Olfactory Stimulation Variables

Which Model Best Predicts the Olfactory Nerve Response?

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ABSTRACT Mozell et al. (1984. *J. Gen. Physiol.* 83:233–267) have examined the traditional manner in which olfactory stimulus-response relationships have been addressed. They developed a model that describes the olfactory nerve response as a function of three factors, viz., the number of odorant molecules (N), the stimulus duration (T), and the stimulus volume (V). In addition, two models derived from this three-variable model were also found to predict the response well. These were the [F, N] model involving flow rate ($F = V/T$) and, ranking closely behind, the [C, T] model involving concentration ($C = N/V$). A model involving the delivery rate ($D = N/T$) and volume was found to predict the response poorly. These models imply very different stimulus-response relationships. The present study was designed to assess the validity of this earlier approach by testing specific predictions drawn from each of the models. Because of the excellence of the [F, N] model, one would predict that the response will not change when F and N are held constant in spite of proportional increases in V and T. Similarly, one would predict from the [C, T] model that the response will be constant when C and T are held constant in spite of proportional increases in N and V. Because of the poor showing of the [D, V] model, one would predict changes in the response even when D and V are held constant while N and T are increased proportionately. It was observed that when F and N were held constant, the response was, in fact, constant. When D and V were held constant, the response increased dramatically. When C and T were held constant, there was a statistically significant, but small, change in the response. These results support the approach taken by Mozell et al. (op. cit.) and highlight the applicability of the [F, N] model to peripheral olfactory processing. The results are discussed in terms of their impact on the traditional manner in which olfactory stimulus-response relationships are conceived.

INTRODUCTION

The standard, oversimplified notion about the initial events in olfaction is that odorant molecules in air dilution enter the nose, contact receptors, and induce

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a level of excitation in accordance with their quantity. This notion overlooks the complicating effects of a formidable number of stimulus access features, such as the volume and flow rate of the carrier airstream, the duration of the flow, and the loss in odorant quantity as the molecules sorb along the respiratory mucosa on the way to the olfactory mucosa. For instance, Stuiver's (1958) approach indicated that the flow rate of odorant through the nasal cavity plays an important role in determining not only the fraction of the inspired air reaching the olfactory mucosa, but also how much of the odorant in that fraction has enough time to interact at the mucosal surface. As another example of how initial olfactory events can be complicated by access features, Hornung and Mozell (1977) found that odorants are not deposited evenly across the olfactory mucosa and that this deposition is consistent with each odorant's mucosa/air partition coefficient (Hornung and Mozell, 1981). This unequal deposition is reflected in the discharges of the olfactory nerve branches supplying different regions of the olfactory mucosa (Mozell, 1966, 1970) and is the basis for suggesting the participation of a chromatographic-like process in olfactory quality coding (Mozell and Jagodowicz, 1973). The present study is part of a continuing series that challenges the standard, simple notion of initial olfactory events by providing descriptions of how the olfactory response is related to the stimulation features with which the odorant molecules gain access to the olfactory mucosa. This study focuses upon six interrelated stimulation variables: the number of odorant molecules (N), the presentation time (T), the volume of the carrier airstream (V), the concentration of the odorant molecules (C), the flow rate of the carrier airstream (F), and the delivery rate of the odorant molecules (D). A previous study (Mozell et al., 1984) derived a number of different models involving different combinations of these six variables and ranked them in terms of how well they predicted the neural response. This study more demandingly tests the adequacy of several of these models by determining whether specific predictions based upon them are indeed observed. In testing the adequacy of these several models, this study, using an approach quite different from the earlier studies, in essence, also tests the validity of this earlier approach and its findings.

Basic to the line of investigation represented by this paper and earlier ones (Mozell et al., 1984, 1985) is the confusion that arises when attempting to determine the contribution of each of the above-listed variables to the magnitude of the olfactory response. For instance, flow rate is a variable very often manipulated in olfactory research but is itself a ratio of two other variables, viz., stimulus volume to stimulus duration ($F = V/T$). Thus, the change in response seen with a change of flow rate could be due to an effect of flow rate per se or to the individual effects of either volume or time. If the change in response were an effect of flow rate per se, a doubling of the flow rate would give the same change in response no matter how that doubling was achieved. That is, there would be the same change in the response for doubling volume at constant time as for halving time at constant volume. If the response does not change in the same way under these conditions, it can be assumed that volume or time must have some separate effect not incorporated in the effect of flow rate. Similar questions apply to the stimulus delivery rate and stimulus concentration, both of

which are also ratios of two different variables. The delivery rate is the number of odorant molecules per stimulus duration ($D = N/T$) and the concentration is the number of odorant molecules per stimulus volume ($C = N/V$).

Because the concentration, delivery rate, and flow rate are ratios of but three other variables (time, volume, and number of odorant molecules), a change in any one of these six variables necessitates changes in the others. To increase the concentration at a constant stimulus volume and stimulus duration, the number of odorant molecules must be increased. This would in turn increase the delivery rate. On the other hand, increasing the concentration by decreasing the stimulus volume, while holding the number of molecules and the time constant, leads to a decrease in the stimulus flow rate. Thus, when a given stimulation variable is manipulated, any resultant change in the response magnitude could be ascribed not only to the variable being singled out, but to other variables as well. The interrelationships of these variables has complicated the interpretation of previous studies. The interpretation of many of these studies has been reviewed by Mozell et al. (1985).

Recently, Mozell et al. (1984) systematically studied, in the bullfrog, the dependence of the magnitude of the summated olfactory nerve discharge upon the six stimulation variables noted above. They designated the concentration, flow rate, and delivery rate as "derived variables," whereas the stimulus volume, stimulus duration, and number of odorant molecules were designated "primary variables." The designation for the two groups of variables was not meant to indicate their relative importance in determining the response but rather to stress that the first set of variables is derived by taking ratios of the second set. Mozell et al. (1984) chose two levels of each of the three primary variables and, by taking all combinations (2^3), defined eight different sniffs that were artifically produced in the frogs by a negative pressure system. By performing an analysis of variance of the logs of the summated multiunit discharges in response to the eight sniffs, Mozell et al. were able to model the response as a multiplicative function of the three primary variables as in Eq. 1:

$$R = A \times N^{0.350} \times V^{-0.279} \times T^{0.216} \times E, \tag{1}$$

where R is the magnitude of the response, A is a constant, E is a log normal error term, and N, V, and T are the three primary variables, viz., the number of odorant molecules, the stimulus volume, and the stimulus duration, respectively. The exponent on each primary variable quantifies the effect of that variable upon the response. Note that the analysis of the data addressed the possibility of interactive effects but none were found.

The effect (i.e., exponent) of each derived variable was, as determined by the method of least squares, the average of the exponents of the primary variables from which that derived variable was generated (Mozell et al., 1984). For example, the estimated exponent on concentration was the average of the estimated exponent on the number of odorant molecules and the negative of the exponent on stimulus volume. (The negative of the exponent on stimulus volume was required since the volume term appears in the denominator of the concentration term.) The actual ability of a derived variable to incorporate the combined

effects of two primary variables depended upon the exponents on the primary variables. That is, if these exponents were equal in magnitude and opposite in sign, the effect of the derived variable adequately incorporated the combined effects of the primary variables. This was nearly the case for both the concentration and the flow rate. Therefore, in the study of Mozell et al. (1984), the three-variable model reduced to two two-variable models: one involving the concentration and the time (i.e., the [C, T] model) and one involving the flow rate and the number of odorant molecules (i.e., the [F, N] model). By further statistical analysis, Mozell et al. (1984) concluded that although the [C, T] model ranked a little lower than the [F, N] model in predicting the observed responses, the two models did not differ significantly. Both models were at least as good as the full three-variable model. In contrast to the [C, T] and [F, N] models, the two-variable model involving the delivery rate and volume (i.e., the [D, V] model) was particularly poor at predicting the observed responses. This occurred because the exponents on N and T were neither equal in magnitude nor opposite in sign. Thus, the effect of the delivery rate did not even come close to incorporating the combined effects of the number of odorant molecules and time. Note that although these three two-variable models and the full three-variable model incorporate all three of the primary variables, they make very different statements concerning the stimulus-response relationship. Each of the reduced models requires that the effects of a different pair of primary variables be equal and opposite, whereas the full three-variable model has no such requirement (Mozell et al., 1984).

This approach led Mozell and co-workers to conclude that the [F, N] and [C, T] models arranged the effects of the three primary variables in such a manner as to best reflect the physiological and physicochemical processes governing the growth of the summated multiunit discharge. It is the purpose of this study to assess the results of Mozell et al. (1984) by testing particular hypotheses drawn from their work.

HYPOTHESES, RATIONALE, AND DESIGN

As discussed above, a major result of the experiment by Mozell et al. (1984) was that the [F, N] and [C, T] models were found to be excellent predictors of the response and, therefore, adequate reductions of the three-variable model. If indeed the [F, N] model predicts the response well, the response should not change when the flow rate and the number of molecules remain constant in spite of changes in volume, time, concentration, and delivery rate. Similarly, if the [C, T] model predicts the response well, the response should not change when the concentration and time remain constant, despite changes in the flow rate, delivery rate, number of molecules, and volume. Both models predict that the response will not change when the two variables included within each model remain constant, regardless of what happens to the other variables. In contrast to the [F, N] and [C, T] models, the [D, V] model was found by Mozell et al. (1984) to be a poor reduction of the three-variable model. Therefore, the response magnitude would be expected to change when the delivery rate and volume are held constant and the remaining variables are altered. These predictions for the [F, N], [C, T], and [D, V] models form the basis for the experiments in the present study.

Three sets of sniffs were developed. In the first set, the flow rate and the number of molecules were held constant while the volume and time were increased proportionately

(the concentration and delivery rate must therefore decrease). In the second set, the concentration and time were held constant while the number of molecules and volume were increased proportionately (the flow rate and delivery rate must therefore increase). In the third set, the delivery rate and volume were held constant while the number of molecules and time increased proportionately (the concentration must therefore increase and the flow rate must therefore decrease). This experimental design allowed a critical test of the results of the Mozell et al. (1984) study using an approach quite different from the former study. Furthermore, this approach might more stringently assess whether there is a significant difference in the predictive ability of the $[F, N]$ and $[C, T]$ models, a question that was left unresolved by Mozell et al. (1984). In addition, the range over which the levels of the primary variables were chosen was expanded beyond that in the Mozell et al. (1984) study. This expanded from two- to eightfold, increasing the range over which the predictive abilities of the various models were tested.

TABLE I

Sniff Variable Combinations for Experiments A and B

		Variables*					
Sniff set	Sniff	N	T	V	C‡	F§	D‖
$[F, N]$	1	2×	0.18	0.22	8×	77	8×
	2	2×	0.35	0.45	4×	77	4×
	3	2×	0.70	0.90	2×	77	2×
	4	2×	1.40	1.80	1×	77	1×
$[C, T]$	5	0.5×	0.35	0.11	4×	19	1×
	6	1×	0.35	0.22	4×	38	2×
	7	2×	0.35	0.45	4×	77	4×
	8	4×	0.35	0.90	4×	154	8×
$[D, V]$	9	1×	0.18	0.45	2×	154	4×
	10	2×	0.35	0.45	4×	77	4×
	11	4×	0.70	0.45	8×	38	4×
	12	8×	1.40	0.45	16×	19	4×

* Sniff variables were defined as follows: N = number of molecules relative to 1.79×10^{16} molecules; T = time in seconds; V = volume in cubic centimeters; C = concentration relative to 1.99×10^{16} molecules/cc; F = flow rate in cc/min; D = delivery rate relative to 2.56×10^{16} molecules/s. The underlining denotes the variables held constant during each sniff set.

‡ $C = N/V$.

§ $F = V/T$.

‖ $D = N/T$.

METHODS

Sniff Definition

As described in the preceding section, three sets of sniffs pertinent to testing the predictive ability of the $[F, N]$, $[C, T]$, and $[D, V]$ models were presented to each animal. These sniffs are listed in Table I. Within each set of sniffs, one primary and one derived variable were held constant (underlined values) by increasing proportionately the two remaining primary variables. In the first sniff set (testing the $[F, N]$ model), the flow rate and the number of molecules were held constant while volume and time were increased by twofold steps. The concentration and delivery rate, by necessity, decreased in twofold steps. In the

second sniff set (testing the [C, T] model), the concentration and time were held constant while the number of molecules and volume were increased in twofold steps. This simultaneously also increased the delivery rate and flow rate in twofold steps. In the last sniff set (testing the [D, V] model), the delivery rate and volume were held constant while time and the number of molecules were increased in twofold steps. By necessity, the concentration increased and the flow rate decreased, again in twofold steps. Note that sniffs 2, 7, and 10 were the same, having identical values for the six variables. The commonality of these three sniffs provided an anchor for the three sniff sets. Thus, there were 10 different sniffs, each having a different combination of values for the primary and derived variables.

The values of the primary and derived variables were chosen from the bullfrog's normal repertoire. That is, the durations, flow rates, and volumes were chosen to be within the range normally produced by the bullfrog, as determined using a hot-wire anemometer mounted at the frog's external naris (Hornung et al., 1980*a*). The number of odorant molecules, and therefore the concentration and delivery rate, were chosen to be within the frog's dynamic range of olfactory responses to *n*-octane as calculated from Mozell's (1970) data.

n-Octane (chromaquality; Matheson, Coleman and Bell, Cincinnati, OH) was chosen as the odorant in this study for a number of reasons. First, Hornung et al. (1980*b*) reported that octane's mucosa/air partition coefficient showed that octane favored the air phase much more than did the coefficients of the other odorants tested. The data suggested that the more an odorant's partition coefficient favors the air phase, the less its molecules will be depleted as it moves across the mucosal surface and the more uniformly will its molecules be distributed across the surface. Indeed, octane, as compared with some other odorants, did show the least such depletion and the least differential distribution across the mucosa (Hornung and Mozell, 1981). Thus, octane seemed to be the odorant of choice to minimize any response variability that might be due to variations in the size or geometry of the olfactory sacs of different frogs. In addition, octane was found to be rapidly removed both from the mucosa (Hornung and Mozell, 1977) and from the tubing of the delivery system. Thus, octane allows repeated presentations with minimal chance of long-term contamination and with minimal interstimulus time required for purging.

Stimulus Delivery

As shown in Fig. 1, two pneumatically driven, Teflon slide valves, S1 and S2 (Altex Automatic Slider Valves, Ranin Instrument Co., Inc., Woburn, MA) were central to the stimulus delivery system. This system was virtually identical to that of Mozell et al. (1984). In the intersniff resting condition, vacuum pump R drew humidified air at 20 cc/min from port 3 to port 2 of valve S1 through the frog's olfactory sac and from port 4 to port 3 of valve S2 (Fig. 1, solid lines). Simultaneously, odorized air was drawn from the output of the olfactometer (tube A) from port 1 to port 4 of valve S1 by the house vacuum line. The flow rate was also 20 cc/min. At the same time, vacuum pump S was set to draw air at the flow rate scheduled for the next sniff. Air traveled through a variable resistance and then from port 2 to port 1 of valve S2. This variable resistance was altered in each experiment to match the resistance to airflow through the frog.

In the stimulation condition (Fig. 1, dotted lines), the ports of the valves were reconnected so that odorized air was drawn through the frog's olfactory sac from port 1 to port 2 of valve S1, and from port 4 to port 1 of valve S2. This was done at the preset flow rate for the sniff by vacuum pump S. In the stimulation condition, humidified air was drawn away from the animal from port 3 to port 4 of valve S1 by the house vacuum, and vacuum pump R drew room air through valve S2 (port 2 to port 3).

The timing of valves S1 and S2 was controlled by solenoid-activated pneumatic valves, which were in turn controlled by an S88 Grass stimulator (Quincy, MA). The timing of the valves was checked before each experiment. A flow dilution olfactometer was used to give the appropriate concentration of *n*-octane, which, with the controlled sniff volumes, produced the required number of odorant molecules for each sniff (Mozell et al., 1984).

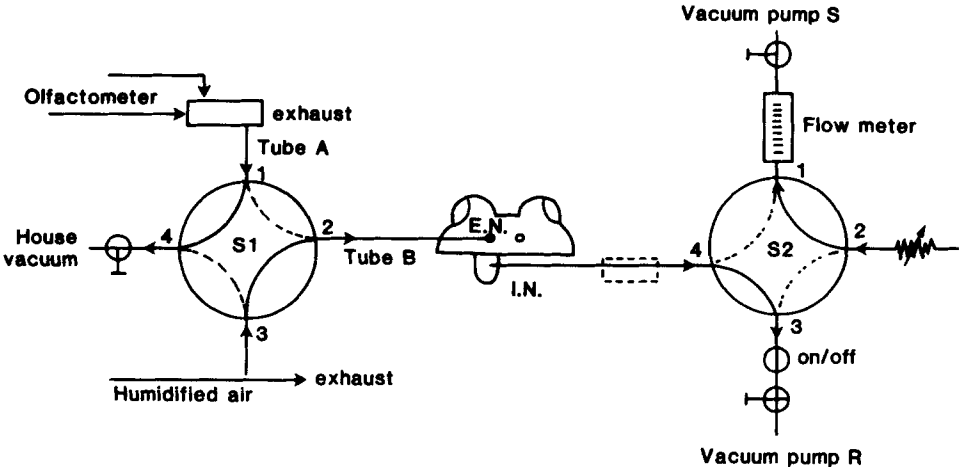


FIGURE 1. Sniff delivery system. Valves S1 and S2 are shown in the "rest" condition by the solid lines and in the "stimulation" condition by the dotted lines. The flow rates produced by the house vacuum and by vacuum pump R were kept constant at 20 cc/min and monitored by in-line flow meters. The dashed-line box shows where the anemometer was inserted in-line to monitor the sniff profiles. Vacuum pump S produced a variable flow rate, which was set in accordance with the flow rate of the next sniff to be presented. The variable resistance (valve S2, port 2) was used to balance the resistance through the valve and the frog. Tube A leads from the mixing chamber of the olfactometer to port 1 of valve S1. Tube B (10.2 mm long) leads from valve S1 (port 2) to the frog's external naris. I.N., internal naris; E.N., external naris.

During the intersniff rest condition, all the tubes and valve ducts coming in contact with the odorized air were deodorized by appropriately connecting valve ports to draw humidified, purified air through the contaminated areas.

Verification of Flows and Durations

The flow profiles for the sniffs in experiments A and B were verified using a hot-wire anemometer (Fig. 2). The placement of the anemometer is shown by the dashed-line box in Fig. 1. Each flow profile in Fig. 2 was in good agreement with the requirements set forth in Table I.

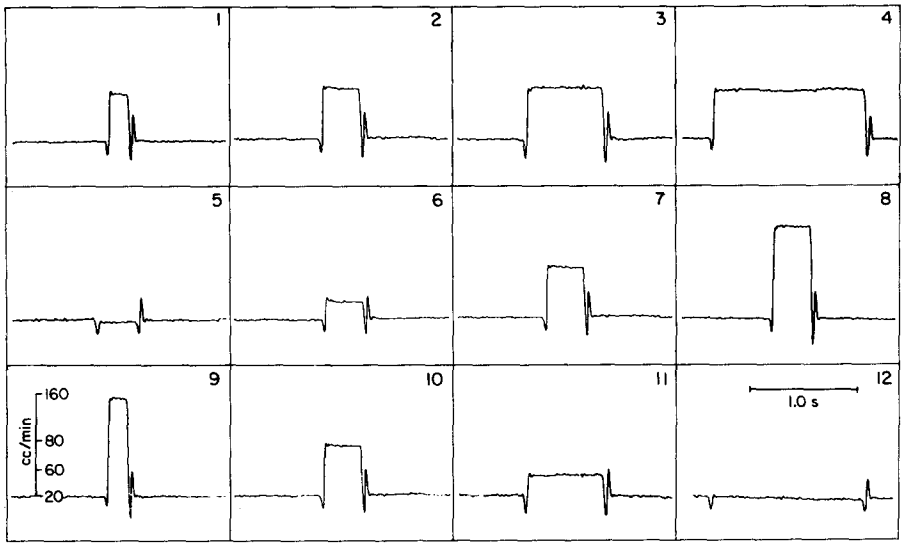


FIGURE 2. Flow profiles for experiments A and B. The figure shows the 10 sniff profiles produced in response to the 10 sniffs described in Table III. These are plotted with flow rate as a function of time. In each case, the sniff profiles are embedded within a constant background flow of humidified air of 20 cc/min. The transients that occur after each sniff are created by the partial vacuum developed during switching to humidified air. They represent no additional source of odorous stimulation. Vertical calibration, cc/min. Horizontal calibration, 1 s.

FIGURE 3. (*opposite*) Chromatograms produced in response to sniffs 1–12. Because stimuli 2, 7, and 10 are identical, the chromatograms for these three sniffs are the same. The number to the right of each chromatogram indicates the relative gain at which each chromatogram was recorded. Chromatograms 1–4 are responses to sniffs with equal number of molecules. Chromatogram 4 is shorter and wider, probably because it was injected into the collection loop in the largest volume and therefore into the gas chromatograph (GC) column over the longest time. Chromatograms 5–8 and 9–12 are in response to sniffs that increase in the number of molecules in twofold steps. Taking the relative gains into account, it can be seen that the number of molecules in each sniff is double its predecessor. By calibrating the gas chromatograph, the average numbers of molecules ($\times 10^{16}$) contained in each sniff (1–12, respectively) were as follows: 3.35, 3.53, 3.77, 3.54, 0.78, 1.56, 3.53, 6.94, 1.79, 3.53, 6.65, 13.56. In no case were these averages significantly different from the requirements listed in Table I. The gas chromatograph was a Varian 940 fitted with a 5% SE-30 column supported on Chromosorb W (6 ft. × ⅛ in. column). All chromatograms were recorded at the same gain of the detector amplifier, with the exception of chromatograms 9, 11, and 12, as noted above. Other operational GC settings were: column temperature, 175°C; injector temperature, 175°C; detector temperature, 225°C; nitrogen flow rate, 30 cc/min; hydrogen flow rate, 30 cc/min; air flow rate, 300 cc/min.

To verify that the correct number of molecules was presented to the animal, the frog was replaced by an odorant collection loop and Teflon slide valve. These were used to first collect the odorant content of the sniffs and then elute that content into a Varian 600 gas chromatograph (Palo Alto, CA). The same sniffs were presented to the collection loop that were presented to the frogs, and the resultant chromatograms are shown in Fig. 3. By calibrating the gas chromatograph with 0.01-μl injections of liquid octane, the actual number of molecules in each sniff was determined. These values are given in the caption to Fig. 3.

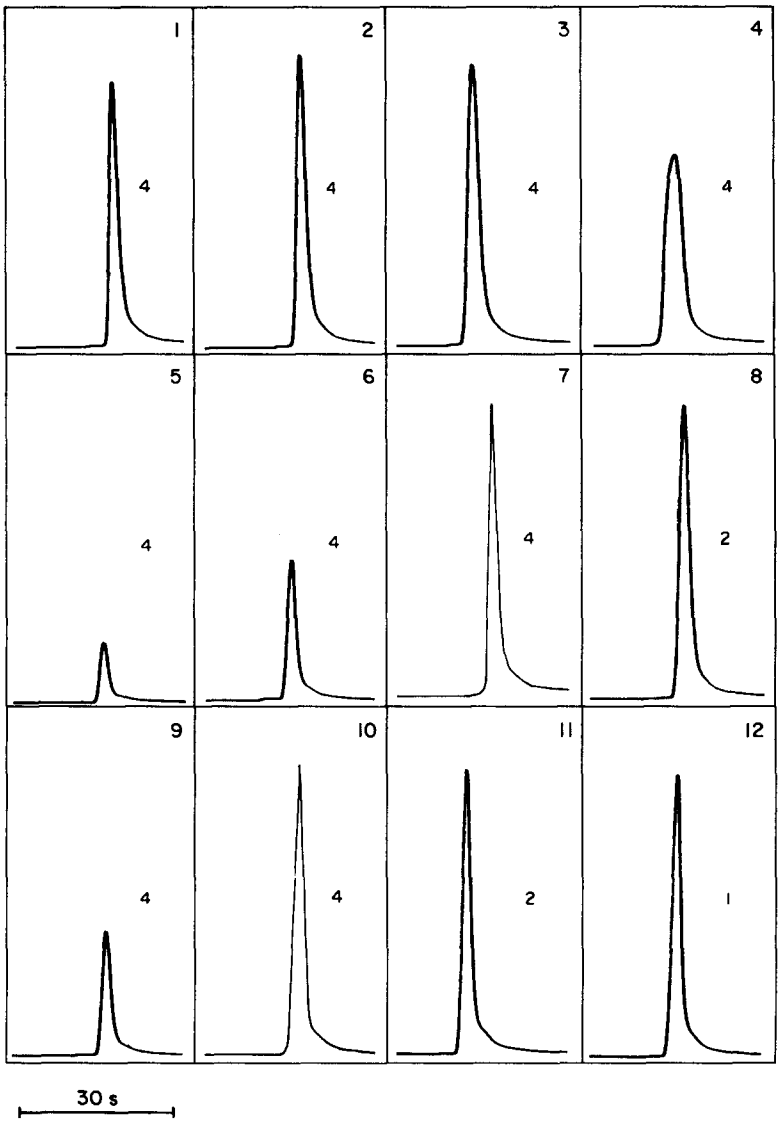


FIGURE 3.

Preparation and Recording Procedures

Bullfrogs (*Rana catesbeiana*) were anesthetized with urethane (1.2 g/100 g body weight). The anesthetized animal was wrapped in a wet towel and fixed in a small-animal head-holder (DKI, Inc., Tujunga, CA) adapted for use with frogs. The olfactory nerves were exposed caudal to the cribriform plate. The tube entering the frog's external naris (tube B, Fig. 1) and a small glass cup made to cover the frog's internal naris were cemented in place using dental cement (Durelon, Premier Dental Products Co., Norristown, PA). The cup was designed as a trap to collect nasal mucus and thus prevented clogging of the vacuum line.

The active electrode was placed on either the medial (experiment A) or the lateral (experiment B) aspect of the olfactory nerve. Six animals were used in each experiment. The indifferent electrode was placed on a piece of cotton, which rested on the skull and was soaked with Ringer's solution. The multiunit electrical activity of the olfactory nerve was recorded and amplified with a P5 AC preamplifier (Grass Instrument Co.) on the push-pull setting. Both the active electrode (63 μm diam) and the indifferent electrode (127 μm diam) were stainless steel and were quadruply coated with enamel to the tip. The amplified electrical activity was filtered below 35 cycles/s and above 500 cycles/s. It was passed through a self-discharging integrator, giving a summated output proportional to the sampled neural activity (Beidler, 1953). The charging and discharging time constants of the self-discharging integrator were 0.250 and 1.9 s, respectively. The summated activity was recorded on magnetic tape using a 5600C FM tape recorder (Honeywell Inc., Fort Washington, PA). This FM recording was then passed through a 10-bit A/D converter and stored on a disk in a PDP 11/34 computer (Digital Equipment Corp., Marlboro, MA). The digitized data were displayed graphically on a graphics computer terminal. The starting and ending points of each response (i.e., the trace of the summated activity) were marked with a light pen and fed into the computer. The computer then determined the area under each trace of summated activity. This procedure was repeated three times for each response and the average area was calculated.

Control for Changes over Time

Each of the 10 different sniffs listed in Table I was presented twice to each frog. The recording sessions lasted for 3–4 h. It was noted in a number of animals that the overall magnitude of the responses gradually changed over time. To prevent this gradual change from having a consistent effect upon the responses to any one sniff within and across animals, the 10 sniffs were presented to each frog first in a randomized sequence and then in the reverse order of that randomized sequence. Standard sniffs were presented at the beginning of the first sequence of sniffs, at the end of the second sequence of sniffs, and between the two sequences. The percentage change in the standards was used in conjunction with linear interpolation to correct for the gradual changes over time.

Protocol

The olfactometer, the vacuum pump, and the timing of the valves were adjusted as described above to produce the sniff called for in the upcoming presentation. During this intersniff rest condition, the nasal passages were flushed with deodorized, humidified air at 20 cc/min. The sniff was presented 3.25 min after the onset of the last clean-out procedure. Immediately after the sniff, the delivery system returned to the rest condition, which again flushed the nasal passages with humidified air. The clean-out of the contaminated tubing and valves was initiated 0.75 min later. This lasted for 1.5 min, after which preparation was made for the next stimulus. The total time between the end of one sniff and the beginning of the next sniff was 5.5 min. Note that, except for the time during

which the sniff was actually being presented, humidified air was drawn through the animal at a rate of 20 cc/min.

Experiments A and B

This study consisted of two experiments, A and B. Experiment B differed from experiment A only in that the summated neural discharge was recorded from the lateral aspect of the olfactory nerve rather than, as in experiment A, from the medial aspect. Mozell et al. (1984) had argued, from previous work (Mozell, 1964*b*, 1966, 1970), that an electrode on the medial aspect probably recorded from receptor cell axons originating near the external naris, and therefore recorded the sniff-evoked activity just as the bolus of odorized air entered the olfactory sac. This was thought to be important because factors such as dilution, sorption, and turbulence could change the profile of the odorant bolus as it passed through the nose, and it is conceivable that these changes could affect the stimulus-response relationships as recorded from different points along the flow path. It was also conceivable that even if the bolus did not change as it moved across the olfactory mucosa, different parts of the olfactory mucosa could respond to the same odorant bolus differently since odorant selectivity varies across the mucosa (Mackay-Sim and Kubie, 1981). To evaluate whether such concerns were warranted, the recording position was moved from the medial to the lateral aspect of the olfactory nerve in experiment B. There was reason to believe, as is indicated below, that the fibers sampled on the lateral aspect of the olfactory nerve originate farther along the mucosal flow path than do those sampled on the medial aspect.

If the results from the two regions of the mucosa do differ, one would be faced with identifying which of the possibilities (a regional difference in sensitivity or one of the factors able to alter the profile of the odorant bolus) underlies the regional variation in the stimulus-response relationship. This identification would be left to future studies; the only question being raised here is whether there should be any concern about a possible dependence of the stimulus-response models upon the mucosal regions sampled.

It should be noted in this regard that one possible contributor to such a dependence (viz., the differential distribution of odorant molecules across the mucosa) is circumvented in this study by using *n*-octane. As discussed previously, octane is an odorant that is not strongly sorbed to the olfactory mucosa so that there is a fairly uniform concentration along the entire flow path. Therefore, if the results of experiments A and B differ, at least one possible explanation for this difference can be ruled out.

Verification of Different Receptor Cell Populations Sampled in Experiments A and B

The comparison of the results of experiments A and B was predicated upon the idea that the sampled responses in the two experiments would originate from different parts of the mucosa. Although Mozell (1964*b*) demonstrated that such differences existed for the branches of the olfactory nerve rostral to the cribriform plate, he did not specifically address whether this topographical organization was continued in the trunk of the olfactory nerve caudal to the cribriform plate. Therefore, it was necessary to verify that the two placements of the electrodes in the trunk of the olfactory nerve did indeed sample activity originating from different mucosal regions. The verification test was based upon the following earlier studies.

Hornung et al. (1980*b*) and Hornung and Mozell (1981) described how the molecules of different odorants were separated along the flow path in accordance with each odorant's mucosa/air partition coefficient. The more the partitioning of the odorant favored the mucosa, the more readily were its molecules sorbed to the mucosal surface at the point of

entry into the olfactory sac and the fewer were the odorant molecules that reached points farther along the flow path. This produced different gradients of neural activity (Mozell, 1970) beginning at the point of entry into the olfactory sac and decreasing to the point of exit. It followed from these observations that the observed gradients across the mucosa depended upon the direction of air flow through the olfactory sac. The test of whether the two electrodes sampled different regions of the mucosa took advantage of these activity gradients.

Electrodes were placed both on the medial and lateral aspects of the olfactory nerve in positions identical to those used in experiments A and B. Puffs of odorants were given either into the external naris or into the internal naris. The odorants used were *d*-limonene, carvone, and butanol. *d*-Limonene was known to produce a near-uniform level of activity across the mucosa, whereas carvone and butanol were known to produce rather steep gradients (Mozell, 1970).

RESULTS

Verification of Mucosal Regions Sampled in Experiments A and B

Fig. 4 verifies that the electrode positions used in experiments A and B did sample activity from different regions of the mucosa. *d*-Limonene produced, as expected, approximately equal responses at the medial (M) and lateral (L) recording sites when presented to either the internal (INT) or external (EXT) naris. This finding presumably reflected the even distribution of *d*-limonene molecules in the olfactory mucosa. The responses to carvone and butanol differed considerably from that to *d*-limonene. When butanol and carvone were presented through the external naris, the response was much larger at the medial recording site than at the lateral recording site. When either odorant was presented to the internal naris, the response recorded was much larger at the lateral recording site. The observation that *d*-limonene gave good size responses at both positions, no matter what the direction of flow, indicated that the airstream carrying either the butanol or carvone molecules had access to both mucosal regions. Relating these results to previous findings discussed above, it appears that the medial recording site sampled activity from a group of receptor cell axons closer to the external naris and the lateral recording site sampled the activity of receptor cell axons nearer to the internal naris.

In addition, this site verification experiment argued against there having been any appreciable electrotonic spread between the recording sites. This appeared to be ruled out by the many cases in which a large response was recorded from one position with no response recorded from the other position.

Experiment A: Medial Aspect of the Olfactory Nerve

Fig. 5 gives the results of experiment A, which tested, at a region near the external naris, the predictive ability of the $[F, N]$, $[C, T]$, and $[D, V]$ models. For each of the tests, the mean $\log_2$ response is plotted as a function of twofold increases of a different pair of primary variables. Therefore, the relationships are shown as double-logarithmic plots. The primary and derived variables held constant are indicated under each curve (see Table I), as are those variables that either increased or decreased in twofold steps. Note that, in accordance with the

experimental design, holding one derived variable and one primary variable constant while proportionately changing the other primary variables, of necessity, also changed the remaining derived variables.

Fig. 5 shows that with the flow rate and the number of molecules held constant, the magnitude of the responses remained essentially constant in spite of changes

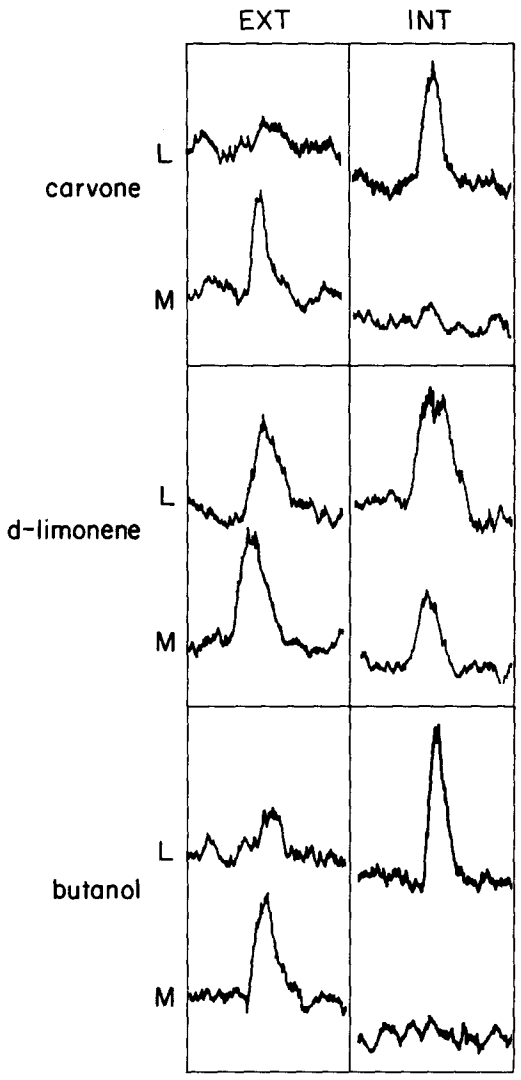
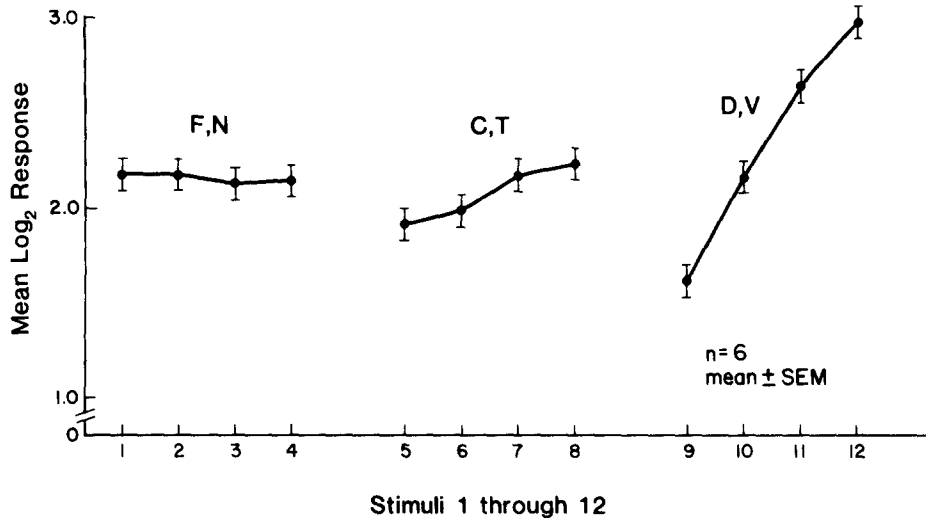


FIGURE 4. Reversal of responses showing separation of recording sites. L, activity recorded from the lateral aspect of the olfactory nerve; M, activity recorded from the medial aspect of the olfactory nerve; EXT, odorant presented via the external naris; INT, odorant presented via the internal naris. See text for explanation.

in the other variables. The sniffs with constant concentration and time tended to produce slightly larger responses as number of molecules and volume increased proportionately. When the delivery rate and volume were held constant, the responses became substantially larger with the proportional increases in the number of molecules and time.

Two procedures were used to test whether the slopes in Fig. 5 were significantly different from zero (Table II). In the first procedure, the least-squares estimates of the three slopes along with their standard errors were calculated. The slope



Constant	F,N	C,T	D,V
Increase	T,V	N,V,F,D	N,T,C
Decrease	C,D	—	F

FIGURE 5. Results of experiment A. Mean $\log_2$ response plotted against the primary variables, which increase in twofold steps (see Table I). The standard error of the mean (SEM) is derived from the ANOVA and is equal to the square root of the error mean square divided by the number of animals (n = 6). See text for further explanation.

of the curve testing the [F, N] model was the only one not significantly different from zero. The curve testing the [D, V] model had a slope that was very different from zero. Although the slope of the curve testing the [C, T] model was different from zero, it was small, especially when compared with the slope of the curve testing the [D, V] model. In the second procedure, the sums of squares due to slope for the three curves were calculated using an analysis of variance. The curves testing the [C, T] and [D, V] models contributed significantly to the total sums of squares, whereas the curve testing the [F, N] model had a sum of squares due to slope not different from that of the error term. Again, this indicated that only the curve testing the [F, N] model had a slope not different from zero.

It appears that, of the three predictions made earlier in the Introduction, two have received outright confirmation: (*a*) when the flow rate and the number of odorant molecules were held constant, the response did not change with changes in the other variables, and (*b*) in spite of holding the delivery rate and volume constant, the responses changed considerably when the other variables were changed. The third prediction was not strictly upheld. That is, when concentration and time were held constant, there was (contrary to the prediction) a small but significant change in the response size with changes in the other variables.

TABLE II

*Slope analysis: Experiment A**

Least-squares estimate of the slope

Curve	Slope	SE
F, N	−0.017	0.036
C, T	0.116	0.036
D, V	0.462	0.036

Analysis of variance of the slope

Curve	Sum of squares slope	Degrees of freedom‡	MS	*F*	*P*
F, N	0.0085	1	0.0085	0.2	NS
C, T	0.40	1	0.40	10.4	<0.005
D, V	6.4	1	6.4	166	≪0.005
Error	1.73	45	0.038		

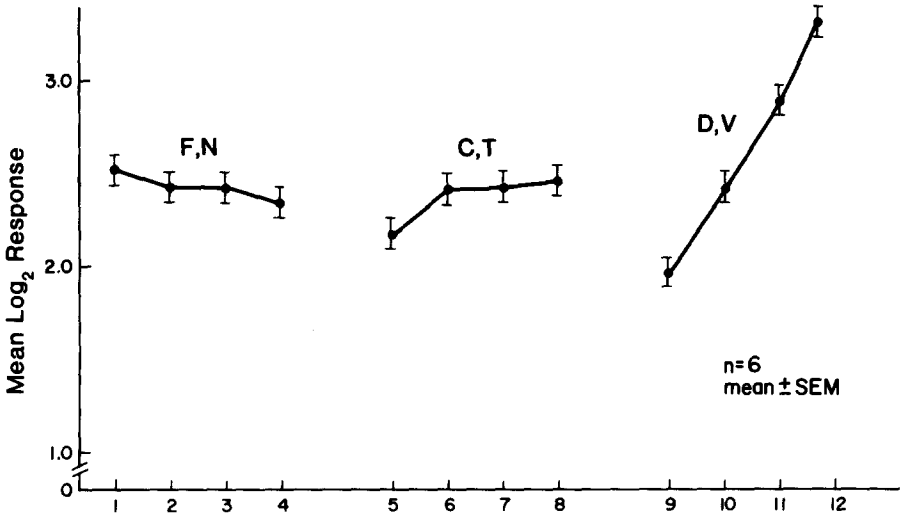
* The comparison of the three curves were not orthogonal in either experiments A (Table II) or B (Table III) since points 2, 7, and 10 were all the same (see Table I). A correlation of $r = 0.05$ existed between each of the sets of stimuli. This led, as calculated by the over-representation of treatment sums of squares in the analysis of variance, to a redundancy of, at most, 0.04%. It is felt that this error is negligible and will not change any of the conclusions drawn.

‡ There were (10 sniffs − 1) × (6 animals − 1) = 45 error degrees of freedom, (10 sniffs − 1) × 6 animals = 54 total degrees of freedom, and (10 sniffs − 1) = 9 treatment degrees of freedom, of which 1 degree of freedom was attributed to each of the three slopes.

However, the apparent failure of the [*C, T*] model did not depart far from the results of the Mozell et al. (1984) study, which ranked the predictive ability of the [*C, T*] model below that of the [*F, N*] model but found no statistically significant difference between them.

Experiment B: Lateral Aspect of the Olfactory Nerve

Experiment B was conducted in the same fashion as experiment A, with the exception that the responses were recorded from the lateral aspect of the olfactory nerve and therefore probably represented activity originating from the mucosal region near the internal naris. The results of this experiment are shown in Fig. 6. Again the curves testing the [*F, N*] and [*C, T*] models showed little if any slope, whereas the curve testing the [*D, V*] model was rather steep. The statistical analysis of these data (Table III) showed that, as in experiment A, the



Constant	F,N	C,T	D,V
Increase	T,V	N,V,F,D	N,T,C
Decrease	C,D	—	F

FIGURE 6. Results of experiment B. See caption to Fig. 5 and the text.

slope of the curve testing the [*F*, *N*] model was not significantly different from zero. The slope of the curve testing the [*C*, *T*] model, although significantly different from zero, was small, whereas the curve testing the [*D*, *V*] model was both very large and very significantly different from zero.

TABLE III

*Slope Analysis: Experiment B**

Least-squares etimate of the slope

Curve	Slope	SE
F, N	−0.056	0.037
C, T	0.081	0.037
D, V	0.467	0.037

Analysis of variance of the slope

Curve	Sum of squares slope	Degrees of freedom	MS	*F*	*P*
F, N	0.094	1	0.094	2.3	NS
C, T	0.12	1	0.12	4.9	<0.05
D, V	6.5	1	6.5	162	≪0.005
Error	1.8	45	0.040		

* See caption to Table II for explanations and cautions.

Exponents: Experiments A and B

Estimates of the exponents on the primary variables were calculated from the data of experiments A and B using the slopes of the curves in Fig. 5 and 6. The slopes of each of these curves, which are on a double-log plot, were the result of the respective effects of the two primary variables being increased. For instance, for the curves testing the $[C, T]$ model, the change in the response (which for unit $\log_2$ changes in the stimulus define the slope) was the result of the summed effects of number of molecules and volume. Therefore, in experiment A the

TABLE IV

*Exponents Estimated in the Two Experiments of the Present Study and in the Earlier Study of Mozell et al.**

Estimated exponent	Experiment A	Experiment B	Mozell et al.‡
$\hat{\beta}_n$	0.297	0.302	0.350
$\hat{\beta}_v$	−0.181	−0.221	−0.279
$\hat{\beta}_t$	0.165	0.165	0.216
SE§	0.031	0.032	0.049
$\hat{\beta}_c$	0.224	0.262	0.315
$\hat{\beta}_f$	−0.173	−0.193	−0.248
$\hat{\beta}_d$	0.066	0.067	0.067
SE§	0.022	0.022	0.034

* Exponents from Mozell et al. (1984) were calculated by the method of least squares, whereas the same exponents for experiments A and B were calculated from linear combinations of three slopes.

‡ Mozell et al. (1984).

§ SE = standard error for each of the three estimated exponents appearing directly above.

slope of this curve (0.116) was equal to the estimated exponent on number of molecules plus the estimated exponent on volume (Eqs. 2 and 3).

$$\log_2(\Delta R) = (\hat{\beta}_v \times \log_2 \Delta V) + (\hat{\beta}_n \times \log_2 \Delta N), \tag{2}$$

where ΔV = twofold increase; ΔN = twofold increase; $\hat{\beta}_n$ = the estimated exponent on the number of molecules; $\hat{\beta}_v$ = the estimated exponent on volume; $\log_2(\Delta R)$ = the slope of the curve testing the $[C, T]$ model for $\log_2$ unit changes in N and V, which is 0.116. Eq. 2 simplifies to:

$$0.116 = \hat{\beta}_v + \hat{\beta}_n. \tag{3}$$

Similarly, the slope of the curve testing the $[F, N]$ model (slope = 0.017) is equal to the sum of the estimated exponents on time ($\hat{\beta}_t$) and volume ($\hat{\beta}_v$) as follows:

$$0.017 = \hat{\beta}_t + \hat{\beta}_v. \tag{4}$$

Finally, the slope of the curve testing the $[D, V]$ model (slope = 0.462) is equal

to the sum of the estimated exponents on number of molecules ($\hat{\beta}_n$) and time ($\hat{\beta}_t$) as follows:

$$0.462 = \hat{\beta}_t + \hat{\beta}_n. \quad (5)$$

By solving Eqs. 3–5 simultaneously, the exponent on each primary variable was estimated. Similar equations were solved simultaneously from the slopes in experiment B. The exponents calculated are presented in Table IV, where they can be compared with those of Mozell et al. (1984).

Lack of Interactive Terms

The curves in Figs. 5 and 6 are approximately linear in these double-log plots. This apparent linearity is supported statistically by the nonsignificant contribution of the interaction sums of squares to the total sums of squares as demonstrated in the analysis of variance.

DISCUSSION

The Relative Predictive Ability of the [F, N], [C, T], and [D, V] Models

As shown by the results of experiments A and B, the hypotheses testing the predictive ability of the [F, N] and [D, V] models were verified. While the flow rate and the number of molecules were held constant, the summated multiunit activity remained unaffected by proportional variations in volume and time. This supported the excellent predictive ability of the [F, N] model. Furthermore, when the number of molecules and time co-varied proportionately, there was a dramatic increase in the response even though the delivery rate and volume were held constant. These findings verify the poor predictive ability of the [D, V] model. On the other hand, the hypothesis concerning the [C, T] model was not strictly verified. As the number of molecules and the volume co-varied, there was a statistically significant increase in the response although the concentration and time were held constant. However, in keeping with the general thrust of the hypothesis, the growth of the response with the concentration and time held constant was relatively small.

The mathematical explanation of the relative predictive abilities of the [F, N], [C, T], and [D, V] models is straightforward. The [F, N] model was excellent because the exponent on volume was, within experimental error, equal to the negative of the exponent on time. Therefore, the effect of flow rate fully incorporated the combined effects of volume and time [$V^{-0.181} \times T^{0.165} = V^{-0.181}/T^{-0.165} \approx (V/T)^{-0.173} = F^{-0.173}$]. The [$D$, V] model was poor because the exponents on time and the number of molecules were neither opposite in sign nor equal in magnitude. Therefore, the effect of the number of molecules and time could not at all be collapsed into a single effect of the delivery rate. The [C, T] model was good but inadequate because the exponents on the number of molecules and the volume were opposite in sign but the difference in their magnitudes was fourfold greater than the exponents on volume and time noted above. Therefore, the effects of the number of molecules and the volume could not be totally incorporated into a single effect of concentration. This conclusion should not be misunderstood. Certainly the concentration is defined as the ratio of the number

of molecules to the volume. However, the above conclusion was not directed toward the definition of concentration but rather to the effect of three variables: the concentration, the number of odorant molecules, and the volume (as discussed by Mozell et al., 1984). The results of the present experiments indicate that either the number of molecules or the volume has an effect not incorporated in an effect of concentration.

Both the excellent predictive ability of the $[F, N]$ model and the inadequate predictive ability of the $[C, T]$ model are consistent with the work of Tucker (1963*a*). He showed that olfactory neural activity in the box turtle increased in response to an increase in the flow rate of the odorant when the stimulus duration and concentration were held constant. Under these conditions, the stimulus volume and thus the number of odorant molecules must have also increased in proportion to the increase in flow rate ($F = V/T$; $V \times C = N$). As is clear from his results, holding the concentration and time constant does not prevent an increase in the magnitude of the response. These findings parallel the findings of the present study. Furthermore, Tucker's data are consistent with the good predictive ability of the $[F, N]$ model. That is, the $[F, N]$ model also predicts, in accordance with its exponents, an increase in the response when, as was the case in Tucker's experiment, the flow rate and the number of molecules were increased by the same ratios. Under these conditions, the $[F, N]$ model predicts an increase in the response because the positive effect of the number of molecules is larger than the negative effect of the flow rate.

Mozell et al. (1984) discussed the effects of these variables in relation to a number of anatomical and physiological properties of the olfactory mucosa and physical properties of the stimuli. For instance, they argued that the exponent on N should vary with different odorants and with different regions of the mucosa because of the differences in sensitivity among various mucosal regions to different odorants (MacKay-Sim and Kubie, 1981). Similarly, the exponents should reflect the differences in the molecular distribution of different odorants across the mucosal surface. These distribution variations result from differences in the odorants' mucosa/air partition coefficients (Hornung and Mozell, 1981) and probably result from variations in the geometry of the olfactory region. Also, as noted in Mozell et al. (1984), the exponents in these studies showing the effects of the variables upon the neural response magnitudes are similar to the exponents relating psychophysically determined magnitude estimates (Berglund et al., 1971; Jones, 1958) to the magnitude of the odorant stimulus.

A topic not previously interpreted, however, is the effect of the stimulus duration upon the magnitude of the neural response. In both this study and in the Mozell et al. (1984) study, the exponent for time was the smallest of all three primary variables, reflecting a relative insensitivity of the summated olfactory nerve discharge to stimulus duration. This exponent for time might be taken to represent the magnitude of temporal integration or summation in the olfactory process at the mucosal level. However, classical temporal integration is usually investigated by presenting a constant-intensity stimulus and examining the magnitude of the response as a function of stimulus duration. In olfaction, it is not clear what represents a constant-intensity stimulus. A reasonable guess might be a stimulus of constant concentration and flow rate. If such a stimulus is presented

for longer periods of time, not only will time increase, but so will the number of odorant molecules and the stimulus volume. The change in response is then a function of changes in all three variables rather than time alone. Using this "constant-intensity stimulus," a doubling of the stimulus duration would give a temporal integration effect equivalent to the sum of the exponents for the time, volume, and number of odorant molecules. This summation is possible because, as discussed previously, each of these exponents expresses the effect of the response of doubling the magnitude of its respective variable. In the present experiments, the resulting exponent would be 0.281 and 0.246 in experiments A and B, respectively, and 0.287 in the Mozell et al. (1984) study. In vision, the exponent on temporal integration is 0.5 (S. S. Stevens, 1966), and in audition, it is 0.345 (J. C. Stevens and Hall, 1966). Thus, it appears that olfaction integrates a constant-intensity stimulus over time less efficiently than either vision or audition.

Temporal integration is also characterized by the critical duration, i.e., the duration after which temporal integration falls to zero. Beyond this, further increases in the stimulus duration do not lead to further increases in the response magnitude. The critical duration for olfactory temporal integration was not measured in this experiment but its magnitude can be inferred from the present data. The stimulus-response curves determined in experiments A and B were very close to being linear over their entire range (Figs. 5 and 6). If the critical duration has been reached, it would be expected that there would be a nonlinearity in the response curves for the $[F, N]$ and $[D, V]$ models, both of which involve increases in T. No appreciable change in the overall slope of these curves was found. Therefore, it can be argued that the critical duration is longer than the longest stimulus duration presented. That is, the critical duration for olfaction must be longer than 1.4 s. As compared to audition and vision, this is a very long critical duration, but the critical duration reported for taste is also quite long (3, 4, and 6 s for sodium chloride, sucrose, and quinine hydrochloride, respectively [Bujas and Ostojcic, 1939, as reported in Marks, 1974]).

Support for the Three-Variable Model

In spite of the statistically significant slope of the curve testing the $[C, T]$ model, the hypotheses of the current study were, for the most part, supported. This result gives credibility to the principles that were earlier proposed by Mozell et al. (1984) to govern the olfactory stimulus-response relationship. These principles describe this relationship as involving the number of odorant molecules, the sniff volume, and the sniff duration in a noninteractive multiplicative function.

A multiplicative noninteractive model would predict linear relationships between the response magnitudes and stimulus magnitudes on the double-log plot. This could not be definitively tested by Mozell et al. (1984) because their experimental design involved but two levels of each of the primary variables. Since interactive effects result in deviations from linearity, the sums of squares unaccounted for by linear regression in the present study were taken to reflect the degree of the nonlinearity in the stimulus-response curves. In no case were the interactive effects found to be significant, which again indicates that the

response is linearly related to the primary variables only through their main effects.

The effects of the primary and derived variables were calculated for experiments A and B by solving a set of simultaneous equations and were found to be similar to those determined in the Mozell et al. (1984) study. In experiments A and B, the absolute magnitudes of the calculated exponents, though smaller than in the Mozell et al. study, ranked the same as in that previous work: $|\hat{\beta}_n| > |\hat{\beta}_c| > |\hat{\beta}_v| > |\hat{\beta}_f| > |\hat{\beta}_t| > |\hat{\beta}_d|$. Again, $\hat{\beta}_v$ and $\hat{\beta}_f$ were negative and $\hat{\beta}_d$ was barely on the positive side of zero.

One may question whether the summated multiunit discharge was the measure of choice to use in this study. There are, of course, other ways to sample the activity of the olfactory mucosa. One can record from single units by penetrating the mucosa or one can record electro-olfactograms (EOGs) from the mucosal surface. In either case, the integrity of the olfactory sac must be compromised, a situation that is countermanded by the present study's requirement of an intact flow path through the olfactory sac. Responses might also be recorded central to the olfactory nerve, but such responses can be complicated by the interactions characteristic of central processing. Therefore, only the olfactory nerve, with its unmyelinated fibers, offers a recording site that neither compromises the olfactory sac nor involves central networks. However, to date, there have been no reports of recordings from single olfactory nerve fibers for periods of time coming even remotely close to the time required by the protocol of this study. Thus, almost by default, the summated multiunit discharge became the response measure. According to Beidler (1953), who first applied this technique to the chorda tympani nerve, and Tucker (1963*b*), who later applied it to the olfactory nerve, this technique gives a measure of the response that is proportional to the number of nerve impulses per unit time. To further test the summated multiunit discharge for olfactory nerve work, Mozell (1964*a*) compared the summated responses to frequency meter responses for several odorants and found no appreciable differences in their relative magnitudes. Although one may still feel a certain amount of trepidation using this measure, one cannot disregard studies like that of Borg et al. (1967), which showed, in humans, that the power function governing the perception of the taste of citric acid is the same whether it is measured using the summated multiunit response from the chorda tympani or using psychophysical magnitude estimations.

Furthermore, in this regard, it is well to point out that although one might expect this study to treat high-amplitude/short-duration responses the same as low-amplitude/long-duration responses, this did not really occur. It did not really occur because, in this study, changes in the areas of the summated responses tended to do so by varying height while duration remained approximately constant. That this study did not confuse the durations and amplitudes of the summated responses is further supported by two additional findings: (*a*) the durations of the responses were not correlated to any of the stimulation variables, and (*b*) the heights of the responses gave stimulus-response relationships that were very similar to those reported for areas.

In summary, this study supports the validity of one of the two-variable reduc-

tions, the [F, N] model, of the full three-variable [N, V, T] model. This study confirms that, as earlier suggested, the magnitude of the olfactory nerve discharge depends solely upon the three variables (N, V, and T) with the proviso that the effects of volume and time are equal and opposite. Moreover, since in the present study the range of levels for each stimulus variable has been increased to cover much more of the frog's dynamic range, the applicability of the model has been greatly extended. It is quite likely that as the ranges of these variables are even further extended, the exponents will change. Obviously, at the point where the response saturates, all the exponents will go to zero. The pursuit of possible changes in exponents with changes in stimulus conditions could give further insight into the role that stimulus access features play in the olfactory process.

The Influence of Different Mucosal Regions upon Octane Stimulus-Response Relationships

Experiments A and B produced results that were remarkably similar to each other, given that there was opportunity for each experiment to produce rather different stimulus conditions. Controls showed that the medial recording site sampled a set of axons that originated in an area near the external naris, whereas the lateral recording site sampled neural activity that originated in a mucosal area nearer the internal naris. Therefore, the neural activity recorded in experiment A was in response to the sniff before such factors as dilution, diffusion, sorption, and possibly turbulence had much chance to alter the character of the sniff in terms of its concentration, volume, flow rate, and duration. On the other hand, the neural activity sampled in experiment B was in response to the stimulus after it had passed some distance into the olfactory sac, thus potentially allowing these factors to have an influence. With the similarity in the results of experiments A and B, it can be hypothesized either that the model is relatively insensitive to these changes in the character of the stimulus or that the characterization of the stimulus does not change appreciably as it passes through the nose. In either case, at least for *n*-octane, the ability of a model to predict responses seems to be independent of the mucosal region from which the responses originated.

As noted earlier a number of times, octane's mucosa/air partition coefficient greatly favors the air phase, so that the small fraction of its molecules that are sorbed along the mucosa are rather evenly distributed from point to point. However, a more strongly sorbed odorant might give very different results. Strongly sorbed odorants show a very steep, decreasing mucosal concentration gradient along the flow path with few, if any, molecules passing through the entire olfactory sac (Hornung and Mozell, 1981). Such strongly sorbed odorants might very well give different stimulus-response relationships at different points along the mucosa, if, as is possible (Mozell et al., 1984), the effect of N is not the same at high and low levels. In testing this concept, it would be most advantageous to choose a highly sorbed odorant for which there is no regional difference in sensitivity across the mucosa. This would minimize the possibility of confounding the influence of the decreasing molecular concentration gradient along the mucosa with changes in sensitivity.

Factors That Could Influence the Predictive Ability of Models

It should be cautioned that a number of different models may be generated, depending on the experimental conditions. It is recognized that the results of experiments A and B might not have been so similar if different odorants had been used, especially odorants that, unlike *n*-octane, are strongly sorbed by the mucosa. If, for one of these odorants, the ratio of the number of molecules reaching the near and far regions of the mucosa differed as a function of the number of molecules presented or the flow rate of the sniff, experiments A and B might have been expected to give different stimulus-response relationships. Similarly, it is possible that the two regions of the mucosa represented in experiments A and B could have given rise to varying stimulus-response relationships because different regions of the mucosa appear differentially sensitive to different odorants (Mackay-Sim and Kubie, 1981).

In addition, the perisniff air flow and the time rate of change of odorant concentration may also affect the model generated. In the present experiments, the perisniff air flow was held at 20 cc/min and the change in odorant concentration was made as close to a square wave as possible. The effect of altering dC/dt was not tested. It is recognized that the relative predictive abilities of the reduced models, and even the three-variable model, might change if concentration ramps were presented. Furthermore, altering the speed at which the odorant is cleared from the nose alters the dynamics of stimulation and could also alter the model generated. Indeed, unpublished observations showed that the $[C, T]$ model becomes an adequate reduction of the three-variable model when, after bringing the sniff into the nose, all air flow is halted before the nose is cleared of odorant. It is also expected that modeling of olfactory stimulation variables would become more complex when describing stimulus-response relationships in animals with nasal cavities more complicated than that of the bullfrog.

Topographical Organization of the Olfactory Nerve

In showing that the medial and lateral recording sites sampled activity originating from near the external and internal nares, respectively, this study also incidentally addressed the topographical organization of the frog's olfactory nerve. It showed that the different odorant-dependent mucosal activity patterns reflected in the relative discharge magnitudes recorded from various branches of the olfactory nerve (Mozell, 1970) are maintained in the trunk of the olfactory nerve as it projects to the olfactory bulb.

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