"Imposed" and "Inherent" Mucosal Activity Patterns

Their Composite Representation of Olfactory Stimuli

MAXWELL M. MOZELL, PAUL R. SHEEHE, DAVID E. HORNUNG, PAUL F. KENT, STEVEN L. YOUNGENTOB, and STEPHEN J. MURPHY

From the Departments of Physiology and Preventive Medicine, State University of New York, Health Science Center at Syracuse, Syracuse, New York 13210

ABSTRACT Both regional differences in mucosal sensitivity and a gas chromatography-like process along the mucosal sheet have been separately proposed in two sets of earlier studies to produce different odorant-dependent activity patterns across the olfactory mucosa. This investigation evaluated, in one study, whether and to what degree these two mechanisms contribute to the generation of these activity patterns. Summated multiunit discharges were simultaneously recorded from lateral (LN) and medial (MN) sites on the bullfrog's olfactory nerve to sample the mucosal activity occurring near the internal and external nares, respectively. Precisely controlled sniffs of four odorants (benzaldehyde, butanol, geraniol, and octane) were drawn through the frog's olfactory sac in both the forward (H_1) and reverse (H_2) hale directions. By combining the four resulting measurements, LN_{H_1}, LN_{H_2}, MN_{H_1}, and MN_{H_2}, in different mathematical expressions, indexes reflecting the relative effects of the chromatographic process, regional sensitivity, and hale direction could be calculated. Most importantly, the chromatographic process and the regional sensitivity differences both contributed significantly to the mucosal activity patterns. However, their relative roles varied markedly among the four odorants, ranging from complete dominance by either one to substantial contributions from each. In general, the more strongly an odorant was sorbed by the mucosa, the greater was the relative effect of the chromatographic process; the weaker the sorption, the greater the relative effect of regional sensitivity. Similarly, the greater an odorant's sorption, the greater was the effect of hale direction. Other stimulus variables (sniff volume, sniff duration, and the number of molecules within the sniff) had marked effects upon the overall size of the response. For strongly sorbed odorants, the effect of increasing volume was positive; for a weakly

Address reprint requests to Dr. Maxwell M. Mozell, Physiology Dept., SUNY Health Science Center at Syracuse, 766 Irving Ave., Syracuse, NY 13210.

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/87/11/0625/26$2.00

Volume 90 November 1987 625–650

sorbed odorant, it was negative. The reverse may be true for duration. In contrast, the effect of increasing the number of molecules was uniformly positive for all four odorants. However, there was little evidence that these other stimulus variables had a major influence upon the effects of the chromatographic process and regional sensitivity differences in their generation of mucosal activity patterns.

INTRODUCTION

There is general agreement that the olfactory receptor cells are to some degree selectively responsive to different odorants and that the ensuing configurations of fiber-to-fiber relative discharges across the entire ensemble of olfactory fibers is a basis for olfactory discrimination. Another possible basis could be different odorant-related topographical activity patterns across the expanse of the mucosal receptor sheet. Several investigators, mainly using salamanders, have reported evidence that receptor cells particularly sensitive to the same odorant cluster together in the same mucosal region, with different regions having different odorant sensitivities (Kubie et al., 1980; Mackay-Sim and Kubie, 1981; Mackay-Sim et al., 1982). That is, the roof of the animal's olfactory sac was removed and different odorants were puffed in punctate fashion directly onto the recording sites in different regions of the exposed olfactory mucosa. Each odorant then produced its greatest response (as measured by the electro-olfactogram [EOG]) over a mucosal region that differed somewhat from those of other odorants. This finding, based upon the EOG, that different regions of the mucosa were differentially sensitive to different odorants, corroborated earlier findings based upon single-unit recordings from the olfactory bulb (Kauer and Moulton, 1974). Extrapolating these findings to the intact olfactory sac, one can project how different odorants passing over the mucosa can establish different spatially organized patterns.

Another putative basis for olfactory discrimination, which would also produce different odorant-related, spatially organized patterns, does not depend upon the selective odorant sensitivity of the receptors, but, instead, depends upon how the molecules of different odorants are spatially and temporally distributed across the mucosal receptor sheet. This was suggested by summated multiunit discharges recorded from two branches of the bullfrog's olfactory nerve, which supplied earlier and later locations along the intact mucosal flow path (Mozell, 1966, 1970). In a given sniff, each odorant gave rise to a characteristic change in activity from the earlier to the later location, ranging from a steep gradient to no change in response at all. These differences could not be adequately explained, as it might first appear, by differences in sensitivity to the odorants at the two locations, because of two other concomitant observations: (*a*) the greater the decrease in activity from the earlier to the later location, the longer was the time interval between the onset of the activity at the two locations (Mozell, 1966, 1970), and (*b*) reversing the flow direction across the mucosa reversed the regions of greater and lesser activity (Mozell, 1964). An explanation that seemed to encompass all the observations centered around the differential sorption of

odorants by the mucosa. The more strongly an odorant's molecules are sorbed, the longer it would take them to be transported from the earlier to later locations, the greater would be their accumulation early in the flow path (regardless of flow direction), and the fewer would be their numbers farther down the flow path. This explanation was later confirmed by using radioactively labeled odorants, which showed that the molecules of different odorants are indeed distributed differently across the mucosal surface (Hornung and Mozell, 1977, 1981). This explanation was also supported when, after the column of a gas chromatograph was replaced with a frog's olfactory mucosa, it was observed that the molecules of different odorants do indeed migrate across the mucosa at different rates (Mozell and Jagodowicz, 1973, 1974). This differential sorption explanation for the activity patterns across the mucosa argued for an analogy between at least one of the processes possibly underlying olfactory discrimination and the process underlying gas chromatography (Mozell, 1970, 1971; Mozell and Jagodowicz, 1973, 1974; Mozell and Hornung, 1985).

Thus, there are two documented mechanisms that appear able to establish different activity patterns across the mucosa. Moulton (1976) has assigned the term "imposed" to the activity patterns stemming from the differential sorption of odorants along the mucosa (i.e., the gas chromatography-like process) and the term "inherent" to those based upon regional differences in mucosal sensitivity. It is the purpose of this study to evaluate, using four odorants, whether and to what relative degree regional sensitivity differences and the chromatographic process influence the generation of mucosal activity patterns. To adequately pursue this topic, it is necessary to regulate and monitor several stimulation variables (number of odorant molecules, sniff duration, sniff volume, and direction of airflow), which, as quantified in earlier studies (Mozell et al., 1984, 1986; Kurtz and Mozell, 1985), define an odorant sniff and are determinants of the magnitude of the olfactory nerve discharge. Thus, in addition to evaluating the relative contributions of mucosal sorption and regional sensitivity to the activity patterns for different odorants, this study also evaluates how these contributions and the activity patterns themselves might change with a variety of stimulation conditions.

METHODS

Preparation and Recording Procedures

Bullfrogs, *Rana catesbeiana* (Arcadian Biological, Rayne, LA), were maintained in groups of a dozen or so in large tanks with constantly flowing tap water. After a frog was anesthetized with urethane (1.2 g/100 g body wt), it was wrapped in a wet towel and secured in a small-animal headholder (David Kopf Instruments, Inc., Tujunga, CA) adapted for use with frogs and modified to accommodate the odorant delivery system, which was connected to the internal and external nares as described below. The olfactory nerve was exposed caudal to the cribriform plate. Care was taken during the surgery and subsequently not to compromise the integrity of the animal's olfactory sac, in order to preserve its normal flow path.

The two active recording electrodes were 63.5-μm stainless-steel wires quadruply

enameled to the tip. After the olfactory nerve had been desheathed, one electrode was pressed against its lateral margin and the other against its medial margin. As shown by Kurtz and Mozell (1985), this sampled the activity originating from two different regions of the olfactory mucosa. The indifferent recording leads were attached to the earbars of the headholder. The multiunit discharges of the olfactory nerve were recorded and amplified by AC preamplifiers (model A-1M, BAK Electronics, Inc., Clarksburg, MD) set with a bandpass region of 100–2,000 cycles/s. To quantify the neural discharges, the preamplifier outputs were passed through self-discharging integrators to give summated outputs proportional to the sampled neural activity (Beidler, 1953). The charging and discharging time constants of these self-discharging integrators were 0.2 and 1.9 s, respectively. The traces of the summated discharges were recorded both on a chart recorder (9176, Varian Associates, Inc., Palo Alto, CA) 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 under the traces, thus giving a magnitude measure to the neural discharges.

Overall Strategy

The odorants, diluted in air, were presented to the bullfrog's intact olfactory sac in either the forward direction (entering the external naris to flow toward and out the internal naris) or in the reverse direction (entering the internal naris to flow toward and out the external naris). These flow directions are referred to as the forward hale (H_1) and the reverse hale (H_2). Important to the strategy was the earlier demonstration (Kurtz and Mozell, 1985) that the discharge recorded from the medial margin (MN) of the olfactory nerve reflects the activity of a mucosal region near the external naris and the discharge recorded from the lateral margin (LN) reflects the activity of a mucosal region near the internal naris. The measurements taken were the medial and lateral nerve discharges with hale in the forward direction (MN_{H_1} and LN_{H_1}) and the medial and lateral nerve discharges with hale in the reverse direction (MN_{H_2} and LN_{H_2}). By comparing the magnitudes of these four measurements in different mathematical expressions, the relative influences of hale direction, regional sensitivity, and the chromatographic process could be indexed. These indexes are as follows:

For the chromatographic process: $$\sqrt{\frac{MN_{H_1}}{LN_{H_1}} \cdot \frac{LN_{H_2}}{MN_{H_2}}}.$$

For regional sensitivity: $$\sqrt{\frac{MN_{H_1}}{LN_{H_1}} \cdot \frac{MN_{H_2}}{LN_{H_2}}}.$$

For hale: $$\sqrt{\frac{MN_{H_1}}{MN_{H_2}} \cdot \frac{LN_{H_1}}{LN_{H_2}}}.$$

The chromatographic process will be used to exemplify the thought process behind these indexes. This expression incorporates two estimates of the effect of the chromatographic process, each of which compares by a ratio the response of the first region contacted by the odorant as it is drawn across the mucosa to that of the second region. For one estimate, the responses are compared with hale in the forward direction (H_1) and MN is the first region contacted. For the other estimate, hale is in the reverse direction (H_2) and the first region contacted is LN. Since these are both ratios, the appropriate average of these two estimates is their geometric mean, i.e., the square root of their product.

The other indexes can be considered in similar terms. For the regional sensitivity index, one estimate of the effect compares the response of the MN region to the response of the LN region with hale in the forward direction (H_1), and the other estimate compares the response of the MN region to the response of the LN region with hale in the reverse direction (H_2). For the hale index, the two estimates compare the responses of hale in the forward direction (H_1) with those of hale in the reverse direction (H_2), with one estimate using the MN responses and the other the LN responses. As with the index for the effect of the chromatographic process, the appropriate average for the two estimates of the effects of either the regional sensitivity difference or the chromatographic process is their geometric mean.

These indexes all have a very important property: each is sensitive to one main effect (hale direction, regional sensitivity, or the chromatographic process) while balancing for the other two. Looking again at the index for the chromatographic process, note that when the responses for the first region reached by the odorant (MN_{H_1} and LN_{H_2}) are greater by some factor than the second ones reached (LN_{H_1} and MN_{H_2}), the ratio will be sensitive to that fact by becoming greater than unity. That happens because both of the first contacted regions are in the numerator and both of the second contacted regions are in the denominator. However, consider what would happen if this index for the effect of the chromatographic process were to be used to compare regional sensitivities. A comparison of regional sensitivities would require that both MN responses be on one side of the ratio and that both LN responses be on the other side of the ratio. This is obviously not the case for the expression of the chromatographic index, since the LN's and MN's appear equally often in the numerator and in the denominator. Similarly, this expression cannot compare the effects of the two hale directions since H_1 and H_2 also appear equally often in its numerator and denominator. In an analogous manner, it can be shown that the index for hale and the index for regional sensitivity are both sensitive to their respective main effects while balancing for the other two.

As shown below, these three indexes can be re-expressed in logarithmic form (base 2), which facilitates the use of statistical procedures. Although, as exemplified below for the chromatographic process, there are for each of these re-expressions several possible algebraically equivalent arrangements of terms, those listed are most convenient for the statistical analysis.

For the chromatographic process:

$$1/2[(\log MN_{H_1} - \log LN_{H_1}) + (\log LN_{H_2} - \log MN_{H_2})]$$

$$= 1/2[(\log MN_{H_1} - \log LN_{H_1}) - (\log MN_{H_2} - \log LN_{H_2})].$$

For regional sensitivity:

$$1/2[(\log MN_{H_1} - \log LN_{H_1}) + (\log MN_{H_2} - \log LN_{H_2})].$$

For hale:

$$1/2[(\log MN_{H_1} + \log LN_{H_1}) - (\log MN_{H_2} + \log LN_{H_2})].$$

Experimental Design

There were five experimental factors that together defined the odorant sniffs presented to the frogs. These were the odorants themselves (O), the number of odorant molecules in the sniff (N), the volume of the sniff (V), the duration of the sniff (T), and the direction of hale or sniff flow across the mucosa (H). There were four odorants and two hale

directions. The number of molecules, sniff volume, and sniff duration were each given at two levels in a 2:1 ratio. Thus, 64 different sniffs were generated ($4 \times 2 \times 2 \times 2 \times 2$).

Previous experience (Mozell et al., 1984) suggested that presenting this many stimuli to a single frog would require a substantial increase in the number of animals used in order to obtain the required number of successful animals needed by the experimental design. To avoid this problem, the number of stimuli presented to each animal was halved by arranging the combinations of N, V, and T levels in one of two possible types of Latin square as follows:

Type 1

N \ V	1	2
1	T_1	T_2
2	T_2	T_1

Type 2

N \ V	1	2
1	T_2	T_1
2	T_1	T_2

The type 1 and type 2 Latin squares were randomly allocated, six each, to 12 frogs. For each frog, each of the $4 \times 2 = 8$ combinations of O and H was combined with each of the four Latin square combinations of N, V, and T to yield $8 \times 4 = 32$ treatments or sniffs. These treatments were randomly allocated to 32 serial time bin subunits of the frog. This yielded a split plot design as follows: (*a*) the 12 frogs were whole experimental units with respect to the two-level factor defined by the type of Latin square; (*b*) the 32 experimental subunits of each frog produced a 32×6 randomized block design within each of the two types of Latin square.

Odorants

The four experimental odorants were geraniol, benzaldehyde (both from Eastman Chemical Co., Rochester, NY), butanol, and octane (both from Aldrich Chemical Co., Milwaukee, WI). In addition, *d*-limonene (Aldrich Chemical Co.) was used as a standard. These odorants were chosen because they represented the best compromise of several specifications. All these odorants had previously been used in pursuing the space-time differential distributions of odorant molecules across the mucosa (Mozell, 1970; Mozell and Jagodowicz, 1973), and all but benzaldehyde had also been used in studies investigating mucosal regions of differential sensitivity (Kubie et al., 1980; Mackay-Sim and Kubie, 1981; Moulton, 1981; Mackay-Sim et al., 1982). In addition, previous experience with these odorants showed them to have little, if any, adverse effect upon the stability of the preparation with repeated presentations. Similarly, previous experience showed them to be easily cleared from the tubing and valves of the delivery system, thus minimizing the interstimulus time for purging.

Sniff Delivery System and Odorant Control

The two levels (in 2:1 ratios) at which N, V, and T were each presented were chosen to fall within the ranges previously determined as normal for the bullfrog's respiratory cycle (Hornung et al., 1987). The two levels of N for each odorant were chosen to fall within

the dynamic range of the electrophysiologically determined stimulus-response relationships observed in an earlier study (Mozell, 1970). The values chosen for each level of N, V, and T with each odorant at each hale are given in Table I.

To control sniff volume and sniff duration (and thus sniff flow rate), the delivery system shown in Fig. 1 was developed. The core of this delivery system, which was based upon an earlier version (Mozell et al., 1984), consisted of three four-port Teflon slide valves. These slide valves were pneumatically driven. They were controlled by solenoids, which were precisely timed and sequenced to open and close by stimulators (S48 and S88, Grass Instrument Co., Quincy, MA). During the "rest" condition (Fig. 1), the four ports of each

TABLE I

Type I						Type II					
Sniff	Odorant	N	V	T	H*	Sniff	Odorant	N	V	T	H*
		molecules	*cc*	*s*				*molecules*	*cc*	*s*	
1	Benzaldehyde	1.74×10^{15}	0.47	0.35	F	1	Benzaldehyde	1.74×10^{15}	0.47	0.70	F
2	"	1.74×10^{15}	0.47	0.35	R	2	"	1.74×10^{15}	0.47	0.70	R
3	"	1.74×10^{15}	0.93	0.70	F	3	"	1.74×10^{15}	0.93	0.35	F
4	"	1.74×10^{15}	0.93	0.70	R	4	"	1.74×10^{15}	0.93	0.35	R
5	"	3.48×10^{15}	0.47	0.70	F	5	"	3.48×10^{15}	0.47	0.35	F
6	"	3.48×10^{15}	0.47	0.70	R	6	"	3.48×10^{15}	0.47	0.35	R
7	"	3.48×10^{15}	0.93	0.35	F	7	"	3.48×10^{15}	0.93	0.70	F
8	"	3.48×10^{15}	0.93	0.35	R	8	"	3.48×10^{15}	0.93	0.70	R
9	Butanol	2.50×10^{16}	0.47	0.35	F	9	Butanol	2.50×10^{16}	0.47	0.70	F
10	"	2.50×10^{16}	0.47	0.35	R	10	"	2.50×10^{16}	0.47	0.70	R
11	"	2.50×10^{16}	0.93	0.70	F	11	"	2.50×10^{16}	0.93	0.35	F
12	"	2.50×10^{16}	0.93	0.70	R	12	"	2.50×10^{16}	0.93	0.35	R
13	"	5.00×10^{16}	0.47	0.70	F	13	"	5.00×10^{16}	0.47	0.35	F
14	"	5.00×10^{16}	0.47	0.70	R	14	"	5.00×10^{16}	0.47	0.35	R
15	"	5.00×10^{16}	0.93	0.35	F	15	"	5.00×10^{16}	0.93	0.70	F
16	"	5.00×10^{16}	0.93	0.35	R	16	"	5.00×10^{16}	0.93	0.70	R
17	Geraniol	1.54×10^{14}	0.47	0.35	F	17	Geraniol	1.54×10^{14}	0.47	0.70	F
18	"	1.54×10^{14}	0.47	0.35	R	18	"	1.54×10^{14}	0.47	0.70	R
19	"	1.54×10^{14}	0.93	0.70	F	19	"	1.54×10^{14}	0.93	0.35	F
20	"	1.54×10^{14}	0.93	0.70	R	20	"	1.54×10^{14}	0.93	0.35	R
21	"	3.09×10^{14}	0.47	0.70	F	21	"	3.09×10^{14}	0.47	0.35	F
22	"	3.09×10^{14}	0.47	0.70	R	22	"	3.09×10^{14}	0.47	0.35	R
23	"	3.09×10^{14}	0.93	0.35	F	23	"	3.09×10^{14}	0.93	0.70	F
24	"	3.09×10^{14}	0.93	0.35	R	24	"	3.09×10^{14}	0.93	0.70	R
25	Octane	2.25×10^{16}	0.47	0.35	F	25	Octane	2.25×10^{16}	0.47	0.70	F
26	"	2.25×10^{16}	0.47	0.35	R	26	"	2.25×10^{16}	0.47	0.70	R
27	"	2.25×10^{16}	0.93	0.70	F	27	"	2.25×10^{16}	0.93	0.35	F
28	"	2.25×10^{16}	0.93	0.70	R	28	"	2.25×10^{16}	0.93	0.35	R
29	"	4.49×10^{16}	0.47	0.70	F	29	"	4.49×10^{16}	0.47	0.35	F
30	"	4.49×10^{16}	0.47	0.70	R	30	"	4.49×10^{16}	0.47	0.35	R
31	"	4.49×10^{16}	0.93	0.35	F	31	"	4.49×10^{16}	0.93	0.70	F
32	"	4.49×10^{16}	0.93	0.35	R	32	"	4.49×10^{16}	0.93	0.70	R

* F, forward; R, reverse.

slide valve were so arranged that deodorized, humidified air was drawn continuously by rotary vane vacuum pump *II* through the frog's olfactory sac at 20 cc/min. During the "rest" condition, rotary vane vacuum pump *I* was set to draw at the flow rate prescribed by the volume and duration scheduled for the next sniff. Also during this "rest" period, the olfactometer was set to generate the concentration required, which, with this sniff volume, produced the next scheduled number of molecules (N/V = concentration [C]) of the next scheduled odorant. The resultant odorant mixture was directed away from the animal, flowing at 250 cc/min from port *3* to port *2* of valve *A*, which led to an exhaust. During the "prestimulation" condition, the ports of valve *C* were so arranged that no air

was drawn into and through the animal's olfactory sac. In addition, the ports of valve *A* were so arranged that the odorant mixture flowed at 250 cc/min past the animal's naris through a specially designed sampling tube (Fig. 2). The purpose of this brief "prestimulation" condition (0.3 s) was to load the short conduit (0.2 cc) through both valve *A* and the sampling tube with the required odorant concentration so that this small volume would not reduce the expected number of molecules in the ensuing sniff. During the "stimulation" condition, the ports of valve *B* were arranged so that rotary vane vacuum pump *I* drew a Mniffof the odorized air from the flow in the sampling tube into the olfactory sac via a T-connection to the naris. As explained with Fig. 2, sampling tubes and

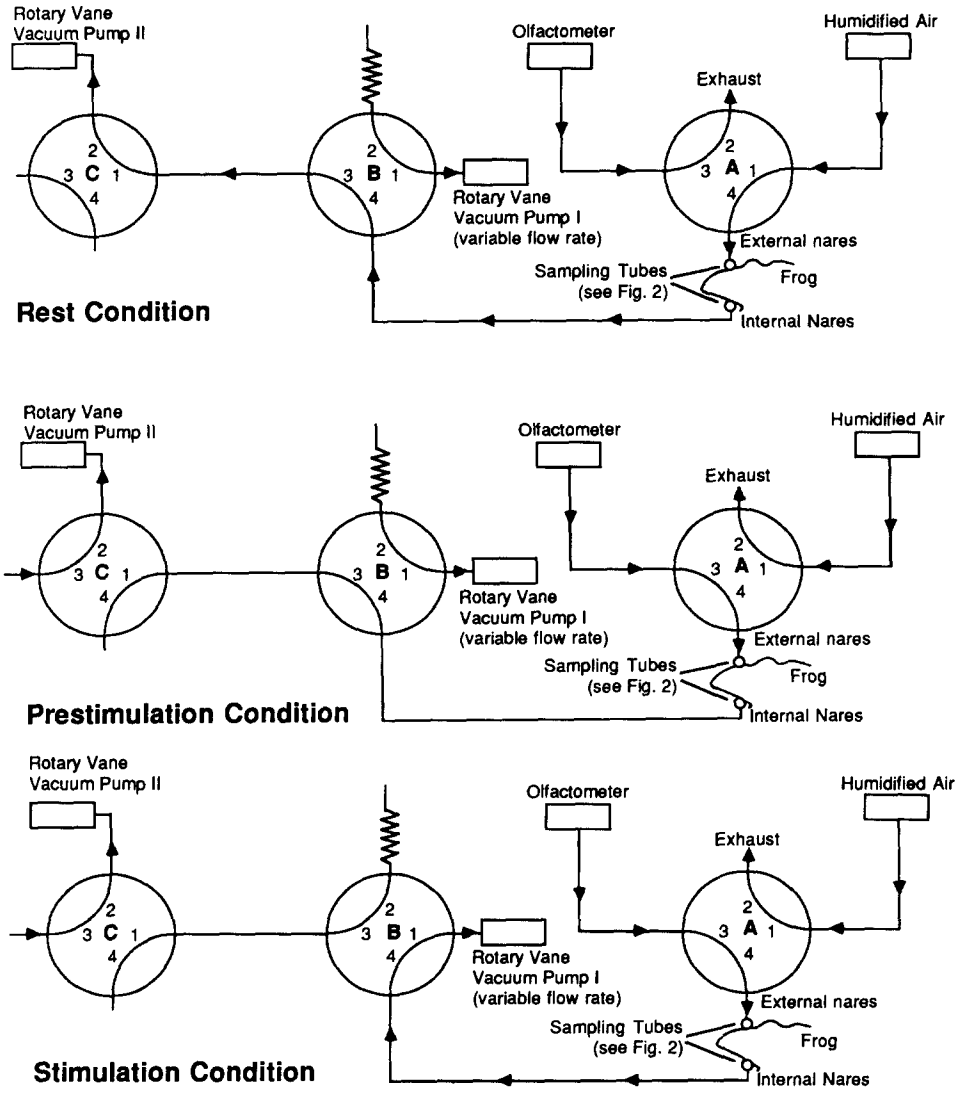

FIGURE 1.

T-connections were attached to both nares, providing stimulus access to the olfactory sac through either one.

The concentration of the odorant (and thus the number of molecules for any given sniff volume [$N/V = C$]) was controlled by an olfactometer that was made entirely of Teflon and glass and was of the flow dilution variety (Mozell, 1970). The flow rate passing through the sampling tubes (Fig. 2) with positive pressure from the olfactometer was always fixed at 250 cc/min for all odorants, concentrations, and sniff volumes.

Verification of Sniff Variable Levels

A hot-wire anemometer (Hornung et al., 1987) was used to verify that the required flows through the olfactory sac were actually generated by the pump and Grass stimulator settings of the odorant delivery system. To monitor this airflow, the hot-wire anemometer was connected in continuity with the bore of the sampling tube in place of valve *A* while the other end of the bore was plugged with a small cork. The circuitry of this device related the instantaneous airflow rate to the amplitude of the galvanometer deflection of a Visicorder (Honeywell, Inc., Denver, CO) (Mozell et al., 1984). Fig. 3 shows a typical profile (instantaneous flow rate over time) before, during, and after a sniff. On the time scale shown, these profiles appear rectangular. Thus, the duration of the sniff was given by the distance between the onset and offset of the sniff trace and the sniff volume was given by multiplying the amplitude of the trace (flow rate) by the duration. There were four different sniff profiles given by the combination of two levels of volume and two levels of duration. In addition, each of these profiles was generated through both the external and internal nares. Such monitoring of the flow also afforded in each animal a test of the patency of the entire flow path, including the valves, the tubing, and the animal's nasal passageway.

To verify the number of molecules of an odorant in a sniff, the output of the olfactometer was led into the gas sampling valve of a gas chromatograph (3700, Varian Associates, Inc.) fitted with a flame ionization detector (Mozell et al., 1984; Kurtz and

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FIGURE 1. (*opposite*) Sniff delivery system under three successive conditions. During the "rest" condition, rotary vane vacuum pump *II* connected to port *2* (in continuity with port *1*) of valve *C* caused the deodorized, humidified air to flow through valve *B* (via ports *3* and *4*) and valve *A* (via ports *1* and *4*) to enter either the frog's external or internal naris at 20 cc/min. (Entrance via the external naris is depicted in this figure.) The odorized air from the olfactometer was directed away from the animal into the laboratory vacuum line via ports *3* and *2* of valve *A*. Rotary vane vacuum pump *I* drew room air from port *2* to port *1* of valve *B* at the flow rate prescribed by the volume and duration scheduled for the next sniff. During the "prestimulation" condition, the ports of valve *C* were rearranged so that rotary vane vacuum pump *II* was shunted away from causing a flow through the animal. At valve *A*, ports *1* and *2* were connected, thus directing the humidified air into the laboratory exhaust, and ports *3* and *4* were connected, thus directing the olfactometer positive pressure output through the sampling tube (see Fig. 2). However, since rotary vane vacuum pump *II* at valve *C* was shunted away from causing a flow through the animal, the output of the olfactometer bypassed the naris. During the "stimulation" condition, ports *1* and *4* of valve *B* were connected, thus allowing rotary vane vacuum pump *I* to draw a sniff of the olfactometer output through the naris from the sampling tube. At the same time, ports *2* and *3* of valve *B* were also connected so that rotary vane vacuum pump *II* at valve *C* simply drew room air. The arrowheads represent flow in the direction shown; the lack of arrowheads represents an absence of flow.

Mozell, 1985). After the concentration of this output was determined by standard techniques, the number of molecules in each sniff volume was calculated.

Protocol

In order to proceed with the running of a given animal, several preliminary tests were administered. First, to test the patency of the delivery system and the frog's nasal passageway, a sniff of deodorized air (at the highest flow rate, the longest duration, and twice the largest volume used; Fig. 3) was presented both in the forward and in the reverse direction with the anemometer in place. The sniff profiles recorded by the Visicorder

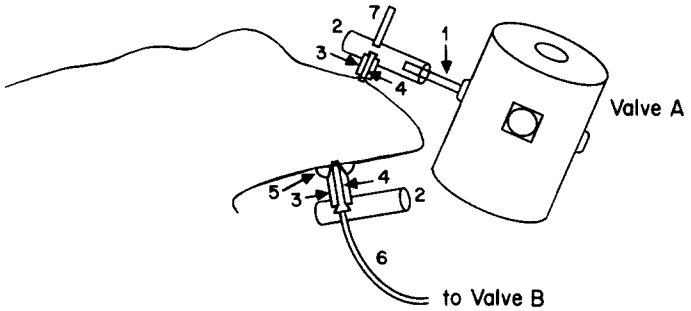

FIGURE 2. Odorant sampling tubes. There was one for each naris and they were both held in place by a dental carboxylate cement (Durelon; Premier Dental Products Co., Norristown, PA). Made entirely of Teflon, these sampling tubes allowed the deodorized or odorized air (as selected through valve *A*; see Fig. 1) to either flow past the naris or be drawn into the naris. The output tube from valve *A* (*1*) inserted snugly into a bore drilled through a ⅛-in. rod (*2*). Another piece of rod (*3*) was drilled to accept a piece of tubing (*4*) that protruded slightly beyond the rod. This protrusion was inserted into the naris but was limited to a certain penetration by the wider annulus of the rod at the external naris or by a tapered Teflon washer (*5*) at the internal naris. This penetration was calculated to keep the tube's opening patent by not allowing it to contact any intranasal tissue. The other end of the rod was fitted as a T-connection into the bore of rod *2*. A small hole was drilled through the wall of rod *2* opposite the T-connection, such that patent tubing (*6*) could be passed through it to fit into tube *4*, as shown, at the internal naris, or nonpatent tubing (*7*) could be backed off from tube *4* as shown at the external naris. This was the arrangement for drawing deodorized or odorized air into the olfactory sac via the external naris. To draw the air into the sac via the internal naris, valve *A* was moved to the internal naris sampling tube and tubes *6* and *7* were exchanged between the two nares. Note that the inside diameters and the lengths of all the tubing and bores were equal for the external and internal nares.

had to be the same in both directions and had to match in volume, duration, and flow rate those which were expected from the pump and Grass stimulator settings. It was also required that this sniff of deodorized air not give any neural discharge, which, among other possibilities, could have been due either to odorant contamination of the system or to mechanically induced artifacts. Furthermore, to determine that the overall responsivities from the two recording sites on the nerve were comparable, *d*-limonene, an odorant that in several previous studies (Mozell, 1966, 1970) elicited responses of equal, or very nearly equal, magnitude from different branches of the olfactory nerve, was presented at the standard levels ($N = 6.76 \times 10^{15}$ molecules; $V = 0.47$ cc; $T = 0.35$ s). With amplifier

gains equal, the summated discharges recorded from the two sites were allowed to differ by no more than 20%. If the difference was greater than this, further desheathing of the nerve and/or repositioning of the electrodes was indicated. Another test before proceeding was to present each odorant at the weakest combination of scheduled sniff variables. It was required that each of these sniffs produce a measurable response. Finally, it was necessary to have some indication that the stimuli to be presented were within the dynamic range of the animal's neural response. To keep the total number of stimulations at a minimum, this indication was pursued with one representative odorant, geraniol. Three consecutive sniffs were presented, with each consecutive sniff having the same volume (V_2) and duration (T_1) as its predecessor but having double the number of molecules ($0.5N_1$, N_1, N_2). A consecutive increase in the traces of the summated discharges that paralleled this increase in N was taken as indicative of the stimuli being within the animal's dynamic range.

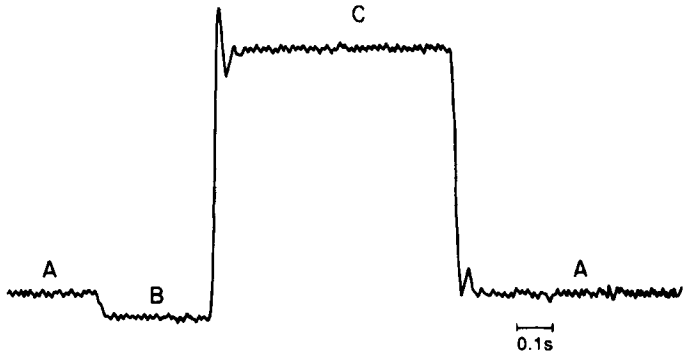

FIGURE 3. Typical airflow profile. During the rest condition (*A*), the flow rate was 20 cc/min. During the prestimulation condition (*B*), the flow rate was zero. During the stimulation or sniff condition (*C*), the flow rate in this case was 160 cc/min, with a duration of 0.70 s and a volume of 1.86 cc. This particular profile was for the nasal patency test done, as described under *Protocol*, at the highest flow rate, longest duration, and twice the largest volume used with odorized sniffs.

The 32 different sniffs presented to each animal were given in random order as determined for each animal with a table of random numbers. Each animal's run began with a presentation of the standard stimulus, followed by a sniff of deodorized air like that described above (Fig. 3). The first eight of the randomized sniffs then followed in order. After the standard stimulus and the deodorized air sniff were again presented, the next group of eight randomized sniffs was given. This cycle was repeated two more times until all 32 randomized sniffs were presented and the run ended with a fifth presentation of the standard and deodorized air.

The standard stimulus was presented at regular intervals in order to control for possible variations over time in the physiology or the recording conditions of the preparation. Such variations might even have affected the recordings from the two nerve sites differentially. (This precaution of presenting a *d*-limonene standard has been used previously [Mozell, 1966, 1970; Mozell et al., 1984; Kurtz and Mozell, 1985].) For each recording site, a computer program calculated the difference between the responses to successive presentations of the standard stimulus. It used this percentage change in conjunction with linear interpolation to correct the responses to the intervening randomized sniffs for any variations over time. Furthermore, the computer program considered,

as experience had taught, that the initial responses to the standard at the two recording sites are equal, and it mathematically proportioned all the other measured responses accordingly. Thus, for all the test odorants, the difference in their responses between the two recording sites was made relative to a *d*-limonene difference taken as zero.

The sniffs were presented at 3–4-min intervals. Note that immediately after the sniff presentation (i.e., the "stimulation" condition), the delivery system went back to the "rest" condition (Fig. 1). Thus, during most of the intersniff interval, the delivery system and the frog's nasal flow path were flushed with deodorized, humidified air. The "prestimulation" condition lasted 0.3 s and the "stimulation" or sniff condition lasted either 0.35 (T_1) or 0.70 (T_2) s.

Statistical Analysis

An ANOVA of the combined data from the two 32 × 6 randomized blocks was applied to each of two dependent variables. The first variable was defined as the sum of log responses ($\log_2$ MN + $\log_2$ LN), and the second as the difference ($\log_2$ MN − $\log_2$ LN) recorded for each treatment. The reason for choosing these two dependent variables relates to the three indexes that have been presented as part of the overall strategy. The logarithmic form of each index involves either the sum of or difference between $\log_2$ MN and $\log_2$ LN neural response measures. That is, the hale index is half the difference between the sums of log responses to the two directions of hale; the index for regional sensitivity, on the other hand, is the mean of the two differences between log responses to the two directions of hale; and the index of the chromatographic process is half the difference between the two differences between log responses for the two directions of hale. Consequently, the various interactions and effects in the ANOVA of these two dependent variables are directly interpretable in terms of the three strategic indexes.

Since multiple tests involving two correlated dependent variables were contemplated in this experiment, nominal p values for variance ratios were somewhat discounted when making statistical inferences. There were some 14 main tests of hypotheses that motivated this study. Consequently, the Bonferroni rule was applied to arrive at a conservative criterion of $p = 0.05/14 = 0.0035$ as the nominal p value for claims of significance at the joint 0.05 level (Tukey, 1977). However, nominal p values <0.05 were considered to be strong indications of association; furthermore, any F ratios >2 flag the items that were worthy of consideration in the interpretation of the manifold relationships observable in this experiment (Mozell et al., 1984).

RESULTS

Responses to the Stimulation Factors

Fig. 4 shows some typical summated multiunit discharges recorded from the MN and LN sites on the olfactory nerve. For each animal, there were 64 such traces, one for each of the 32 combinations of treatment factor levels at each of two recording sites. The logarithms (base 2) of the areas under these traces (adjusted to the *d*-limonene standard) were averaged across preparations and are presented in Table II. In order to evaluate these data in terms of the indexes for the direction of hale, regional sensitivity, and the chromatographic process, the sums of the log MN and log LN regional responses, as well as their differences, were subjected to analyses of variance, the results of which are summarized in Table III. In this table, the indexes are tested as follows. The main effect of hale direction is tested with 1 degree of freedom by whether the sum of the $\log_2$

summated multiunit discharges from MN and LN differs on the average between H_1 and H_2 ($F = 209.9$, $p \simeq$ nil). The main effect of the chromatographic process is tested with 1 degree of freedom by whether the difference of the $\log_2$ summated multiunit discharges from MN and LN differs on the average between H_1 and H_2 ($F = 405.5$, $p \simeq$ nil). The overall regional sensitivity for preparations in general is tested with 1 degree of freedom by whether the grand mean of the

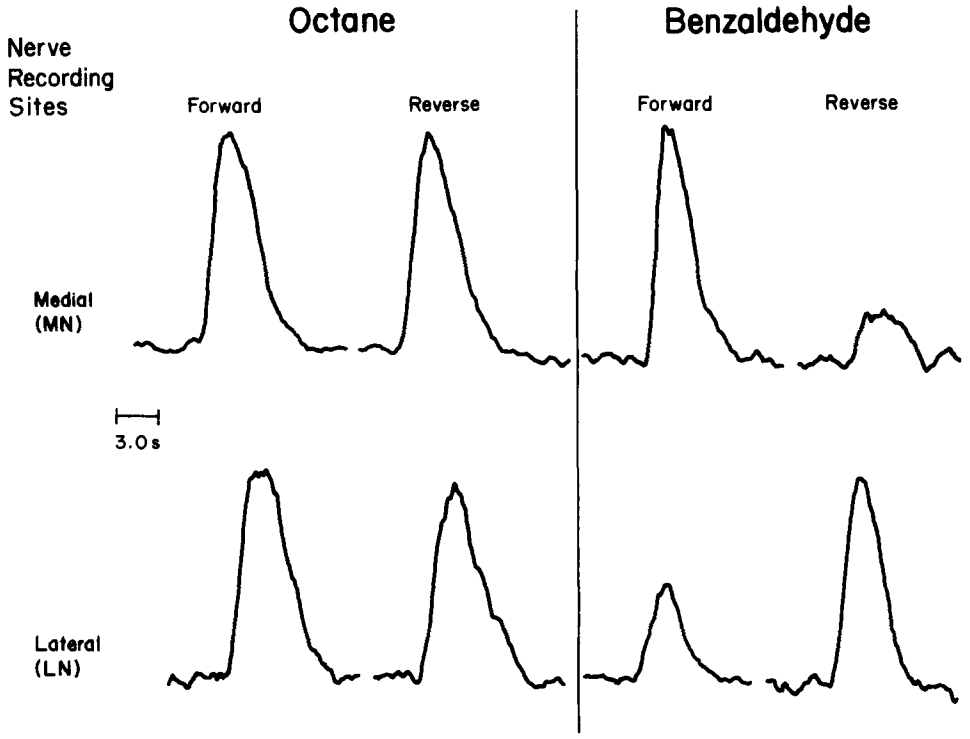

FIGURE 4. Typical summated multiunit discharges recorded from the medial (MN) and lateral (LN) margins of the olfactory nerve in response to two of the four odorants presented in the forward and reverse flow directions. All these traces came from the same frog. Not including the *d*-limonene standards, there were 64 such responses (32 from each recording site) representing the 32 combinations of treatment factor levels. The N, V, and T levels for the octane discharges shown here were: $N = 4.49 \times 10^{16}$ molecules, $V = 0.47$ cc, and $T = 0.35$ s. For benzaldehyde, the levels were: $N = 3.48 \times 10^{15}$ molecules, $V = 0.93$ cc, and $T = 0.70$ s. These responses were traced from those originally recorded by a Varian 9176 chart recorder.

difference between $\log_2$ MN and $\log_2$ LN is different from zero ($F = 6.5$, $p < 0.05$). Other main effects and interactions test whether the individual indexes are affected by the odorant, the number of molecules, sniff volume, sniff duration, or some combination thereof. However, before examining these effects in more detail, it seems appropriate to provide a general overview of the formal ANOVA in Table III.

As indicated by the p values for the F ratios in the analysis of the sums, the main effects of odorants (O), direction of hale (H), and number of molecules (N) were particularly strong ($p \simeq$ nil); the main effects of duration (T) and volume (V) were also significant ($p < 0.001$). Moreover, the first-order interactions, $O \times H$ and $O \times V$, were both highly significant ($p \simeq$ nil). For the analysis of regional differences, it is noteworthy that the grand mean regional difference was indicated to be different from zero ($p < 0.05$). The main effect of the direction of hale (H) on the regional difference was particularly strong ($F^1_{310} = 405.5$, $p \simeq$ nil) and even the weaker effects of odorants (O) were highly significant ($F^3_{310} = 8.8$, $p \simeq$ nil). The very strong $O \times H$ interaction term ($p \simeq$ nil) shows that the

TABLE II

Mean log_2 MN and log_2 LN Response Magnitudes to Experimental Combinations of the Factor Levels

			T:	Short				Long			
			V:	Low		High		Low		High	
Odorant	Hale direction	Recording site	N:	Small*	Large‡	Small‡	Large*	Small‡	Large*	Small*	Large‡
Benzaldehyde	Forward	MN		5.09	5.41	4.80	5.52	4.69	5.64	5.23	5.20
		LN		4.61	4.61	4.37	5.32	3.57	5.06	4.88	4.51
	Reverse	MN		3.37	3.09	3.38	4.21	2.43	3.55	3.64	3.36
		LN		4.77	4.41	4.32	5.05	3.61	4.68	4.68	4.85
Butanol	Forward	MN		4.10	4.13	4.52	4.33	4.04	3.54	4.06	4.73
		LN		3.26	3.62	3.60	3.85	2.84	3.41	3.60	3.87
	Reverse	MN		3.24	2.90	3.25	3.20	2.51	2.81	2.79	3.28
		LN		3.35	3.74	3.97	3.65	2.86	3.55	3.45	4.34
Geraniol	Forward	MN		5.54	5.77	5.44	5.65	5.62	5.81	5.58	5.71
		LN		5.47	5.57	5.21	5.61	5.20	5.69	5.38	5.44
	Reverse	MN		4.69	4.80	4.45	5.33	3.94	4.56	4.56	4.94
		LN		5.35	5.38	4.94	5.49	4.68	5.52	5.26	5.32
Octane	Forward	MN		4.97	5.19	4.29	4.98	4.81	5.08	4.80	4.88
		LN		5.25	5.28	4.59	5.22	4.88	5.49	5.27	4.95
	Reverse	MN		5.15	5.03	4.47	5.14	4.66	5.15	4.76	4.81
		LN		5.19	5.25	4.69	5.34	4.72	5.33	5.15	5.06

* Mean of six frog preparations randomized to Latin square 1.
‡ Mean of six frog preparations randomized to Latin square 2.

effects of the direction of hale on the regional differences varied markedly, depending on which odorant was being used.

In each analysis of sums and differences, the multivariate 32 degrees of freedom term for interactions with the type of Latin square is not formally testable in this design, since the number of response variables (32) exceeds the number of preparations (12). However, judging from the fact that the mean square for the interactive term is well below the preparations mean square in each analysis, these interactions with the type of Latin square appear to be negligible.

This summary analysis points up the need to examine the main effects of all

five experimental factors, as well as some of their more salient interactions, in more detail. This will be facilitated by Tables IV and V.

Comparison of Odorants with Respect to Indexes of Hale Effect, Regional Sensitivity, and Chromatographic Process

As indicated in Table III, by far the greatest variation in both dependent variables was due to the four odorants (O) in the two directions of hale (H) and their interactions ($O \times H$). The strength and variety of these relationships are summarized in Table IV.

TABLE III

Analysis of Variance of Experimental Response Combinations

Source	Degrees of freedom	log MN + log LN				log MN − log LN			
		Sum of squares	Mean square	F	P‡	Sum of squares	Mean square	F	P‡
Grand mean	1	31,931.67	31,931.67	4,912.6	Nil	10.91	10.91	6.5	<0.05
Preparations*	10	64.98	6.50	—	—	16.94	1.69	—	—
Treatments	31	863.98	27.87	56.9	Nil	141.64	4.57	21.8	Nil
Main Effects	7	787.49	112.50	229.6	Nil	91.55	13.08	62.3	Nil
O	3	632.97	210.99	430.6	Nil	5.54	1.85	8.8	Nil
H	1	102.84	102.84	209.9	Nil	85.15	85.15	405.5	Nil
T	1	5.30	5.30	10.8	<0.001	0.10	0.10	0.5	≫0.10
V	1	7.66	7.66	15.6	<0.001	0.11	0.11	0.5	≫0.10
N	1	38.72	38.72	79.0	Nil	0.65	0.65	3.1	<0.10
Interactions	24	76.49	3.19	6.5	Nil	50.09	2.08	9.9	Nil
$O \times H$	3	43.47	14.49	29.6	Nil	42.49	14.16	67.4	Nil
$O \times T$	3	1.91	0.64	1.3	>0.10	0.32	0.11	0.5	≫0.10
$O \times V$	3	20.78	6.93	14.1	Nil	1.00	0.33	1.6	>0.10
$O \times N$	3	2.46	0.82	1.7	>0.10	2.15	0.72	3.4	<0.02
$H \times T$	1	1.61	1.61	3.3	<0.10	0.96	0.96	4.6	<0.05
$H \times V$	1	2.15	2.15	4.4	<0.05	0.40	0.40	1.9	>0.10
$H \times N$	1	0.17	0.17	0.3	≫0.10	0.02	0.02	0.1	≫0.10
$O \times H \times T$	3	0.41	0.14	0.3	≫0.10	0.37	0.12	0.6	≫0.10
$O \times H \times V$	3	2.15	0.72	1.5	>0.10	2.07	0.69	3.3	<0.02
$O \times H \times N$	3	1.38	0.46	0.9	≫0.10	0.31	0.10	0.5	≫0.10
Error*	310	152.32	0.49	—	—	66.32	0.21	—	—
Interactions with Latin squares	32	58.90	1.84	—	—	10.44	0.33	—	—
Total	384	33,071.85	86.12	—	—	246.25	0.64	—	—

* Within type of Latin square.
‡ Nominal p value.

As shown in the first column, the responses in the forward direction of hale were substantially greater than in the reverse direction for geraniol, benzaldehyde, and butanol, but there was virtually no effect of hale for the weakly sorbed odorant, octane. Differences among hale effects of the four odorants in column 1 were highly significant (ANOVA $F^3_{310} = 14.49$, $p \simeq$ nil, for $O \times H$ effects on $\log_2$ MN + $\log_2$ LN). The strongest hale effect was for benzaldehyde, several standard errors above the nearly equivalent hale effects for geraniol and butanol. The hale effect for benzaldehyde was 0.95, or nearly 1 $\log_2$ unit, which indicates that the responses to this odorant in the forward direction were, on the average, nearly double those in the reverse direction.

The indexes of relative sensitivity (column 2) in the regions represented by MN and LN responses differed among the four odorants (ANOVA $F^3_{310} = 8.8$, $p \simeq$ nil, for O effects on $\log_2$ MN $-$ $\log_2$ LN). As indicated by a log index value of -0.30, the *d*-limonene–normalized MN discharges were only 81% ($2^{-0.30} \times 100$) those of the LN discharges when benzaldehyde was used. The log indexes for geraniol and octane were nearly the same, -0.20 and -0.22, or 87 and 86%, respectively, while for butanol there were nearly equal responses at the two sites, so that the log index was $+0.03$, or 102%.

Column 3 provides the details for the very strong evidence that the different odorants are differentially sorbed across the mucosa (ANOVA $F^3_{315} = 67.4$, $p \simeq$ nil, for $O \times H$ effects on $\log_2$ MN $-$ $\log_2$ LN). Except for some moderate shifting in the relative positions of geraniol and butanol, the pattern of differential sorption among the four odorants very much follows the pattern of differential hale effects. That is, the chromatographic process index was strongest for benzaldehyde, intermediate for geraniol and butanol, and practically zero for octane, closely resembling the pattern of effects for hale in column 1.

TABLE IV

Comparison of $\log_2$ Indexes of Responses to the Four Odorants

Odorant	1 Forward vs. reverse hale*	2 MN vs. LN sensitivity‡	3 Chromatographic process (mucosal sorption)‡
Benzaldehyde	0.95	−0.30	0.88
Butanol	0.54	0.03	0.64
Geraniol	0.60	−0.20	0.39
Octane	0.01	−0.22	−0.03

* SE = ±0.07.
‡ SE = ±0.05.

As to the relative importance of regional sensitivity differences and mucosal sorption in the generation of mucosal activity patterns, this is indicated by comparing the absolute magnitudes of the sensitivity and sorption log indexes (columns 2 and 3) in Table IV. When the odorant is geraniol, for example, the mucosal sorption index is twice as important as the MN vs. LN sensitivity index in determining the patterns of log response (standardized to *d*-limonene) for the two regions. In the forward direction of hale for geraniol, the chromatographic process would result in an estimated MN response 1.31 ($2^{0.39}$) times that of the LN response, were it not for a partially offsetting differential sensitivity index, which reduces the actual MN/LN ratio to 1.14 ($2^{0.39-0.20}$). In the reverse direction of hale, the differential sensitivity index combines with, rather than offsets, the sorptive influence to produce an estimated LN/MN ratio of 1.51 ($2^{0.39+0.20}$). For benzaldehyde, mucosal sorption is even more dominant over the sensitivity index differential, the log sorption index being nearly triple the absolute value of the log sensitivity index. For benzaldehyde, as for geraniol, the chromatographic

process is partially offset by the sensitivity index differential to produce an estimated MN/LN ratio of 1.49 ($2^{0.88-0.30}$) in the forward direction of hale; the two factors again combine in the reverse direction of hale to yield an estimated LN/MN ratio of 2.27 ($2^{0.88+0.30}$). For butanol, the chromatographic process is almost completely unaffected by the differential sensitivity index, so that the MN/LN ratio in the forward direction is 1.59, and the LN/MN ratio in the reverse direction is 1.53, nearly the same. Finally, octane exhibits practically no chromatographic process influence, so that the response pattern is dominated by the differential sensitivity index, with an MN/LN ratio of only 0.84 ($2^{-0.03-0.22}$) for forward hale and an LN/MN ratio of 1.14 ($2^{-0.03+0.22}$) in the reverse direction.

Effects of Varying Sniff Duration, Volume, and Number of Molecules

Other than the effects of odorants (O), hale (H), and their interactions ($O \times H$), which were summarized in the foregoing paragraphs, Table III shows that most of the remaining significant or suggestive relationships reside in the effects on

TABLE V

Estimates of Effects (i.e., Response Exponents, Base 2) per Doubling of Sniff Duration, Volume, and Number of Molecules

	1			2			3		
Odorant	MN	LN	Average*	MN	LN	Average*	MN	LN	Average*
Benzaldehyde	−0.14	−0.20	−0.17	0.26	0.29	0.28	0.42	0.46	0.44
Butanol	−0.24	−0.14	−0.19	0.32	0.46	0.39	0.06	0.38	0.22
Geraniol	−0.12	−0.07	−0.10	0.18	−0.03	0.08	0.34	0.31	0.33
Octane	−0.03	+0.01	−0.01	−0.24	−0.14	−0.19	0.17	0.32	0.25
All odorants	−0.13	−0.10	−0.12‡	0.13	0.15	0.14‡	0.25	0.37	0.31‡

* SE = ±0.07.
‡ SE = ±0.04.

$\log_2$ MN + $\log_2$ LN values for sniff duration (T) ($F^1_{310} = 10.8, p < 0.001$), volume ($V$) ($F^1_{310} = 15.6, p < 0.001$), and number of molecules (N) ($F^3_{310} = 79.0, p \simeq$ nil) for the various odorants (O) ($F^3_{310} = 430.6, p \simeq$ nil), including the first-order interactions $O \times T$ ($F^3_{310} = 1.3, p > 0.10$), $O \times V$ ($F^3_{310} = 14.1, p \simeq$ nil), and $O \times N$ ($F^3_{310} = 1.7, p < 0.10$). Estimates of the detailed effects (i.e., response exponents, base 2) of these factors are given in Table V.

As might have been expected, all four odorants showed a positive effect (positive exponent) for the number of molecules, although the effectiveness of N differed somewhat among the odorants (Table V). For butanol, which showed the least effect, a doubling of N multiplied the response magnitude by a factor of 1.16 ($2^{0.22}$), whereas for benzaldehyde, which showed the greatest effect, a doubling of N multiplied the response magnitude by a factor of 1.36 ($2^{0.44}$). The average for all four odorants was 1.24-fold ($2^{0.31}$) for a doubling of N. This display for the effects of N across all four odorants was relatively uniform compared with the effects of changes in T and V (Table V). While the duration

exponents for geraniol, benzaldehyde, and butanol were all negative, the near-zero exponent for octane was quite different from the average of the others ($p < 0.001$). Indeed, looking at the standard errors, the octane exponent might even be a random departure from positivity, but in any event it is quite different from the overall estimate for the four odorants taken together, which was significantly negative.

The most outstanding contrast between octane and the other odorants occurs in respect to the effect of doubling the volume of a sniff. The effect of increasing the volume of an octane sniff was to significantly reduce responses. In sharp contrast, the exponents on each of the other three odorants are positive, with this being especially true of benzaldehyde and butanol. These reversals of the octane volume effect cannot reasonably be ascribed to chance fluctuations even in the context of multiple tests, because, as given in the analysis of variance (Table III), the evidence for the interaction of odorants with volume effects is very strong ($F^3_{310} = 14.1$, $p \simeq$ nil).

DISCUSSION

Chromatographic Process

This study supports the findings of previous studies that the molecules of different odorants are sorbed in different gradients across the mucosal sheet. That is, the indexes for the chromatographic process differ considerably among the four odorants, the closest ones being separated by >2 SE. Recall that the chromatographic process index is an average drop across the mucosa in response to the two directions of hale. Assuming, then, that the "inherent" sensitivities at the MN and LN sites are unaffected by the direction of hale, any sensitivity differential between them would be offset by the two directions of hale, leaving an index that is sensitive only to sorption effects. Furthermore, the order of the indexes for the four odorants fell exactly as would be predicted from earlier studies, most directly from the study in which the column of a gas chromatograph was replaced with the intact olfactory sac of the bullfrog (Mozell and Jagodowicz, 1973, 1974). The purpose of the earlier study was to measure, in relative terms, how long it takes the molecules of different odorants to migrate across the frog's olfactory mucosa. This would, in analogy to gas chromatography, depend upon the partitioning of the odorant molecules between the moving phase (the airflow) and the stationary phase (the mucosa). This partitioning would, in turn, depend upon how strongly an odorant's molecules are sorbed to the mucosa. Focusing upon the four odorants in the earlier study, which are also common to the present study, their order, from the shortest time to the longest time to migrate across the mucosa, was octane, geraniol, butanol, and benzaldehyde. Thus, in the time frame of a given sniff, the ratio of the number of molecules sorbed at the earlier position in the flow path to the number reaching and being sorbed at the later position would also increase in the order of octane, geraniol, butanol, and benzaldehyde, and this is exactly the same order as the indexes for the chromatographic process in the present study.

Hale Direction

The indexes for the effect of hale showed a very similar order for the four odorants, as did the indexes for the chromatographic process, with the only exception being a shift in the relative positions of geraniol and butanol. However, when one takes into consideration the standard errors of the hale indexes for these two odorants relative to the difference between them, one cannot rule out the possibility that error-free hale indexes for the four odorants would indeed be in the exact same order as the cross-mucosal sorption indexes. This similarity suggests that perhaps the same mechanism, differential sorption by the mucosa, underlies both effects. That is, the more strongly an odorant's molecules are sorbed by the mucosa, the fewer will be its molecules reaching points farther down the flow path in a given time. Taking this lead, several aspects of the hale effect can be explained by further suggesting that the flow path through the external naris to the mucosal region supplied by the medial margin of the nerve covers less absorbent area than does the flow path through the internal naris to the mucosal region supplied by the lateral margin. Thus, a greater number of molecules would reach the earlier and later flow path positions when flowing in the forward direction through the external naris than when flowing in the reverse direction through the internal naris. This would give, as shown by the positive hale indexes in Table IV, bigger responses in the forward direction than in the reverse direction. Note that the one odorant that does not show a decided difference in response magnitudes between the two flow directions is octane. However, octane also shows very little, if any, effect of the chromatographic process and, from studies using radioactively labeled molecules (Hornung and Mozell, 1977), it is known to be poorly but rather uniformly sorbed across the mucosal surface after a sniff. Thus, there is only a minimal loss of octane molecules by sorption to the mucosa as they migrate along the surface. Therefore, for octane, the amount of mucosa crossed makes little difference and the response magnitude remains relatively independent of hale direction.

This relative independence from hale direction would not, as confirmed in Table IV, be expected of the other odorants since they are more readily sorbed by the mucosa and would lose more molecules before reaching the recorded sites when flowing in the reverse direction than when flowing in the forward direction. In this regard, the close correlation between the hale effects for these odorants and the effect of their chromatographic process has already been noted. The sharper the decrement in response from the earlier flow path position to the later position (i.e., the larger the index for the chromatographic process), the larger is the hale effect. It is argued here that both of these effects depend upon the propensity of the odorant to lose molecules by sorption to the mucosa as the molecules move along the mucosal surface. The effect of the chromatographic process then comes from the loss that occurs between recorded sites, whereas the hale effect comes from the losses that occur before reaching the recorded sites. It is interesting to note in this regard that, unlike for the MN responses, there was some tendency for the LN responses for any given odorant to approach equality for the two flow directions (Table II). This suggests that the absorbent

surface area passed over by the odorant as it flowed in the forward direction to the later position in the flow path was nearly equal to the absorbent surface area passed over to the earlier position in the reverse direction. Unfortunately, it is not yet possible to verify this suggestion since methods to chart the exact flow path through a frog's olfactory sac have not yet been adequately developed.

It has been argued above that the hale effect could come from a smaller amount of sorption in the segment of the external naris before reaching the MN site in the forward direction of hale than the amount of sorption in the internal naris segment before reaching the LN site in the reverse direction of hale. However, even if the amount of sorption in the external and internal segments were to result in the same responses at the earlier sites in the two directions, there remains the possibility of a residual hale effect attributable to differential sorption between the MN and LN sites, depending on the direction of hale. For example, less sorption in going from MN to LN than in the reverse direction would increase the hale index from what it would otherwise be; this is true because the numerator of the index of hale effects would tend to be higher and the denominator would tend to be lower than would be the case if sorption were the same in each direction. Unfortunately, there is no way in the present experiment to test directly for this possible sorption–hale direction interaction. Following the same logic as for the index of the effect of hale, the index for the effect of the chromatographic process was also not designed to detect any possible differences in the influence of sorption in the two directions of hale.

Regional Differences in the Mucosa's Sensitivity to Different Odorants

Just as this study supports previous findings (Mozell, 1966, 1970; Mozell and Jagodowicz, 1973; Mozell and Hornung, 1984, 1985; Hornung and Mozell, 1977, 1981, 1986) that the molecules of different odorants are sorbed in different gradients across the mucosa, so too does this study support previous findings (Kauer and Moulton, 1974; Kubie et al., 1980; Mackay-Sim and Kubie, 1981; Moulton, 1981; Mackay-Sim et al., 1982) that different mucosal regions differ in their odorant-selective sensitivities. Indeed, this is the first published study to extend the documentation of regional sensitivity differences to olfactory nerve recordings, since the earlier publications reported either EOGs or single-unit recordings in the olfactory bulb. However, although this study, as evidenced by the log indexes listed in column 2 of Table IV, certainly agrees with other studies showing regional differences in selective sensitivity, it is somewhat less concordant with the earlier work when specific odorants are linked with specific regions. For instance, the earlier EOG studies showed both butanol and geraniol to be more effective stimuli to the anterior mucosa than to the posterior mucosa. In the present study, neither of these odorants could be clearly considered more effective for the anterior mucosa since, in accordance with the log indexes of column 2 of Table IV, butanol gave little, if any, evidence of a regional sensitivity difference, and geraniol was actually more effective for the LN response (more posterior) than the MN response (more anterior). It should be noted, however, that these studies are methodologically dissimilar in many respects, including the type of activity sampled, whether the olfactory sac was intact or not, and the

species used. Furthermore, it must also be re-emphasized that the regional sensitivities in the present experiment are all relative to that of *d*-limonene, requiring them to be interpreted in relative rather than absolute terms. Thus, relative to *d*-limonene, there was no regional difference in mucosal sensitivity for butanol, but this does not rule out the possibility of some absolute difference, which, if it were the same as that for *d*-limonene, would not be reflected in the regional sensitivity index. In addition, whereas in the earlier studies, which for the most part used salamanders, the mucosa is simply classified as either anterior or posterior, in the present frog study, the LN region is as far lateral to the MN region as it is posterior. Therefore, not only is there a difference between the studies in the type of activity recorded, the species used, and the analysis made, but the mucosal regions sampled might also not be analogous. In the one earlier study that did report frog data (Kauer and Moulton, 1974), the results for the only common odorant, butanol, were not reported sufficiently to allow any comparisons with the present study. On the other hand, there is some agreement between the earlier studies and the present study for octane. In all studies, the sensitivity to this odorant is least at the more anterior regions of the mucosa and increases posteriorly. It should be emphasized again in this discussion that although some details may differ between the methods and results of the present study and those that preceded it, the present study, even though sampling a different type of activity, still supports the earlier studies, which show that different mucosal regions do indeed differ in their odorant-selective sensitivities.

Joint Representation of Odorants by the Chromatographic Process and Regional Sensitivity Differences

It has been shown in this one study that regional sensitivity differences and the chromatographic process, which in earlier studies were investigated separately, actually operate together to establish different activity patterns across the mucosa. Although both these mechanisms were observed to contribute to the different activity patterns, the relative importance of each depended upon the particular odorant used. At one extreme (for butanol), regional sensitivity differences made little contribution and mucosal sorption by itself apparently accounted for the cross-mucosal activity pattern. At the other extreme (for octane), it was the regional sensitivity difference that accounted for the cross-mucosal activity pattern, with essentially no contribution from the chromatographic process. For the other two odorants, geraniol and benzaldehyde, the contribution of mucosal sorption was, respectively, double and triple that of the regional sensitivity differential.

It is admittedly difficult to make generalizations covering all odorants when only a sample of four has been used, but perhaps some tentative suggestions can be made. It appears by comparing the magnitudes of the indexes for regional sensitivity and the chromatographic process that the latter, though dependent upon the odorant, can generate a substantially greater effect upon the mucosal activity patterns than the former. This is not to say that regional sensitivity cannot make the greater contribution, but only suggests that when it does make the greater contribution, as for octane, its potential impact is still less than what

can be achieved by mucosal sorption. Of course, this implication must be tempered by the realization that the most sensitive mucosal regions for the odorants used may not have been sampled.

It should be noted that although the contribution of the chromatographic process to the cross-mucosal activity patterns is rather considerable for benzaldehyde, geraniol, and butanol, it is probably still less than for other odorants that are more readily sorbed by the mucosa. Although the molecules of these three odorants took an appreciable time to cross the frog's olfactory mucosa when it replaced the column of a gas chromatograph, the molecules of other odorants took even longer. It would be expected that these other odorants, being more strongly sorbed by the mucosa, would produce even larger values for the mucosal sorption index, thus giving this mechanism even greater weight relative to regional sensitivity differences in generating mucosal activity patterns.

Because the gradients of activity across the mucosa can depend upon both regional sensitivity differences and mucosal sorption, the steepness of the activity gradient for any given odorant can differ depending upon the flow direction. This was mathematically demonstrated in the Results. For example, in one direction, a decreasing number of molecules from the earlier to the later position in the flow path fell upon a parallel decrease in sensitivity, so that the two mechanisms reinforced each other to give a sharper decrement in activity from the earlier to the later position than would have been true for either one alone. However, for flow in the opposite direction, a decreasing number of molecules fell upon an increasing sensitivity, thus moderating somewhat the gradient of activity across the mucosa. To explain an earlier observation of this direction-dependent difference in activity gradients (Mozell and Hornung, 1985), several more complicated mechanisms were proposed, including a possible variation in the flow path for the two directions. These other explanations ought not to be ruled out altogether, but now that the combined roles of mucosal sorption and regional sensitivity differences have been demonstrated, parsimony would suggest that their interplay is a sufficient basis for producing the activity gradient differences seen with changes in flow direction.

These two mechanisms, when acting in concert, can help in the differentiation of odorants more effectively than can either one acting alone. Working together, they can produce a larger number of alternative activity patterns by which different odorants can be analyzed and encoded. At one extreme, the odorants having similar mucosal regions of greatest sensitivity could still establish different cross-mucosal activity patterns if one odorant is sorbed more readily than the other, so that their molecules are differentially distributed across the mucosa. At the other extreme, two odorants similarly sorbed by the mucosa could still be differentiated if for each one there was a different maximally sensitive region of the mucosa.

Sensitivity of Mucosal Activity Patterns to Other Stimulation Variables

It has been proposed earlier a number of times (Mozell, 1970, 1971; Mozell and Jagodowicz, 1973, 1974; Mozell and Hornung, 1985) and has been implied here

that the cross-mucosal activity patterns (be they established by the chromatographic process, regional sensitivity differences, or a combination of both) can be a basis for olfactory quality discrimination. The justification for this proposal has been discussed elsewhere (Mozell, 1971; Mozell and Jagodowicz, 1974; Mozell and Hornung, 1984, 1985), but it should be noted here that if these cross-mucosal activity patterns are to underlie the discrimination of different odorants, they should be predominantly sensitive to changes in the odorants per se and show little variation with such aspects of the stimulus as its intensity or duration of presentation. It is noteworthy in this regard that there were no significant p values for any of the ANOVA main effects or interactions that would have signaled a dependence of the mucosal sorption effect or the effect of regional sensitivity differences upon sniff volume, sniff duration, or the number of odorant molecules within the sniff. The statistics did flag a few of these possible relationships as, perhaps, suggestive (most notably an interaction of odorant and number of molecules with regional sensitivity [$p < 0.02$] on the one hand and an interaction of odorant and sniff volume with the effect of the chromatographic process [$p < 0.02$] on the other), but none came particularly close to reaching the established criterion of 0.0035. This result differs somewhat from at least one earlier study (Mozell, 1970), which did show some relationship between cross-mucosal activity patterns and intensitive stimulus factors. It might be argued that the increments used in the present study for the number of molecules, volume, and duration were not large enough for their influence upon the effects of mucosal sorption and regional sensitivity differences to be seen. However, as explained in the Methods, the two sniff durations and the two sniff volumes used in this study fell within, and near the limits of, the bullfrog's normal repertoire and to go beyond these limits would be to become aphysiological. On the other hand, although the numbers of molecules chosen did fall within the dynamic range of the frog's stimulus-response relationship, the twofold increment called for by the strategy of this experiment is admittedly rather small. However, this twofold increment was large enough to significantly multiply the average response magnitude by a factor of 1.24 ($2^{0.31}$), as shown in Table V. One would think that if there were a dependence of mucosal sorption and regional sensitivity upon the number of molecules robust enough to rebuff these mechanisms for olfactory discrimination, it would surface under these circumstances. Apparently this dependence is not large, if it exists at all, since, even in the previous study cited, the changes in the cross-mucosal activity patterns brought about by changes in stimulus concentration were small in spite of stimulus increments of several orders of magnitude. It is interesting to speculate that whatever changes do occur in the cross-mucosal activity patterns as a result of increments in the number of molecules might help explain the common observation that the qualities of some odorants, at least, change with concentration.

Effect of Number of Molecules, Volume, and Duration upon Response Magnitude

Octane is common to this experiment and a previously published one (Mozell et al., 1984), which also evaluated the effects of N, V, and T upon the magnitude

of the olfactory nerve discharge. Although only the MN region was tested in the previous experiment and only the forward direction of hale was used, it is nevertheless of interest to compare the N, V, and T effects for octane as given by the estimated exponents in the two experiments. They are, for the previous experiment, +0.35 for N, −0.28 for V, and +0.22 for T; these compare with the exponents for the present experiment, which are +0.25 for N, −0.19 for V, and −0.01 for T. Considering that there was no formal control between these two distinct experiments, the estimates for N and V are quite close to each other. Moreover, the disparity between a positive exponent for T in the first experiment and a near-zero exponent for T in the present experiment is not significant, even without allowing for procedural differences involving directions of hale and regions sampled. In fact, if the estimates for the exponents for N, V, and T in the present experiment are based only on MN responses in the forward direction of flow, as was the case in the earlier experiment, the values for the two experiments are even more consistent. That is, the values for N, V, and T in the present experiment, as calculated from the MN responses to the forward direction of octane, are, respectively, +0.31, −0.27, and +0.03 and the corresponding values in the earlier experiment were, respectively, +0.35, −0.28, and +0.22.

With all the absolute values of the exponents being less than unity, both studies concur that for octane, at least, the relationship between the summated multiunit discharge and each of the three variables is either negatively accelerating (in the case of N and T) or negatively decelerating (in the case of V). Of course, there is a discrepancy in that the present experiment sees much less effect of sniff duration upon the response magnitude than did the earlier study, but the relationship is still in the same direction. Thus, except for this one quantitative difference, the two studies are basically in agreement.

The two clearcut, unexpected findings in the present experiment, which, unlike the earlier one, used three odorants in addition to octane, were: (*a*) the negative effect of volume for octane as compared to positive effects for benzaldehyde, butanol, and geraniol; and (*b*) the negative effects of increasing sniff duration for all odorants except, perhaps, for octane. The statistics bear out that, with the possible exception of geraniol, the signs of the exponents for volume on all four odorants are not chance variations from zero. In addition, as discussed above, when the techniques and strategies applied to all four odorants in the present study are followed for octane alone, the results corroborate those of a previous independent study. Thus, it appears that the difference in the volume effect between octane and the other three odorants is not due to some chance variation or to differences in experimental procedures but is due to some difference in the odorants themselves. In keeping with the thrust of this article, one obvious difference is the greater mucosal sorption of benzaldehyde, butanol, and geraniol than of octane.

Examination of the data shows that the increase in response that occurs for the highly sorbed odorants with an increase of sniff volume results mainly from a marked increase of the response at the later position along the flow path. On the other hand, the reduced response with increased sniff volume observed for octane, the poorly sorbed odorant, results from a reduced response at both the

earlier and later positions along the flow path. One can only speculate. Perhaps the increased flow rate generated by the larger-volume sniffs reduces the probability of a given molecule's sorption by the mucosa, so that for poorly sorbed odorants there is still less sorption across the entire mucosa. On the other hand, highly sorbed odorants, which at lower flow rates could have the bulk of their molecules sorbed before the later flow path position, could at the higher flow rate present a greater number of molecules to that later position. A similar argument can be advanced for the negative effects of increased duration, seen especially for the three highly sorbed odorants. That is, increasing the duration slows the flow rate so that there may be an increased probability of a given molecule's sorption before reaching the sampled positions. However, regardless of the explanation, it is interesting and challenging that the same change in a sniff variable can produce opposite changes in the response for different odorants.

This research was supported by National Institutes of Health grant NS-03904.

Original version received 9 February 1987 and accepted version received 6 July 1987.

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