Adaptation and Cross-Adaptation to Odor Stimulation of Olfactory Receptors in the Tiger Salamander

FRANK BAYLIN and DAVID G. MOULTON

From the Monell Chemical Senses Center and the Departments of Physics and Physiology, University of Pennsylvania, and the Veterans Administration Hospital, Philadelphia, Pennsylvania 19104. Dr. Baylin's present address is Solar Energy Research Institute, Golden, Colorado 80401

ABSTRACT We have used the effects of self- and cross-adaptation on the unitary responses of olfactory receptors of the tiger salamander to odor stimulation to investigate the stimulus-specific components of these responses and to provide information about the cross-cell variations in the numbers and numbers of types of constitutent receptive sites. An olfactometer delivered sequential odorous pulses, either juxtaposed or separated by a variable time delay. We used four pairs of odorants judged to be similar within a given pair. The unitary response to the test stimulation relative to that of the conditioning stimulation varied from being unchanged to being completely eliminated. We sometimes observed substantial poststimulus increases in the firing rate following stimulation with juxtaposed odorous pulse. Except in the case of one odorant pair, cross-adaptation occurred both with juxtaposed pulses and with pulses separated in time. With the methyl butyrate/ethyl butyrate odorant pair, however, statistically significant cross-adaptation appeared only with juxtaposed pulses. We propose a simple model to aid in explaining these phenomena. The experimental observations in conjunction with this model are used to obtain estimates of the maximal and minimal number of receptive site types available for interaction with the chosen odorants.

INTRODUCTION

The role of the olfactory receptor in the coding of olfactory information is poorly understood. Receptor-to-receptor differences in selectivity and responsiveness to odorants are marked (Gesteland et al., 1965; O'Connell and Mozell, 1969). We need to know what chemoreceptor structure accounts for these variations. Several workers have postulated that entities called receptive sites, located somewhere on the apical receptor membrane, interact with the odorants. The resulting current flow initiates impulse activity (e.g., Moulton and Tucker, 1964; Gesteland et al., 1965; O'Connell and Mozell, 1969; Beets, 1971; Getchell and Gesteland, 1972). The cell-to-cell differences in responsiveness observed in the unitary studies on the olfactory epithelium would then be accounted for by variations in receptive site composition of the receptors (Gesteland et al., 1965; Mathews, 1972; Moulton, 1976; Baylin, 1979). However, it is difficult to quantify the effects that access factors such as local variations in mucus thickness and

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geometrical structure of the receptors might have on the unitary response. Consequently, these cell-to-cell response differences may not be entirely a result of variations in intrinsic receptor structure.

As a first step in investigating these questions, we have designed an experiment using an approach analogous to that used in functionally similar systems. For example, research on certain hormone and drug receptors has been conducted first, by deriving a measure of the strength of the interaction between the substrate and the acceptor and second, by manipulating the receptive surface in a controlled fashion designed to provide information about the mechanisms of this interaction. There are various possible methods of modifying the receptors including reaction with group-specific protein reagents (see Getchell and Gesteland, 1972) and application of a transepithelial current (Higashino and Takagi, 1963). Of these, we have chosen one which is most consistent with the normal physiological functioning of the olfactory organ, namely, self- and cross-adaptation. A conditioning odorous pulse, hypothesized to alter the state of the receptive sites, is followed shortly after by a test stimulation of the same or a similar odor.

Evidence presented below shows that this approach has two additional advantages: first, it partially obviates the need to consider some of the access factors in olfactory function and second, it allows clarification of some of the details of the hypothesized receptive site composition of these chemoreceptors. The previous paper (Baylin, 1979) examined the single unit responses of olfactory receptors in the tiger salamander. This study extends these data and conclusions.

Both self-adaptation and cross-adaptation have been observed in studies on human subjects (Stuiver, 1958; Köster, 1971). However, no systematic study of the responses of single receptors conclusively demonstrates that these effects may originate partially in the olfactory periphery. This paper presents such evidence.

MATERIALS AND METHODS

Animal Preparation and Recording Techniques

Tiger salamanders (*Ambystoma tigrinium*) were used in this study. The preparation of this animal and the recording techniques have been described by Baylin (1979).

Stimulation Methods

An all glass, Teflon, and stainless steel olfactometer, previously described by Baylin (1979, see Figs. 1 and 2), was constructed. This apparatus was designed to deliver sequential stimuli of either similar or dissimilar odorants. These pulses were either separated by a variable time delay or juxtaposed by using the mode 1 and the mode 2 olfactometer, respectively.

Sequential standardized pulses separated by a time delay were delivered using the mode 1 olfactometer (refer to Fig. 1 in Baylin, 1979). Details of the procedure for switching valves are described below. Switching V_{A1} and V_{A2} simultaneously directed the contents of loop l_{A2}, odorant A, onto the mucosa. 3 s or later, reswitching both valves delivered an identical stimulation from loop l_{A1}. If, instead of reswitching V_{A1} and V_{A2}, T

and then V_{B1} and V_{B2} were simultaneously switched, odorant B played onto the epithelium. Alternatively, two sequential pulses of odorant not separated in time were delivered using the mode 2 olfactometer (refer to Fig. 2 in Baylin, 1979) as follows: switch V_{A2} at $t = 0$, V_{B1} at $t = \delta t$, V_{A2} at $t = 5$ s, V_{B2} at $t = 5 + \delta t$, and V_{A2} at $t = 10$ s. This sequence gave two juxtaposed 5-s stimulations of A and B. Reversing this sequence reversed the order of A and B.

While recording the activity from a single receptor, a random search was made for a pair of chemicals which were both effective stimuli. This search was facilitated by the use of Teflon puff bottles partly filled with liquid odorant. If such a pair, A/B, was found, the following standard stimulation sequence was delivered from the olfactometer: A; A–*t* seconds–A; A–*t* seconds–B; B; B–*t* seconds–B; and B–*t* seconds–A. Here *t* is either 0, 5, or 10 seconds (Fig. 1). The first of each of the paired stimulations was the conditioning pulse; the second was the test pulse. Each step was separated by a recovery period of at least 2 min to minimize olfactory fatigue (the nonspecific loss of responsiveness). Odorant concentrations were chosen so that the impulse output of each unit for each stimulus was near the maximal response (Holley et al., 1974; Baylin, 1979) but below those intensities which evoked any substantial spike decrements. When possible, this sequence was immediately repeated to check that each response was reproducible.

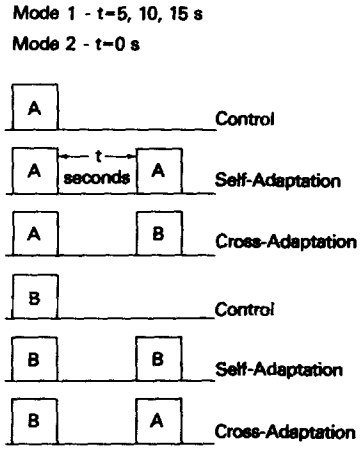

FIGURE 1. Odor presentation sequence. A and B are pulses of variable concentration of two different odorants.

When using the mode 1 olfactometer, this repetition also had the advantage of averaging out small differences in volume between the odorant loops. This is because the order in which the loops were used in the two sequences of six stimulations were reversed: $A(l_{A2})$, $A(l_{A1})$–$A(l_{A2})$, $A(l_{A1})$–$B(l_{B2})$, $B(l_{B1})$, $B(l_{B2})$–$B(l_{B1})$, $B(l_{B2})$–$A(l_{A2})$ and then reversing the order of the loops $A(l_{A1})$, $A(l_{A2})$–$A(l_{A1})$, $A(l_{A2})$–$B(l_{B1})$, $B(l_{B2})$, $B(l_{B1})$–$B(l_{B2})$, $B(l_{B1})$–$A(l_{A1})$. Whenever possible, an additional pair of odorants or a different concentration of the same odorants was tested on the olfactory receptor cell. In those cases, when both members of an odorant pair caused a noticeable change in the spontaneous impulse activity of a unit, or when some individual odorants stimulated the cell, various types of stimulus sequences were tested. These included both phasic and tonic stimulations and testing for self-adaptation with, when possible, two or three different concentrations of the same odorant.

Odorant Selection

Seven odorants, grouped into four pairs of similar stimulants, were used. These four pairs, were methyl butyrate (MNB) and ethyl butyrate (ENB), butanol (BUT) and propanol (PROP), benzaldehyde (BZA) and nitrobenzene (NB), and benzaldehyde and acetophenone (ACP). They were the same pairs used by Baylin (1979).

Calibration of Olfactometer

The flame ionization detector (FID) from a Varian 1520 gas chromatograph (Varian Associates, Palo Alto, Calif.) was used to calibrate the olfactometer odorant concentrations and to monitor pulse waveshape (see Baylin, 1979). It was essential that the sequential odorous pulses were identical because the difference in the response frequency and the number of evoked spikes were used as measure of adaptation and cross-adaptation.

The wave forms of two sequential standard pulses delivered by the mode 1 olfactometer are shown in Fig. 2. In general, both pulses have essentially identical wave forms.

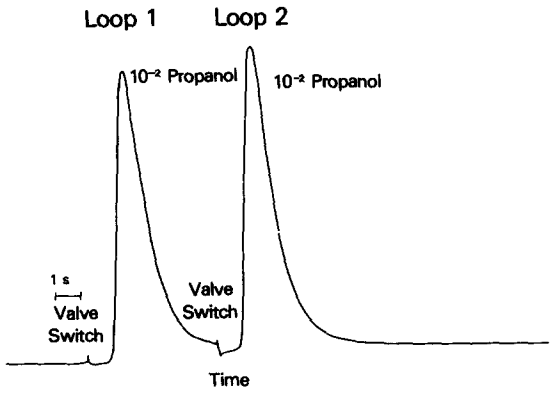

FIGURE 2. Sequential odorous pulses: mode 1 olfactometer. The trace represents odorant concentration vs. time as determined by FID monitoring.

However, the second pulse is slightly larger, as determined by measuring the curve areas, because it is initiated on the non-zero tail end of the first pulse. For example, the volumes of two pulses of 10^{-4} propanol delivered by loop 1 followed by loop 2 were, in arbitrary units, 10.0 ± 0.16 and 11.1 ± 0.18, respectively. When loop 2 was followed by loop 1, these volumes were 10.0 ± 0.11 and 10.5 ± 0.14 units. This implies that in those cases where adaptation is observed the reduction in olfactory response to the second pulse in the sequence may be slightly understated. The wave form of sequential pulses delivered by the mode 2 olfactometer are shown in Fig. 3.

Data Analysis

To enable a comparison of unitary responses, various measures such as maximum frequency of response, response rise time, and several other scalars were considered. Of these measures the most consistent (judged by relative constancy across stimulations) was a scalar description of the total number of spikes expected for this period. Therefore, we used this quantity as a measure of outcome in all our statistical tests. In most cases, the total number of spikes in a response was much larger than the expected spontaneous rate. In addition, although the wave form of the response varied from cell to cell and

from stimulus to stimulus in each unit, the response duration was usually clear-cut. These features facilitated identification and quantification of the response. In those few cases where response was 2–3 times longer than the duration of the electroolfactogram recorded simultaneously (see Baylin, 1979), only the total number of spikes generated during the phasic component of the response was measured.

10 numbers describing the olfactory response were generated each time the entire stimulus delivery sequence was applied to a unit. Three numbers quantified the responses to each of odorants A and B. In addition, these data included one measure of self-adaptation and one for cross-adaptation for each odorant. Whenever possible, the entire stimulus delivery sequence was repeated. These data allowed statistical comparisons of the measures of self- and cross-adaptation for each stimulus with the three or more numbers describing the individual responses. Probabilities computed from the t distribution served as tests of the null hypothesis that the responses used to measure cross- and self-adaptation were from the same statistical sample as the responses of the receptors to the conditioning stimulations. Finally, all the t test probabilities describing both self- and cross-adaptation for each odorant were combined and χ^2 probabilities were computed. These measured the statistical significance of self- and cross-adaptation across the entire collection of receptors sampled.

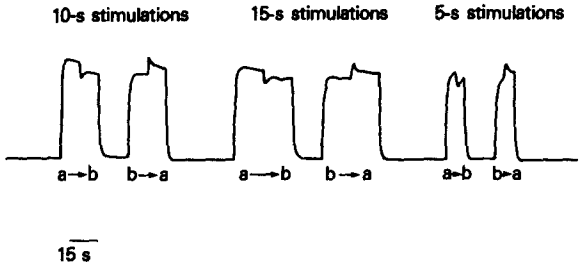

FIGURE 3. Sequential odorous pulses: mode 2 olfactometer. The trace represents odorant concentration vs. time as determined by FID monitoring.

The reproducibility of the olfactory response is one of the basic assumptions underlying the use of these statistical methods. For all those units where data were obtained for two or more repetitions of the stimulus delivery sequence, a t test comparing the first three response magnitudes with the second group of three generated probabilities indicating the degree of reproducibility. In those cases where the stimulus delivery sequence was presented only once, the last response was compared with the first two. Subsequently, χ^2 values, both for each type of stimulus and across all stimuli, served as measures of reproducibility across the sample of receptors. Without exception, all responses were reproducible by both of these criteria.

All data derived when using the mode 2 olfactomer were presented in the form of PST histograms (see Baylin, 1975, for a complete presentation of these data).

RESULTS

A total of 100 units was recorded from various positions on the ventral olfactory epithelium of the tiger salamander for periods of time ranging from 10 min to 3 h. 56 receptors were responsive to at least one of the seven odorants. Both adaptation and cross-adaptation were studied in 30 units which responded to both members of at least one of the stimulus pairs. In an additional 20 units (1A-20A), for which no effective stimulatory pair of odorants could be found,

we studied only self-adaptation using the mode 1 olfactomer. Units 1–21 and 1C–9C were stimulated by the mode 1 and mode 2 olfactometers, respectively.

Adaptation—Observations

We often observed adaptation in the response of a unit to stimulation with a test odorant shortly after stimulation with an identical conditioning odorant. The effect was manifested by either a reduced relative number of impulses generated or a change in the distribution of spikes in time relative to a control stimulation (see Fig. 4 A, B, and C). Similar effects were often observed when the test and conditioning stimuli were dissimilar, i.e., cross-adaptation (Fig. 2 D and E). For example, in unit 1 (Fig. 5), ENB evoked a response with an initial phasic burst

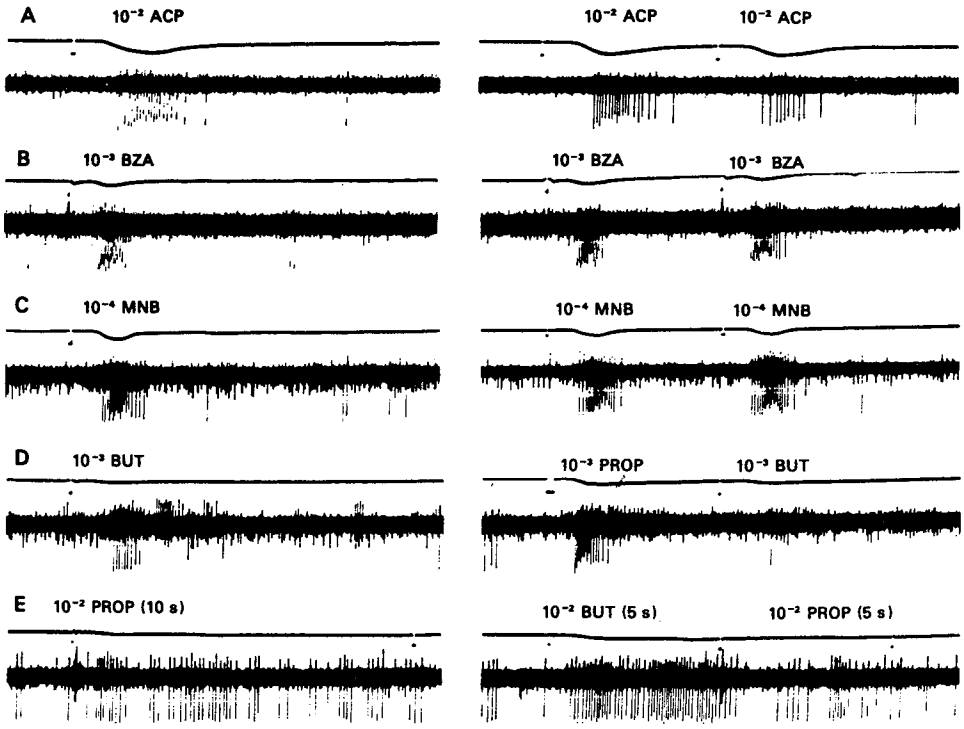

FIGURE 4. Receptor responses. Tests for adaptation (A-C) and cross-adaptation (D and E). (A) Unit 15A-Tri-SRI; (B) unit 19A-Tri-SR14; (C) unit 18A-Tri-SR18; (D) unit 16-Tri-SR12; (E) unit 4C-Bi-SR108.

consistently followed by a small afterburst. However, a second puff of ENB following 10 s after an initial ENB stimulation evoked essentially the same number of impulses, although this afterburst was consistently absent. All conclusions in this study are based on the statistical analysis. By this criterion, self- and cross-adaptation were observed in the five responses displayed in Fig. 4. A visual inspection of the records in Fig. 5 suggests that the second response is partially adapted. However, in this case, no statistically significant differences were found among these four responses to ENB.

In unit 16 (Fig. 6), stimulation by 10^{-3} butanol elicits a vigorous response. However, when an identical stimulation followed 5 s after a propanol pulse, almost no response was elicited.

The response to the test stimulation varied across the receptor population from being completely abolished to being unchanged. Figs. 7 and 8 illustrate

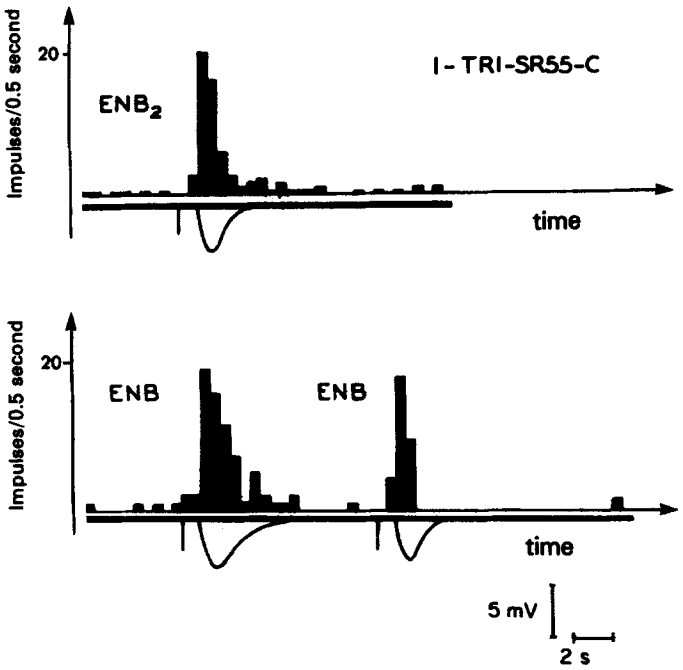

FIGURE 5. Self-adaptation. Responses of unit 1-Tri-SR55 to sequential stimulation by ENB. The subscripts indicate multiple stimulation; in this case, two responses to ENB are averaged.

this observation for the BUT/PROP and the MNB/ENB odorant pairs. The percent reductions in the test response relative to the control response were ordered according to the percent *t*-test probability, i.e., ordered according to the criteria used to judge whether this reduction was significant.

When the two odorous pulses were juxtaposed (mode 2 stimulation) we observed other phenomena as well as the graded effects of cross-adaptation. For example, MNB and BUT almost completely abolished the responses to subsequent stimulation by ENB and PROP, respectively (Fig. 9, units 2C and 4C). (The MNB/ENB and the BUT/PROP odorant concentrations here were relatively low and high, respectively.). After the second stimulation a strong afterburst was observed in both cases. Such an afterburst is also seen in unit #7C (see Fig. 10). The number and the frequency of spikes generated after the 5- or 10-s test stimulation increased as the odorant concentrations increased. These afterbursts occurred in 44% of the units tested for cross-adaptation using juxtaposed odorous pulses. It is interesting that this effect was never observed following (*a*) the responses to a single stimulation of an odorant or (*b*) the

responses to the test pulse when using the mode 1 olfactometer, viz., odorous puffs separated in time.

Another phenomenon was observed in unit 5C (Fig. 11). Here MNB caused a marked reduction in the response to ENB. However, as the concentrations of both odorants were increased, a partial recovery of the ENB test response following the MNB stimulation occurred, although this response was smaller relative to the control at these successively higher concentrations.

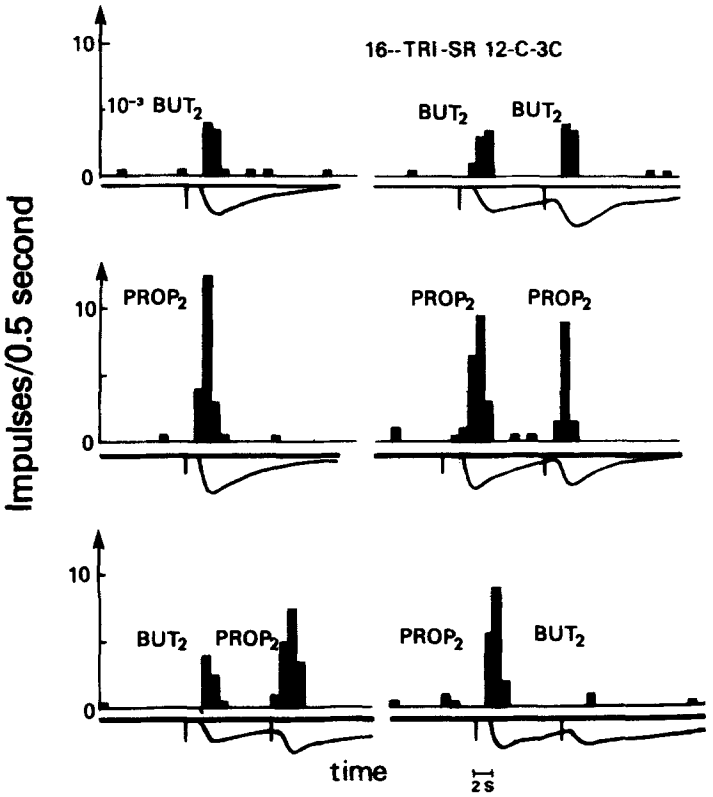

FIGURE 6. Self- and cross-adaptation. Responses of unit 16-Tri-SR12 to BUT and PROP.

In general, as the odorant concentrations were increased, the effects of cross-adaptation and adaptation became more pronounced (Figs. 10 and 11). In unit 4C, stimulation with a conditioning pulse of 10^{-2} butanol reduced the propanol response by roughly 50%. However, at a concentration of 0.5×10^{-1}, the propanol response was totally abolished. This observation does not necessarily imply that relatively low concentrations of a given odorant will not cause significant adaptation. It does suggest that less adaptation will occur as the odorant concentration is lowered. For example, in unit 6A, although self-adaptation occurred in the response to a test pulse of 0.25×10^{-3} BZA, reducing the stimulus concentration by a factor of 10 eliminated this effect.

In general, olfactory receptors samples in this study maintained tonic firing in the presence of prolonged stimulations. Repetitive stimulation of units 8 and 4A by ENB resulted in a very gradual, small decrease in the phasic unitary response.

Adaptation—Statistical Tests

A summary of the statistical tests used to estimate the significance of the effects of adaptation and cross-adaptation is presented in Table I. An arbitrary 5% significance level has been chosen. t probabilities $< 5\%$ are considered to refute

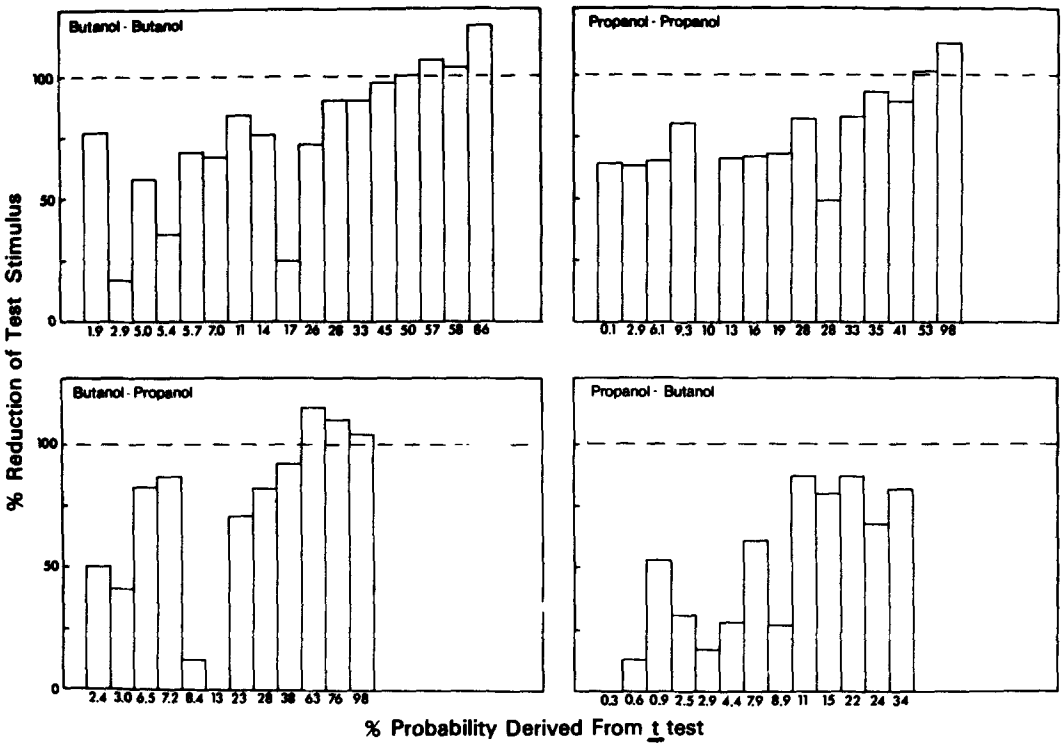

FIGURE 7. Summary of responses to the BUT/PROP odorant pair. The percent reduction of the test stimulus, the second member of each pair indicated in the upper left-hand corners, relative to the conditioning pulse are ordered according to the percent significance levels obtained from the t test.

the null hypothesis that the responses after cross- and self-adaptation were from the same statistical sample as the responses of the receptors to the individual stimulations. Listed for each odorant are (*a*) the number of units for which significant adaptation occurred as well as the total number of units tested and (*b*) the cross-unit cumulative χ^2 probability and the associated number of degrees of freedom (twice the number of t test probabilities used). This latter probability serves as a measure of the significance of each effect for each odorant across the total population of receptors sampled. If we assume that the

receptors studied are a representative sample, a simple interpretation of the χ^2 test is possible. When this probability is $< 5\%$ (0.05) we acknowledge that the total number of impulses in the response to the test stimulation relayed by the olfactory nerve to the olfactory bulb is less than the number in the response to the control stimulation.

Self-adaptation occurred in responses to all four pairs of odorants. Cross-adaptation occurred in response to all but the MNB/ENB pair when delivered by the mode 1 configuration, and in all four pairs when delivered by the mode 2 configuration. In all cases, when cross-adaptation was observed it was nonreciprocal, i.e., if A reduced the response to B, then a conditioning stimulus

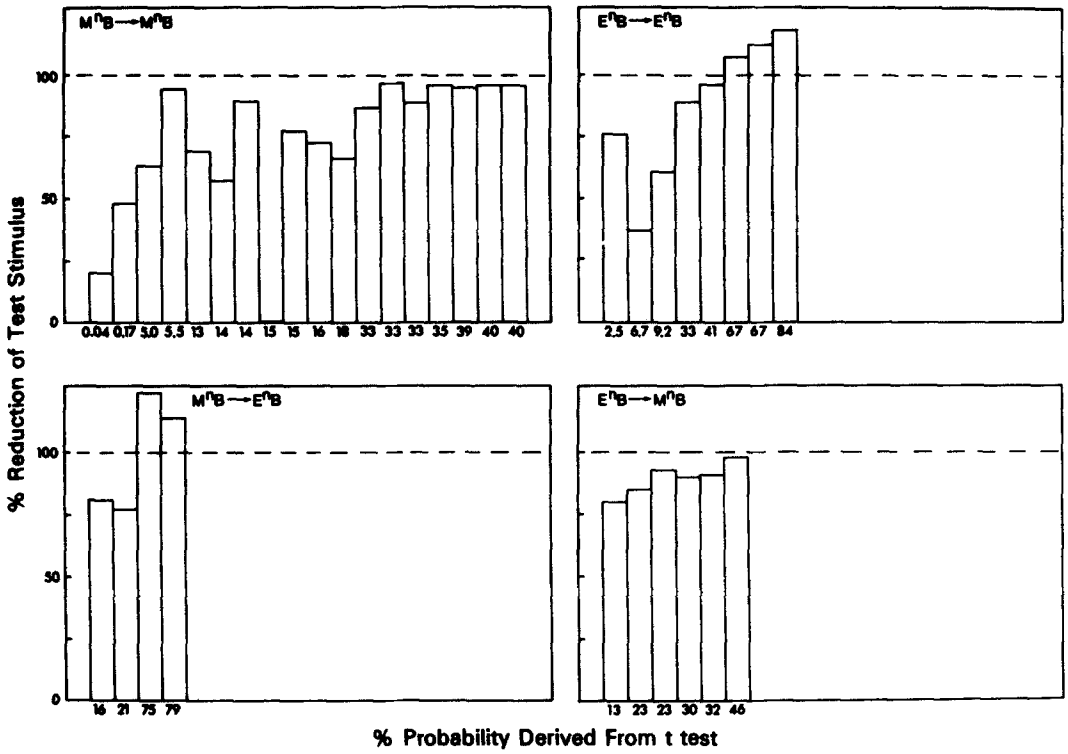

FIGURE 8. Summary of responses to the MNB/ENB odorant pair. See legend to Fig. 7.

of B had little effect on the response to A. These effects were not isolated to odorants having a particular type of chemical structure. Butanol, nitrobenzene, and methyl butyrate have quite different molecular shapes, weights, polarities, and functional groups.

Table II summarizes various aspects of the statistically significant unitary data for the BUT/PROP pair, the pair for which the data are the most complete (also see Fig. 12). We observed no correlation between the effects of self- and cross-adaptation and the spike generating effectiveness of an odorant. For example,

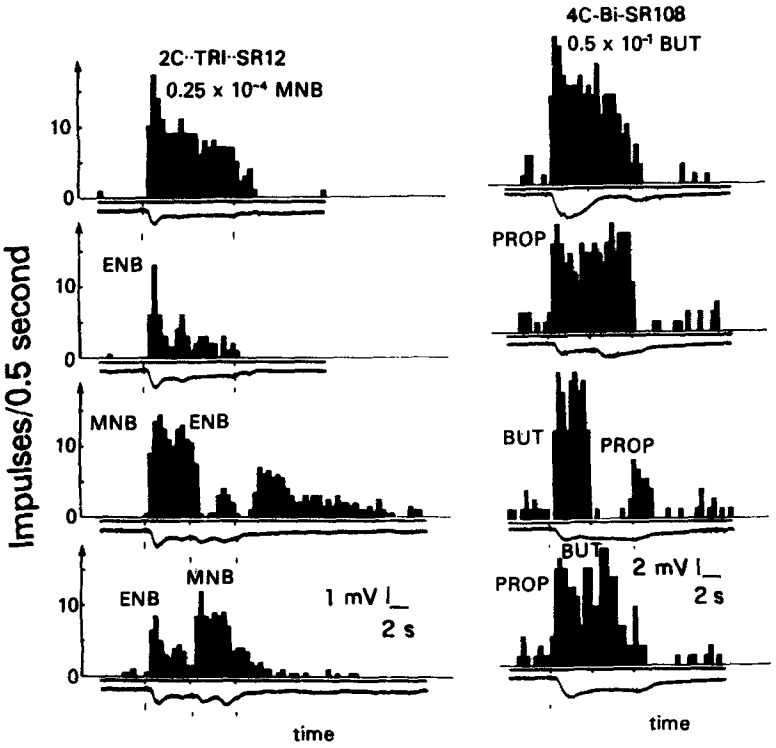

FIGURE 9. Responses to sequential odorous pulses: mode 2 olfactometer. The responses of unit 2C-Tri-SR12 (left column) and unit 4C-Bi-SR108 (right column) are indicated.

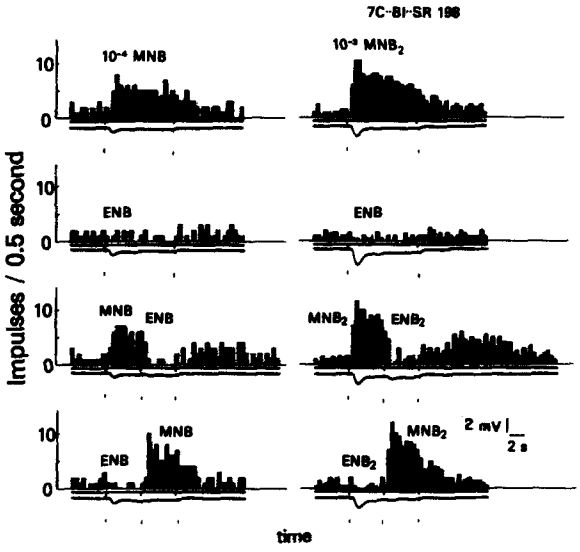

FIGURE 10. Responses to sequential stimulations: mode 2 olfactomer. Unit 7C-Bi-SR198.

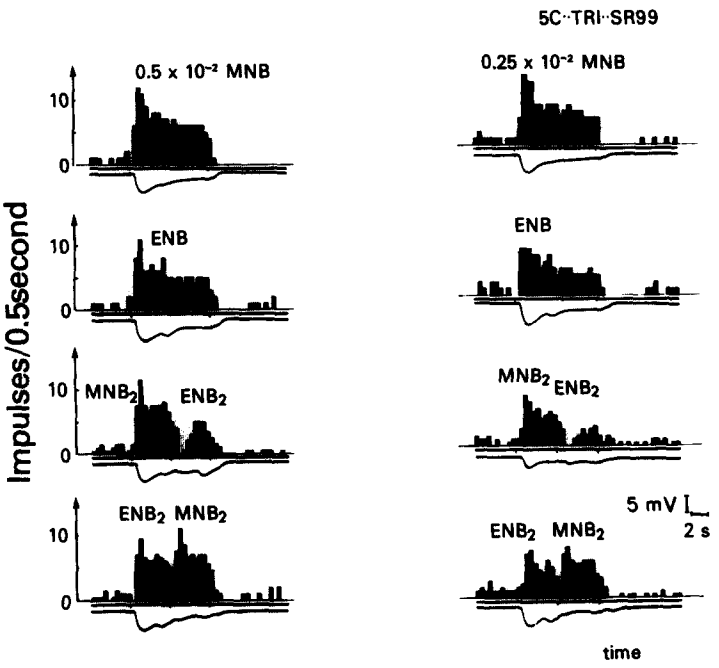

FIGURE 11. Responses to sequential stimulations: mode 2 olfactometer. Unit 5C-Tri-SR99.

TABLE I

STATISTICAL TESTS AND UNITARY DATA

	t test* No. of units with probability < 0.05		χ^2 test‡ Cumulative probability	
	Adaptation	Cross-adaptation	Adaptation	Cross-adaptation
	n		*P* *n*	*P* *n*
BUT	4 of 16	5 of 13	<0.01 (32)	<0.001 (26)
PROP	3 of 15	3 of 13	<0.001 (30)	<0.005 (26)
MNB	3 of 18	0 of 6	<0.001 (36)	0.19 (12)
ENB	1 of 8	0 of 4	<0.05 (16)	0.46 (8)
BZA	3 of 3	1 of 1	<0.001 (20)	<0.05 (2)
ACP	5 of 10	0 of 1	<0.001 (6)	0.44 (2)
BZA	5 of 10	1 of 2	<0.001 (20)	<0.01 (2)
NB	0 of 2	1 of 2	0.14 (4)	0.18 (4)

* Number of units with significant adaptation relative to the total number of units tested.

‡ Number of degrees of freedom in parentheses.

propanol cross-adapted the butanol response in units 3 and 16. Butanol and propanol stimulation caused the generation of 6.5 and 17.3 spikes, respectively, in unit 16. However, in unit 3, butanol evoked more than twice as many

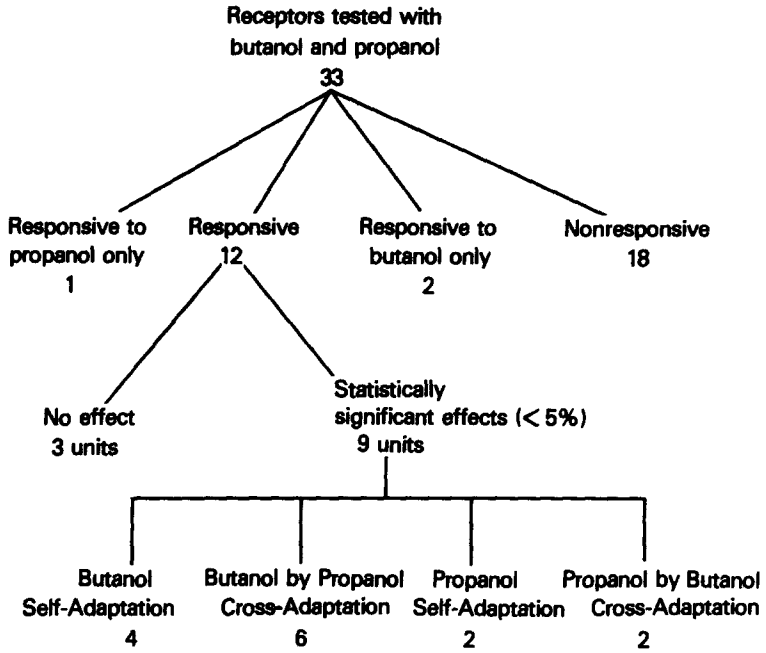

FIGURE 12. Summary of the statistically significant effects observed with the BUT/PROP odorant pair.

TABLE II

SIGNIFICANT ADAPTATION EVENTS—BUTANOL/PROPANOL PAIR

Unit number	Concentration (fraction of saturated vapor)	*t* test probabilities: Butanol Self-adaptation	Butanol Cross-adaptation	Propanol Self-adaptation	Propanol Cross-adaptation	Average number of spikes evoked: Butanol	Propanol
			P			*n*	
3-Tri-SR14	0.5×10^{-2}		0.006			17.6	6.5
4-Bi-SR6	0.5×10^{-3}	0.050			0.024	22.8	13.6
5-Bi-SR34	0.5×10^{-2}		0.025			17.9	16.3
6-Bi-SR56	0.5×10^{-2}			0.029		20.2	9.9
8-Tri-SR32	0.5×10^{-2}	0.050	0.044			10.0	3.3
11-Tri-SR86	0.5×10^{-2}	0.054				21.4	62.9
15-Q-Bi-SR24	10^{-4}				0.030	25.2	15.2
16-Tri-SR12	0.25×10^{-2}	no data	0.029	no data		11.5	19.5
	10^{-3}		0.003	0.001		6.5	17.3
19-Tri-SR94	0.5×10^{-2}	0.019	0.009		no data	12.8	36.3

The interpulse interval is 5 and 10 s in the first and last five rows, respectively.

impulses (17.6) as did propanol (6.5). An examination of the data reveals that each of the four effects could and did occur independently.

DISCUSSION

Self- and cross-adaptation were observed in a portion of the receptors sampled in response to odorous stimulation. These effects did not depend on the

particular choice of odorants; each of the three different odorant pairs was quite dissimilar.

Olfactory adaptation may be mediated in a variety of peripheral locations, not all necessarily localized at the immediate vicinity of the receptive site. Some important parameters may be: odorant solubility in the mucus; odorant solubility in the lipid membranes of the receptor and supporting cells; the strength and the time-course of odorant-receptive site interaction; and the time-course of removal and the mechanism of removal of odorant from the mucus and the environment of the receptor. The current generating mechanism may also be sensitive to the immediate history of the receptor, perhaps as a result of accumulation of excess intracellular Na^+ and Cl^- or extracellular K^+ (Takagi et al., 1968).

Any generalized reduction in the responsiveness of a receptor, perhaps caused by cellular damage or excessive odorous stimulation, is termed a nonspecific fatigue. In contrast, a stimulus-specific sensitivity reduction, resulting from localized and reversible changes in a receptor resulting from interaction with a particular odorant, can serve as a useful tool in elucidating details of the receptive site structure of the olfactory receptor.

Our results indicate that these effects are odorant specific and are mediated at the receptive sites. Self- or cross-adaptation occurs in the response of a particular unit to both or to either one of a pair of odors (e.g., compare units 4C and 16, Figs. 6 and 9). These effects are not correlated with the number of impulses evoked during a response to a particular odorant; we observed statistically significant effects in receptors which responded to a given odorant with relatively few and with many impulses. Thus, a nonselective fatigue of the current or spike-generating mechanisms probably does not account for this adaptation. These observations, the variability of the effects across the receptor population, and the graded nature of this sensitivity reduction suggest that there are cross-cell variations in the numbers and types of receptor sites. Further support for these conclusions is provided by evidence that (*a*) cross-adaptation was nonreciprocal; (*b*) cross-adaptation could occur independently of self-adaptation; and (*c*) in some receptors these effects did not occur even when using the higher odorant concentrations. This last result is an unusual finding; it strongly suggests that different types of receptive site types responsive to a given odorant can coexist on a given receptor cell.

Thus, it proves difficult to understand how a nonspecific mechanism, such as the competitive accumulation of the conditioning stimulus in the mucus phase, could account for the experimental observations. However, access factors are likely to have a second order influence on these phenomena. For example, when self- or cross-adaptation did occur, these effects became more pronounced at the higher odorant concentrations. As the number of molecules in the conditioning pulse is increased, more receptive sites are engaged but also more odorant is absorbed in the mucus and lipid phases, and therefore, more odorant may be retained in the vicinity of these sites.

Temporal Course of Adaption

No cross-adaptation was observed when the two sequential pulses of the ENB/MNB odorant pair were separated by a time delay of either 5 or 10 s. However,

eliminating this time delay often resulted in appreciable reductions in the test stimulation responses. How can we account for this difference between the ENB/MNB pair and remaining pairs? A conditioning pulse of MNB or ENB may render the receptive sites unavailable for interaction for a shorter period of time than any of the other five odorants. These substances may simply have been more quickly removed from the vicinity of the receptive sites or perhaps may have interacted with the available sites for a relatively short period of time. For example, in unit 5C (Fig. 11), a partial recovery of the cross-adapted ENB response occurred at the higher odorant concentrations. As the number of molecules of ENB was increased, the sites interacting with the MNB which was in the process of being removed, may have more easily bound the test odorant. To explain this absence of cross-adaptation one might also hypothesize that these substances interact with only a portion of the many available sites on a given receptor. However, it would be difficult to reconcile this suggestion with the near total abolition of the test pulse response that often occurred (e.g., Fig. 9, unit 4C).

How can we account for the high sensitivity of the receptors to the methyl and ethyl butyrate molecules? We observe that the probabilities of the MNB/ENB and the BUT/PROP odorant pairs not stimulating a given receptor were nearly equal (see Table II, Baylin, 1979). Therefore, it is not likely that the stimulatory effectiveness of the MNB/ENB pair could be accounted for by the existence of relatively many more different types of receptive sites distributed among the receptors available for interaction with these two odorants. If firing frequency is a function of rate of odorant-receptive site interaction and not solely of number of bound molecules, then a rapid rate of arrival of these substances, perhaps coincident with a rapid rate of odorant removal, would account for the high responsiveness of the receptors to methyl and ethyl butyrate.

The temporal structure of the response may be quite a complicated function of odorant concentration waveform at the receptive sites. We hypothesize that the wide variability observed in the phasic-tonic structure of the PST histograms in response to longer duration stimulations by a given odorant may be a result of variable access to or rate of removal (or inactivation) of the odorant from the vicinity of the receptive sites. However, these differences may also reflect the particular set of interaction dynamics of each substance with each receptive site type.

Often, when using mode 2 stimulation, a poststimulus increase in receptor firing was observed after the test odorant pulse. This effect, an expression of the interaction of the two odorants at the epithelial surface, appears to be similar to a postinhibitory rebound. In unit 7C (Fig. 10), the ENB response may be an inhibitory one and thus may account for the sudden termination of the response to the conditioning pulse of MNB. However, in units 2C and 4C (Fig. 9), for which the test pulse odorant was an effective stimulant, such a straightforward explanation was not available. Perhaps the presence of the test pulse caused the conditioning odorant to be retained on or near the receptive sites. When the test odorant was subsequently removed from the air just above the mucus, the retained conditioning odorant may have been desorbed and, in the process, evoked a response. Perhaps consistent with this hypothesis that the rate of departure from the receptive surface is a determinant of the response is the

speculation that the rate of odorant arrival at the receptive site, and not simply the presence of an odorant site complex, may be an important parameter. This hypothesis is further supported by the observation that these effects occurred only using mode B stimulation; viz., juxtaposing the two odorous pulses.

Simple Model

A general model of the receptive site composition of an olfactory receptor is suggested by these data (others have advanced very similar models, e.g., Beets, 1971; Polak, 1973). A finite number of receptive site types exist in the salamander olfactory epithelium. Each odorant is capable of interacting with a subset of this total population of sites. A particular receptor has a variety of different receptive site types and numbers of these types on the apical membranes. The odorant-receptive site interaction generates a current which, in turn, initiates spikes. No assumptions need be made about the structure of these sites or the nature of the odorant site interaction.

Thus, whether or not a receptor will respond to a particular odorant is determined by the presence or absence of receptive sites which recognize this chemical. Receptors which are highly sensitive to a given odorant have either site types which generate a relatively large current or have many more available sites of interaction than the norm or both. Each stimulant molecule has a greater probability of contacting and interacting with a receptive site if these entities are packed more densely. (In addition, dense spacing would perhaps result in a cooperative interaction among these receptive sites.) The similarity observed in temporal patterns of response of both highly sensitive and insensitive receptors may be more congruent with the existence of variable numbers of receptive sites from cell to cell rather than receptive sites which generate currents larger than the norm. Cross-odorant differences in unitary temporal response patterns may be an expression of variable odorant site interaction dynamics.

According to the model a conditioning odorous pulse interacts with some of the available receptive sites. Therefore, a subsequent identical test pulse evokes either (*a*) no response if all or most of the receptive sites are occupied or in a state which renders them unavailable for interaction or (*b*) a full response if all the sites had totally recovered. The degree of adaptation would thus depend on odorant access to the receptors, strength of odorant binding, duration of interaction, rate of odorant removal or inactivation, number of available receptive sites, and odorant concentration.

If two odorants, A and B, are employed, a conditioning pulse of either interacts with a portion of the available sites from subsets {A} and {B}, respectively, of the total population of olfactory receptive site types. ({A} signifies $A_1, A_2, \ldots, A_n$, i.e., n different types of receptive sites.) Each receptor cell has variable types and numbers of types from each of {A} and {B}. Let us label the receptive site composition of a given cell r, relative to odorants A and B as $[A_r]$ and $[B_r]$, respectively.

From the experimental observations we know that, if either A or B self-adapts, neither necessarily evokes the largest unitary response. A sufficient condition for adaptation is that a substantial portion of the sites in either $\{A_r\}$ or $\{B_r\}$ cannot generate a current in response to stimulation during the test pulse.

This effect is then odorant-specific, determined by the particular receptive sites involved in the response.

The following mechanism accounting for the nonreciprocal cross-adaptation in a particularly simple case suggests a possible explanation for more complex situations. If $\{A_r\}$ is contained in $\{B_r\}$, response to a test pulse of A will be reduced because the preceding conditioning stimulation by B potentially interacted with all the receptive sites in $\{A_r\}$. However, a conditioning pulse of A will not affect all the sites in $\{B_r\}$ and hence, probably not substantially cross-adapt the response to B.

Estimate of the Number of Types of Receptive Sites

In an attempt to estimate a rough upper limit to the number of different types of receptive sites available for interaction with a particular odorant, let us assume that MNB and ENB interact with two sets of chemoreceptive sites, $\{M_j; j = 1, \ldots, m\}$ and $\{E_j; j = 1, \ldots, e\}$, respectively. From Table II in the previous paper (Baylin, 1979), one can deduce that these sets probably have many members in common. For example, if MNB (ENB) evokes a response in a given receptor, the probability that ENB (MNB) will do likewise is 63% (88%).

In this study, receptors both responsive and nonresponsive to MNB and ENB have been found throughout the ventral olfactory epithelium. Kauer and Moulton (1974) have suggested that diffuse topographic maps may represent the geographic distribution of receptor cell selectivity (see also Mustaparta, 1971). However, the claim is not made that in some epithelial regions no cells responsive to any particular odorant can be found. Most likely, any particular epithelial region does contain at least some of the members of {M} and {E}. This speculation is further supported by our observation that no gross topographic preference is apparent for those cells which exhibit effects of self- and cross-adaptation or both.

The following argument implicitly assumes that the statistical distributions of each member of {E} and {M} are invariant from receptor to receptor. The preceding discussion suggests that this assumption is weak. However, it suffices in a rough order of magnitude estimate of the maximal number of receptive site types available for interaction with these odorants. In view of the meager data available, the very simplest statistical treatment is warranted. The chance of sampling a receptor with none of the members of {M}, namely a receptor which is nonresponsive to MNB, is 0.65 (see Table II in Baylin, 1979). Assuming that all types of receptive sites have equal probability, P, of being found on a receptor, then $P^m = 0.65$ when m different types of sites respond to MNB (or $P^e = 0.75$ for ENB). Thus, if 4, 8, and 32 types of receptive sites respond to MNB, then each site type has 0.90, 0.95, and 0.99 chance, respectively, of not being found on any particular receptor.

The arguments are further complicated because (*a*) the minimal number of each receptive site type necessary for a response to be evoked is not known and (*b*) we have assumed that each individual site, when interacting with an odorant, generates a current independently of the other sites. However, the above reasoning suggests that the trend towards increasing numbers of site types is punctuated by the increased rarity of each site type. Incorporating into this

argument the possibility that large numbers of each site type may be situated on any given cell further strengthens the conclusions that the existence of rare site types is unlikely.

The minimal number of receptive site types responsive to each member of a typical odorant pair can also be estimated. Receptors which respond to either butanol or propanol, but not to both, have been observed. Thus, at least two different receptive site types recognize these substances. In fact, our data suggests that, with the possible exception of NB, receptive sites exist which respond, in particular, to each of the seven odorants tested (see Getchell and Getchell, 1975, for discussion of this point).

The simplest explanation of the cross-adaptation data is as follows: we can assume the existence of one site which responds to only A (we label this site A_1), one which responds to only B (B_1) and one which responds to both A and B (A_0B_0). The similarity in receptor selectivity to both members of the odorant pairs suggests that sites responsive to both odorants exist. Nonreciprocal cross-adaptation would be observed in response to stimulation by odorant A and B if a given receptor lacked either A_1 or B_1 sites. For example, if only A_1 sites were absent, stimulation with either A or B would evoke a response. A conditioning pulse of A would not affect response to B while a conditioning pulse of B would reduce subsequent response to A.

Therefore, we speculate that a minimum of at least two site types responsive to each of the odorants employed in this study exist. Odorants which are similar in structure (as defined perhaps by the molecular shape, types of functional groups, or other parameters) most likely interact with some of the same site types. For example, at least one and probably two or more sites can trigger a response to butanol and propanol. It should be noted that these arguments do not preclude the possibility that substances with quite different molecular structures may also interact with a given type of receptive site.

Much extensive study remains before the mechanisms underlying the coding of olfactory information by the aggregate of primary receptors are grasped (see Moulton, 1976, for a recent review of the subject). A study of adaptation and cross-adaptation in the responses to groups of three odorants may provide additional insight into this problem.

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Requests for reprints should be directed to D. G. Moulton, Department of Physiology, G4, University of Pennsylvania, Philadelphia, Pa. 19104.

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