A Parametric Study of the Stimulation Variables Affecting the Magnitude of the Olfactory Nerve Response

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ABSTRACT The magnitude of olfactory responses can be related to three primary variables [number of odorant molecules (N), sniff volume (V), and sniff duration (T)] and three derived variables [concentration ($C = N/V$), flow rate ($F = V/T$), and delivery rate ($D = N/T$)]. To evaluate the effects of these interdependent variables upon the olfactory response, the summated multiunit discharges were recorded from the olfactory nerves of nine frogs in response to octane presented at two levels (in 2:1 ratio) of each primary variable. This presentation defined eight "sniff" combinations representing three levels of each derived variable. In an ANOVA of the logs of the responses, the effect of each primary variable was highly significant, with no significant interactions. A multiplicative regression model incorporating the effects of the three primary variables represented responses exceedingly well, with positive effects of N and T and a negative effect of V. When, with this model, the effect of each of the derived variables was isolated from the effects of all other variables, the analysis showed a positive effect for C, a near-zero positive effect for D, and a negative effect for F. Placing certain constraints upon the model parameters generates 13 distinct one- and two-variable models (e.g., the [C, T] model requires N and V to have equal but opposite effects). In ranking these reduced models in terms of their ability to predict the neural response, the predictive ability of [F, N] and [C, T] was at least as good as that of the three-variable model.

INTRODUCTION

Other than changing the odorants themselves, the stimulation variable to which the responsiveness of the olfactory system has most often been related is the concentration of the odorant in a sample of the carrier gas (usually air). Less often, olfactory responsiveness has been related to other stimulation variables, most notably to the flow rate at which the odorized gas is sampled and, to a lesser degree, to the odorant delivery rate, i.e., the amount of odorant sampled

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per unit time. However, these three variables [concentration (C), flow rate (F), and delivery rate (D)] are actually derived from different combinations of three primary variables,[1] namely: the number of odorant molecules in the sniffed sample (N), the volume of the sniffed sample (V), and the duration or time of the sniff (T). That is, the concentration is given by the number of molecules per unit sniff volume ($C = N/V$); the delivery rate is given by the number of molecules per unit sniff time ($D = N/T$); and the flow rate is given by the volume sniffed per unit time ($F = V/T$). With such interrelationships of these three primary and three derived variables, an experimental variation in any one of them will of necessity produce variations in others. This has made it difficult to interpret the previous studies, which attempted to assess the relative effects of the different stimulation variables upon olfactory responsiveness (Tucker, 1963; Beidler, 1961; Teghtsoonian et al., 1978; Rehn, 1978; Schneider et al., 1966; Le Magnen, 1944–1945). Often in such studies only the stimulation variable under investigation is manipulated in a controlled manner, whereas the other primary and derived variables not so singled out, but still necessarily varying, remain unmonitored and often disregarded. For instance, in several of the above studies (Table I), the effect of flow rate upon responsiveness was assessed by increasing the sniff flow rate while holding the sniff time and odorant concentration constant. However, to increase the sniff flow rate while holding the sniff duration constant, sniff volume must also be increased. Furthermore, this increase in volume at constant concentration will increase the number of molecules, which, at constant duration, will in turn increase the delivery rate. Thus, any change in responsiveness cannot a priori be ascribed solely to the manipulated variable since the effects of other variables, either alone or in combination, have not been ruled out. Apparently then, any study attempting to evaluate the individual or combined effects of the three primary (N, V, T) and three derived (F, C, D) variables upon olfactory responsiveness must satisfy, among other more general experimental requirements, two rather special requirements. First, it must incorporate techniques and procedures to regulate, control, and monitor all six stimulation variables simultaneously. Second, it must incorporate in its experimental design and in its data analysis a strategy that both recognizes and addresses the confusions that can occur in attempting to disentangle the effects of such interrelated variables. Both of these requirements are a prime concern in this present study, which uses as a measure of that responsiveness the magnitude of the summated multiunit discharges recorded from the frog's olfactory nerve. The first requirement is met by an odorant delivery system which produces sniffs of precise quantitative dimensions. The second requirement is met by developing a number of mathematical models, each of which relates the response magnitude to a different combination of the six stimulation variables, and then comparing how well these different models predict the responses.

[1] The phrase "primary variable" is used here only to indicate that these variables, when taken in different combinations, produce the derived variables. The phrase is not intended to imply anything about the relative importance of these or the derived variables in determining the responsiveness of the olfactory system.

METHODS

Sniff Definition: a Balanced Design of Primary Variables

In defining the sniffs presented to the frogs, two levels (i.e., magnitudes) in a 2:1 ratio were chosen for each of the three primary variables. As can be seen in Fig. 1, with each volume of the sniff (V_1 and V_2) paired with each of two numbers of molecules (N_1 and N_2), and with each combination of volume and number of molecules paired with each of two sniff durations (T_1 and T_2), eight different sniffs are generated. These eight different sniffs generate among themselves three levels of the three derived variables (C, F, and D), with each level double its predecessor. Note that with such a design, some of the variables can be changed while keeping the other variables constant. For instance, concentration is kept constant at the C_2 level by doubling both sniff volume and the number of molecules

TABLE I

How Stimulation Variables Changed in Previous Studies

	Tucker (1963)	Teghtsoonian et al. (1976)	Rehn (1978) A	Rehn (1978) B
Primary variables				
N	Increase	Increase	Increase	No change
V	Increase	Increase	Increase	No change
T	No change	No change	No change	Decrease
Derived variables				
C	No change	No change	No change	No change
F	Increase	Increase	Increase	Increase
D	Increase	Increase	Increase	Increase

All the experiments referred to in the table attempted to study the consequence of increasing flow rate on the magnitude of either psychophysical or neurophysiological olfactory responses. In all cases concentration was held constant. In most cases the flow rate was increased by increasing the volume of the sniff while holding the duration constant. As listed in this table, this approach also increased the number of molecules and the delivery rate. Note that Rehn, besides following the more common approach (A), also increased flow rate by decreasing time while holding volume constant (B). This approach also increased the delivery rate. Although these experiments were designed to study the influence of flow rate, other variables which varied along with flow rate were not ruled out as being the possible precursors for the observed change in response.

within the sniff ($C = N_1/V_1 = N_2/V_2$). Similarly, by doubling the time, the volume remains constant at the V_1 level in spite of halving the flow rate (since $F_1 = V_1/T_2$ and $F_2 = V_1/T_1$, then $V_1 = F_2 \cdot T_1 = F_1 \cdot T_2$).

The levels for each of the six variables were chosen in accordance with previous work, from which normal values could be estimated for the bullfrog. That is, the volumes, times, and flow rates of the sniffs were chosen to fall well within the ranges previously determined for the flow of air entering and leaving the bullfrog's nasal cavity during normal respiration cycles. These were monitored by a hot-wire anemometer sealed over the animal's external naris (Hornung et al., 1980). The two levels for the number of molecules were chosen from previous work in which the electrophysiologically recorded response magnitude was related to the intensity of the stimulus (Mozell, 1970). The stimulus intensities chosen for this purpose were taken from the dynamic range of the stimulus-response curve. The actual values chosen for each of the six variables at each combination of N, V, and T are given in Fig. 2.

Sniff Delivery System

To control sniff volume, sniff time, and thus sniff flow rate, the delivery system, the core of which is shown in Fig. 3, was used. This core consisted of two four-port Teflon slide valves (A and B), which were pneumatically driven by solenoids controlled by a Grass S88 stimulator (Grass Instrument Co., Quincy, MA). Between sniffs ("rest" condition in Fig. 3), the four ports of each valve were so arranged that deodorized, humidified air drawn by an exhaust pump continuously flowed through the frog's olfactory sac at 20 cc/min.

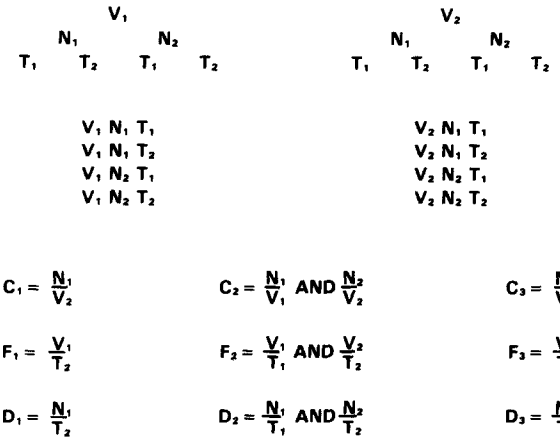

FIGURE 1. The generation of eight different sniffs from two levels each of three primary variables. Primary variables: *V*, volume; *N*, number of molecules; *T*, duration or time. Derived variables: *C*, concentration; *F*, flow rate; *D*, delivery rate. Subscripts 1, 2, and 3 represent increasing levels of the variables, with each succeeding level double its predecessor. Starting at the top of the figure, each of the two levels of *V* is paired with each of the two levels of *N*, and each of the four resulting combinations of *N* and *V* is combined with each of the two levels of *T*. This yields the eight different combinations of sniff variables given in the middle of the figure. Each of these eight primary variable combinations describes a particular level of each of the three derived variables. Furthermore, across all eight primary variable combinations, as shown at the bottom of the figure, there are three levels for each derived variable. Note that there are three derived variable levels rather than four since two of the primary variable combinations, being in the same ratio, give the same level of the derived variable.

During the rest condition, the variable-speed withdrawal pump (Harvard Apparatus Co., Inc., South Natick, MA) was set at the flow rate prescribed by the scheduled volume and time for the next sniff. The olfactometer was set to generate the concentration required, which, with this sniff volume, produced the next scheduled number of molecules. During a sniff ("stimulation" condition in Fig. 3) the ports of the slide valves were so arranged that a sample of the odorized air flowing from the olfactometer was drawn through the frog's olfactory sac. This air was drawn at the pre-set flow rate generated by the variable-speed withdrawal pump, giving the animal an artificially produced sniff with the next scheduled *N*, *V*, and *T*.

Odorant Control

The olfactometer controlling the partial pressure of the odorant was made completely of Teflon and glass and was of the flow dilution variety, where one stream of air (Ultra Zero; Matheson Division of Searle Medical Products, East Rutherford, NJ), having first been saturated with odorant, is then mixed with another stream of air. By varying the flow

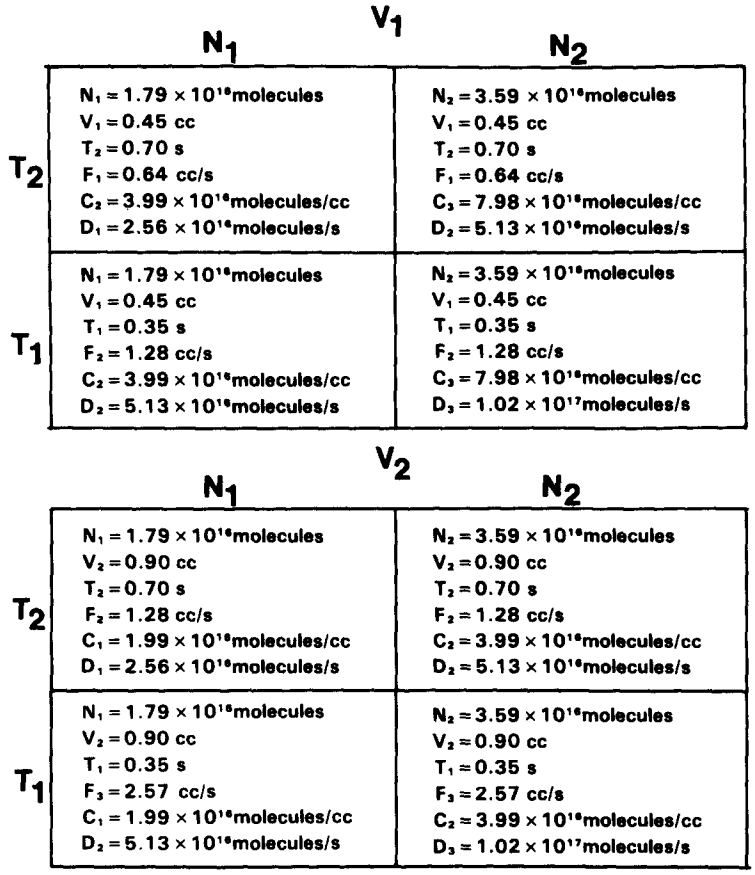

FIGURE 2. The values expected for the three primary and three derived variables, which describe each of the eight different sniffs. Each square represents a different sniff resulting from a particular combination of N, V, and T levels as shown. Primary variables: V, volume; N, number of molecules; T, duration. Derived variables: F, flow rate; C, concentration; D, delivery rate. Subscripts 1, 2, and 3 represent increasing levels of the variables. Note that, for any given variable, each succeeding level is double its predecessor.

rates of these two streams, the desired partial pressure is reached (Mozell, 1970). The Ultra Zero air was further purified through columns of silica gel and activated charcoal.

The odorant used in this study, *n*-octane (chromaquality; Matheson, Coleman and Bell, Cincinnati, OH), was chosen for several reasons. First, Hornung et al. (1980) observed, while comparing the mucosa/air partition coefficients of several different odorants, that

the partitioning of octane favored the olfactory mucosa several orders of magnitude less than did the partitioning of the other odorants. The less an odorant's partition coefficient favors the mucosa (or, conversely, the more it favors the air), the more uniform should be its point to point mucosal sorption, i.e., the less will a sample of its molecules be

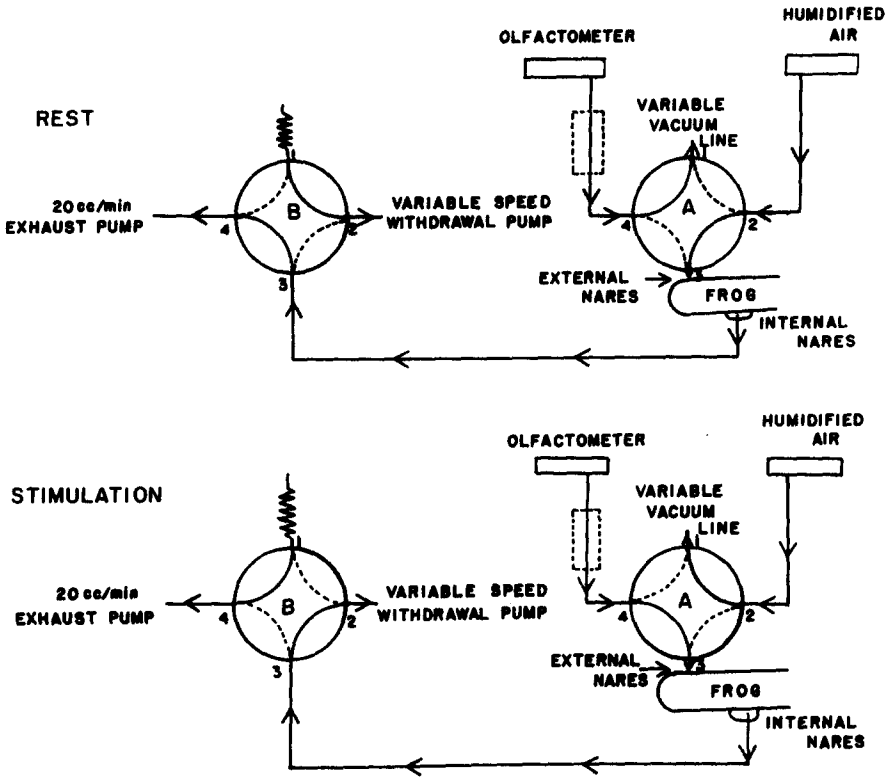

FIGURE 3. Sniff delivery system. During the rest condition, the exhaust pump connected to port 4 of valve B caused the air to flow through valve A from port 2 to port 3 and to enter the frog's external naris. The odorized air from the olfactometer was directed away from the animal into the laboratory vacuum line via ports 4 and 1 of valve A. The withdrawal pump drew room air from port 1 to port 2 of valve B. During the sniff ("stimulation") condition the exhaust pump was switched to draw room air at 20 cc/min from port 1 to port 4 of valve B and the humidified air was drawn into the laboratory vacuum line via ports 2 and 1 of valve A. During both rest and stimulation conditions, the internal naris was connected to a specially designed miniature trap which maintained the patency of the delivery system for long periods of time in spite of the continued production of mucus in the olfactory sac.

depleted as it moves across the mucosal surface. Of the odorants tested, octane showed the least such depletion (Hornung and Mozell, 1981). Thus, the use of octane could reduce the response variability that may be traceable to variations in the size and geometry of the olfactory sacs of different frogs. A second reason for choosing octane was that previous experience showed it to be one of the more rapidly cleared odorants both from

the mucosa (Hornung and Mozell, 1977) and from the tubing of the delivery system (unpublished observation). Thus, repeated presentations could be given without fear of long-term contamination and with a minimal interstimulus time required for purging.

Verification of Sniff Variable Levels

To verify that the desired sniffs were actually produced by the delivery system, a hot-wire anemometer (Hornung et al., 1980) was inserted into the line as shown by the dashed box

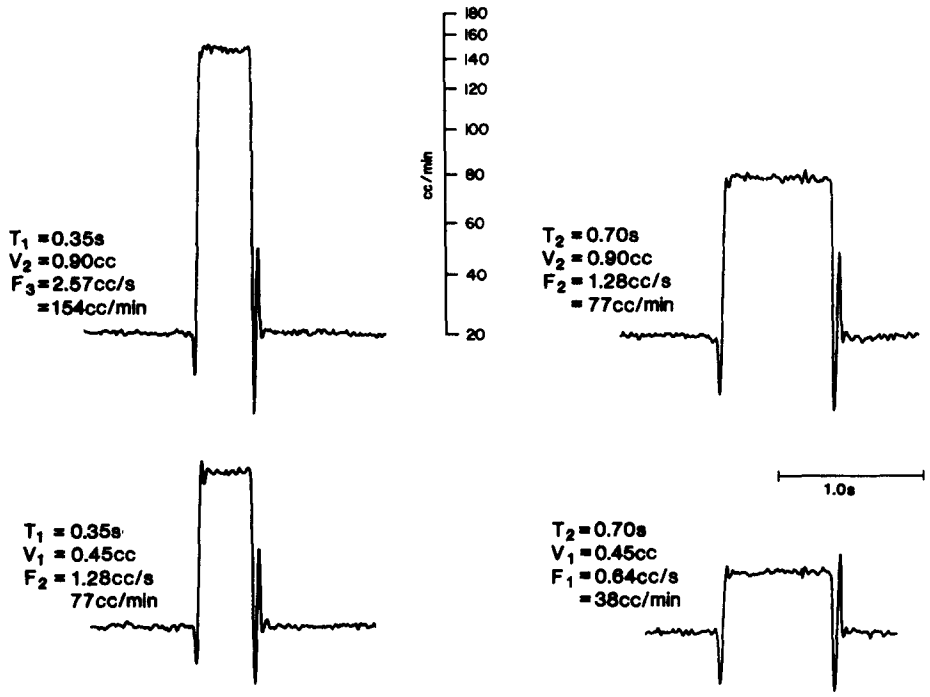

FIGURE 4. Sniff profiles monitored by the hot-wire anemometer. These profiles are copies of the tracings originally recorded on ultraviolet-sensitive Visicorder paper. The amplitude of the trace at any given time is the instantaneous flow rate. The time (duration), volume, and flow rates measured for each sniff are given to the left of each trace. The trace before and after the sniff is the air flow during the "rest" condition (20 cc/min). The transients following the sniffs occurred in the line passing through the anemometer after the line was shunted away from the animal. See text for further explanation.

in Fig. 3. The circuitry of this device related the air flow rate to the galvanometer deflection of a Honeywell (Denver, CO) Visicorder; this relationship, represented by the flow rate calibration line in Fig. 3, had a somewhat positive acceleration. By monitoring the flow rate, this hot-wire anemometer recorded the sniff profiles generated by four different combinations of volumes and durations (Fig. 4). The amplitudes of these profiles represent the flow rate. The resting flow rate was measured to be 20 cc/min, as expected from the setting of the exhaust pump (Fig. 3). Similarly, all of the flow rates during the sniffs read as predicted in Fig. 2 and these levels are given with the sniff profiles in Fig.

4. Note in this regard that the four combinations of two volumes and two times do indeed produce four different sniffs having among them three different flow rates. Furthermore, each successively higher flow rate is indeed double its predecessor.

On this time scale the profiles appear essentially rectangular. The desired durations of the sniffs were verified by measuring the distance between the onset and offset of the sniff traces, and as can be seen in Fig. 4, the durations of the four sniffs were equal to the values expected. Finally, since the sniff profiles were essentially rectangles, the volumes of the sniffs were given by multiplying the amplitudes of the traces (flow rates) by their duration. The four sniffs show the two expected volumes, one double the other.

A gas chromatograph (Varian [Palo Alto, CA] 940 with a flame ionization detector) was used to verify the numbers of octane molecules within the sniffs by fitting its sampling valve with a specially designed sample loop. This loop allowed the odorant delivery system to draw odorized air into the gas chromatograph at the same volumes, times, flow rates, and olfactometer settings as were drawn into the frog's olfactory sac. Fig. 5 shows one set of typical chromatograms for the eight different combinations of N, V, and T, and the numbers of molecules given along the top of the figure are the averages for six sets of such chromatograms. Using liquid injection techniques the output of the chromatograph was calibrated so that the areas of the chromatograms could be converted into numbers of molecules. Within the limits of experimental error, the numbers of molecules match the two expected values given in Fig. 2 and are therefore close to a 2:1 ratio. Furthermore, note that the number of molecules entering the sample loop (i.e., the surrogate for the frog's olfactory sac) was not altered by the flow rate, an effect which had to be assessed because of a possible interplay between the flow rate and the adsorption of octane molecules to the Teflon of both slide valve A and the tube leading to the external naris. Earlier work (unpublished) had suggested that the adsorption of octane molecules to Teflon, though not nearly as great a problem as with some other odorants, still required some vigilance. Thus, the distance from port 4 of slide valve A to the external naris (Fig. 3) was kept as short as the geometry of the situation would allow. At any rate, whatever residual adsorption there might have been, it was not great enough, as shown by Fig. 5, to detectably affect the consistency with which the numbers of molecules could be controlled. The total sample volume from port 4 of valve A to the tube from port 3 to the frog's external naris (Fig. 3) was 0.0096 cc. This volume was such a small percentage of the total sniff volume that its added effect on the response was probably quite small.

Recording Procedures

The active electrode was a stainless-steel wire 63.5 μm in diameter quadrupally enameled to the tip. The inactive electrode was a similar wire 127 μm in diameter. The neural activity was recorded differentially with the active electrode pressed lightly but securely against the desheathed olfactory nerve (see below) and the inactive electrode contacting a piece of cotton soaked in Ringer's solution resting nearby on the exposed skull. The multiunit discharge of the olfactory nerve (Mozell, 1962) was amplified with a Grass P5 AC preamplifier. To quantify the neural activity, the preamplifier output was passed through an electronic summator (integrator) circuit like that described by Beidler (1953). The charging and discharging time constants were 0.25 and 1.9 s, respectively. The resulting summated curves were recorded both on a Honeywell Visicorder and on magnetic tape. The latter was used as input to a PDP 11/34 computer (Digital Equipment Corp., Marlboro, MA), which was programmed to calculate the areas (in arbitrary units) of the summated curves, thus giving a measure of the neural response magnitude.

Preparation

The frogs, *Rana catesbeiana*, were procured from Jacques Weil Co., Rayne, LA, and were

maintained in groups of several dozen in large tanks with constantly flowing tap water. After each frog was anesthetized with urethane, it was secured in a headholder, and its olfactory nerve, still ensheathed, was exposed. During this surgery and in all other aspects of the study, care was taken not to compromise in any way the integrity of the olfactory sac in order to preserve its normal flow path. The miniature trap, which, as described in

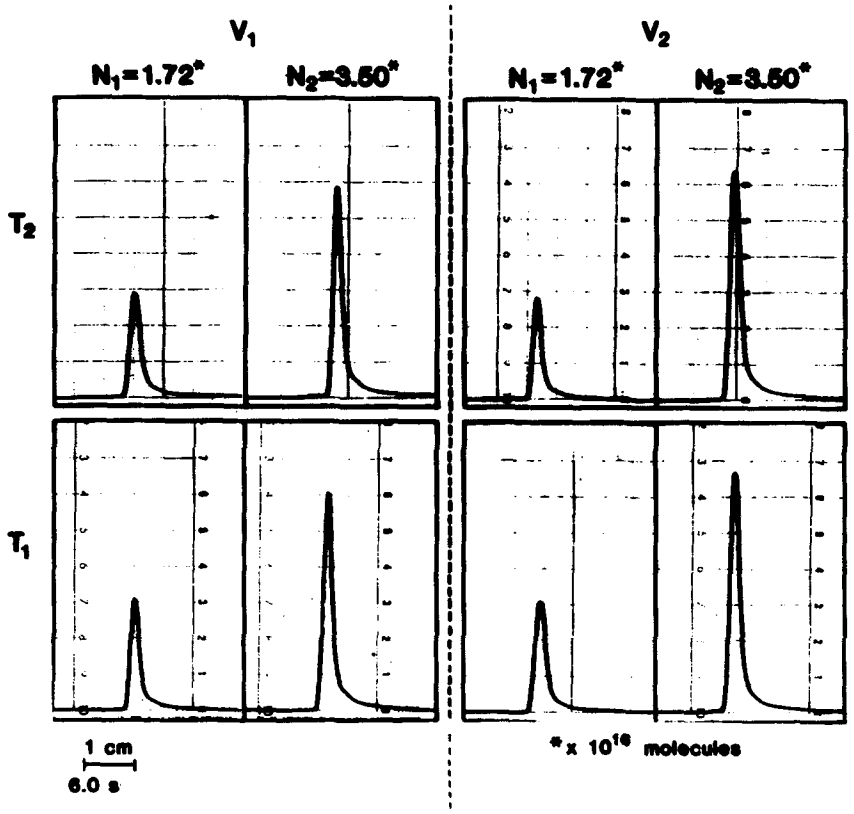

FIGURE 5. One set of six such sets of chromatograms for octane at the eight different combinations of N, V, and T, each at two levels (see Fig. 2). The gas chromatograph was a Varian 940 fitted with a 5% SE-30 column supported on Chromosorb W (6 ft × ⅛ in. column). All chromatograms were recorded at the same gain of the detector amplifier. Other operational chromatograph 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. See text for further discussion.

Fig. 3, kept the nasal mucus from plugging the delivery system, was then cemented in an airtight fashion around the frog's internal naris using a dental carboxylate cement (Durelon; Premier Dental Products Co., Norristown, PA). After the olfactory nerve was desheathed, the recording electrode was positioned to rest on that part of the nerve known from previous studies (Mozell, 1964, 1966, 1970) to supply a region of the olfactory mucosa near the external naris where the incoming odorized air makes its initial mucosal contact. This was done to further counteract the previously noted sorption

phenomenon along the mucosal surface and to ensure that the recorded activity would be in response to the maximum number of molecules available in a given sniff.

At this time, a probe stimulation of *d*-limonene was puffed into the external naris from a squeeze bottle, and if activity was recorded from the nerve the animal was then connected to the odorant delivery system (Fig. 3). The patency and integrity of the entire system, including the frog's nasal airway, was then tested by inserting the anemometer into the line, as shown in Fig. 3, and by setting the olfactometer to deliver only non-odorized Ultra Zero air. The withdrawal pump was then set to draw sniffs at each of the three flow rates to be used in the experiment, and the S88 Grass stimulator was set to produce the two sniff durations scheduled for use. In order to proceed with a given animal, all six of the resultant anemometer recordings had to show, as in Fig. 4, the correct rise and fall times, flow rates, and durations. In addition, these sniffs were not to give neural discharges since this would suggest non-olfactory, mechanically induced artifacts. A further requirement to proceed was an indication that the stimuli to be presented were within the dynamic range of the animal's neural response. Therefore, the frog was presented with three consecutive sniffs having the same volume (V_1) and duration (T_1), but with each successor having double the number of molecules of its predecessor (0.5 N_1, N_1, N_2). A consecutive increase in the summated multiunit discharges paralleling these increases in the number of molecules was taken as indicative of the stimuli being within the animal's dynamic range.

Protocol

Each animal's run began with two presentations of a standard sniff (viz., T_1, V_1, and N_1). These were followed by the eight different test sniffs (Fig. 2). These eight sniffs were presented in a randomized order which was determined separately for each animal. Following this series of the eight test sniffs, two presentations of the standard sniff were again presented. These, in turn, were followed with the series of the eight test sniffs, but this time the order of their previous presentation was reversed. In concluding an animal's run, there were two final presentations of the standard sniff.

The standard sniffs were given to control for variations that might occur over time in either the physiological status or the recording conditions of the preparation. Such variations over time could mask or confuse the response effects caused by the sniff variables per se. Thus, the two responses to each set of standard sniffs were averaged, yielding for each animal three such averages. The changes over time in the average response from the first set of standard sniffs to the second set of standard sniffs and from the second set to the third were calculated. The value of the response to each test sniff was then adjusted by linear interpolation.

The sniffs were given at 5.5-min intervals. For 1.5 min of these intersniff intervals the delivery system was flushed with deodorized, humidified air. That is, after the valves returned to the "rest" condition (Fig. 3) following a stimulation, the line that had connected the olfactometer to valve A at port 4 was connected instead to the source of humidified, deodorized air. With this connection in place, the ports of valve A were then reset to the arrangement of the "stimulation" condition, whereas the ports of valve B were kept set in the "rest" condition arrangement. With this setting of the valves, deodorized air passed for 1.5 min at 20 cc/min from port 4 to port 3 of valve A, thus purging any remaining octane from the lines accessing the frog's external naris. Gas chromatography was used to verify that this had been accomplished. Note that deodorized air continuously passed through the frog's olfactory sac during the 5.5 min between sniffs. This was true both for the 4.0 min during which the valves were arranged, as shown in the "rest" condition of Fig. 3, as well as for the 1.5 min during which valve A was purged as described above.

Statistical Procedures

An ANOVA was applied to the log summated multiunit discharges to determine the significance of the individual and interactive effects of each of the three primary variables in determining the magnitude of the neural response. Further statistical analyses were performed to determine how well different combinations of the primary variable effects could predict the neural response. These analyses include the effects of the derived variables since the effects of these variables can be described by certain combinations of the effects of the primary variables.

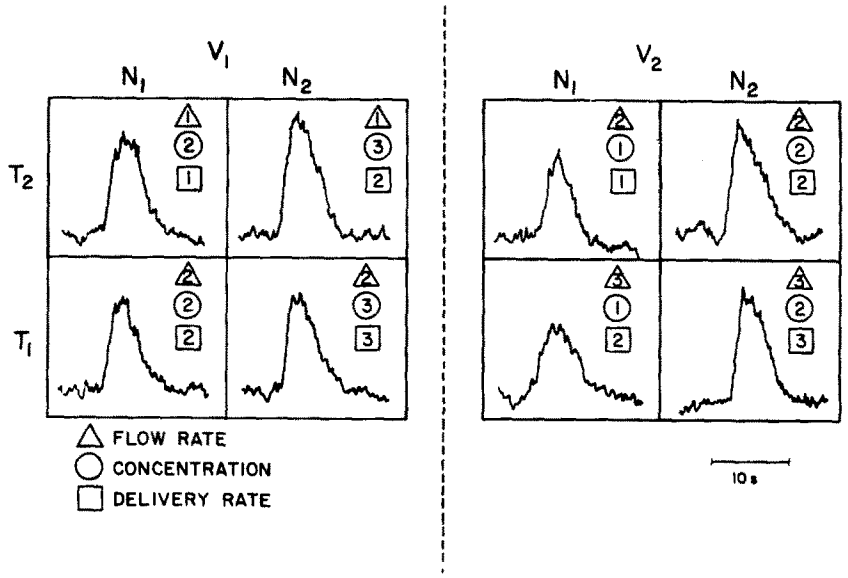

FIGURE 6. One array of typical summated multiunit discharges recorded from the olfactory nerve in response to the eight combinations of *N*, *V*, and *T*, each at two levels (see Fig. 2). All eight traces came from the same series of eight test sniffs in the same animal. There were 18 such arrays with 2 arrays from each of 9 animals. The number (1, 2, or 3) within the symbol representing a given derived variable designates the level of that derived variable for the particular combination of *N*, *V*, and *T* levels (see Fig. 2). These responses were traced from those originally recorded on ultraviolet-sensitive Visicorder paper.

RESULTS

Responses to the Stimulation Variables

An array of typical summator curves of the multiunit discharges in response to a single run of the eight combinations of primary variable levels is given in Fig. 6. There were 18 such arrays, 2 from each of 9 animals. For each animal the areas (in arbitrary units) under the two curves were adjusted to the standard sniff responses (see *Protocol* above) and were averaged. The logarithms (base 2) of these are presented in Table II. To determine whether each of the primary variables had a significant effect upon the magnitude of the neural response,

these data were subjected to an analysis of variance. This analysis, summarized in Table III, showed highly significant ($P \simeq 0$) increases in the summated multiunit discharges as a result of: (*a*) an increase in the number of odorant molecules, (*b*) an increase in sniff duration, and (*c*) a decrease in sniff volume. In addition, this analysis of variance showed no significant interactive effects.

Mathematical Models

The initial, most general mathematical model of the above experimental observations was:

$$R_{ij} = A_j\,(N_i^{\beta_n})(V_i^{\beta_v})(T_i^{\beta_t})E_{ij}, \tag{1}$$

where R_{ij} = the mean of the two standard-adjusted, summated multiunit discharges (see *Protocol*) attributable to the ith combination of stimulation variables (see Fig. 1) presented to the jth frog preparation, $i = 1, 2, \ldots, 8$ and $j = 1, 2, \ldots, 9$; A_j = responsivity characteristic of the jth frog preparation; N_i = the number of molecules of odorant, V_i = volume of sniff, and T_i = duration of sniff, as outlined in Fig. 1 and specified in Fig. 2; β_n, β_v, and β_t are exponents that quantify the effect of each respective variable upon the response; and E_{ij} is an approximately log normal error coefficient.

TABLE II

Responses (Log_2) to Experimental Combinations of the Primary Variables

Duration (*T*) Volume (*V*) Number of molecules (*N*)	Short				Long					
	Low		High		Low		High			
	Small	Large	Small	Large	Small	Large	Small	Large	Total	Mean
Preparation										
1	2.79	3.53	2.25	3.47	3.21	3.34	3.12	3.03	24.74	3.092
2	3.42	3.79	3.49	3.79	4.11	4.18	3.73	3.93	30.44	3.805
3	3.47	3.75	3.36	3.99	3.69	4.11	3.23	3.90	29.50	3.688
4	3.36	3.56	3.30	3.63	3.73	4.19	3.38	3.75	28.90	3.612
5	3.58	4.19	3.21	3.46	4.00	4.04	3.70	3.74	29.92	3.740
6	3.27	3.19	2.35	2.65	2.89	3.03	2.49	3.29	23.16	2.895
7	2.63	2.69	2.28	2.78	2.97	3.37	2.66	2.99	22.37	2.796
8	2.86	2.96	2.47	2.51	2.61	3.10	2.59	3.09	22.19	2.774
9	3.63	3.76	3.08	3.62	3.76	4.29	3.04	3.63	28.81	3.601
Total	29.01	31.42	25.79	29.90	30.97	33.65	27.94	31.35	240.03	—
Mean	3.223	3.491	2.866	3.322	3.441	3.739	3.104	3.483	—	3.334

Since the stimulation variables were in a 2:1 ratio, logarithms to the base 2 are convenient to express the model in its linear additive form, i.e.,

$$r_{ij} = \alpha_j + \beta_n n_i + \beta_v v_i + \beta_t t_i + \epsilon_{ij}, \tag{2}$$

where the lowercase symbols with subscripts refer to the logarithms of the corresponding uppercase terms in Eq. 1. Furthermore, since the derived variables are ratios of the primary variables, they can be expressed in logarithms to the base 2 as follows:

$$c_i = n_i - v_i; \qquad f_i = v_i - t_i; \qquad \text{and} \qquad d_i = n_i - t_i, \tag{3}$$

where c, d, and f are the logarithms of the derived variables. Consequently, the

general model can be expressed in a number of equivalent three-variable ways so as to include derived variables. For example, by substituting $c_i + v_i$ for n_i in Eq. 2, the model is expressed with a concentration term along with those for volume and duration, $[C, V, T]$,[2] as follows:

$$r_{ij} = \alpha_j + \beta_n c_i + (\beta_n + \beta_v)v_i + \beta_t t_i + \epsilon_{ij}. \quad (4)$$

If $n_i - c_i$ were substituted for v_i in Eq. 2, the model would again be expressed with a concentration term, but along with duration it would include the number of molecules rather than volume, $[C, N, T]$:

$$r_{ij} = \alpha_j + (\beta_n + \beta_v)n_i - \beta_v c_i + \beta_t t_i + \epsilon_{ij}. \quad (5)$$

Other substitutions of derived variables for primary variables lead to a total of 16 expressions of the three-variable model. These 16 expressions of the three-

TABLE III

Analysis of Variance of Experimental Responses (Log_2)

Source	Degrees of freedom	Sum of squares	Mean square	F	P
Preparations	8	12.1178	1.5147	—	—
Treatments	7	4.5622	0.6517	—	—
Main Effects	3	4.4597	1.4866	—	—
Duration (T)	1	0.8428	0.8428	19.7	Nil
Volume (V)	1	1.4084	1.4084	32.9	Nil
Number of molecules (N)	1	2.2085	2.2085	51.6	Nil
Interaction	4	0.1025	0.0256	0.6	$\gg 0.10$
$V \times T$	1	0.0048	0.0048	0.1	$\gg 0.10$
$N \times T$	1	0.0026	0.0026	0.1	$\gg 0.10$
$N \times V$	1	0.0820	0.0820	1.9	> 0.10
$N \times V \times T$	1	0.0131	0.0131	0.3	$\gg 0.10$
Error	56	2.3985	0.04283	—	—
Total	71	19.0785	—	—	—

variable model are equivalent, since they can all be algebraically derived from each other with the exponents in any one expression determining the exponents in any of the other expressions (see Table AI in Appendix A).

Estimates of the exponents (with their standard errors) for the three primary variables were obtained by least-squares applied to the logarithmic version of the full model. These estimated exponents, which characterize the dependence of the response magnitude upon number of molecules, sniff volume, and sniff duration, are shown in Table IV.

These estimated exponents were used to determine how accurately this non-interactive, three-variable model represents the observed neural responses. Estimates of the response magnitudes were calculated from these least-squares estimates of the exponents, and the corresponding geometric means are presented in the "Expected" column of Table V. For purposes of comparison, the

[2] Brackets indicate models.

"Observed" column in Table V gives the geometric means of the actually observed neural responses elicited by each of the eight combinations of primary variable levels. The ratios of the observed to the expected values are also given. As can be seen, the observed values did not differ from the expected values by >4%, and for most of the treatments the difference was only ≤2%. This illustrates the high degree of accuracy with which the non-interactive, three-variable model (Eqs. 1 and 2) describes the experimental results.

With the balanced design of this study, the effect (exponent) of any given derived variable was actually an average of the effects (exponents) of the primary variables incorporated by that derived variable. In explanation, recall first that two ways to double concentration (C), for instance, are: (a) doubling the number of molecules (N) while holding volume (V) constant, or (b) halving V while holding N constant. In the design of this study the changes in response magnitude were induced equally often by a and b. Therefore, the estimated exponent for C is the average of the estimated exponent on N and the negative of the estimated exponent on V. (Note again that, in accordance with a and b above, it is the negative of the effect of V that drives the response in the same direction as that

TABLE IV

Estimates of the Effects (i.e., Exponents) for the Primary and Derived Variables

Primary variable exponents ± standard error
$\hat{\beta}_n = 0.3503 \pm 0.0488$
$\hat{\beta}_v = -0.2797 \pm 0.0488$
$\hat{\beta}_t = 0.2164 \pm 0.0488$
Derived variable exponents ± standard error
$\hat{\beta}_c = 0.3150 \pm 0.0345$
$\hat{\beta}_d = 0.0670 \pm 0.0345$
$\hat{\beta}_f = -0.2480 \pm 0.0345$

of N.) Similarly, the estimated exponent for flow rate (F) is the estimated exponent on V averaged with the negative of the estimated exponent on duration (T), and the estimated exponent for delivery rate (D) is the exponent on N averaged with the negative of the estimated exponent on T. From this logic, the estimated exponents that characterize the dependence of the response magnitude upon the derived variables of concentration, delivery rate, and flow rate are shown in Table IV.

As discussed in the Methods section, the next higher level of each of the stimulation variables was twice the lower level, and Table VI gives for each of the primary and secondary variables the percent change in the response brought about by this doubling. This table, based upon the estimated exponents (Table IV), further demonstrates the effects that changes in each of the variables had upon responses under the conditions imposed by the design of this study.

An Adequate Model

There are several reasons to take the initial three-variable model given in Eq. 1 as an adequate model. Most important is its fairly accurate representation of the neural responses, with no indication of any interactive effects when the data

were subjected to the analysis of variance shown in Table III. The additive model that results from transformation into the logarithmic form (Eq. 2) required no additional interactive terms to describe the dependence of the neural response upon the stimulation variables. This gave strong support to the adequacy of the multiplicative model of Eq. 1, i.e., the response magnitude is related to the stimulation variables simply by the product of their separate powers. Somewhat less compelling but still giving added confirmation to the adequacy of the model is the fact that in a preliminary analysis of residuals (not shown here), the magnitudes of the standard deviations of the log observed responses correlated only to a low degree ($r = -0.26$) with the log average response levels elicited by changing from one treatment combination of variables to another. Thus, with no consistent change in the magnitudes of the errors as different treatments give larger and larger responses, the model's performance seems to be essentially uninfluenced by the treatment chosen. Finally, as tested by chi-square in the

TABLE V

Responses to Experimental Combinations of the Primary Variables

Duration (T)	Volume (V)	Number of molecules (N)	Geometric mean response		
			Observed* (O)	Expected‡ (E)	Ratio (O/E)
Short	Low	Small	9.34	9.13	1.02
		Large	11.24	11.64	0.97
	High	Small	7.29	7.52	0.97
		Large	10.00	9.59	1.04
Long	Low	Small	10.86	10.61	1.02
		Large	13.35	13.52	0.99
	High	Small	8.60	8.74	0.98
		Large	11.18	11.14	1.00

* Anti-logs of the mean log responses presented in Table I.

‡ Anti-logs of what the $\log_2$ responses would be as a result of only the main effects of N, V, and T, with no interaction effects.

previously mentioned preliminary analysis, the distribution of the log errors was found to fit the log normal distribution exceedingly well ($\chi^2 = 4.90$ with 6 degrees of freedom, $P > 0.5$). Therefore, it can be argued that in this model's representation of the data, the errors are like the resultants of chance factors rather than of some consistent misrepresentation by the model.

It must be emphasized that the apparent adequacy of this model does not rule out the possibility of other adequate models. However, one major advantage of this model, above and beyond the statistical considerations given above, is its harmony with the current conceptualization of the stimulus variables involved in olfaction. Such a multiplicative model is implied by the concepts of flow rate (V/T), concentration (N/V), and delivery rate (N/T).

Reduced Models: One- and Two-Variable Models

If one places constraints upon the exponents of the three-variable model, additional models are generated with reduced numbers of variables. For instance, if

one of the three primary variable exponents were set to zero, the stimulus-response relationship would depend only upon the other two primary variables. Three such two-variable models ($[N, V]$, $[N, T]$, $[V, T]$) are obtained as each of the exponents on the three primary variables is set to zero. These three models are not equivalent to each other because they are not algebraically interchangeable and the exponents in any one do not define the exponents in the other two. However, as shown in Appendix A (Eqs. A10–A12 and Table AI), each of these models does have two other equivalent expressions.

There are four other two-variable models. Three of these require the exponents on two of the primary variables to be equal and opposite in sign. These are the $[F, N]$, $[C, T]$, and $[D, V]$ models, none of which is equivalent (Appendix A, Eqs. A13–A15). Each of these three models implies that although the response depends upon all three primary variables, the combined effects of two of the primary variables are entirely incorporated into the effect of one of the derived variables. For instance, in the $[F, N]$ model, where $\beta_v = -\beta_t$, the effects of volume and time are entirely incorporated into an effect of flow rate. The fourth two-variable model, $[F, D]$ or equivalent expressions, requires combinations of the exponents to be equal and opposite in sign and implies that the effects of the three primary variables can be entirely incorporated into the effects of two derived variables (Appendix A, Eqs. A16–A18 and Table AI).

TABLE VI

Percent Change in Response as Each Variable Is Doubled

Variable	Percent change per doubling
Number of molecules (N)	+27.5
Volume of gas (V)	−17.6
Duration of presentation (T)	+16.2
Concentration ($C = N/V$)	+24.4
Delivery rate ($D = N/T$)	+4.8
Flow rate ($F = V/T$)	−15.8

The percent changes were calculated from the exponents reported in Table IV by determining their anti-logs, subtracting 1, and multiplying by 100.

Finally, there are three one-variable models ($[N]$, $[V]$, $[T]$), which require that the exponents on two of the primary variables be equal to zero. Three other one-variable models ($[F]$, $[C]$, $[D]$) require one of the exponents on the primary variables to be equal to zero and the other two to be equal and opposite in sign. The latter models imply that although the response depends upon two primary variables, the effects of these two primary variables are completely incorporated into an effect of one derived variable (see Appendix A, Eqs. A19 and A20 and Table AI).

Predictive Abilities of the Reduced Models

From the estimated exponents for the three primary variables shown in Table IV, it appears that, of the several constraints upon the full three-variable model (Table AI) required by the various reduced models, two were fulfilled. Since the absolute values of the estimated exponents for V and T were quite close to each

other and of opposite sign, the constraint for the [F, N] model (viz., that $\beta_v = -\beta_t$) was fulfilled. In the same way, the constraint for the [C, T] model (viz., that $\beta_n = -\beta_v$) was also fulfilled. Thus, it appeared that these two reduced models might compare rather favorably to the three-variable model in describing the relationship between response magnitude and stimulation variable magnitudes, whereas the other reduced models would compare less favorably.

To more formally compare the various models, two closely related statistical indexes were used. To obtain the first index for each reduced model, the estimated average predictive error variance (Appendix B) of the reduced model was divided by that of the three-variable model. This ratio facilitated making comparisons of the predictive abilities of the various reduced models. The second

TABLE VII

Relative Predictive Abilities of Reduced Models

Main effect variable(s) in reduced model	Rank of predictive ability	Estimated predictive error variance ratio*	Partial F ratio‡
[F, N]	1	0.989	0.89
[C, T]	2	0.990	1.05
[C, F] or [C, D] or [D, F]	3	1.172	11.56
[C]	4	1.294	10.37
[N, V] or [C, V] or [C, N]	5	1.314	19.68
[N, T] or [D, T] or [D, N]	6	1.543	32.90
[F]	7	1.828	26.22
[N]	8	1.832	26.29
[V, T] or [F, V] or [F, T]	9	1.868	51.58
[D, V]	10	2.143	67.48
[V]	11	2.145	35.63
[T]	12	2.369	42.23
[D]	13	2.637	50.18

* The estimated average predictive error variance for each reduced model is expressed as a ratio to that of the full model [N, V, T]. The full model estimate of the average predictive error variance is 0.0523, slightly greater than for the [F, N] and [C, T] models, but smaller than all others.

‡ The partial F ratio, as discussed in Appendix B, is the variance attributable to the reduced model constraints, relative to the error variance estimated in the full model.

index of predictive ability, the partial F ratio, compares the partial variance attributed to the deleted variable(s) in a given reduced model to the error variance in the full three-variable model (Appendix B). The smaller this F ratio, the less evidence there is that the deleted variable(s) affects the response. In Table VII the ratios representing the relative predictive abilities of the 13 distinct reduced models are presented in rank order. These results indicate that the [F, N] model and the [C, T] model were, respectively, as suggested above, the first and second best reduced models in predictive ability. Moreover, since their estimated average predictive error variance ratios were both lower than that of the three-variable model, and since their partial F ratios were both lower than the critical value (Appendix B), the [F, N] and [C, T] models appeared even better than the three-variable model in predicting neural responses.

One might have expected, on the contrary, that better predictions of the response would be provided by all three primary variables rather than by the two variables of the [*F*, *N*] or [*C*, *T*] models. However, it should be noted (see Appendix B) that the enhancement in predictive ability contributed by including a given variable hinges upon the balance between two opposing effects. On the one hand, the inclusion of a variable improves the prediction of the response, provided the correct value of its exponent is used. On the other hand, estimation of the exponent involves an error which, depending on its size, may reduce the model's ability to predict the response. Only in the case of the [*F*, *N*] and [*C*, *T*] models is the potential gain from adding a third variable more than offset by the error introduced in estimating that variable's exponent.

The third best two-variable model was that which included any two derived variables having the equivalent expressions of [*F*, *D*], [*F*, *C*], or [*C*, *D*]. This model, like the [*F*, *N*] and [*C*, *T*] models (although under different constraints), uses all three primary variables. It was considerably better in predictive ability than the remaining two-variable models, each of which completely disregards one of the three primary variables. In regard to these remaining two-variable models, predictive ability increased as the estimated magnitude of the deleted variable's effect decreased ($|\hat{\beta}_n| > |\hat{\beta}_v| > |\hat{\beta}_t|$). Thus, as emphasized by the partial *F* ratios, the greater the evidence of a variable's effect, the more its deletion reduced the predictive ability of the two-variable models.

The best one-variable model was [*C*], but it was only the third (by the estimated predictive error variance ratio) or fourth best (by the partial *F* ratio) of all 13 reduced models. It was considerably better than either the [*N*] or [*V*] models, a result which would be expected since the independent effects (i.e., the exponents) of both *N* and *V* were significant and in opposite directions. Therefore, the ratio of the two primary variables would be expected to predict the responses better than either primary variable alone. One might also have expected that the [*C*] model would be at least as good as the [*N*, *V*] model since $\hat{\beta}_n \simeq -\hat{\beta}_v$. In fact, the one-variable [*C*] model actually surpassed the two-variable [*N*, *V*] model, and this occurred for the same reason that the two-variable [*F*, *N*] and [*C*, *T*] models surpassed the full three-variable model.

Significance of Comparisons Between Models

In the previous section the various models were ranked in accordance with the estimates of their ability to predict neural responses. When the reduced model is superior to the full model, the partial *F* ratio for the deleted or constrained variables is, as shown in Appendix B, <2. However, note that this partial *F* criterion is not being used in a test of significance; this criterion does not imply a probability statement about responses under one model vs. another. Therefore, tests of significance, which require a much more stringent criterion than the foregoing analysis, were undertaken in order to determine which of the reduced models could be proven to be superior to others at conventional significance levels. Scheffé's multiple test criterion (Steel and Torrie, 1980*a*) was employed in testing the significance of two-variable reductions compared with the full model and in testing one-variable reductions compared with two-variable models.

Appendix C contains a detailed description of this statistical procedure as well as the t values for the specific comparisons. At the joint 5% level of significance, all the one-variable models were rejected in favor of one or more of the two-variable models. Of the two-variable models, only the $[F, N]$ and $[C, T]$ models are not rejected in favor of the three-variable model in describing the relationship between the neural response and the stimulation variables.

Of these two models, $[F, N]$ ranked higher than $[C, T]$ (Table VII), but whether the $[F, N]$ model is significantly superior to the $[C, T]$ model remained to be determined. This determination required the statistical analysis developed in Appendix D. Although the $[F, N]$ model ranked higher than the $[C, T]$ model in the estimation of its ability to predict responses, the difference between these two models in relating responses to the stimulation variables was not found to be statistically significant at the joint 0.05 level.

DISCUSSION

Same Variables, Different Statements

This study gives formal consideration to various mathematical models relating the magnitude of the olfactory response to olfactory stimulation variables. This formal consideration brings out a number of points concerning the olfactory stimulus-response relationship and the interplay of the variables involved, which, although not totally disregarded in olfactory studies, are at least not often brought to attention. First, this study systematically highlights the variety of very different statements involving different numbers and combinations of variables, which could possibly describe the olfactory intensity stimulus-response dependence. Furthermore, it demonstrates that even when the olfactory response is conceived to depend upon the same three primary stimulation variables, it can still do so in a number of very different ways. This point is important enough to this study to justify its further emphasis by summarizing the comparison of the $[F, N]$, $[C, T]$, $[D, V]$, and three-variable models.

As demonstrated in Appendix A, these four models incorporate, as do some others, all three primary variables, but each model involves distinctly different requirements. The $[C, T]$ model requires that N and V have effects that are equal in absolute magnitude and opposite in sign (except when their effects are zero). This gives a description of the stimulus-response relationship in which the effect of sniff duration may take on any real value and in which the effects of number of molecules and sniff volume are regarded as being totally incorporated into an effect of one derived variable, viz., concentration. On the other hand, in the $[F, N]$ model the effect of the number of molecules is unconstrained, whereas the effects of sniff volume and sniff duration are required to be equal and opposite. These latter effects can therefore be regarded as totally incorporated into an effect of another derived variable, flow rate. In the $[D, V]$ model the effect of volume is unconstrained and it is the effects of the number of molecules and sniff duration that must be equal and opposite in sign, thereby allowing their combined effects upon the response to be reflected completely as an effect of the third derived variable, delivery rate. In distinction to these three two-variable

models, the full three-variable model has no constraints placed upon the effects of N, V, and T. That is, the exponent on any one primary variable can take on a value unrelated to those of the others. It is, of course, possible in the full three-variable model to take that part of the effect of one variable, such as V, which is equal and opposite to the effect of another variable, such as N, and consider the combined effect as an effect of a derived variable, in this case C. However, unlike in the $[C, T]$ model, this use of the three-variable model still recognizes the possibility of a separate effect of N or V. Thus, among the 16 equivalent expressions of the three-variable model, $[C, V, T]$ and $[C, N, T]$ are included. As stated above, the point being emphasized here is that this study identifies and differentiates in a formal and systematic way those mathematical models which, though they have a basic impact upon olfactory research, are rarely articulated.

Relative Predictive Abilities of the Models

Several governing factors seem to emerge from the rankings of the predictive abilities of the 14 distinct models evaluated in this study. First, the closer a model comes to incorporating the effects of all three primary variables, the better its predictive ability. This is consistent with the finding that all three primary variables had a highly significant effect on the magnitude of the neural response. Second, if the effect of a primary variable is to be excluded from a model, the smaller the absolute value of the exponent of that variable, the less reduction there will be in the predictive ability of the model ($|\hat{\beta}_t| < |\hat{\beta}_v| < |\hat{\beta}_n|$).

Finally, those models whose constraints (Appendix A) require either that $\beta_n = -\beta_t$ or that $\beta_n = -\beta_v$ (i.e., models incorporating the total combined effects of V and T into an effect of F or the total combined effects of N and V into an effect of C) have the better predictive abilities. On the other hand, models requiring that $\beta_n = -\beta_t$ (i.e., models incorporating the total combined effects of V and T into an effect of D) have the poorer predictive abilities. This is consistent with the finding that $\hat{\beta}_v$ came close to equaling $-\hat{\beta}_t$, and $\hat{\beta}_n$ came close to equaling $-\hat{\beta}_v$, but $\hat{\beta}_n$ was very far from equaling $-\hat{\beta}_t$. Obviously, each model evaluated in this study presents a different mix of these factors. The $[F, N]$ and $[C, T]$ models represented the two best combinations. Although these two models require that the effects of the three primary variables be arranged differently (i.e., they impose different constraints upon the effects of the primary variables), the two arrangements account for the observed response magnitudes almost equally well. No single function with a particular set of terms has clearly excelled in describing the dependence of the response magnitude on the stimulation variables. Instead, this study identifies at least two reduced models with different sets of terms, $[F, N]$ and $[C, T]$, which are viable contenders for giving the best description of the stimulus-response relationship. Apparently, under the conditions of this study, the constraints required by both the $[F, N]$ and $[C, T]$ models best reflect those chemical, physical, and physiological processes that underlie the growth of the neural response. The design of this experiment was developed without bias toward any expected influences from these underlying processes. Nevertheless, one might have expected the $[C, T]$ model to have been clearly superior because the concentration gradient drives the odorant molecules into the mucosa, and

the longer this process continues, the greater would be the odorant molecular density at the receptor cells. Since the [C, T] model was not clearly superior, further investigation is required to determine how basic physicochemical processes should be applied in attempting to understand the growth of the olfactory response.

Further Consideration of the Derived Variable Effects

Recall (Results section) that the estimated exponent for a derived variable is the average of the estimated exponent on one primary variable and the negative of the estimated exponent on the other primary variable. As an example, the estimated exponent for C is the average of the estimated exponent on N and the negative of the estimated exponent on V. As mathematically shown in the narrative following Table AI in Appendix A, if the estimated effect of a derived variable had completely incorporated the combined effects estimated for the two primary variables, it would equal the estimated effect of one primary variable, which in turn would equal the negative of the estimated effect of the other primary variable. Thus,

$$\hat{\beta}_c = \hat{\beta}_n = -\hat{\beta}_v; \qquad \hat{\beta}_f = \hat{\beta}_v = -\hat{\beta}_t; \qquad \hat{\beta}_d = \hat{\beta}_n = -\hat{\beta}_t.$$

Although for the data of this study none of these three relationships held exactly, the first two, as discussed above, came close. The third, however, was not at all upheld. In the latter case the exponents on N and T were both positive. Thus, the negative of the effect of T did not drive the response in the same direction as did the positive effect of N. Instead, it drove the response in the opposite direction. Consequently, the combination expressed in the experiment for the delivery rate was not greatly different from zero. In other words, the delivery rate did not provide an efficient way to describe the relationship between the olfactory response magnitude and the stimulation variables. Thus, from yet another point of view, the poor showing of the [D] model in predicting the response magnitudes is underscored.

These relationships can be visualized by the three graphs in Fig. 7, each of which plots the relative response magnitude as a function of one of the derived variables. Since each derived variable can be altered by varying either one of two primary variables, the responses are plotted with three curves—one for each way to vary the derived variable and one for their average. It can be seen in Fig. 7*A* that when C was doubled either by doubling N and holding V constant or by halving V and holding N constant, the response was substantially increased. Although the slopes given by these increases were not exactly equal, they were sufficiently close so that the average slope was a reasonably good representation of the two. The magnitudes of the responses to N_1/V_1 and to N_2/V_2, both of which are at the same concentration, were also quite close, so that their average was a very good representation of the two. This demonstrates that the effect of concentration adequately represented the combined effects of number of molecules and volume. Similar remarks apply to flow rate and its primary variables, volume and time (Fig. 7*B*).

However, in Fig. 7*C* it can be seen that very different responses were elicited

by doubling the delivery rate, depending upon whether N was doubled or T was halved. Not only did the slopes differ, they were actually of opposite signs, so that the average slope (i.e., the slope for D) was quite small. Furthermore, the responses to N_1/T_1 and to N_2/T_2, both of which are at the same delivery rate and might be expected to give the same response (if the model were accurate), actually gave very different responses. Thus, the combined effects of N and T

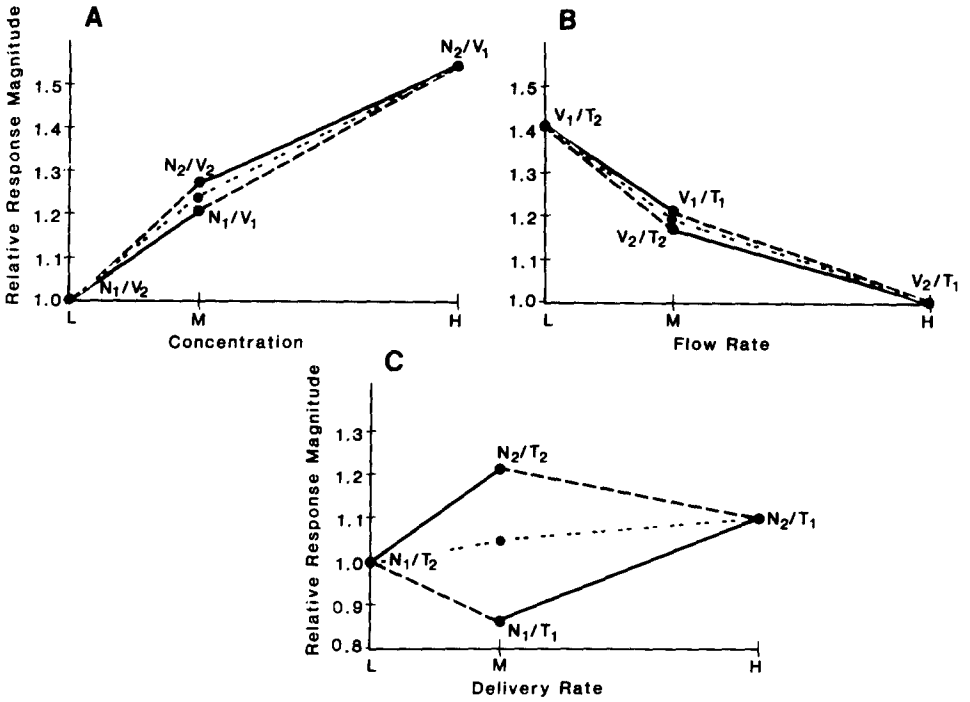

FIGURE 7. Relationship between the relative response magnitude and each of the derived variables: concentration (A), flow rate (B), and delivery rate (C). The responses in each curve are made relative to that given by either the smallest (curves A and C) or largest (curve B) level of the respective derived variable. In each plot the dashed line represents the change in response as a function of one of the primary variables defining the derived variable while the other primary variable is held constant. The solid line represents the change in response when the operations on the two primary variables are reversed. The double dotted line is the average of the other two. See text for detailed explanation.

cannot be regarded as *the* effect of delivery rate, since a given level of delivery rate has been shown to give more than one level of response.

Influences on the Effects of the Variables

In interpreting these exponents it must be cautioned that the ranges over which the variables were varied were rather restricted, being only twofold for the primary variables. However, as stated in the Methods section, there was an effort to present stimuli likely to be within the dynamic range of the animal's summated

multiunit response. This effort was made to reduce the chance that response saturation might materially influence the exponents. Wider ranges or different levels of primary variable values might very well have given different estimated exponents. Certainly there is no necessity that the exponent on a given variable over one range of values be the same as that over another range of values. Furthermore, if the exponents do vary in this way, the derived variables might completely incorporate the combined effects of primary variables at some levels but not at others. In a study currently being run, which uses wider ranges of N, V, and T, there is a strong indication that the concentration effect will indeed not fully incorporate the combined effects of number of molecules and volume, a conclusion that cannot be drawn here because the observed N and V effects more nearly offset each other in this present study.

There are a number of other experimental conditions, which, if altered, might also change the effects of the variables. Several of these might particularly influence the effect of number of molecules. First, the effect of N might vary with different odorants because the receptor cells have different sensitivities to different odorants (Gesteland et al., 1965). The greater the sensitivity, the greater would be the likely effect of a given increment in the number of molecules. Secondly, the effect of N might be further influenced by the sensitivity differences of various mucosal regions to different odorants (MacKay-Sim et al., 1982). Thus, the effect of the number of molecules of some odorants at some mucosal regions might be greater than the effect of the same odorants at other regions. Consequently, different exponents might be estimated depending both upon the odorants used and upon the region of the mucosa sampled. Another mechanism that might have an impact upon the effect of N is the pattern in which the incoming molecules of different odorants are sorbed across the mucosal sheet (Mozell, 1970). In accordance with their mucosa/air partition coefficients, different odorants establish different gradients of surface concentration along the flow path from the external naris to the internal naris (Hornung and Mozell, 1981). If, as suggested above, the effect of N is not the same at low and high levels, the exponents for some chemicals will change as the position of the mucosal region sampled moves farther along the flow path. For odorants with lesser gradients of surface concentration, such changes will be less marked.

Still other experimental conditions might influence the exponents on the various variables. The manner in which the odorized air is drawn over the mucosal region sampled by the neural recording might make a difference in the exponents on flow rate, volume, and duration. That is, it might make a difference whether, as in this experiment, the odorized air is presented as a transient slug drawn in its entirety over the sampled mucosal region or whether the slug is so large or so long as to approach being a continuous stream, which may or may not be entirely drawn over the sampled region. Finally, when one considers the differences in the geometry, nasal locations, airway convolutions, etc., of the olfactory regions in various species, species differences in the effect that each stimulation variable has upon the magnitude of the olfactory response would not be unexpected. For instance, in frogs almost all of the air entering the external naris must pass through that part of the nasal cavity housing the olfactory

mucosa, so that changes in flow rate would not be expected to alter the number of molecules gaining access to the nasal olfactory region. The effect of flow rate might then be due to its influence in determining the number of molecules striking the mucosal surface. As emphasized by Stuiver (1958), the faster the odorant molecules in a slug of air pass over a given locus on the mucosa's surface, the smaller would be the fraction of those molecules expected to diffuse from the slug to the locus. This is a likely basis for the negative exponent for flow rate found in this study. However, in humans, where the olfactory mucosa is not in the main nasal flow path, an increased flow rate could influence the response by several other mechanisms. First, unlike in the frog, it could have the positive effect of bringing more odorant-laden air to the olfactory mucosa by establishing eddy currents or by contributing to a shunt of the air stream. It could have a second positive effect by decreasing the loss of odorant molecules that occurs by sorption to the walls of the non-olfactory nasal pathway prior to reaching the olfactory mucosa. Third, even though more air with more odorant molecules might be brought to the olfactory mucosa, there could be, as in the frog, a negative influence by reducing the fraction of the number of molecules striking the mucosal surface. Thus, the final effect of flow rate in humans could be given by some balance of its positive effect of getting molecules to the olfactory mucosa and its negative effect of reducing the relative number of molecules making contact with the mucosa (de Vries and Stuiver, 1961).

This difference between frog and human in the mechanisms by which the flow rate could affect the response magnitude may be responsible for the apparent discrepancy between the negative effect found in this study for flow rate and the positive effect found in some (Rehn, 1978; Le Magnen, 1944–45), but not all (Teghtsoonian et al., 1978), human studies.

A more appropriate comparison of the flow rate effect would be with the findings of Tucker (1963). Tucker also recorded olfactory nerve summated multiunit discharges in the tortoise, which, like the frog, passes almost all of the incoming odorized air through that part of the nasal cavity housing the olfactory mucosa. He observed that the response to a presentation of amyl acetate at constant concentration and constant duration increased as the flow rate increased. Taken at face value, the positive effect of increased flow rate appears to be the antithesis of the findings in the present study, but actually it is just what the present study would predict considering the circumstances of the experiment. In order to increase flow rate while keeping the duration of the presentation constant, Tucker must have increased the volume of the presentation, and with concentration being held constant, this increase in volume would have had to increase the number of molecules presented. As shown by the size of the exponents in this study, increasing the number of molecules will increase the response more than the decrease expected from increasing either the flow rate or volume. Consequently, Tucker's observation of an increasing response with increasing flow rate is, under the conditions of the experiment, wholly in accord with the present study. Furthermore, Tucker attributed the increased response with increased flow rate, just as indicated by the present experiment, to an increase in the number of molecules. However, the data of Tucker's

experiment have been interpreted to mean that there is a direct relationship between response magnitude and flow rate, whereas the data of the present experiment indicate that the effect of flow rate upon the response magnitude was negative and that the positive effect seen in Tucker's experiment was due to the particular way in which the variables were manipulated. That is, increasing the flow rate at a given concentration by increasing volume does what Tucker proposed. Namely, it works toward replenishing the molecules that are continuously being sorbed out of the odorized air in the nasal cavity, thereby maintaining the driving force (i.e., the concentration gradient) across the mucus to the receptors.

The above comparison of the present study with that of Tucker may focus too much upon one aspect to the exclusion of others. There are several other major differences in technique that might affect the comparative interpretation of their results. For instance, the durations of Tucker's presentations (5, 10, and 30 s) were considerably longer than those of the sniffs in the present study (0.35 and 0.70 s), and it is conceivable that the effects of the various variables could themselves be affected by such large differences in presentation time. The long durations may begin to approach continuous stimulation relative to the response characteristics of the receptors, bringing to mind such terms as saturation and adaptation. Finally, as is so often true, there is also a difference in the odorants used in the two studies, and, as discussed earlier, the effects of the variables may very well depend upon such physicochemical properties of the odorants as their mucosa/air partition coefficients.

Magnitude Estimation, Similar Exponents

It is clear from the analysis of variance (Table III) that each of the three primary variables (number of molecules, sniff volume, and sniff duration) had a significant partial effect upon the magnitude of the summated multiunit discharge. How much effect each of these primary variables had upon the response is indicated by its estimated exponent in the original three-variable model. It is to be noted that the absolute values of these exponents were all less than unity, so that the relationship describing the dependence of the summated multiunit discharge upon each of the primary variables was not linear. Instead, the relationship was either negatively accelerating (in the case of N and T) or negatively decelerating (in the case of V). Furthermore, since the absolute values of these exponents were considerably less than unity, the growth (N and T) or fall-off (V) of responses as a function of arithmetic increases in stimulus magnitude would be substantially diminished. Similarly, the absolute values of average estimated exponents for the derived variables were considerably less than unity. These relationships are reminiscent of the many psychophysically determined power functions relating the olfactory sensory magnitude to the amount of odorant presented (Cain, 1969; Jones, 1958*a*, *b*; Berglund et al., 1971). Although the exponents in these power functions vary from odorant to odorant and although the overall levels of the exponents seem to vary from study to study, for the vast majority of cases the exponents are considerably less than unity (see Berglund et al., 1971). Jones (1958*b*), who used octane, as in the present study, reported 0.55 as the exponent

relating the sensory magnitude to the amount of octane presented; Berglund et al. (1971) reported 0.24. Considering the major differences in technique, these exponents are not too far from the ones which in the present study relate the magnitude of the neural discharge to the magnitudes of the various stimulation variables. Thus, the present study and psychophysical studies seem to describe similar stimulus-response relationships. It would, of course, be presumptuous to compare human psychophysically determined sensory magnitudes to electrophysiologically recorded discharges from the frog's olfactory nerve, but it is intriguing that the functions relating these two measures of response magnitude to the magnitude of the stimulus are so similar.

APPENDIX A

Derivation of the Mathematical Models

Three-Variable Models: No Constraints

In the Results section of this paper, the full three-variable multiplicative model involving the three primary variables [number of molecules (N), volume (V), and duration (T)] was presented, and the logarithm to the base 2 additive form of this model was given as follows:

$$r_{ij} = \alpha_j + \beta_n n_i + \beta_v v_i + \beta_t t_i + \epsilon_{ij}, \tag{A1}$$

where the lowercase symbols with subscripts refer to the logarithms of the uppercase terms, N, V, and T. The logarithm to the base 2 form of the primary variable ratios defining the derived variables of concentration (C), flow rate (F), and delivery rate (D) were also given as follows:

$$c_i = n_i - v_i; \qquad f_i = v_i - t_i; \qquad \text{and} \qquad d_i = n_i - t_i. \tag{A2}$$

The [C, V, T] and [C, N, T] equivalent expressions of the three-variable model were derived by substituting the appropriate relation in Eq. A2 into A1, giving, respectively:

$$r_{ij} = \alpha_j + \beta_n c_i + (\beta_n + \beta_v) v_i + \beta_t t_i + \epsilon_{ij} \tag{A3}$$

and

$$r_{ij} = \alpha_j + (\beta_n + \beta_v) n_i - \beta_v c_i + \beta_t t_i + \epsilon_{ij}. \tag{A4}$$

Note that in both Eqs. A3 and A4 the three-variable model is expressed in terms of two primary variables and one derived variable. There are four more such expressions, two including a term for flow rate, [F, N, T] and [F, N, V], and two including a term for delivery rate, [D, V, T] and [D, N, V].

For flow rate:

$$r_{ij} = \alpha_j + \beta_n n_i + \beta_v f_i + (\beta_v + \beta_t) t_i + \epsilon_{ij}; \tag{A5}$$

$$r_{ij} = \alpha_j + \beta_n n_i + (\beta_v + \beta_t) v_i - \beta_t f_i + \epsilon_{ij}. \tag{A6}$$

For delivery rate:

$$r_{ij} = \alpha_j + \beta_n d_i + (\beta_n + \beta_t) t_i + \beta_v v_i + \epsilon_{ij}; \tag{A7}$$

$$r_{ij} = \alpha_j + (\beta_n + \beta_t) n_i + \beta_v v_i - \beta_t d_i + \epsilon_{ij}. \tag{A8}$$

This three-variable model can also be expressed in terms of two derived variables and one primary variable. There are nine such expressions, each of which includes a different combination of the three variables: [C, F, N], [C, F, T], [C, F, V], [D, F, N], [D, F, V],

[*D*, *F*, *T*], [*D*, *C*, *N*], [*D*, *C*, *V*], and [*D*, *C*, *T*]. The [*C*, *F*, *N*] expression can be used as an example of how all nine of these expressions derive from the original model. Begin with Eq. A6, which, as shown above, already is the [*F*, *N*, *V*] expression of the original model. One then arrives at the [*C*, *F*, *N*] expression by substituting $n_i - c_i$ for v_i in Eq. A6 as follows:

$$r_{ij} = \alpha_j + \beta_n n_i + (\beta_v + \beta_t)n_i - (\beta_v + \beta_t)c_i - \beta_t f_i + \epsilon_{ij},$$

so that

$$r_{ij} = \alpha_j + (\beta_n + \beta_v + \beta_t)n_i - (\beta_v + \beta_t)c_i - \beta_t f_i + \epsilon_{ij}. \tag{A9}$$

Two-Variable Models: One Primary Exponent Constrained to Be Zero

Note that in all of the above expressions describing the dependence of the response upon the various stimulation variables, there are no constraints placed upon any of the exponents, i.e., none of the exponents has to have a particular value either alone or in relation to any of the other exponents. With no such restrictions, each of these expressions can be algebraically derived from any of the others, with, as shown above, the exponents in one expression determining the exponents in any other expression. All these expressions having such a one-to-one correspondence constitute a group of equivalent alternative three-variable expressions for the original three-variable model. However, by placing constraints upon one or more of the exponents in the original expression of the three-variable model (or any of its equivalent expressions), new models, not equivalent to the others previously described, are generated. That is to say, these new models describe a more limited range of relationships between the response and the stimulation variables. For instance, if β_t were taken as being equal to zero (i.e., a constraint is placed upon β_t), the relationship between the response and the stimulation variables would be conceived as depending only upon the number of molecules and the volume, with no effect being due to the duration. This [*N*, *V*] model would not be an equivalent expression for the original three-variable model but rather a distinct reduced model incorporating but two variables.

There are three mutually distinct two-variable models in which the exponent of one of the primary variables is constrained to equal zero. For each of these models there are two additional equivalent expressions which include a derived variable. These are: [*N*, *V*], [*C*, *V*], and [*C*, *N*]; [*V*, *T*], [*F*, *V*], and [*F*, *T*]; and [*N*, *T*], [*D*, *T*], and [*D*, *N*]. The [*N*, *V*] model will be used to exemplify the derivation of these models and their equivalent expressions.

If in Eq. A1, β_t is set equal to zero, the [*N*, *V*] model is obtained:

$$r_{ij} = \alpha_j + \beta_n n_i + \beta_v v_i + \epsilon_{ij}. \tag{A10}$$

Substituting $c_i + v_i$ for n_i in Eq. A10 and gathering terms gives the [*C*, *V*] expression of this particular two-variable model:

$$r_{ij} = \alpha_j + \beta_n c_i + (\beta_n + \beta_v)v_i + \epsilon_{ij}. \tag{A11}$$

Substituting $n_i - c_i$ for v_i in Eq. A10 and gathering terms gives the [*C*, *N*] expression of this particular two-variable model:

$$r_{ij} = \alpha_j + (\beta_n + \beta_v)n_i - \beta_v c_i + \epsilon_{ij}. \tag{A12}$$

Inspection of Eqs. A10–A12 shows them to be equivalent expressions of the same model since they all result from the same constraint upon exponents in the three-variable model. They are all algebraically interchangeable, and the exponents in any one of them define the exponents in the other two. Similarly, the [*V*, *T*] model with its equivalent expressions results when $\beta_n = 0$ and the [*N*, *T*] model with its equivalent expressions results when $\beta_v = 0$.

Two-Variable Models: Two Equally Valued Primary Exponents with Opposite Signs

Clearly, in the foregoing three two-variable models, the exponents for one could not define the exponents for the others (and are therefore not equivalent) because each ignores the effect of a different variable on the response. However, the distinctions are more subtle for several other two-variable models which take cognizance of variations in all three primary variables. Even though none of the exponents for the three primary variables is equal to zero in these models, different constraints are placed upon them which result in distinct two-variable models; that is, the models do not fall into the same equivalence groups. Three such mutually distinct models are $[C, T]$, $[F, N]$, and $[D, V]$. Each of these models places a different constraint upon the three-variable model. Expression A3, an equivalent expression ($[C, V, T]$) of the three-variable model, reduces to the $[C, T]$ model only when $\beta_n = -\beta_v$, giving:

$$r_{ij} = \alpha_j + \beta_v c_i + \beta_t t_i + \epsilon_{ij}. \tag{A13}$$

Eq. A5, another equivalent expression ($[F, N, T]$) of the three-variable model, reduces to the $[F, N]$ model only when $\beta_v = -\beta_t$, giving:

$$r_{ij} = \alpha_j + \beta_n n_i + \beta_v f_i + \epsilon_{ij}. \tag{A14}$$

Eq. A7, still another equivalent expression ($[C, T, V]$) of the three-variable model, reduces to the $[D, V]$ model only when $\beta_n = -\beta_t$, giving:

$$r_{ij} = \alpha_j + \beta_n d_i + \beta_v v_i + \epsilon_{ij}. \tag{A15}$$

Since each of these models is obtained by a different constraint, none is equivalent to any other. To further exemplify that none of these three models is equivalent to any other, consider the possible equivalence of the $[F, N]$ and $[D, V]$ models. An equivalent expression of the $[F, N]$ model can be obtained by substituting $v_i - t_i$ for f_i in Eq. A14, giving:

$$r_{ij} = \alpha_j + \beta_n n_i + \beta_v v_i - \beta_v t_i + \epsilon_{ij}.$$

It is clear that these terms can be rearranged to give the $[D, V]$ model only by adding another constraint, i.e., $\beta_v = \beta_n$. Thus, the $[F, N]$ model cannot be considered equivalent to the $[D, V]$ model since the exponents of their variables correspond only in the special case that $\beta_v = \beta_n = -\beta_t$.

Two-Variable Models: the Sum of Two Primary Variable Exponents Equals the Negative of the Third Primary Variable Exponent

There is yet another two-variable model. This one has three equivalent expressions of two derived variables, i.e., $[F, D]$, $[C, F]$, and $[C, D]$. For all of these the same constraint, $\beta_n = -(\beta_v + \beta_t)$, must be placed upon the three-variable model. With this constraint, Eq. A5, which is an equivalent expression of the three-variable model, reduces to the $[F, D]$ model as follows:

$$\begin{aligned} &\text{Eq. A5 (rearranged) is} \quad r_{ij} = \alpha_j + \beta_n n_i + (\beta_v + \beta_t) t_i + \beta_v f_i + \epsilon_{ij}; \\ &\text{if} \quad \beta_n = -(\beta_v + \beta_t), \qquad r_{ij} = \alpha_j + \beta_n n_i - \beta_n t_i + \beta_v f_i + \epsilon_{ij}; \\ &\text{replacing } n_i - t_i \text{ by } d_i\text{:} \quad r_{ij} = \alpha_j + \beta_n d_i + \beta_v f_i + \epsilon_{ij}. \end{aligned} \tag{A16}$$

Similarly, with the same constraint, Eq. A6 reduces to the $[C, F]$ expression of the same model and Eq. A8 reduces to the $[C, D]$ expression of the same model:

$$r_{ij} = \alpha_j + \beta_n c_i - \beta_t f_i + \epsilon_{ij}; \tag{A17}$$

$$r_{ij} = \alpha_j + (\beta_n + \beta_t)c_i - \beta_t d_i + \epsilon_{ij}. \quad \text{(A18)}$$

Since $[F, D]$, $[C, F]$, and $[C, D]$ are all derived from equivalent expressions of the three-variable model by imposing the same constraint, they are equivalent expressions of the same model, which itself is distinct from the three-variable model and other two-variable models.

One-Variable Models: Two Primary Exponents Equal to Zero

There are six one-variable models, three involving only a primary variable and three involving only a derived variable. Two constraints are placed upon the general three-variable model to give the one-variable models. To obtain a primary one-variable model, the exponents for two of the primary variables are set equal to zero. If, for instance, β_v and β_t both equal zero, the result is the $[N]$ model:

$$r_{ij} = \alpha_j + \beta_n n_i + \epsilon_{ij}. \quad \text{(A19)}$$

Similarly, the $[V]$ model and the $[T]$ model are given when $\beta_t = \beta_n = 0$ and $\beta_v = \beta_n = 0$, respectively.

One-Variable Models: One Primary Exponent Equal to Zero, Others Equally Valued with Opposite Signs

To obtain a derived one-variable model, one of the constraints is that one of the exponents is set equal to zero. The other constraint is that the two remaining primary variable exponents are equal and opposite. For instance, Eqs. A10–A12 describe three equivalent expressions ($[N, V]$, $[C, V]$, and $[C, N]$) of the same model. This model is given when β_t in the three-variable model is constrained to equal zero. The additional constraint that $\beta_n = -\beta_v$ then gives the $[C]$ model:

$$r_{ij} = \alpha_j + \beta_n c_i + \epsilon_{ij} \quad \text{or} \quad r_{ij} = \alpha_j - \beta_v c_i + \epsilon_{ij}. \quad \text{(A20)}$$

Similarly, the $[F]$ model is given when $\beta_n = 0$ and $\beta_v = -\beta_t$. The $[D]$ model is given when $\beta_v = 0$ and $\beta_n = -\beta_t$. Since the constraints given each of these models are different, the models are not equivalent to each other.

TABLE AI

Summary of Constraints and Resultant Models

Below are the three-, two-, and one-variable models categorized in accordance with the constraints giving rise to them. All those listed under a single constraint are equivalent to each other, whereas those listed under different constraints are distinct from each other.

Constraint	*Model*
None	$[N, V, T]$, $[C, V, T]$, $[C, N, T]$, $[F, N, T]$, $[F, N, V]$, $[D, T, V]$, $[D, N, V]$, $[C, F, N]$, $[C, F, T]$, $[C, F, V]$, $[D, F, N]$, $[D, F, T]$, $[D, F, V]$, $[D, C, N]$, $[D, C, V]$, $[D, C, T]$
$\beta_t = 0$	$[N, V]$, $[C, V]$, $[C, N]$
$\beta_v = 0$	$[N, T]$, $[D, T]$, $[D, N]$
$\beta_n = 0$	$[V, T]$, $[F, V]$, $[F, T]$
$\beta_n = -\beta_v$	$[C, T]$
$\beta_v = -\beta_t$	$[F, N]$
$\beta_n = -\beta_t$	$[D, V]$
$\beta_n = -(\beta_v + \beta_t)$	$[F, D]$, $[F, C]$, $[C, D]$
$\beta_t = 0$, $\beta_n = -\beta_v$	$[C]$
$\beta_n = 0$, $\beta_v = -\beta_t$	$[F]$

TABLE AI (*continued*)

$\beta_v = 0$, $\beta_n = -\beta_t$	[D]
$\beta_v = \beta_t = 0$	[N]
$\beta_t = \beta_n = 0$	[V]
$\beta_v = \beta_n = 0$	[T]

A note on these models:

It is well to further point out that the olfactory response can depend upon the same set of stimulation variables in a number of distinct ways. For example, the [*N*, *V*] model and the [*C*] model both involve the same primary variables, because both regard sniff duration as having no effect upon the response and $C = N/V$. However, the [*C*] model regards the effects of *N* and *V* to be equal with opposite signs, i.e., $\beta_n = -\beta_v$ (Eq. A20). [Mathematically, if the effect of *C* is given by any exponent, *x*, the absolute magnitude of that exponent, *x*, must be equal to the exponents on *N* and *V*: $C^x = (N/V)^x = N^x/V^x$.] Another way of stating the [*C*] model relationship is to say that the magnitude of the olfactory response depends only upon the ratio of *N* to *V* and that any combination of effects by changing *N* and *V* can be entirely incorporated by their effect on concentration. On the other hand, the [*N*, *V*] model makes no statement as to how the effects of *N* and *V* must be related to each other since their exponents can take any values independently (Eq. A10). It is only apparently contradictory that in the [*N*, *V*] model part of the effect of *V* can be regarded as equal and opposite to the effect of *N* so that an equivalent expression for the [*N*, *V*] model is [*C*, *V*] (Eqs. A10 and A11). Note that unlike in the [*C*] model, this model recognizes an effect of sniff volume on the response which is not incorporated by the effect of concentration. Since there is no constraint placed upon the volume effect, this model, in spite of regarding part of the volume effect as equal and opposite to the effect of the number of molecules, still makes no statement as to how the entire effects of *N* and *V* must be related to each other.

Thus, although various models involve the same set of variables, each model can make a distinct statement as to how the response is related to these variables.

APPENDIX B

Derivation of Indicators of Predictive Ability

The least-squares linear model estimate of the response for an observed individual with known regressor (e.g., *n*, *v*, *t*) values may be regarded as a prediction of the response that would be observed in the next randomly selected individual that has the same regressor values. The expected squared deviation of that response from the predicted response is defined as the predictive error variance (Steel and Torrie, 1980*b*). The average predictive error variance (averaged over all individuals in the available sample) can be defined as a measure of the predictive ability of the fitted model: the smaller the average predictive error variance, the greater the predictive ability.

It has been shown (Hocking, 1976) that the average predictive error variance depends not only on the variance of the error term in the linear model, but also on the number of coefficients estimated in the model, relative to the available sample size. That is,

$$\sigma_k^{*2} = \sigma_k^2(m + k)/m, \tag{A21}$$

where σ_k^{*2} = average predictive error variance; σ_k^2 = error variance; m = sample size; and k = number of coefficients in the model. The error variance is estimated by routine analysis of variance procedures as

$$\hat{\sigma}_k^2 = \mathrm{RSS}_k/(m - k), \tag{A22}$$

where RSS_k = the minimized residual sum of squared deviations of observed from

estimated responses. Consequently,

$$\hat{\sigma}_k{}^{*2} = \frac{\mathrm{RSS}_k(m + k)}{m(m - k)}$$

estimates the predictive ability of the model. If a set of variables, numbering $h \leq k$, is deleted or if some other set of h constraints is specified, the estimate becomes

$$\hat{\sigma}_{k-h}{}^{*2} = \frac{\mathrm{RSS}_{k-h}(m + k - h)}{m(m - k + h)}. \tag{A23}$$

Ratios of reduced to full model estimates as determined by these expressions were used as the basis for ranking predictive abilities.

The superiority or inferiority of a reduced model would hinge upon whether the foregoing ratio is less than or greater than unity and this criterion is related to the customary F in the analysis of variance as follows:

$$\text{If } \hat{\sigma}_{k-h}{}^{*2} = \hat{\sigma}_k{}^{*2}, \text{ then from Eq. A23, } \frac{\mathrm{RSS}_{k-h}(m + k - h)}{(m - k + h)} = \frac{\mathrm{RSS}_k(m + k)}{(m - k)}. \tag{A24}$$

From conventional analysis of variance procedures (Kempthorne, 1952), the partial variance attributable to the h constraints, relative to the error variance estimated in the full model, is

$${}_hF_{m-k} = \frac{(\mathrm{RSS}_{k-h} - \mathrm{RSS}_k)}{\mathrm{RSS}_k} \cdot \frac{(m - k)}{h}. \tag{A25}$$

By using Eq. A25 we can re-express Eq. A24 in terms of ${}_hF_{m-k}$ to obtain:

$${}_hF_{m-k} = 2m/(m + k - h). \tag{A26}$$

When $(k - h)$ is small relative to m, we obtain a convenient approximate critical value of ${}_hF_{m-k} = 2$. Thus, when the partial F ratio equals or exceeds 2, the predictive ability of the full model is estimated to exceed that of the reduced model. When the partial F is <2 or, more precisely, less than the critical value in Eq. A26, the predictive ability of the reduced model is estimated to be superior. It should be emphasized that the criterion is not used as a test of significance. When $F \geq 2$, the data indicate that the potential gain from including the additional regressors in the full model more than offsets the loss that can be expected from errors in estimating their coefficients, but in the neighborhood of $F = 2$, the apparent advantage is very slight. In contrast, significance at the 5% level, as exemplified in Appendix C and as presented in the Results section (*Significance of Comparisons Between Reduced Models*), requires that a much more stringent criterion be met.

APPENDIX C

Statistics Testing the Significance of Comparisons Between Models

With three treatment parameters involved in the multiple testing procedure and with 56 degrees of freedom for error, the Scheffé criterion for significance at the joint 5% level was determined from an F table of critical values for 3 numerator and 56 denominator degrees of freedom:

$$t_s = (3 \cdot {}_3F_{56,0.05})^{1/2} = 2.88.$$

Thus, only calculated t values >2.88 in absolute value were declared significant at the joint 5% level.

Testing the significance of one-variable reductions of two-variable models is straightforward because each one-variable model is equivalent to the hypothesis that the exponent for one of the variables in a two-variable model is zero, i.e., that the variable with the zero coefficient has no effect on the response. For example, testing model $[N]$ vs. $[N, V]$ is equivalent to testing that $\beta_v = 0$. From the results given earlier concerning the exponents, the calculated t in this example is:

$$t = (\hat{\beta}_v - 0)/s_{\hat{\beta}_v} = \frac{-0.2797}{0.0488} = -5.73.$$

Similarly, certain two-variable models are obtained by hypothesizing that the exponent for one of the variables in the three-variable model is equal to zero. However, other two-variable models are obtained by imposing the constraint that a certain linear combination of exponents be zero. As an example of the latter type of model, Eq. 5 in the Results section gives an alternative expression for the $[N, V, T]$ model, which by substitution includes a term for concentration (C). From this equation it can be seen that testing the $[C, T]$ vs. the $[N, V, T]$ model is equivalent to testing that the linear combination, $\beta_n + \beta_v$, is zero. If $\beta_n + \beta_v = 0$ (alternatively expressed as $\beta_n = -\beta_v$), neither N nor V would in the $[C, T]$ model have any effect on the response other than through its respective effect on N/V (i.e., the concentration). Using the results for the exponents given earlier, the calculated t for testing this hypothesis is:

$$t = (\hat{\beta}_n + \hat{\beta}_v - 0)/(s_{\hat{\beta}_n}^2 + s_{\hat{\beta}_v}^2)^{1/2} = \frac{0.3503 - 0.2797 - 0}{[(0.0488)^2 + (0.0488)^2]^{1/2}} = 1.02.$$

The absolute values of the calculated t for specific comparisons involving each of the six single factors are: for $[N]$ vs. $[N, V]$, $|t| = 5.73$; for $[V]$ vs. $[N, V]$, $|t| = 7.18$; for $[T]$ vs. $[V, T]$, $|t| = 5.73$; for $[C]$ vs. $[C, T]$, $|t| = 4.44$; for $[F]$ vs. $[F, N]$, $|t| = 7.18$; and for $[D]$ vs. $[D, V]$, $|t| = 5.73$. These calculated t values were compared to the critical value ($t_s = 2.88$) given by Scheffé's multiple test as the criterion for the 5% level of significance in the present experiment. This provided a conservative test of each of the above hypotheses, i.e., that the exponent for a deleted variable in a particular one-variable vs. two-variable test equals zero. Since each calculated $|t|$ is much greater than 2.88, this hypothesis in all cases must be rejected; in every case there is conclusive evidence that the deleted variable does have an effect upon the response. Thus, all the one-variable models are rejected in favor of one or more of the two-variable models.

Similarly, in testing each of the seven distinct two-variable models vs. the full model, $[N, V, T]$, the calculated test values are: for $[N, V]$, $|t| = 4.43$; for $[N, T]$, $|t| = 5.73$; for $[V, T]$, $|t| = 7.18$; for $[D, V]$, $|t| = 8.21$; for $[C, F]$, $|t| = 3.40$; for $[C, T]$, $|t| = 1.02$; for $[F, N]$, $|t| = 0.92$. Comparing these values to the Scheffé criterion value, it is clear that for most of the two-variable models the constraints placed upon the three-variable model to achieve the proper reduced model did indeed neglect significant effects upon the response. Therefore, these reduced models must be rejected in favor of the three-variable model in describing the relationship between the response and the stimulation variables. However, there are two exceptions to this general finding. These are the $[F, N]$ and $[C, T]$ models. The constraints which were imposed upon the three-variable model to derive these two two-variable models did not neglect significant effects upon the response. Therefore, the $[F, N]$ and $[C, T]$ models are not rejected in favor of the $[N, V, T]$ model in describing the relationship between the response and the stimulation variables.

APPENDIX D

Test of the Hypothesis of Equal Mean Squared Residuals for Models $[F, N]$ and $[C, T]$

In the logarithmic expression of the general model, the main effects and error terms appear as follows:

$$\beta_n n_i + \beta_v v_i + \beta_t t_i + \epsilon_{ij}. \tag{A27}$$

These terms can be expressed as

$$\beta_n n_i + \beta_f f_i + [\tfrac{1}{2}(\beta_v + \beta_t)(v_i + t_i) + \epsilon_{ij}], \tag{A28}$$

where the least-squares main effects of the $[F, N]$ model are given in the first two terms by β_n and by $\beta_f = (\beta_v - \beta_t)/2$, and where the residual term of the $[F, N]$ model is in brackets. [The equivalence of Eqs. A28 and A27 is established by substituting $(\beta_v - \beta_t)/2$ for β_f and $(v_i - t_i)$ for f_i in Eq. A28 and collecting terms to get Eq. A27.] Similarly, an equivalent expression of Eq. A27 is given by

$$\beta_c c_i + \beta_t t_i + [\tfrac{1}{2}(\beta_n + \beta_v)(n_i + v_i) + \epsilon_{ij}], \tag{A29}$$

where the least-squares main effects of the $[C, T]$ model are given in the first two terms by $\beta_c = (\beta_n - \beta_v)/2$ and by β_t, and where the residual term of the $[C, T]$ model is in brackets. Now, it is hypothesized that the expected values of the squared residuals of $[F, N]$ in Eq. A28 and of $[C, T]$ in Eq. A29 are equal:

$$E[\tfrac{1}{2}(\beta_v + \beta_t)(v_i + t_i) + \epsilon_{ij}]^2 = E[\tfrac{1}{2}(\beta_n + \beta_v)(n_i + v_i) + \epsilon_{ij}]^2. \tag{A30}$$

In the subsequent derivation it is convenient to center the variables about means of zero, with $v_i = -\tfrac{1}{2}$ or $+\tfrac{1}{2}$ for low or high volume, $t_i = -\tfrac{1}{2}$ or $+\tfrac{1}{2}$ for a short or long duration, and $n_i = -\tfrac{1}{2}$ or $+\tfrac{1}{2}$ for a small or large number of molecules, respectively. Squaring the bracketed terms and taking expected values in Eq. A30 gives:

$$\tfrac{1}{4}(\beta_v + \beta_t)^2 E(v_i + t_i)^2 + (\beta_v + \beta_t)E(v_i + t_i)\epsilon_{ij} + E(\epsilon_{ij})^2$$

$$= \tfrac{1}{4}(\beta_n + \beta_v)^2 E(n_i + v_i)^2 + (\beta_n + \beta_v)E(n_i + v_i)\epsilon_{ij} + E(\epsilon_{ij})^2.$$

That is, $\tfrac{1}{8}(\beta_v + \beta_t)^2 + \sigma^2 = \tfrac{1}{8}(\beta_n + \beta_v)^2 + \sigma^2$, so that

$$(\beta_v + \beta_t)^2 = (\beta_n + \beta_v)^2. \tag{A31}$$

Taking the square roots of each side, Eq. A31 is satisfied by two relationships:

$$\begin{aligned} &(i)\ \beta_t - \beta_n = 0 \\ \text{and}\quad &(ii)\ \beta_t + \beta_n + 2\beta_v = 0. \end{aligned} \tag{A32}$$

Therefore, the hypothesis that $[F, N]$ and $[C, T]$ have the same residual variability is equivalent to either condition *i* or *ii*.

Using least-squares estimates of the coefficients and their standard errors (with zero covariances), the calculated t values are:

$$(i)\ t = \frac{\hat{\beta}_t - \hat{\beta}_n}{(s_{\hat{\beta}_t}^2 + s_{\hat{\beta}_n}^2)^{1/2}} = \frac{0.2164 - 0.3503}{[(0.0488)^2 + (0.0488)^2]^{1/2}} = 1.94$$

$$\text{and}\quad (ii)\ t = \frac{\hat{\beta}_t + \hat{\beta}_n + 2\hat{\beta}_v}{(s_{\hat{\beta}_t}^2 + s_{\hat{\beta}_n}^2 + 4s_{\hat{\beta}_v}^2)^{1/2}} = \frac{0.2164 + 0.3503 + 2(-0.2797)}{[(0.0488)^2 + (0.0488)^2 + 4(0.0488)^2]^{1/2}} = 0.061.$$

Since the absolute values of both calculated t's do not exceed the Scheffé criterion of 2.88, the hypothesis of equal residual variability is not rejected at the joint 0.05 level. Thus, the performances of the $[F, N]$ and $[C, T]$ models are not significantly different.

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