Temporal Patterns and Selectivity in the Unitary Responses of Olfactory Receptors in the Tiger Salamander to Odor Stimulation

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ABSTRACT Temporal patterns and selectivity in unitary responses of 100 single olfactory receptors in the tiger salamander to odor stimulation were investigated. An olfactometer which permitted control of stimulus concentration, duration, and flow rate was calibrated with a gas chromatograph. Stimulus pulses were monitored by recording the electroolfactogram from the surface of the olfactory epithelium. Both diphasic and triphasic spikes were recorded extracellularly. No discernible differences in types of responses, reproducibility of responses, and cross-unit distribution of spontaneous rates distinguished diphasic from triphasic units. The cross-unit selectivity in responses to the seven olfactory stimulants used and the range of odorant concentrations which effectively evoked these responses suggest variations in types and number of types of receptive sites on each cell. Temporal patterns in the unitary responses were generally less complex than those observed in the olfactory bulb. Phasic stimulations evoked phasic patterns. Tonic stimulations evoked phasic/tonic patterns. Occasionally poststimulus depressions or elevations in firing rates were observed. The nature of these patterns varied somewhat with odorant concentration for a particular unit.

INTRODUCTION

Single unit studies of the vertebrate olfactory epithelium demonstrate that the receptors have broad response spectra characterized by unpredictable differences in cell-to-cell responsiveness (Gesteland et al., 1963, 1965; Shibuya and Shibuya, 1963; Takagi and Omura, 1963; Altner and Boeckh, 1967; Shibuya and Tucker, 1967; O'Connell and Mozell, 1969; Shibuya, 1969; Mathews, 1972; Getchell, 1973; Blank, 1974; Holley et al., 1974; Getchell and Getchell, 1975). However, a generally accepted account of the basis for differential sensitivity to odors is still lacking.

In this study responses of olfactory receptors to stimulation with four pairs of similar odorants are used to investigate (a) unit response spectra across the population of receptors and (b) temporal patterns in these responses. Kauer (1974) has observed complex firing patterns in olfactory bulb neurons in the tiger salamander. These patterns, perhaps an important element in the olfactory code, may partially originate in the peripheral organ.

A knowledge of physical characteristics of the stimulus is critical when investigating sensory systems. Accordingly, an olfactometer which was designed to permit control of stimulus onset, concentration, and duration was built and calibrated with a gas chromatograph. The electroolfactogram (EOG), the DC-summated activity recorded at the epithelial surface, served as a stimulus monitor. These results are used to elucidate some details of the structure and function of olfactory receptors in the tiger salamander.

METHODS

Tiger salamanders (*Ambystoma tigrinium*) were immobilized with subcutaneous doses of *d*-tubocurarine, 6 mg/kg body weight, and the wound areas were anesthetized with liberal applications of xylocaine. The dorsal skin, bone, and cartilage over the nasal cavity were unilaterally removed. Finally, the dorsal olfactory tissue roofing one nasal cavity was cut on a line from the external to near the internal nares and was carefully folded back on either side. During this procedure no blood was permitted to flow onto the ventral epithelium. (The use of the tiger salamander for single unit coding studies was developed by Kauer [1973, 1974] and Kauer and Moulton [1974].)

Recording Techniques

The electroolfactogram (EOG) (Ottoson, 1956) was recorded with an Ag/AgCl electrode bridged to the epithelial surface with a Ringer-gelatin micropipette of tip diameter 20–40 μm. Receptor cell activity was recorded with a platinum-black, metal-filled microelectrode (Gesteland et al., 1963; Getchell, 1973). A chlorided, silver wire was inserted into a small cut in the dorsal skin and functioned as the preparation ground. The EOG electrode was always placed sufficiently caudal to the microelectrode in order to minimize perturbations to mucus flow near the receptor cell in question.

The metal microelectrode was lowered along an angle of at least 45° from perpendicular to the epithelial surface. This relatively large angle of entry minimized perturbations to the receptive surface of the cell being investigated. Upon touching the mucus, baseline noise of well-functioning electrodes would decrease from ~40 to 20 μV or less. As the probe was advanced at speeds ranging from 1-5 μm/min through the mucus, through the cell body layer, and subsequently through the basement membrane, spontaneously active units were encountered. Periodically, test stimuli were delivered in order to aid in locating those units with low or no spontaneous firing rates. When stable single-unit activity could be recorded for at least a 10-min period, odor stimulation was begun. The electrode position was readjusted whenever necessary to optimize signal-to-noise ratio.

Single Unit Criteria

Single unit recordings from individual olfactory receptors are a relatively direct measure of output of these transducers. In order to extract meaningful information, it is essential that impulse activity is minimally perturbed by surgery, by olfactory stimulation, and by microelectrode penetration of the epithelium. To these ends, criteria were developed to ensure (*a*) that the unit in question was truly a single unit and not a measure of multiple receptor cell activity; (*b*) that voltage wave forms of individual impulses were consistent with the existing experimental and theoretical models; (*c*) that the spontaneous rate of impulse generation in the absence of odor, and the impulse activity in response to odor stimulation were within normal physiological bounds; and (*d*) that these responses were reproducible.

Three criteria were used to ascertain whether the electrical activity was truly that of a

single receptor. First, if one or more units were being recorded, small movements of the microelectrode often resulted in differential variations in single-to-noise ratios. Second, if two spikes with nearly equal magnitudes were separated in time by approximately less than the relative refractory period of spike generation (Katz, 1966), it was considered unlikely that this was unitary activity (see Fig. 5). Third, differences in impulse wave shape distinguished impulses emanating from two or more cells.

Stimulus Selection

Seven chemically stable compounds, grouped into four pairs, were used as olfactory stimuli. The odorant pairs were (1) propanol (PROP) and butanol (BUT), (2) methyl butyrate (MNB) and ethyl butyrate (ENB), (3) benzaldehyde (BZA) and nitrobenzene (NB), and (4) benzaldehyde (BZA) and acetophenone (ACP) (see Table I).

TABLE I

PHYSICAL PARAMETERS OF THE ODORANTS

Compound	Structure	Molecular weight	Saturated vapor at $20°$ C
			mm Hg
Butanol	C—C—C—C—OH	74.1	39.0
Propanol	C—C—C—OH	60.1	100.6
Methyl butyrate	C—C—C—$\overset{\overset{O}{\|\|}}{C}$—O—C	102.1	154.0
Ethyl butyrate	C—C—C—$\overset{\overset{O}{\|\|}}{C}$—O—C—C	116.2	84.3
Benzaldehyde	*b—$\overset{\overset{O}{\|\|}}{C}$—H	106.1	5.44
Acetophenone	*b—$\overset{\overset{O}{\|\|}}{C}$—CH$_3$	120.2	2.48
Nitrobenzene	*b—$\overset{\overset{O}{\|\|}}{N}$=O	123.1	1.57

* b is the benzene base.

Both members of each odorant pair were chosen so that responses at the receptor level would be similar. Two selection techniques were used. First, a number of a priori criteria including molecular shape, chemical structure, and range of vapor pressures were considered to be determinants of response. Second, results from previous electrophysiological and psychophysical experiments (e.g., Stuiver, 1958; Daval et al., 1972; Holley et al., 1974; and Getchell and Getchell, 1975) were used as a guide in the selection process.

Stimulation Methods

Whenever a single unit was isolated for at least 10 min, all seven odorants were successively delivered by hand-held puff bottles in order to determine which evoked responses. Those substances which changed the receptor firing rate were then delivered by the controlled route, the olfactometer. Successive odorant presentations were separated in time by a minimum of 90 s.

The constant-flow, air-dilution olfactometer permitted stimulation with pulses of

odors of chosen concentration, duration, purity, and flow rate (see Fig. 1 for schematic diagram and explanation of operation).

The valve system was designed to permit delivery of a pulse of odorant of variable or of standard duration without a concomitant change in flow rate at the mucosa. It was operable in one of two configurations (Fig. 2). Mode 1 was designed so that either a pulse of standard length, a pulse of variable length, or two sequential standard pulses of
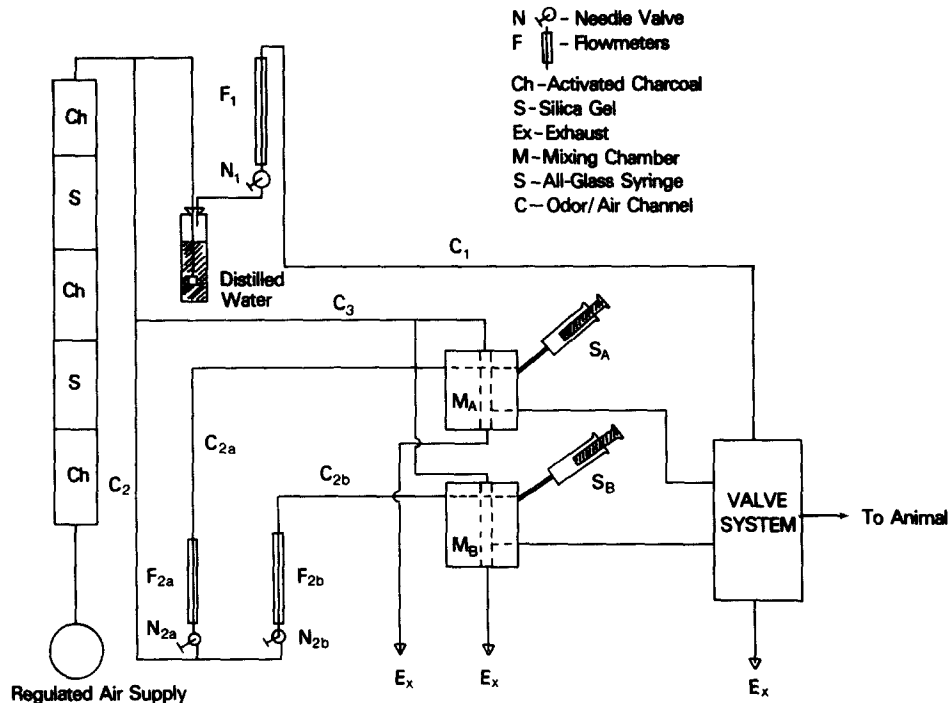

FIGURE 1.   Constant-flow air-dilution olfactometer. The air stream from a pressure-regulated source is dehumidified, cleaned, and split into three streams, C_1, C_2, and C_3. C_3, closed during normal use is used exclusively for flushing out the mixing chambers, M_A and M_B, when changing odorant syringes, S_A and S_B. Saturated odorant vapors from S_A and S_B, driven simultaneously by a Sage model 355 syringe pump (Sage Instruments Div., Orion Research, Inc., Cambridge, Mass.), are mixed with the clear air from C_{2a} and C_{2b} in the mixing chambers, M_A and M_B. The fractional concentration relative to the saturated vapor pressure is $f_s/(f_{2a,b} + f_s)$. Flows are adjusted to minimize pressure transients when switching valves, $f_{2a} = f_{2b}$ and $f_1 = f_{2a,b} + f_s$. Setting $f_1 = 40$ ml/min allowed delivery of concentrations ranging from 2.25×10^{-4} to 0.5 relative to the saturated vapor.

odorants separated by a variable time delay could be delivered. Mode 2 was constructed so that either variable length pulses or juxtaposed variable length odorous pulses could be delivered. (Small all-Teflon, four-port Hamilton valves (Hamilton Industries, Two Rivers, Wis.) were used to facilitate rapid switching and, hence, to minimize any transients in the flow of air or odorant bathing the mucosa.) Only those valve operations resulting in single odorous pulses are described here. Sequential pulses are used in the experimental paradigm in the following paper (Baylin and Moulton, 1979).

Mode 1 Operation

Air Input → Bypass loop   Air/Odor Output

Mode 2 Operation

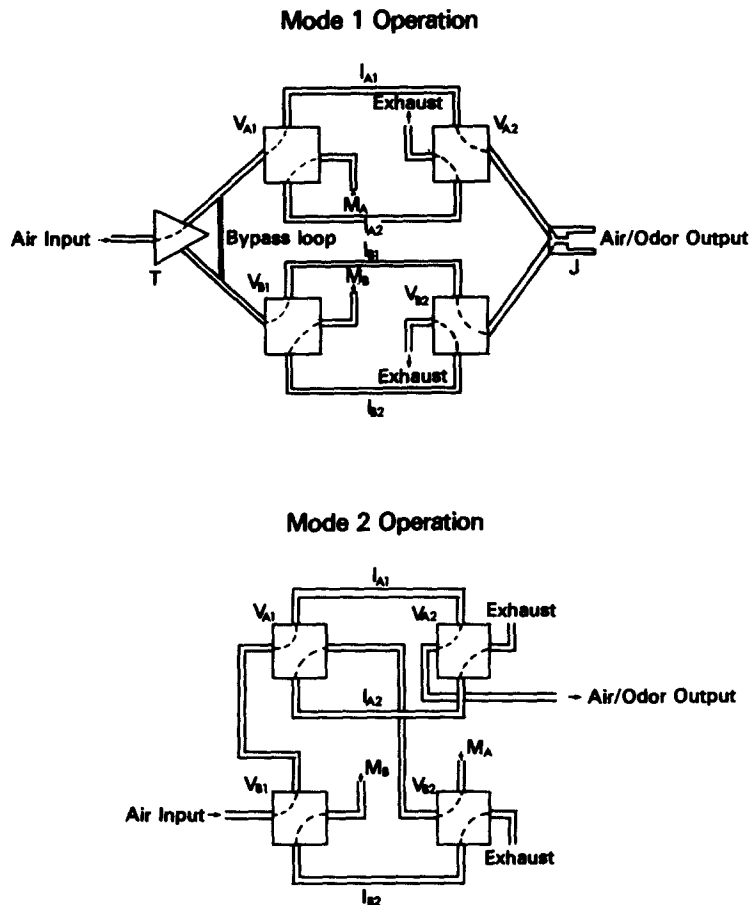Air Input →   Air/Odor Output

FIGURE 2.   Olfactometer valve systems. Expansion of abbreviations: I, Teflon tubing loop; V, four-port two-way valve; M, mixing chamber outputs; T, two-way distribution valve; J, output port. Mode 1. Switching V_{A2} directs odor from M_A onto the mucosa. Simultaneously switching V_{A1} and V_{A2} delivers a standard pulse, the contents of loop l_{A2}. Reswitching both valves stimulates with the contents of loop l_{A1}. Volume of l_{A1} = volume of l_{A2} The bypass loop is high resistance pathway which creates a small forward pressure at J to minimize any backwards leakage of odorant into the nonoperating loops. Mode 2. Switching V_{A2}, similarly, delivers a continuous pulse from M_A. Switching V_{B2} readies the system so that a continuous pulse of M_B is delivered when switching V_{A2}.

The olfactory epithelium was bathed with clean humidified air at a flow rate of 40 ml/min delivered by a 2-mm i.d. Teflon nozzle held at about 4 mm directly above the area of the mucosa containing the cell under study. This stream was gentle enough to cause no pressure distortion of the mucosa, but rapid enough to deliver an odorous pulse with a concentration rise time shorter than the minimal EOG rise time. Unavoidable mixing occurs at the leading and trailing edges of an odorant pulse, if the flow rate of the carrier stream is too low, or if the distance the pulse must travel before reaching the mucosa is too long. In order to avoid delivering pulses with an overly rounded wave shape, the valve system was placed as close as possible to the exposed epithelium.

Chromatographic Analysis of Olfactometer Output

To monitor odorant pulse waveshapes for the two modes of olfactometer configuration and to calibrate odorant concentrations, the olfactometer output was directly connected into the base of the flame ionization detector (FID). The detector had been removed from the body of a Varian 1520 gas chromatograph (Varian Associates, Palo Alto, Calif.) and installed close to the output valves.

The wave shapes of pulses from the mode 1 and 2 olfactometers are shown in Fig. 3 *a* and *b*, respectively. The FID, heated as in normal operation, was installed at the same distance from the olfactometer output as was the epithelial tissue. Thus, odorant pulse wave shapes at the surface of the epithelium are considered to be reasonably represented by these measurements.
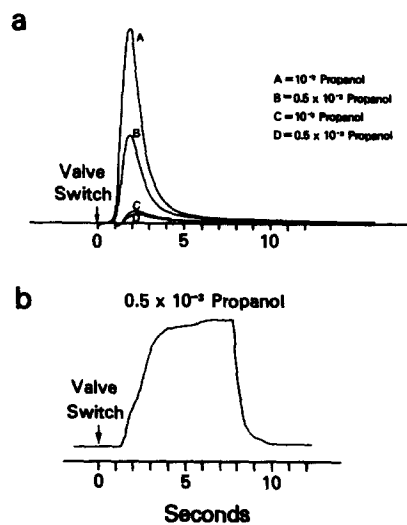

FIGURE 3.   Flame ionization detector records of olfactometer output. (*a*) Variable concentration pulses delivered by the mode 1 olfactometer. (*b*) 10-s stimulation delivered by mode 2 olfactometer.

When changing odorants by introducing a new syringe, the concentration of the diluted odorant in the mixing chambers reached equilibrium rather slowly. When standard stimulations were delivered at 10-sec intervals after the start of the syringe run, a plateau in the concentration was attained in roughly 2 min. Therefore, when changing odorants, at least 2 min were allowed for equilibration.

Concentration calibration curves for both modes of olfactometer function were constructed by plotting the averaged areas under the FID curves vs. the calculated concentration (Fig. 4). These curves are normalized to the 10^{-2} point, i.e., ordinal and abscissal values coincide at this point.

RESULTS

Types of Spikes

A total of 100 units was recorded from various locations on the ventral olfactory epithelium of the tiger salamander for durations ranging from 10 min to 3 h. In some animals, vigorous reproducible responses to odors were recorded up to 20

h after the initial surgery. 44 of these 100 units were nonresponsive to all seven odorants tested and to odorous air drawn from the salamander aquarium. A total of 677 stimulations, including all repetitions, was applied via the olfactometer to the 56 responsive units. On two separate occasions, two units were recorded simultaneously and analyzed.

Most unitary spikes had either diphasic (+,−) or triphasic wave forms (Fig. 5). Getchell (1973) correlated these two voltage configurations with recordings from dendrites and axons of receptor cells, respectively. In these experiments, little or no activity was observed at a depth of less than 20–30 μm from the surface of the mucus. As the microelectrode penetrated deeper into the epithelium, a region of diphasic spikes was first encountered. At deeper levels a
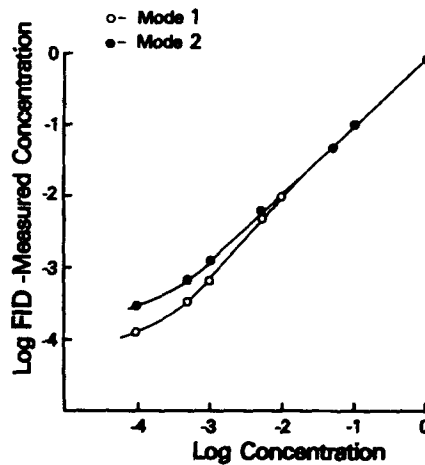

FIGURE 4.   Flame ionization detector calibration of olfactometer concentrations.

gradual transition into a region of only triphasic spikes occurred. Often, both types of impulses were observed simultaneously. No diphasic spikes with a negative/positive transient sequence, as have been reported by Getchell (1973) in the frog, were observed. Occasionally, diphasic or triphasic spikes gradually developed an additional phase (see Fig. 5 E), a slow negative afterpotential reminiscent of those described by Patton (1965) in C fibers. These afterpotentials may have a similar origin. Perturbations caused by the presence of an electrode within the epithelium may retard the diffusion of K^+, extruded during the action potential, away from the immediate vicinity of the fibers.

Spontaneous Rates

Stability in the electrical activity of a unit was considered to be a major factor in deciding whether or not damage had occurred. All units included in the data sample were observed for at least 10 min. Units whose spontaneous rate (SR) was altered with electrode movement were always discarded; units with high and regular impulse frequencies or whose spontaneous rates altered with passage of time were usually discarded. Although this procedure may have
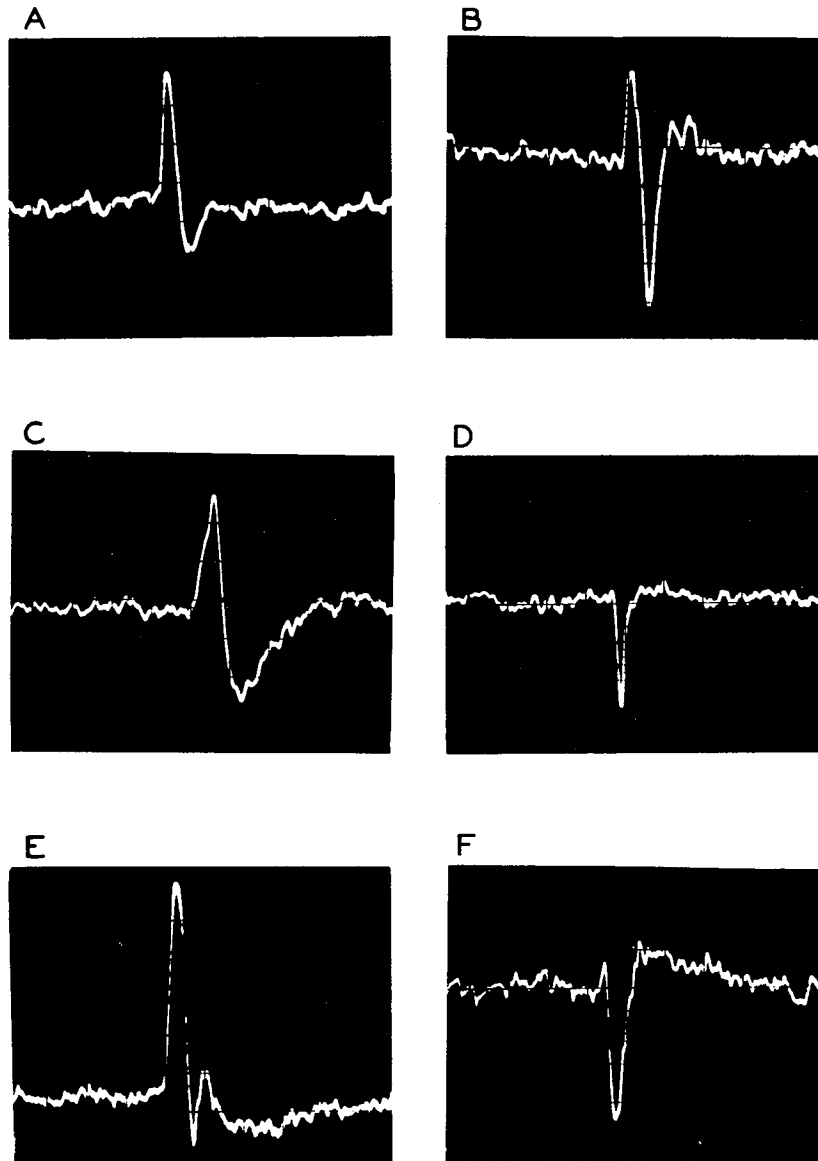
FIGURE 5.   Spike wave shapes. (A, B, and C) biphasic spikes; (D, E, and F) triphasic spikes. The negative components of the unit depicted in (E), which was recorded from among the filia olfactoria, are small relative to the positive component. Positive is in the upward direction.

slightly biased the sample population, it is justified because rejected units probably were injured or were being unduly perturbed by the microelectrode.

Histograms of the distribution of SRs for only biphasic units, for only triphasic units, and for all units were constructed (Fig. 6). A Kolmogorov-Smirnov test indicated that these distributions were non-Poisson. No statistically

significant differences between the distribution of SRs of biphasic and triphasic units could be detected using a nonparametric, two-tailed, two-sample Kolmogorov-Smirnov test. These SRs have an overall average, calculated from 100 units, of 39.1 impulses per minute. These range from one unit which had nearly no spontaneous activity to one with a SR of 166. 56% of all receptors observed had spontaneous rates of less than 50 impulses per minute. These results are consistent with those of other studies: Gesteland et al. (1965) and O'Connell and Mozell (1969) found average spontaneous rates of 3.5 and 60 impulses per minute, respectively, in the frog; Getchell (1974) and Holley et al. (1974) reported that 68 and 70% of all units sampled, also in the frog, had spontaneous rates of less than 25 and 20 spikes per minute, respectively. The distribution was skewed in favor of units with lower SRs. 56% of all the neurons sampled had SRs of less than 50 impulses per minute.
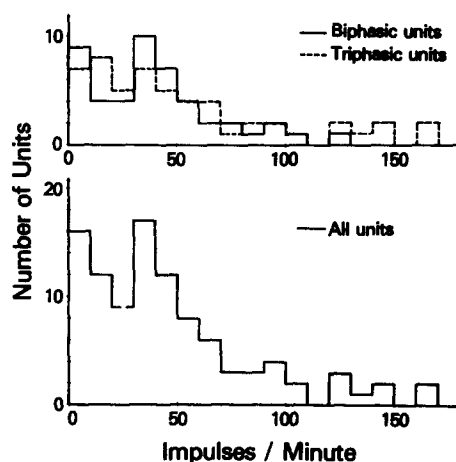

FIGURE 6.   Distribution of spontaneous rates of the sampled receptors.

Selectivity of Response

Each unit was stimulated with at least two and occasionally all seven odorants. In general, it was clear whenever a response was evoked. If a cell was not responsive to any concentration of a given odorant the event was classified as a null response. This criterion was somewhat subjective because the difficulty inherent in isolating and recording stable unitary activity usually did not allow sufficient time to deliver a complete concentration series for each odorant. However, a unit which did not respond to either a small or a large puff of odorant from hand-held puff bottles and was therefore judged to be nonresponsive, was never observed to respond to any concentration delivered via the olfactometer. As a control, pulses of pure air drawn from the olfactometer line were delivered to some receptors. In no cases were either EOGs or impulses generated.

Occasionally, wide variations in receptor responsivity to a given odorant were observed. For example, whereas 10^{-4} butanol evoked a vigorous response in two simultaneously recorded units (Fig. 7 G), 10^{-3} butanol barely stimulates another

cell (Fig. 7 A–D). Fig. 11 A and B demonstrates a case where stimulation by MNB at two concentrations differing by a factor of 200 evoked nearly the same response in two different units.

The cross-cell responsiveness to the odorant pairs is shown in Table II. Both members of the PROP/BUT odorant pair effectively stimulated 29% of the 65 receptors sampled. Viewed from this perspective this pair had the most closely
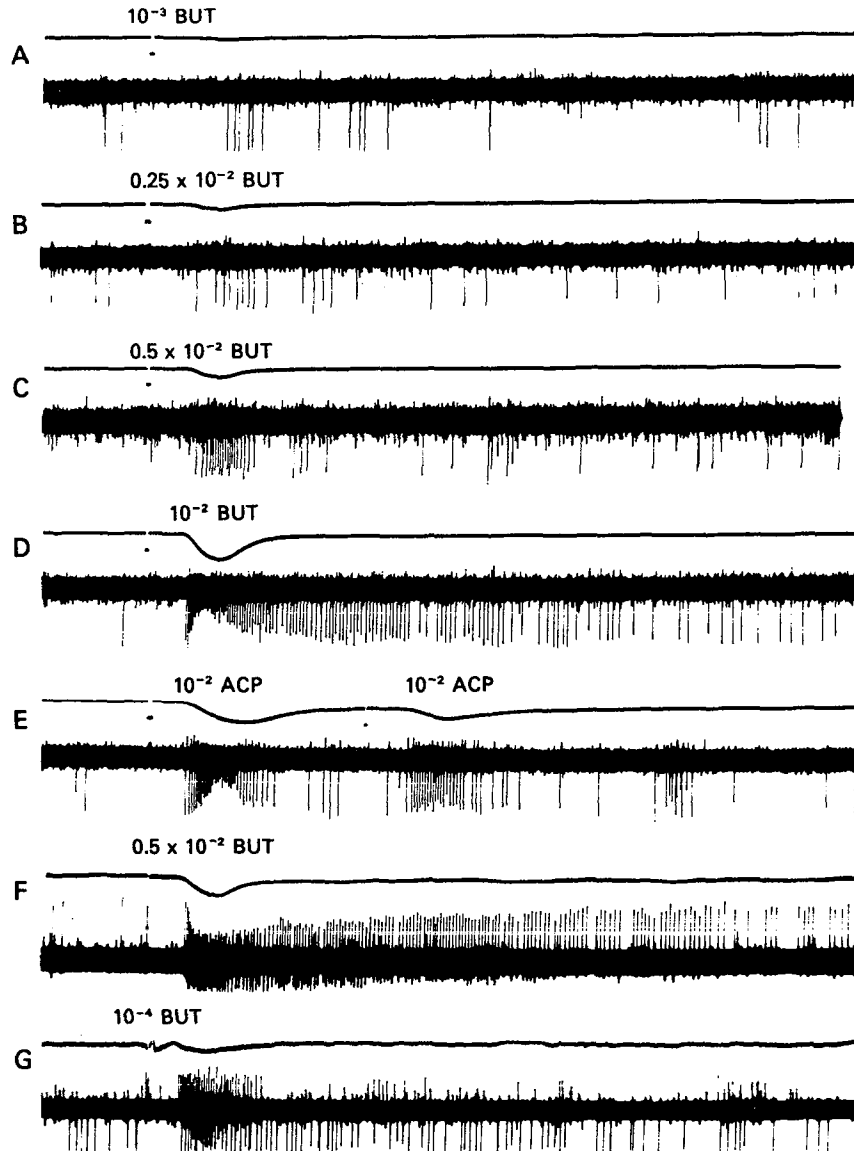

FIGURE 7.   Unitary responses. The top trace is the EOG; the bottom is the spike data. (A, B, C, and D) Unit 12A-Tri-SR60; (E) unit 16A-Tri-SR36; (F) unit 7-Bi-SR88; (G) units 15P-Tri-SR72 and 15Q-Bi-SR24.

matched odorants. In general, if a receptor responded to one member of a pair, it was probable that it would respond to the second member. However, receptors which responded to only one of the odorant pairs were often found. 26% of the 68 cells sampled with the MNB/ENB pair responded to either ENB or MNB, but not to both.

Reproducibility of Response

One must ensure that each response of a given olfactory receptor is independent of all preceding ones; i.e., that a nonspecific fatigue, a general decrease in sensitivity of the receptor caused by excessive stimulation with any odor, does not occur. Therefore, as often as was possible (see Fig. 1 in Baylin and Moulton, 1979), two or more repetitions of a given concentration of a given odorant were delivered. The responses were reproducible as judged by both a visual inspec-

TABLE II

RECEPTOR RESPONSIVENESS TO ODORANT PAIRS

	Both A and B	A, not B	B, not A	Neither A nor B	Total units
					n
A = MNB B = ENB	15 (22%)	9 (13%)	2 (3%)	42 (62%)	68
A = PROP B = BUT	19 (29%)	4 (6%)	1 (2%)	41 (63%)	65
A = BZA B = NB	3 (7.5%)	3 (7.5%)	0 (0%)	33 (85%)	39
A = BZA B = ACP	3 (8%)	1 (2%)	3 (8%)	32 (82%)	39

Numbers in parentheses are the percentages of units relative to the total number in which the indicated combination of responsiveness is observed.
* Number of receptors which were tested with both members of a given pair.

tion of successive record of response and a t test (which employed, as a measure of response, the total number of spikes evoked corrected for the expected spontaneous rate during the response). The same conclusion held when all the t test probabilities describing responses of different receptors to a given odorant were combined in a X^2 test, i.e., nonspecific fatigue did not occur in the receptor responses to odorants delivered at 90-s intervals.

Responses to Changes in Concentration

Both frequency of impulse generation and total number of spikes evoked increased from threshold to maximal response over one to three log units of concentration (see Fig. 7). Also, responsiveness to a given odorant varied from cell to cell (cf. Fig. 11 A and B and Fig. 7 A and G); the MNB/ENB odorant pair was generally effective from 10^{-4} to 10^{-3} of saturated vapor and the other three pairs from 10^{-3} to 10^{-2}. Oscillatory EOGs (initially observed by Ottoson, 1956) were always evoked, whereas decrementing spikes were often evoked by odorant concentrations greater than 10^{-2} and 10^{-1} for the MNB/ENB and the BUT/PROP pairs, respectively.

Some typical response-concentration functions are plotted in Fig. 8. Curves for units 5C and 9C were derived as follows: corrected odorant concentration,

calculated from the GC calibration of the mode 2 olfactometer, was plotted vs. the total number of spikes evoked during the 10 s the odorants were presented at the epithelial surface. The ordinates for the other two curves are the number of spikes evoked during the standard pulse of BZA. The form of these response-concentration curves is similar to those observed elsewhere (see Figs. 3 and 4 in Holley et al., 1974).

Decrementing Spikes

A reduction of impulse amplitudes of both triphasic and biphasic spikes was often observed during responses to each of the seven odorants. In many cases
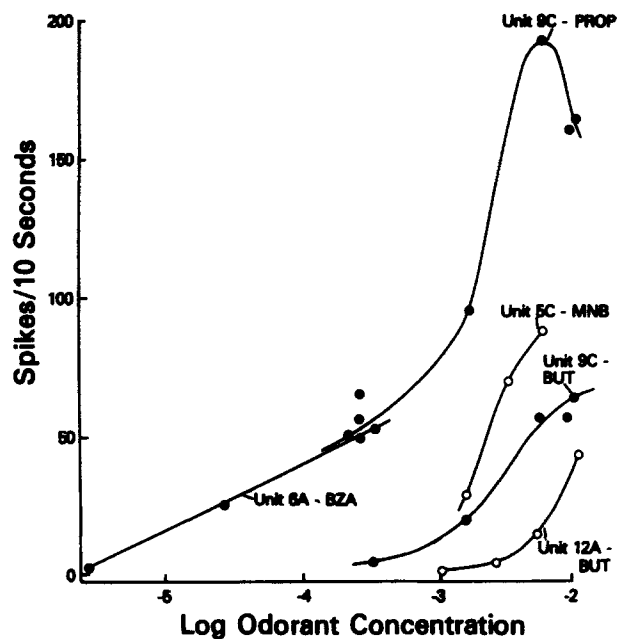

FIGURE 8.   Response-concentration curves. Many of these points represent the averaged response of two or more stimulations.

this activity could be induced by increasing the odorant concentration (Fig. 7 A–D). With two successive presentations of this same odorant, decrementing spikes present in the first response were often absent in the second. This phenomenon is observed in the response to the second puff of ACP in Fig. 7 E. These data suggest that the presence of decrementing spikes may be correlated with the frequency of spike generation.

A shift in spike position relative to background noise was occasionally observed. An example of this phenomenon is seen in the response of a unit to butanol (see Fig. 7 F). This continuous variation in the relative magnitudes of positive to negative spike components may be a reflection of the changing site of spike generation during odorous stimulation. However, as seen by the recording electrode, movement of the animal tissues during stimulation or self-shunting

due to stimulus activation of conductance increase, thus mediating the generator
current, may provide a more parsimonious explanation.

Temporal Patterns of Response

Traditionally, responses to odorous stimulations have been classified as being
inhibitory, excitatory, or null. All unitary data here have been summarized in
the form of peri-stimulus-time (PST) histograms and have not been prejudged
into these three categories. Some examples are presented in Figs. 9, 10, and 11.
These histograms are plots of total number of spikes observed each half-second
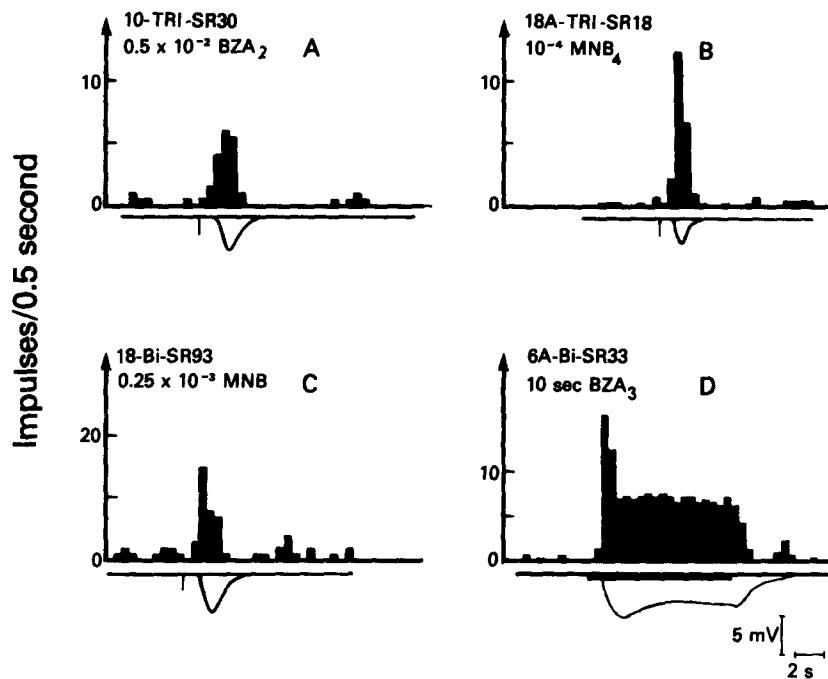

FIGURE 9.   Receptor responses: PST histograms. (A) Unit 10-Tri-SR30; (B) unit
18A-Tri-SR18; (C) unit 18-Bi-SR93; and (D) unit 6A-Bi-SR33.

vs. time. This particular unit of time was chosen as being short enough to show
detail but long enough to minimize noise in the PST histograms.

A number of details should be noted in interpreting these figures. Standard
puffs delivered by the mode 1 olfactometer are indicated by a single valve switch
artifact on the EOG trace. Longer pulses are indicated either by a bar or two-
valve switch artifacts on the DC trace, when using mode 1 and mode 2
stimulation, respectively. The unit number, type (either biphasic or triphasic),
and the spontaneous rate are coded with each histogram (e.g., 16-Tri-SR27).
The suffix on the odorant name indicates the number of stimulations used to
construct the averaged histogram (e.g., MNB_3). Finally, the line below the time
axis on all 12 histograms indicates the time period for which the impulses were
counted and displayed.

In general, the shape of the PST histograms roughly followed that of the EOG (see Figs. 9, 10, and 11) which closely resembled the stimulus concentration wave forms. The impulse frequency was usually higher on the rising phase of the EOG relative to that on the falling phase. Longer stimulations evoked responses with phasic and tonic components whose relative magnitudes varied from cell to cell. Often a given unit exhibited differing phasic/tonic patterns in response to different odorants. Temporal patterns of response were often similar in receptors which were both highly sensitive and insensitive to a given odorant (eg., see Fig. 11 A and B).
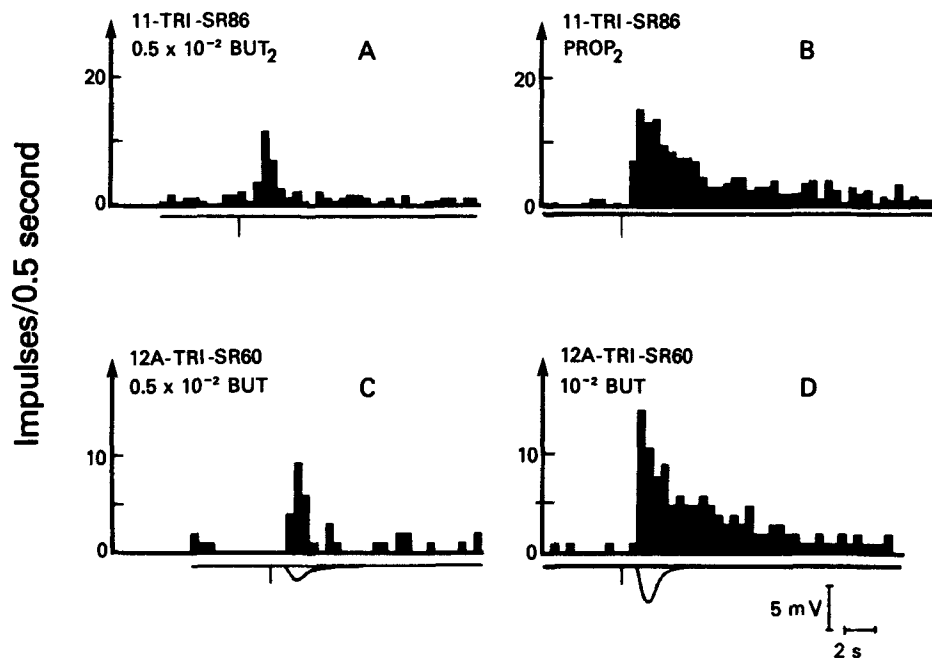

FIGURE 10. Receptor responses: PST histograms. (A and B) Unit 11-Tri-SR86; (C and D) unit 12A-tri-SR60.

Occasionally, response frequency decreased slowly to the spontaneous rate even after the EOG had returned to base line. This behavior is termed a post-stimulus firing. For example, in Fig. 7 G, even though both the diphasic unit and the EOG have returned to the prestimulus state, the triphasic unit was still quite active. In unit 11 (Fig. 10 A and B), response to a puff to butanol was short and predominantly phasic while that to propanol had a relatively long duration (see also Figs. 11 C and 7 F). This maintained activity apparently is related to the degree of activation of a cell; as odorant concentration was increased, the effect was accentuated (see Fig. 7 A–D and Fig. 10 C and D).

A second poststimulus response pattern was also observed. Often, after the initial rapid phasic burst of activity, no impulses were evoked. The identification of this decrease in firing rate as a poststimulus depression was often difficult,

because the prestimulus spontaneous firing rates observed (see Figs. 7 A–C, 9 A–C, and 11 B and D) were generally quite low in comparison.

The nature of the poststimulus firing observed varied with a number of factors. Responses of a given unit to two different odorants as well as responses of two different units to the same odorant occasionally were tentatively identified as both poststimulus firings and depressions. Also, the type of temporal response patterns varied with concentration. For example, as the concentration of butanol is raised in Fig. 7 A–D, the apparent poststimulus depression disappears and a tonic firing is maintained.
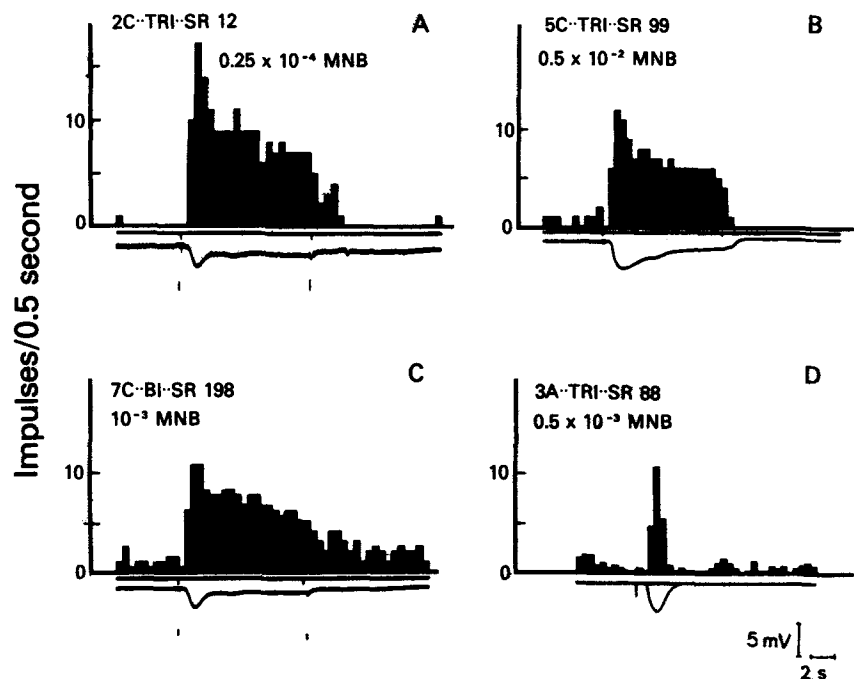

FIGURE 11. Receptor responses: PST histograms. (A) Unit 2C-Tri-SR12; (B) unit 5C-Tri-SR99; (C) unit 7C-Bi-SR198; and (D) unit 3A-Tri-SR88.

DISCUSSION

The purpose of this study has been to provide further information to aid in an understanding of olfactory coding. To accomplish this task one must first ascertain whether unitary activity being recorded is minimally perturbed by the recording technique and thus closely approximates the naturally occurring events. In this respect, the consistency of these data with those observed in other unitary studies (Gesteland et al., 1965; O'Connell and Mozell, 1969; Mathews, 1972; Getchell, 1973, 1974; Holley et al., 1974, Getchell and Getchell, 1975) is encouraging.

In general, the difference between odor-evoked firing frequencies and spontaneous rates was higher than those observed in some of the other unitary

studies. For example, we observed a maximal instantaneous response frequency of 52 impulses per second, but Holley et al. (1974) reported a maximum rate of 30 impulses per second in the frog (during excitation, this rate rarely exceeded 15 spikes per second). One possible explanation may be that the salamander receptors, whose cell body diameters are generally more than twice as large as those in the frog (see Getchell, 1975), may be more effective spike generators.

No discernible difference in types of response, reproducibility of responses, and cross-unit distribution of spontaneous rates distinguished diphasic from triphasic units. If, as is assumed here, receptors behave as functionally independent units, these results suggest that triphasic recordings are from individual axons, not from groups of axons acting in concert.

Also, these results suggest that the relatively large Pt-black microelectrodes do not damage the receptors from which activity is being recorded (see also the physiological criteria developed by Getchell, 1973). Similarly, the statistical tests, which in all units indicate that the distribution of interspike intervals is probably Poisson, suggests that no perturbation has consistently altered these rates.

The Physiological Stimulus

Stimulation of receptor cells during normal behavior in the salamander is certainly different from the experimental mode of odorant presentation via the olfactometer. However, care may be exercised in choosing the type of stimulation so that normal physiological function of the olfactory organ is approximated.

Stuiver (1958) has calculated that, for humans, only 2% of those molecules entering the nose make contact with the olfactory epithelium. Although the structure of and the access to the salamander nasal cavity is much less complicated, many molecules may be adsorbed en route to the receptors during a sniff (see also Mozell and Jagodowicz, 1973; Moulton, 1976). Thus, odorant concentrations normally encountered by the salamander may be reduced before odorant molecules contact the epithelium. Furthermore, odorant concentrations encountered in nature are most often low. Therefore, those odorant concentrations which evoke decrementing spikes may seldom, if ever, be attained in the majority of receptors. Consequently, odorant concentrations were chosen to be below that level which caused decrements except in those cases when a complete concentration series was presented.

Decrementing Spikes

Decrementing spikes have been observed in the olfactory bulb (Kauer, 1974; Meredith, 1974) and the olfactory epithelium (Shibuya and Tucker, 1967 [Getchell, 1975, has observed decrementing spikes in a very few instances]) using electrolyte-filled glass micropipettes, and in the olfactory epithelium using the Pt-black microelectrode (see Fig. 2 in Mathews, 1972). These considerations and the fact that both diphasic and triphasic spikes show decrements suggest that this phenomenon might not be artifactual and may actually reflect variations in temporal patterns of impulses relayed to higher olfactory centers.

Others have reached different conclusions. It has been suggested that the location of impulse generation may simply vary with firing frequency. However,

it is conceivable that, if this were the case, one could observe incrementing spikes when this locus occasionally would move closer to the recording electrode during a response.

Holley et al. (1974) have concluded that "this phenomenon probably represented a trite case in small fibers and could be tentatively ascribed to the ionic variations taking place in extracellular and intracellular spaces that were too restricted." However, one may still envisage situations where some of the impulses were completely blocked.

The reductions in amplitude were sufficiently large to merge the impulses into the background noise in only a few of the olfactory receptors in the salamander. In the frog (Holley et al., 1974; Sigwart and Gesteland, 1975), such a reduction occurs more frequently. As suggested earlier, this difference may perhaps be a reflection of differences in receptor cell size or spike generation capacity between these two species.

Selectivity of Response

Can we define the specificity and types of responses of receptors to a particular odorant in terms of certain physiochemical parameters (e.g., molecular weight, lipid solubility) of this odorant? None of the seven odorants had better than, at most, a 35% chance of stimulating a given receptor (see Table II). In many cases, a given odorant evoked a response in only one of two closely spaced receptors. Assuming that the odorant accessibility to these receptors is not vastly different, we conclude that whether or not a receptor responds to a particular odorant is determined by an intrinsic property of the cell in question. For all four odorant pairs, receptors which were responsive to either member, but not to both, were observed. Therefore, it appears that the types of response are also determined largely by intrinsic structural features of the receptors, i.e., receptor molecules.

We have occasionally observed differences in responsiveness during odorous stimulation to be a large as a factor of 200 (e.g., compare Fig. 11 A and B). One might possibly attempt to account for the cross-cell variability of sensitivity to a given odorant on the basis of factors such as the overall site accessibility, as determined by the details of the cellular geometry, or the "penetrability" of an odorant through the mucus to these sites, or both. However, there is no experimental evidence to suggest that such large cell-to-cell differences in sensitivity could be a result of these accessibility factors. As in other vertebrates, olfactory receptors in the tiger salamander are of a relatively homogeneous size. They are also rather uniformly distributed throughout the epithelium (Getchell, 1975; Graziadei and Graziadei, 1976). Thus, no apparent morphological differences would seem to account for these variations in sensitivity. One reasonable interpretation would be that there are some intrinsic differences in the receptive membranes.

Temporal Patterns

The time-course of an olfactory stimulation cannot be controlled with nearly the same precision as that of visual or auditory stimuli. However, even if a square pulse of odorant could be delivered to the mucosal surface (see Kauer and

Shepherd, 1975), cell-to-cell differences in geometrical location and accessibility of the receptive sites on each receptor cell, and varying diffusion times of individual odorants through the mucus to these sites (Bostock, 1974) would permit only a gradual buildup of odorant at each sites. In spite of these limitations, an investigation of the general features of the temporal patterns of response is warranted.

The phasic/tonic patterns of response evoked by tonic stimulations suggest that the rate of odorant binding, which is higher at the beginning of a pulse when few sites are occupied, may be an important parameter in the generation of current in the receptors (Heck and Erickson, 1973). Other possible mechanisms, such as a cooperative interaction among receptors, may be invoked and should not be overlooked. In addition, the PST histograms illustrate the variability of these patterns in response to a given odorant across the receptor population. In a single unit, two different odorants apparently can evoke two dissimilar poststimulus firing patterns. Also, a given odorant may sometimes evoke either a maintained poststimulus firing or a poststimulus depression in two different receptors. The poststimulus inactivation process possibly occurs in only a portion of the subset of receptive sites with which a given odorant can interact. This hypothesis may then account for the variability observed in these temporal patterns.

Similarities in the temporal patterns of response of receptors, which are either relatively sensitive or insensitive to a given odorant, may possibly be accounted for by cross-cell variations in numbers of receptive sites. However, whether or not a given concentration of a given odorant evokes a maintained poststimulus firing may or may not be a reflection of the time-course of odorant-receptive site interaction alone. When a sufficient number of odorant molecules are dissolved in the mucus, a current may be generated in the more sensitive receptors by the residual odorant which is sufficiently intense to cause this long-lasting firing.

Kauer (1973, 1974) and Kauer and Moulton (1974) have observed rather complex temporal patterns in olfactory responses of cells in the tiger salamander olfactory bulb. However, temporal sequences of impulses from primary receptors are, by comparison, simple. The temporal sequences in secondary neurons therefore likely arise as a result of bulbar synaptic interactions.

The electrical recordings presented here are assumed to be from single olfactory receptors. An analogy with some observations in visual research indicates that caution may be necessary in interpreting these results. For example, Owen and Copenhagen (1975) have conclusively demonstrated that functional connections exist between turtle photoreceptors, a result not necessarily apparent from an analysis of single unit intracellular responses. Although there is no convincing evidence for electronic coupling between olfactory receptors—tight junctions but not gap junctions have been observed (de Lorenzo, 1970; Graziadei, 1971; Altner, 1975), speculations about the numbers and types of hypothesized receptive sites would have to be viewed with caution should functional interactions be shown to occur.

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