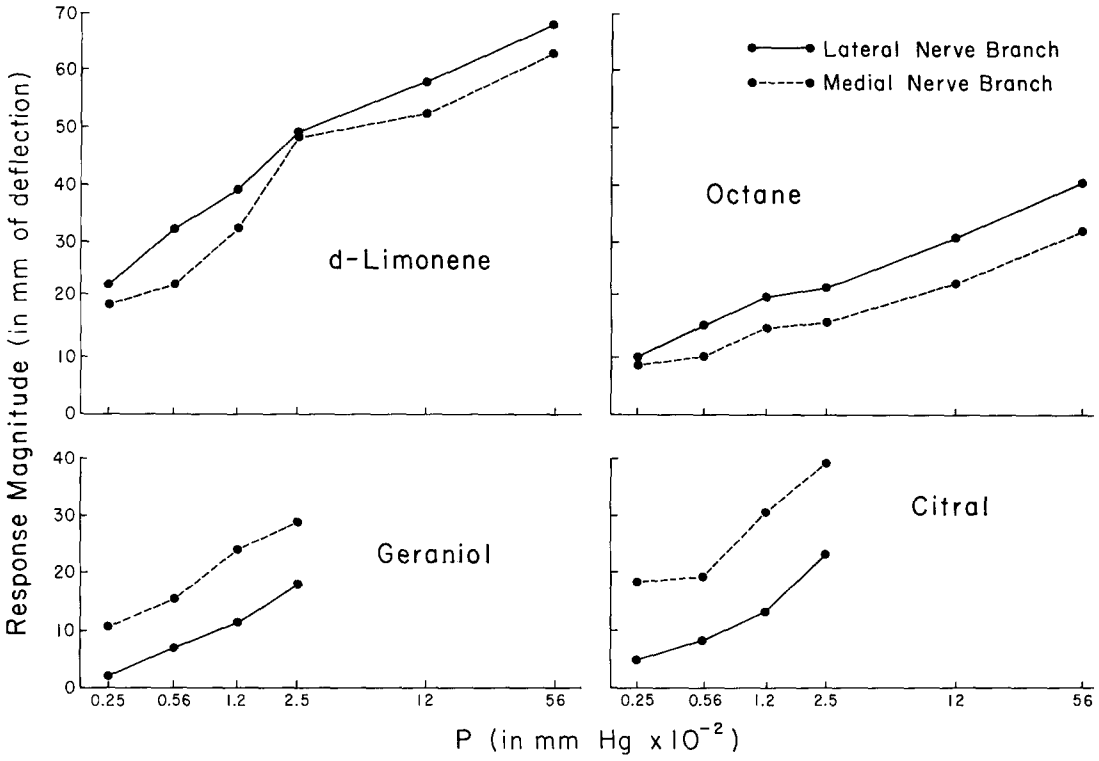


FIGURE 3. Median response amplitudes as a function of concentration. Each point is the median of at least thirty determinations; i.e., at least three presentations of each stimulus to each animal (see footnote 2). The first four partial pressures are the same for each chemical but $12 \times 10^{-2}$ and $56 \times 10^{-2}$ mm Hg could not be attained with geraniol and citral at the room temperature (23°C) because of their lower vapor pressures.

active electrodes rested on nearby bone wetted with Ringer's solution, and the preparation was grounded through the head holder.

The neural activity was amplified by Grass P5 ac preamplifiers. In order to quantify this activity the preamplifier outputs were led through summator (integrator) circuits in which the charging time constants were set at 0.25 sec and the discharging time constants were set at 1.9 sec. The summator outputs drove the galvanometers of a Honeywell Visicorder, thus yielding traces proportional to the total area of the neural impulses per unit time (3).

Two direct measurements of the Visicorder responses (Fig. 2) were made: (*a*) the height of the peak amplitude,[3] (*b*) the latency between the onset of the stimulus and the onset of the response.[4]

In order to quantify the relative activity elicited by a stimulus across the receptor sheet, two further measures derived from the direct measurements were computed: (*a*) the ratio of the response amplitude recorded from the lateral nerve branch (LB) to that recorded from the medial nerve branch (MB), i.e., LB/MB ratio, (*b*) the latency difference (or the time interval) between these two responses. If different stimuli do indeed yield different spatiotemporal patterns of activity across the mucosa, these differences will be reflected both in a comparison of LB/MB ratios and in a comparison of latency differences.

At the beginning of each experiment the gains of the preamplifiers were adjusted so that *d*-limonene at a partial pressure of $2.5 \times 10^{-2}$ mm Hg yielded equal response amplitudes on the two nerve branches; i.e., the LB/MB ratio was made equal to 1.00.[5] Once these initial preamplifier gains were set, they were not readjusted during the rest of the experiment.

## RESULTS

To illustrate the data which are analyzed in the following sections, the records taken from one frog in response to a single presentation of each stimulus are shown in Fig. 2.

### A. *Spatial Differentiation of Different Odorants*

Several of the factors determining the amplitude of the recorded olfactory neural discharge can be seen in Fig. 3. First, in most cases the median response amplitude approximates a linear function of the logarithm of the partial pressure. Second, the response amplitude differs for different chemicals. This can be seen for the responses which are elicited on the same nerve branch by equal concentrations of different stimuli. Third, the amplitude depends upon the region of the receptor sheet sampled since each chemical at a given concentration yields different responses on the two nerve branches. Finally, and most important to this study, is the interaction of different chemicals with different regions. For instance, at equal concentration the discharge from the medial branch is greater than that from the lateral branch in response to citral and geraniol whereas the reverse is true in response to *d*-limonene and octane. Such a difference in relative regional activity suggests a spatial analysis. If, indeed, this type of analysis exists, the patterns produced

[3] The base line for this measure was the straight line which by visual estimate best ran through the middle of the prestimulus activity.

[4] The onset of the response was defined by the intersection of the base line (see footnote 3) with a line drawn tangential to a point on the response 1.5 times the height of the average prestimulus deflection.

[5] *d*-Limonene was chosen as the standardizing chemical because at equal amplifications the responses on the two nerve branches were already nearly equal.

by the same chemicals would be expected to be consistent from animal to animal.

The previously described LB/MB ratio was used in testing for this consistency of pattern between animals. First, the median ratio of each animal was determined for each concentration of every chemical. Then, with each

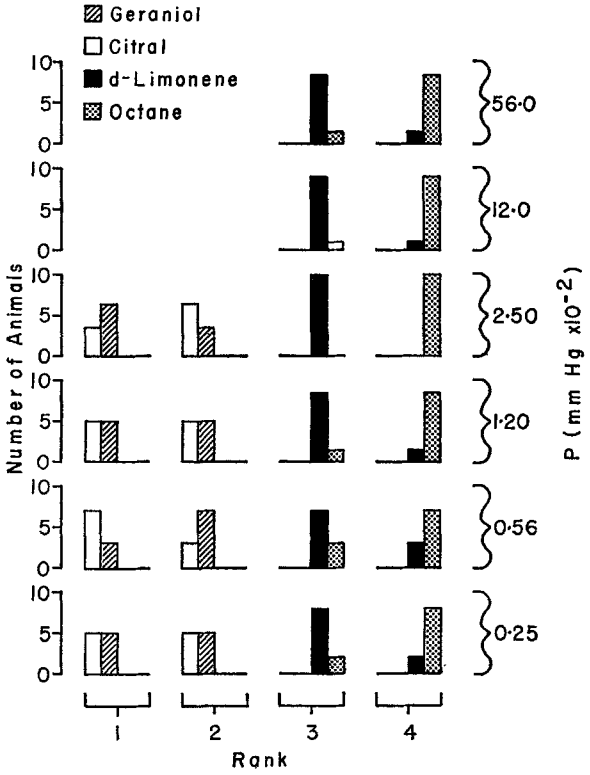


FIGURE 4. The number of animals in which the LB/MB ratio of a given chemical falls into a given rank at a given partial pressure. The total number of animals is ten. A rank of "1" represents the smallest ratio and "4" the largest. The larger the ratio the greater is the lateral nerve branch discharge relative to the medial nerve branch discharge.

concentration taken separately, the chemicals were ranked in the order of the size of their median ratio. Finally, at each concentration the number of animals in which a given chemical occupied a given rank was determined (Fig. 4). The greater the number of identical rankings for each chemical, the greater is the consistency between animals. In a similar way consistency of pattern from concentration to concentration was determined. This test of the consistency between concentrations is relevant to the question of whether the pattern is dependent upon the molecular species or molecular concentration.

Fig. 4 shows that at any given concentration there is a strong tendency toward a consistent ordering of the chemicals although no chemical always fell into the same rank. In addition, this order appears to be quite similar at all concentrations. For instance, at all partial pressures octane most often yielded the highest ranking ratios whereas *d*-limonene most often yielded the next highest. Furthermore, at all concentrations neither the octane ratios nor

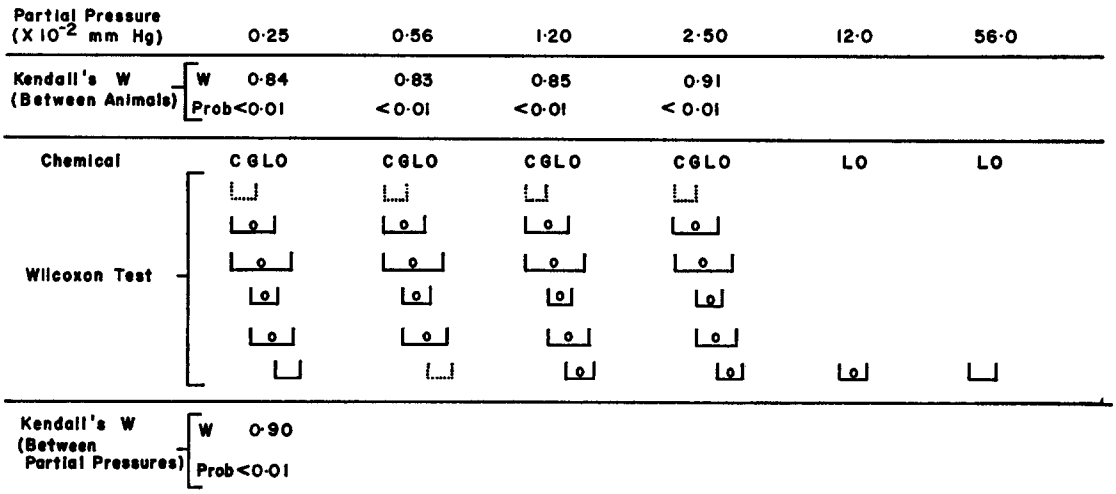


FIGURE 5. Summary of the statistical analysis of the LB/MB ratio data by Kendall's coefficient of concordance (Kendall's *W*) and by the Wilcoxon matched-pairs signed-ranks test (Wilcoxon test, 13). The analysis by Kendall's *W* of the correspondence of the ranks attained by the four chemicals both between animals and between partial pressures is given. As *W* approaches 1.00 from 0.0 the correspondence increases. The Wilcoxon test tests the difference of the ratios between each chemical and every other chemical at any given partial pressure. *G*, geraniol; *C*, citral; *L*, *d*-limonene; *O*, octane. The dotted brackets signify no statistically significant differences (i.e., probability $> 0.05$). The solid line brackets without circles signify differences to the 0.05 probability (i.e., probability $< 0.05 > 0.01$). The solid brackets with circles signify the 0.01 probability (i.e., probability $< 0.01$).

the *d*-limonene ratios ever fell into the two lowest ranks. In contrast, the ratios of geraniol and citral consistently shared these two lowest ranks, and at no concentration did either of them ever attain the two highest ranks. Statistical tests confirmed the high degree of consistency in the rank attained by the ratio of a given chemical both between animals and between concentrations (Fig. 5). Statistical testing of the difference in rank between each chemical and every other chemical revealed that of the six possible pairs of chemicals only one, citral vs. geraniol, consistently showed no significant differences in rank (Fig. 5).

The method of ranking used above does not give any indication of the absolute size of the ratio for different chemicals, nor does it show how this

may vary with concentration. A plot of the median ratio for each chemical as a function of partial pressure is given in Fig. 6. It can be seen that the median ratios for *d*-limonene and octane do not vary in a consistent manner with concentration and this was confirmed statistically.[6] The curves for geraniol and citral show that, although the ratios are not very different from concentration to concentration, there is a gradual increase of the median ratio with the partial pressure. This increase, though slight, is consistent across animals ($P < 0.01$).[6] However, as can be seen from this figure, this small

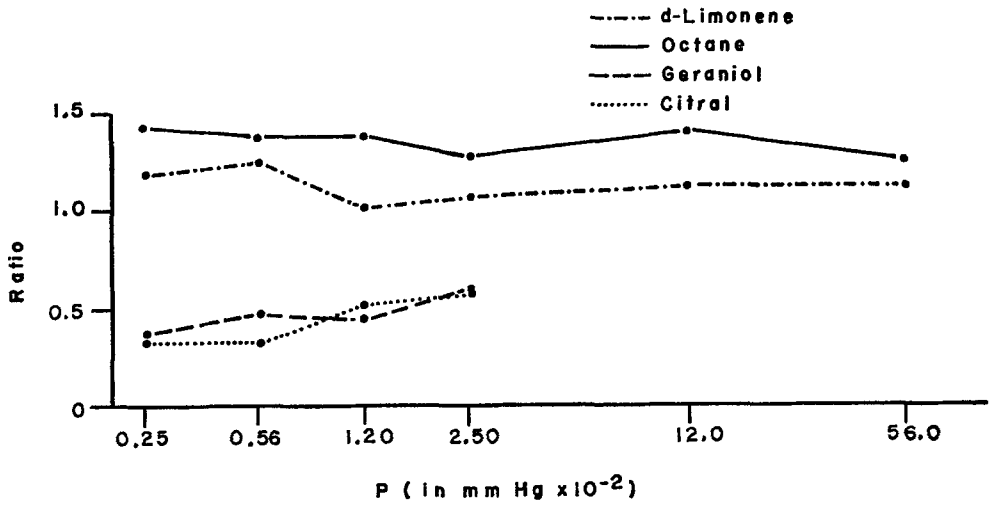


FIGURE 6. The median LB/MB ratio as a function of partial pressure for each chemical. Each point is the median of at least thirty determinations; i.e., at least three from each animal (see footnote 2).

increase with concentration is not enough to alter the basic pattern since at none of the concentrations tested do the median geraniol and citral ratios equal any of the *d*-limonene and octane ratios. Likewise, none of the median octane ratios ever equals or surpasses the *d*-limonene ratios.

### B. *Temporal Differentiation of Different Odorants*

The same techniques which were used to rank the chemicals according to their LB/MB ratios were also used to rank the chemicals according to the magnitude of the latency differences between their responses on the two nerve branches. As can be seen in the graphical and statistical presentations (Figs. 7 and 8), there is a tendency toward orderliness across concentrations and across animals within concentrations, but this consistency, especially at the lower partial pressures, is less than it was with the ratio data. A major con-

[6] By Wilcoxon matched-pairs signed-ranks test (13).

tribution to this variability is that the per cent error involved in measuring the latencies of very small Visicorder responses is much greater than in measuring the amplitudes alone (see Fig. 2). Indeed, at the lowest concentration ($0.25 \times 10^{-2}$ mm Hg) the lateral branch responses were so small that no attempt was made to determine the latencies. At the next highest concentration ($0.56 \times 10^{-2}$ mm Hg) some consistency in order becomes apparent with

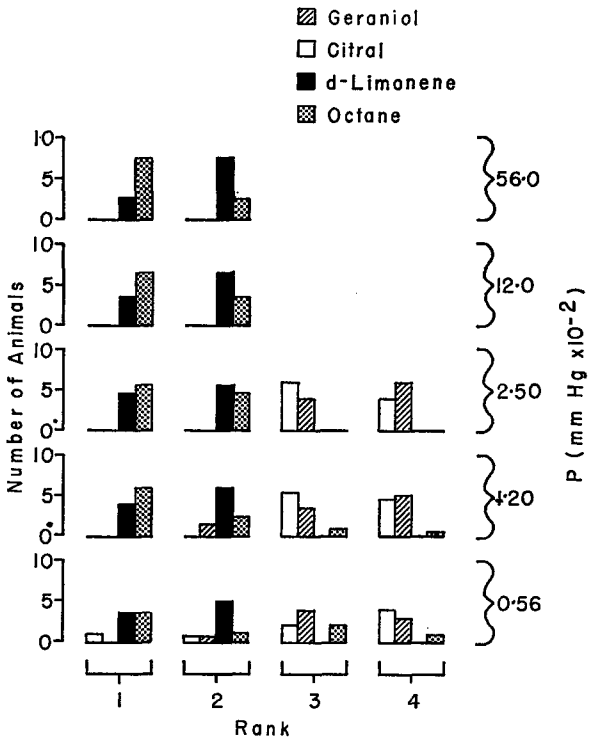


FIGURE 7. The number of animals in which the latency difference between the two nerve branches produced by a given chemical falls into a particular rank at a given partial pressure. The total number of animals is ten. A rank of "1" represents the shortest latency difference and "4" represents the longest.

*d*-limonene and octane falling more often into the lower ranks in each animal and citral and geraniol falling more often into the higher. This separation of the four chemicals into two pairs is most consistent at $2.5 \times 10^{-2}$ mm Hg where *d*-limonene and octane fall only into the two smaller ranks and geraniol and citral fall only into the two larger ranks. Only at the very highest concentrations is the variability reduced enough to show a statistically significant difference between octane and *d*-limonene. Nevertheless, of the six different possible comparisons that can be made of the four different chemicals, four of them show statistical significance at all concentrations measured. Thus,

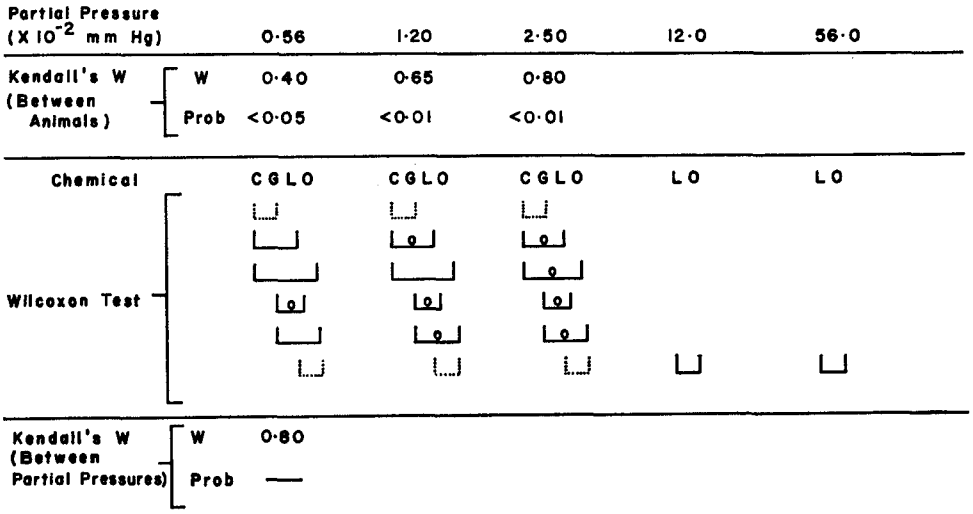


FIGURE 8. Summary of the statistical analysis of the latency difference data by Kendall's coefficient of concordance and the Wilcoxon matched-pairs signed-ranks test. See caption of Fig. 5 for an explanation of symbols, etc. (Probability for $W$ between partial pressures is not available because $N$ and $k$ are too small.)

the latency differences of these chemicals, like their LB/MB ratios, were consistently ordered, and this general order did not change with concentration.

A plot of the median magnitude of the latency differences as a function of concentration for different chemicals is given in Fig. 9. Only the citral

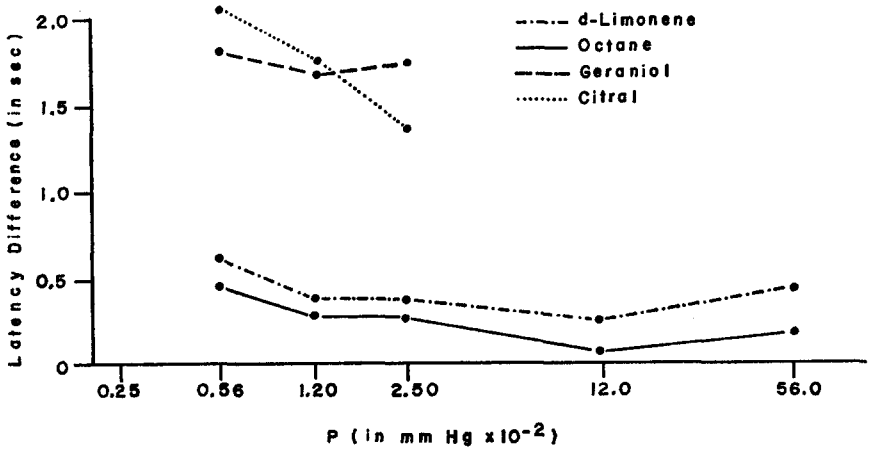


FIGURE 9. The median latency difference as a function of the partial pressure for each chemical. Each point is the median of at least thirty determinations; i.e., at least three from each animal (see footnote 2).

curve shows any consistent trend with partial pressure, and only the points at the lowest partial pressure ($0.56 \times 10^{-2}$ mm Hg) of both *d*-limonene and octane are statistically different from the other points on their respective curves ($P < 0.05 > 0.01$).[6] Nevertheless, even at these points the overriding relation between the magnitude of the latency difference and the molecular species is not masked; i.e., none of the median latency differences produced by octane and *d*-limonene equals or surpasses any of those produced by citral or geraniol.

## DISCUSSION

As was expected, the absolute magnitude of the responses, in distinction to the relative magnitude in different regions, was found to encode olfactory stimulus intensity. It was related to the logarithm of the stimulus partial pressure. Of greater interest to the present discussion is the demonstration of different spatiotemporal representations of different odorants at the level of the receptor sheet. This could provide a basis for olfactory quality encoding. Whether the animal actually uses this available spatiotemporal representation to discriminate odorants remains to be seen. However, a human observer watching only the traces coming from the Visicorder can easily identify the odorant eliciting any given response once he knows the code. If a foreign nervous system can discriminate and decode these patterns, one might expect the frog's own nervous system to do so.

The data suggesting a spatiotemporal pattern as a basis for olfactory quality discrimination are not in conflict with those data which have suggested selectively tuned receptors (2, 6) since the two mechanisms need not be mutually exclusive. Indeed, conceivably these two mechanisms can be superimposed (2). They might then complement each other by increasing the available number of neural discharge patterns with which to encode the vast number of discriminable odorants. The spatiotemporal encoding mechanism, if unsupported by tuned receptors, can take advantage of a comparison of discharge characteristics between mucosal regions in order to encode quality differences. However, these same discharge characteristics without such an interregional comparison could not by themselves encode quality. For instance, the response latency in one region alone could not be used to develop a code since the animal has no measure of the stimulus onset time. However, the time lapse between responses in different regions (i.e., the latency difference) can act as a differential coding symbol. Likewise, the magnitude of the response in any one given area would result in a poor code for quality since this same magnitude could be duplicated with other chemicals by varying their concentration. It is not the magnitude per se that appears unique to different chemicals but rather the relative magnitudes between different regions.

The number of different alternative spatiotemporal patterns with which to encode different stimuli is potentially much greater than that indicated by the present experiment. This experiment only sampled the activity of two regions of the receptor sheet on the dorsal wall of the olfactory sac. The receptor sheet taken as a whole would offer what could amount to a continuum of spatiotemporal patterns. These would depend upon the speed and the magnitude gradient of the wave of activity spreading across it. Indeed, geraniol and citral might have been differentiated from each other in this experiment if a larger group of nerve branches surrounding the entire olfactory sac and supplying a finer mosaic of mucosal areas were sampled.

The chemicals ordered according to their LB/MB ratios are in the exact reverse sequence as when they are ordered according to latency differences. Since there is a perfect negative correlation, there is the possibility that these two measures are actually measuring the same fundamental processes. But what is the fundamental process by which different chemicals elicit different spatiotemporal patterns? The spatial code alone could possibly be explained by a difference in the regional density of selectively tuned receptors. For instance, receptors highly sensitive to geraniol might be more concentrated in the areas subserved by the medial nerve branch, whereas *d*-limonene receptors might be more evenly distributed across the whole mucosa. However, although the regional concentrations of selectively sensitive receptors might explain the different LB/MB ratios for different chemicals, they cannot easily explain the different latency differences. A previously proposed discrimination mechanism (1, 4, 11, 12) that might explain these effects is that the molecules of different chemicals are differentially adsorbed or absorbed across the mucosa in accordance with their attraction to the media of the mucosa. In other words, the system would be analogous to gas chromatography. This mechanism would predict that some vapors would arrive at various points along the receptor sheet in a faster time and with relatively more molecules than would other vapors. This speculation would go on to suggest that the receptors are the detectors and they merely signal the relative activity in different regions without necessarily having any specificity of their own. To test this, the direction of the air flow through the sac was reversed and the ratio pattern was found to also reverse (12); i.e., geraniol and citral now gave larger responses on the lateral nerve branch than on the medial nerve branch. If the pattern depended upon the regional placement of selectively sensitive receptors, such a complete reversal of ratio pattern would not be expected. However, a sorption process could explain these data since the region absorbing or adsorbing the greatest number of molecules would change.

A sorption process might also explain the observed slight increase in ratios and slight decrease in latency differences as the partial pressure of geraniol

and citral is increased. This may start to overload the mucosa "sorption column" thus reducing the time for some of the molecules to travel to the more distal points and increasing the number of molecules getting there in a given unit of time.

One of the arguments against a gas chromatograph model for the olfactory mucosa is its comparatively short "column length." However, the surface devoted to odorant separation may be considerably larger than it appears. First, the surface of the nonolfactory nasal mucosa may also act as part of the column. Second, both the olfactory mucosa and nonolfactory mucosa are in some species highly convoluted. The apparent anecdotal positive correlation between the nasal surface area and the olfactory discrimination prowess of animals is brought to mind in this connection. Third, the olfactory gas chromatograph column may have several stationary phases for sorption. Not only is there a mucous phase, but there is also a vast number of cilia which, either on their own account or as supports for mucus, provide a very large surface area.

If the nose operates like a gas chromatograph, one might expect the latency differences and the LB/MB ratios produced by different chemicals to be correlated with their chromatographic retention times. One would predict that the greater the retention time for a given chemical, the greater would be the latency difference since it would take a longer time for the molecules to move between two given regions. On the other hand, one would predict that the LB/MB ratio would be smaller in the period represented by one "sniff" since fewer molecules of a high retention time chemical would be likely to reach the more distant region which, as stated previously, is subserved by LB. The relative retention times on a Carbowax column have been determined by Fuller et al. (5) for a large group of chemicals several of which are pertinent to the present study and are given below:

| | |
|---|---|
| Geraniol | 3.65 |
| Alpha-citral | 2.43 |
| Beta-citral | 2.00 |
| Limonene | 0.27 |
| Nonane | 0.064 |
| Pentane | 0.007 |

Assuming that octane would fall between pentane and nonane, the order of the above relative retention times predicts the order of the LB/MB ratios and the latency differences almost perfectly. Only the overlap between the ratios and between the latency differences for geraniol and citral is not predicted; however, the relative retention times of these two chemicals, as seen above, differ by a smaller factor than those of any other two chemicals

used in the present study. Thus a gas chromatographic model may actually explain why geraniol and citral would be more likely to overlap than the other combinations.

The apparent fit of the retention times with the latency differences and the LB/MB ratios requires some further clarification. The order of the relative retention time on a polar stationary phase, such as Carbowax, increases with both the dipole moments of the chemicals being separated and their boiling points (7). Thus, geraniol and citral, having the greater values in both of these properties, possess longer retention times than do *d*-limonene and octane. However, on a nonpolar stationary phase, boiling points and dipole moments operate in opposite directions in determining retention times. Thus, although the differences in the boiling points of these four chemicals may be large enough to keep the retention times in a nonpolar column in the same order as given above in spite of their dipoles, it is not as certain as on a polar column. Therefore, for a polar column the retention times of these four chemicals fit well with the LB/MB ratios and with the latency differences, and although the same conclusion is not precluded for a nonpolar column, it cannot be asserted conclusively. Consequently, even assuming the model of gas chromatography to be valid, it would not be possible to predict from the data of this paper alone which type of column the nose possesses, although one might lean toward the polar stationary phase.[7] However, as stated previously, there is the possibility of several stationary phases, each with its own sorption characteristics.

A word of caution is needed. Only four chemicals were used in this experiment and, therefore, it is quite possible that all the similarities noted between the animal data and the gas chromatograph data are fortuitous. However, when vapors pass over any surface, they will do so in different spatiotemporal patterns. It does not seem too unrealistic to think that the nervous system, in all its evolutionary wisdom, could come to take advantage of this common physical phenomenon.

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[7] One would expect that at least the mucus would be polar due to its glycoproteins and water.

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