Patterned Response to Odor in Single Neurones of Goldfish Olfactory Bulb: Influence of Odor Quality and Other Stimulus Parameters

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ABSTRACT Responses of 75 single units in the goldfish olfactory bulb were analyzed in detail for their relationship to the time-course of the change in odor concentration during each odor stimulus. Odor stimuli were controlled for rise time, duration, and peak concentration by an apparatus developed for the purpose. This apparatus enabled aqueous odor stimuli to be interposed into a constant water stream without changes in flow rate. The time-course of the concentration change within the olfactory sac was inferred from conductivity measurements at the incurrent and excurrent nostrils. Temporal patterns of firing rate elicited by stimuli with relatively slow rising and falling phases could be quite complex combinations of excitation and suppression. Different temporal patterns were produced by different substances at a single concentration in most units. Statistical measures of the temporal pattern of response for a small number of cells at a given concentration were more characteristic of the stimulus substance than any of three measures of magnitude of response. The temporal patterns change when the peak concentration, duration, and rise time of the stimuli are varied. The nature of these changes suggests that the different patterns are due primarily to the combined influence of two factors: (a) a stimulus whose concentration varies over time and (b) a relationship between concentration and impulse frequency which varies from unit to unit. Some units produce patterns suggestive of influence by neural events of long time constant. The importance of temporal patterns in odor quality and odor intensity coding is discussed.

INTRODUCTION

The relationship between the time-course of an odor stimulus and the time-course of neural response in the olfactory bulb has not been thoroughly investigated, often because the time-course of the stimulus was not well defined. Some previous reports of odor response in the bulb have been simple descriptions of responses and response types (Mancia et al., 1962; Mathews, 1972b; Nanba et al., 1966; Döving, 1966a; Zippel and Breipohl, 1975). Other workers have addressed more complex problems by using as a tool quantitative measurements of the similarity of responses, recorded electrophysiologically (Döving, 1966b, 1966c, 1974; Higashino et al., 1969; Mathews, 1972a; Pfaff and Gregory,
Many investigators have observed temporal patterns of response to odors, and some have thought them to be characteristic of the odor quality (Døving, 1966a; Nanba et al., 1966). In assessing similarity of response, however, complex patterned responses, which include both enhancement and suppression of ongoing activity, have generally been categorized as excitatory, inhibitory, or zero (+, −, 0), or have been expressed as an average firing rate for a single fixed period. Several authors have commented that these methods may not adequately represent all responses (Døving, 1966b; Higashino et al., 1969). They have been used, however, because they allow response similarity to be analyzed by standard statistical methods.

There have been some reports in which the relation between stimulus time-course and response has been more carefully defined. Macrides and Chorover (1972), for example, found discriminable patterns of neural activity related to the phase of their cyclic odor delivery. Døving (1964) and Kauer (1974) describe the relationship of neural activity to the time-course of the electro-olfactogram. Kauer, in addition, proposed that the time-course of the change in stimulus concentration could be a factor in producing some of the temporal patterns seen in the salamander olfactory bulb. None of these authors, however, make any quantitative measurements of response similarity.

This paper has two purposes. Firstly, it attempts to relate the details of each odor response to parameters of the stimulus other than odor quality, such as concentration, duration, and rise time, in order to assess the contribution of these parameters to the genesis of temporal patterns. Secondly, a measure of response is developed which emphasizes the entire response and especially its temporal pattern. This response measure, which has been found to be suitable for use in the statistical analysis of response similarity, is compared with other response measures.

The fine control of stimulus parameters required for these analyses was achieved by the use of a stimulator which delivered aqueous stimuli of calibrated and reproducible rise time, duration, and concentration. Compared to airborne stimulation in air breathing vertebrates, aqueous stimulation in fish is less likely to be affected by sorption from the flowing stream of odorous material. For most aqueous stimuli, partition coefficients between water and the mucus lining the olfactory chamber will be closer to one than would be the case for partition between air and mucus for airborne stimuli. The sorptive effect can greatly influence the distribution (Mozell, 1964; Hornung et al., 1975) and time-course (Tucker, 1963; Mozell and Jagadowicz, 1974) of odors arriving at the receptor surface through the nasal passages. Amino acids were selected as stimuli to allow a more meaningful analysis of response similarity than would be possible using odorous chemicals selected at random. They provide a variety of molecular arrangements within a basic chemical structural plan and probably have some biological significance for fish (Idler et al., 1956; Hashimoto et al., 1968).

Goldfish have advantages over many other species in that: (a) natural delivery of odor to the olfactory epithelium is by a continuous nonreciprocal flow which is simple to duplicate; (b) this unidirectional flow through separate incumbent and excurrent nostrils allows the time-course of the stimulus to be measured.
after its passage through the olfactory sac, as well as before it enters the
incurrent nostril; and (c) the olfactory bulbs are connected to the forebrain by
long stalks which can be cut to eliminate centrifugal influences on bulbar activity
(Moulton and Tucker, 1964; Doving, 1966d).

The control and monitoring of stimulus parameters is complemented here by
a more rigorous definition of the response itself. Firing rate patterns during
stimulation were averaged over several repetitions, and each putative response
was tested to ensure that it was, in fact, a statistically significant change from the
pattern of activity before stimulation.

The experiments reported here are also part of an effort to assess similarity
of response in single neurones to odors at more than one concentration. This
paper describes the response types encountered and their relationship to certain
stimulus parameters. The similarities of response to different substances are
described elsewhere (Meredith, 1974, and in preparation).

METHODS

Animal Preparation

3-6-in goldfish *Carassius auratus* were kept in spring water at room temperature
(~21°C). All experiments were conducted at this same temperature. The animals were
immobilized by i.p. injection of 2.5 mg/kg tubocurarine HCl, or 0.5 mg/kg succinyl
choline. Animals were clamped in a tank and respirated with recirculated, aerated water.
Aerated, bottled spring water was passed into one incurrent nostril at 4 ml/min via a
Teflon tube (Teflon, E. I. DuPont de Nemours & Co., Wilmington, Del.).

The cranial cavity was opened and the olfactory bulb carefully exposed. The olfactory
stalk (crus) containing the ascending and descending olfactory tracts was cut on the side
to be stimulated (see Fig. 1). Care was taken to avoid damage to the large vein running
along the wall of the crus which drains the olfactory bulb. The bulbs and crura were then
covered with mineral oil.

Stimuli

The stimuli were seven high-purity amino acids at 10^-2, 10^-4, and 10^-6 M concentration
in commercial bottled spring water. These were stored between experiments at ~ 4°C in
Teflon-capped glass flasks and allowed to warm to room temperature before use. The
substances used were glycine, L-alanine, L-phenylalanine, L-serine, β-alanine, taurine,
and L-arginine, all of which except L-arginine have near neutral pH. Two additional
stimuli were used: (a) arginine HCl produced by titrating arginine solutions with HCl to
the same pH as spring water (pH 7.3); and (b) water from the tank in which the animals
were kept. Although initially spring water, this tank water must have contained
numerous odorous chemicals when used as a stimulus.

Stimulus Delivery

Stimuli were controlled for flow rate, duration, for one of two rise times, and for
concentration and pH. Stimuli were delivered via the apparatus shown in Fig. 2 which
was constructed from Teflon tubing (2 mm ID) and valves made of Teflon and KEL-F
(3M Company, St. Paul, Minn.). A constant flow of spring water passed from the
reservoir through the carrier loop to a 1-mm ID delivery tube in the animal's incurrent
nostril. Without disturbing the carrier flow, the stimulus loop could be filled with
solutions transferred from storage bottles in 20-ml all glass syringes (stimulus solutions
thus contacted only Teflon and glass until they reached the nostril). During stimulation the carrier flow was redirected through the stimulus loop by rotating valves 1 and 3 through 90°. This flushes the stimulus down the delivery tube to the animal. The flow rate through stimulus and carrier loops was equalized at the start of the experiment by adjusting valve 2. Stimulation was terminated by returning the carrier flow to the carrier loop. The stimulus loop could then be refilled from the same syringe or flushed out and filled with a new solution. Stimuli were routinely presented three times (sometimes more.

Figure 1. Animal preparation. The hatched area of the figure is diagrammatically cut away for clarity. The animal is clamped to a frame in a tank on the recording table. Water for respiration flows through the mouth and over the gills to fill the tank up to the level of the pupil. The back of the animal is covered with wet paper towel. Stimulus carrier flow passes into the incurrent nostril (shown in section) and out of the excurrent nostril (not shown). The flow path through one of the interlamellar grooves is indicated by an arrow. The nasal bones are dissected away to expose the olfactory bulbs and the olfactory stalk is cut on the stimulated side.

mean number 4.4) at 2-min intervals forming a block of stimulus presentations. Stimulus blocks were also repeated periodically to check the reproducibility of the response. (Second and subsequent blocks were often limited to two stimulus presentations, unless the response pattern deviated markedly from that observed in the first block).

Stimuli are identified in the text by their “nominal concentration” and “nominal duration” (i.e., the concentration of the standard solution used and the duration of flow through the stimulus loop). The stimulus substance did not reach the nominal concentration instantaneously, however, and remained at above threshold concentration for considerably longer than the nominal duration (see Results). It is important to distinguish between the change in concentration which occurs during a single stimulation at one
nominal concentration and the change to a new nominal concentration, both of which could be referred to as "a change in concentration."

The insertion of an additional valve (3A) and a 10-cm length of larger diameter (4 mm ID) Teflon tubing allowed stimuli with slower rise times to be given (insert Fig. 2). The

Figure 2. Stimulus control apparatus. Continuous flow through the nasal sac is maintained at all times. Between stimulations, spring water passes from the reservoir through the carrier loop to the animal. (A) The stimulus loop can be filled from a syringe without interrupting the flow to the animal. (B) During stimulation the carrier flow is redirected through the stimulus loop, flushing the stimulus solution down the delivery tube to the animal. Inset at top: rise time control. Insertion of a length of larger diameter tubing filled with water ("mixing tube" shown in insert) results in a slower rate of rise of stimulus concentration. The length of the mixing tube is not drawn to scale.

stimulus loop was filled in the normal manner, but the added mixing tube was flushed out with water via valve 3A before stimulation. Turbulent mixing of the leading edge of the stimulus pulse with water in the mixing tube produced the slower rise time.

Measurement of Stimulus Time-Course

The time-courses of the stimuli entering and leaving the olfactory sac were measured by using stainless steel conductivity electrodes in the tip of the delivery tube and in the
excurrent nostril. Conductivity measurements were not made during the recording of odor responses to avoid direct electrical stimulation by any current leakage. To correct for nonlinearities, the output voltage produced for each concentration level was determined by passing long pulses of known concentrations through the system. The output for each stimulus could thus be plotted as a true concentration-time profile from which duration, rise time, and instantaneous concentration could be derived (see Results and Fig. 3).

Recording Methods and Data Analysis
Conventional AC single unit recording methods were used with glass micropipettes of 5-25 MΩ filled with 3 M NaCl. Unit activity was filmed with a continuous recording camera during playback from a magnetic tape recorder and FM adaptor. Spikes were counted from film run at 10 mm/s (i.e., twice the film speed shown in the records of Fig. 5). A second oscilloscope trace, triggered by each spike and run at a sweep speed much faster than the film speed (~3.2 mm/ms on the film) was used routinely to display spike shape on the filmed records. In this way the shape of any spike could be checked while counting.

The spike waveform was one of the criteria used in discriminating single units. Another criterion was that of a minimum interspike interval > 20 ms. This was assessed by superimposing 50 sweeps on a storage oscilloscope, each triggered by a spike and run at 39 ms/cm division. In multiunit records, the spike which follows the triggering spike may appear at any point in the trace, but in single unit records this second spike is always delayed by some minimum interval. This leaves a spike-free period, which does not appear in multiunit records, after the triggering spike.

The spike trains were analyzed so as to preserve information on the temporal pattern of response and its relation to the time-course of stimulation. Spikes were counted in 1-s time-bins for 30 s before and 30 s after the mark which indicates stimulus valve movement. (These individual bin counts were used to produce a measure of variance and in calculating the significance of changes in firing rate.) Post-stimulus time was divided into successive periods according to the time-course of the stimulus concentration change. For a stimulus with a 10-s nominal duration, these stimulus periods would be as follows: (a) the fast rising phase of the concentration change (RI; 2 s); (b) the slower rise to peak concentration (RII; 3 s); (c) the plateau of maximum concentration (P; 5 s); (d) the initial fast falling phase (FI; 4 s); (e) the slow falling phase (FII; 6 s); and (f) the final "recovery" phase (RE; 9 s), during which there may still be some low concentration of stimulus substance in the olfactory sac. The relationship of these periods to the time-course of the stimulus is shown in Fig. 3 C. Periods RI and FI start 1 s after the marks indicating the opening and closing of the stimulus loop because the odor pulse takes a little over 1 s to traverse the delivery tube to the animal. The 1-s period after the opening of the stimulus loop is not included in either pre- or post-stimulus time. The number of spikes in each period (pre-stimulus time counted as one period) was totalled for all repetitions within a stimulus block and a normalized average firing rate calculated for each period as:

\[
NA_i = \frac{S_i/T_i - S_p/T_p}{n},
\]

where \(NA_i\) = normalized average for the period \(i\); \(S_i\) = sum of spikes for period \(i\); \(T_i\) = duration of period \(i\) (seconds); \(S_p\) and \(T_p\) = sum of spikes and duration for the pre-stimulus period and \(n\) = number of stimulus repetitions.

The normalized averages (\(NA_i\)) are plotted against time as a stimulus period histogram.
(SPH). For each unit and stimulus, this plot represents the average change from pre-stimulus firing rate during successive phases of the change in stimulus concentration. In Figs. 4-9, two or more SPH's for the same unit are plotted on the same base line to demonstrate the changes in response which occur with changes in stimulus parameters. The dotted base line of the SPH represents zero change from pre-stimulus rate. Points above and below the line are firing rates above and below pre-stimulus average. The vertical bars show 1 SE above and 1 SE below each period average; those at the left are for the pre-stimulus periods of the responses shown. The number of repetitions is shown bracketed at the right. Note that intermediate positions on the line connecting points have no real meaning; they simply delineate points belonging to the same response. In each figure the time course of the stimulus is also shown as a concentration-time profile.

The similarity between responses to pairs of stimulus substances was measured for each unit by cross correlation of the list of stimulus period averages $S_i/T_i$ for the response to one stimulus, with the equivalent list for the other stimulus. The pre-stimulus 30 s is treated as a stimulus period for this purpose. The Spearman rank correlation value ($r_s$) is used and referred to as a similarity coefficient. It is used as a descriptive and not an inferential statistic. The method is very sensitive to differences in temporal pattern of response but insensitive to relative magnitude. The similarity of temporal pattern evoked by two stimuli in a large population of units was estimated by averaging the individual $r_s$ values across all units ($Av r_s$). This $r_s$ measure of response pattern was compared with three measures of response magnitude, in a test of discrimination between responses to different stimuli. In these three measures of response a single number represents the magnitude of response. Each is normalized for spontaneous activity and number of repetitions, and they are as follows: (a) the average firing rate during the rise and plateau phases of the stimulus ($R + P$); (b) the overall average firing rate during the 29 s of post-stimulus time (Overall); and (c) the average firing rate for the 2 s which show the greatest departure (+ or -) from the pre-stimulus rate (Peak). Similarities of response for those measures consisting of a single number were expressed in a form having the same range as $r_s$ (+1 -- 1) by dividing the smaller normalized firing rate by the larger. We have called this measure the "fractional change" in response. The $r_s$ and fractional change values were averaged over many units for each comparison.

To assess the significance of each response, the probability associated with each period average was calculated by one of two methods (Kolmogorov-Smirnov test, Siegel, 1956; and a test based on the mathematical relation between the Poisson and binomial distributions devised by Dr. Warren Ewens, University of Pennsylvania; see Meredith, 1974). Fisher's (1950) method was used to combine the probabilities for the separate stimulus periods and assess the significance of the whole response. Only SPH's which had a combined probability < 5% were considered as real responses.

**RESULTS**

**Stimulus Concentration-Time Profile**

The time-course of the stimulus concentration change at the incurrent nostril during a 10-s stimulation is shown in Fig. 3 A and D, whereas the corresponding time-course at the excurrent nostril is shown in Fig. 3 B and E. Passage through the olfactory sac delayed the arrival of the odor pulse and slowed the rise time. Both locations experienced a long tail of low concentration on the falling phase of the stimulus which is seen more clearly in the log plot (Fig. 3 C). The graphs of Fig. 3 are plotted from conductivity measurements corrected for nonlinearity (see Methods). The monitor system could not be used with all stimuli, especially
FIGURE 3. Stimulus concentration time profile. Conductivity of fluid at the nostrils is measured by imbalance of a DC bridge or by an AC phase lock amplifier. Flow rate is 4 ml/min throughout. (A–C) Signals are corrected for nonlinearity, calibrated for concentration, and plotted as concn vs. time. Concentration scales (ordinate) are labeled for a $10^{-2}$ M stimulus; numbers may be substituted for other concentrations. Zero on the time scale (abscissa) is the point at which the stimulus valves were switched. (A) Three superimposed plots of concentration change at the incurrent nostril during the standard 10-s stimulus. Linear concn axis, labeled at log M intervals. (O, □) DC measurement using tank water. (△) AC measurement using $10^{-2}$ M arginine. (B) Two plots for 10-s stimulus and two for 5-s stimulus of concn change at the excurrent nostril after the odor pulse has passed over the olfactory mucosa. Linear concn axis, labeled at log M intervals. (O, □) DC measurement using tank water, 10-s stimulus. (△) DC measurement using $10^{-2}$ M arginine, 5-s stimulus. (C) The curves through the points of A and B (fitted by eye) are replotted on a logarithmic concentration scale. The vertical lines and symbols show the division of post-stimulus time into stimulus periods (see Methods). (D–F) Profiles photographed from the oscilloscope screen. Vertical lines in D and E are valve switching marks. (D) Incurrent profiles for $10^{-2}$ M arginine (upper profile, gain $\times \frac{1}{2}$) and for $10^{-3}$ and $10^{-4}$ M arginine (AC measurement). (E) Excurrent profile for $10^{-2}$ M arginine (DC measurement). (F) Superimposed incurrent profiles for 10-s normal rise-time stimulus and the equivalent slow rise-time stimulus. (Note different time scale from B and D.)

Those of low concentration. All those which could be measured (both different substances and different concentrations) however, gave identical profiles when adjusted for the same peak height and corrected for nonlinearity. For example, in Fig. 3 A and B the circle and square symbols were data from one substance
and the triangles from another. Fig. 3 B also shows excurrent nostril profiles for a 5-s stimulus. A 5-s profile is not shown for the incurrent nostril but would have rising and falling phases identical to those of a 10-s stimulus but with a falling phase starting 5 s sooner.

With the slow rise-time modification to the stimulator, the maximum rate of change of concentration is decreased to approximately one-third of normal and occurs some 6 s later (see Fig. 3 F). The conductivity monitor shows that there is a low concentration, estimated at $10^{-4}$ of the nominal concentration, present at the incurrent nostril during the extra delivery time, i.e., before the period of maximum rise. Because the valve switching times were adjusted to give comparable stimulus duration, the slow rise stimuli did not reach the same peak concentration as normal rise stimuli. A "10-s" stimulus (actual stimulus-loop flow, 15 s) reached only 75% of normal and a "5-s" stimulus (8-s stimulus-loop flow) only 40% of normal peak concentration.

One of the unique features of this experimental preparation is the ability to place limits on the inferred concentration within the olfactory chamber close to the receptors. Such information is not available for an intact nasal chamber in the higher vertebrates although it is especially critical in animals such as mammals which have sorptive structures interposed between the stimulus delivery system and the olfactory receptors. In the present experiments the delivery tube was positioned to provide flow between lamellae and not across their free upper borders. Unless diffusion of aqueous solutes through the thin mucus layer is unexpectedly slow, the concentration time profile at the receptors must be presumed to lie between that of the incurrent and excurrent nostrils.

**Cell Properties and Spontaneous Activity**

Approximately 200 units were recorded during these experiments. Of these, 75 were quantitatively analyzed and are presented here. Units were recorded from all regions of the bulb but with a majority from the lateral and ventro-lateral regions where the electrode was mechanically stabilized by its passage through the bulb (see Meredith [1974] for further details of spike type and location). Units were generally slow firing, the mean pre-stimulus rate for 75 units being 1.70 ± 0.20 SE spikes per second.

Many units such as that shown in Fig. 5 B tended to fire in irregular bursts and often had spikes which declined in amplitude during a burst. We have been unable to discover any artificial cause for this behavior and must conclude that it is a normal firing pattern for these cells. Similar patterns of spontaneous activity have been observed in olfactory tract fibers by Nanba et al. (1966) in the goldfish, by Døving and Hyvarinen (1969) in burbot, as well as by MacLeod (1976), recording with microelectrodes, in trout. In the results reported here, both the decline in amplitude and the tendency to fire in bursts appeared to be related to overall firing rate inasmuch as both disappeared at lower rates whether the decrease in rate was in response to odor or to electrical stimulation, or was unrelated to experimental operations. Declining amplitude units almost invariably had a large-negative/small-positive wave shape with an A/B notch (Fuortes et al., 1957; Eccles, 1964) on the initial negative going phase. This suggests that they were recorded from cell somata or dendrites (Nelson and Frank, 1964). The calculated location of these cells was consistent with their
being mitral cells. Electrode depth and angle measurements indicate that most such cells were located between 150 and 350 μm from the nearest surface of the bulb, where the somata and dendrites of mitral cells are found (Sheldon, 1912; Andres, 1970; Macleod and Lowe, 1976; Ichikawa, 1976).

Declining amplitude spikes were accepted as single units only after careful examination for (a) irreducible minimum interspike interval >20 ms, (b) constant spike duration and wave form, and (c) no change for long periods of time in the firing pattern and relative amplitude of the spikes in a burst. Evaluation on the first criterion, using 50 spike-triggered oscilloscope traces (see Methods) was routinely made before data collection. This semi-random sampling of 50 interspike intervals was repeated as necessary during the course of recording (e.g., during odor response or if any changes in spontaneous activity or signal/noise ratio became apparent). Evaluation on the second criterion was routinely made on the storage oscilloscope before data collection and again during spike counting by reference to the spike shape displayed on the filmed records (see Methods; for further discussion, see Meredith, 1974).

There were no observable differences in background activity or responses between animals immobilized with curare or with succinyl choline but MS222 anesthetic appeared to depress both spontaneous activity and response. The tendency to fire in bursts was also reduced. This finding is different from the observations of Døving and Hyvarinen (1969) who found many fewer units showing cyclic variations in firing rate when recording from paralyzed animals than when recording from anesthetized animals.

**Mechanical Sensitivity**

Zippel and Breipohl (1975) have suggested that interaction of mechanical and odor sensitivity may have had a major influence on the results of previous studies of odor response. The design of the odor delivery apparatus used here allows the effects of flow disturbance, which is transmitted almost instantaneously down the water-filled tube to be separated in time from the arrival of the odor which occurs ~1.2 s later. The spike count for the 1-s period immediately after the initial valve switching was not included in the response analysis but the firing rate did not show any significant response to the switching artifacts. There were also no significant responses to blank stimulation using water in place of an odor stimulus. It is thus improbable that mechanical sensitivity influenced the results of this study.

**Odor Response and Classification of Response Patterns**

Maximum rates of firing during odor response were, in general, not high. The average rate for a 1-s time bin never exceeded 30 impulses per second. Responses were quite consistent when the same stimulus was repeated, especially in terms of the temporal pattern of response but were not precisely duplicated in response magnitude. If responses are defined as excitation or suppression, on the basis of the most obvious phase of response, excitation exceeded suppression by about 2:1, but such a definition is often misleading because many responses were combinations of excitation and suppression.

To demonstrate consistency of temporal pattern, Figs. 4 and 5A and B include stimulus period histograms for the same stimulus delivered at different
times during the unit recording. Each SPH is the average for a block of several repetitions of the same stimulus (bracketed numbers at right). The variation within blocks is indicated by the SE bars and the variation over longer periods is shown by the different SPH's. The time between the first and last blocks in Figs. 4 and 5 may be as long as 2 1/2 h during which time other stimuli were presented. In Fig. 5 A and B are shown an excitation and a suppression whose time-course appeared to follow the stimulus concentration change (shown schematically above [b]). Responses of this type and also those with a small rebound (see Fig. 7 B) are termed simple responses. Complex responses, having both excitatory

Figure 4. Consistency of temporal pattern and variation in magnitude of response. Temporal pattern is consistent over long periods of time. The upper part of the figure (B and C) shows one unit, the lower part (E and F) another. In each case, two different stimulus substances were used. Each substance was presented several times and stimulus period histograms (SPH’s) were constructed from the responses. New SPH’s for each substance were obtained at a later time during the single unit recording by presenting another block of stimulations with each substance. The SPH’s for the same substance presented at different times are plotted on the same axes (B, C, E, F: solid lines). Broken lines are the average between blocks. To the right of the SPH’s are shown the order in which blocks were given (Roman numerals) and the time between blocks. Bracketed numbers are the number of repetitions contributing to each block. (A and D) Idealized concentration-time profiles for the stimuli used in B and C and E and F, respectively. (B and C) Responses of unit 1 to $10^{-2}$ M alanine and $10^{-2}$ M arginine, respectively. 5-s stimulus. (E and F) Responses of unit 2 to $10^{-2}$ M arginine and $10^{-2}$ M alanine, respectively. 10-s stimulus.
FIGURE 5. Simple patterns: firing rate related mainly to concentration. Filmed records (a) of two repetitions each, for the responses of two units showing simple patterns. The bars indicate the period of flow through the stimulus loop. The SPH (c) for two (A) or three (B) blocks of stimulus presentations are shown with the idealized concn/time profile (b). The time-base is the same for filmed records and SPH's. SPH's are labeled as in Fig. 4. (A) Response of unit 3 to 10^{-2} M glycine. This is a unit with very little spontaneous activity which gave definite and consistent responses without a great change in firing rate. 5-s stimulus. (B) Response of unit 4 to tank water. The simple suppression follows the stimulus concn/time profile. The SE during maximum concentration is zero because no spikes occurred during those periods. This single unit with higher spontaneous activity than unit 3 fired in irregular bursts and declined in amplitude during a burst. The amplitude is not absolutely determined by the firing rate. For single unit criteria see text. The second trace on the films in B is the simultaneously recorded stimulus valve monitor.
and (or) suppressive phases were also seen. The consistency of temporal pattern of response and the variation is response magnitude shown in Figs. 4 and 5 was found in all units. Units were not restricted to showing any particular response type and rarely gave the same response to different odors at the same molar concentration (Fig. 4). Units were often excited by some substances and suppressed by others. Even when responses to two substances could be classified as both excitatory or both suppressive, the temporal pattern of firing rate was often quite different.

Table I gives the results of a comparison between different methods of quantifying responses. The similarity of temporal pattern (Av \( r_\beta \); see Methods) was compared to the similarity of response-magnitude calculated as the fractional change for three different response measures. Data from 22 units were obtained in the following form: (a) responses of the 22 units to a block of three presentations of a stimulus (not necessarily the same stimulus for each unit); (b) a repetition of the first set, the same units responding to another block of presentations of the same stimulus; and (c) responses of the same units to stimuli different from those used in the first and second sets. The individual similarity estimates for the comparison of sets 1 and 2 and sets 1 and 3 were averaged over all 22 units, for each method. Comparison of sets 1 and 2 (Table I, line A) thus shows the degree of consistency of response with stimulus repetition. Perfect reproducibility would produce a value of +1.0. Comparison of sets 1 and 3 (Table I, line B) shows the degree of discrimination between stimuli, best discrimination being shown by the lowest number. The most efficient measure of response in terms of consistency and discrimination can be found by dividing consistency figures by discrimination figures (line C of Table I).

The two matrices of responses, to the same substance and to different substances, were subjected to Friedman two-way analysis of variance (Siegel, 1956). In both cases the test was significant at the 0.01 level. The variance between response measures was therefore greater than the variance between units within response measures. The superiority of average \( r_\beta \) over the next most efficient measure was highly significant for responses to same stimuli (\( P < 0.001 \)) but was not significant for the responses to different stimuli (Wilcoxon signed ranks test; Siegel, 1956). When the two measures are combined in Table I, however, the efficiency of \( \text{Av } r_\beta \) in characterizing responses is evident.
Responses were classified according to the form of the SPH; that is, according to the temporal pattern of response and its relation to the stimulus time-course. Fig. 6 shows schematic response patterns, in SPH form, representing the major classes of response observed. This classification is an expansion of that used by Kauer (1974) to classify the responses of salamander olfactory bulb cells and allows the results of the two studies to be compared. SPH's for excitatory response only are shown in Fig. 6; suppressive responses would be numbered and described equivalently. $E_0, E_1, S_0,$ and $S_1$ are termed simple patterns. Other classes in which the firing rate does not follow concentration and in which both excitatory and suppressive phases occur are termed complex patterns. Responses with the additional subscript "A" in Fig. 6 show the same sequence of firing rate changes as those without the subscript, but the phases of response follow each other more rapidly and cannot be correlated with the concentration change in the same way (see Fig. 9 E, Arg.-6; type $E_{2A}$). Subscript "A" responses made up 19% of all responses with almost half belonging to the $S_{2A}$ class. 98% of all the statistically significant responses could be classified according to Fig. 6.

Table II shows the distribution of classes of response within and between substances and concentrations. The differences in the distribution of excitatory, suppressive, and null responses among the different substances are not statistically significant ($3 \times 9 \chi^2$ test). This is in surprising contrast to individual unit

![Hypothetical Response Patterns](image-url)
results which show great differences in response to different substances and different concentrations. It appears that the different substances and concentrations do not differ in the range of possible response classes that they can elicit, but within this range two substances generally elicit different response patterns from the same unit.

**Figure 7.** Effect of duration of stimulus. Responses to the longer stimulus are shown in broken lines. A dot-dash line is used for response to the longest duration stimulus in D. Duration is identified at the right. Vertical scale mark equals 1 spike/s for D, 0.2 spike/s for B and F. (A) Concentration/time profiles for 5- and 10-s stimuli. (B) Response of unit 5 to $10^{-4}$ M alanine, 5- and 10-s stimuli (same unit as Fig. 5A). (C) Concentration/time profile for 5-, 10-, and 15-s stimuli. (D) Response of unit 5 to $10^{-2}$ M alanine. 5-, 10-, and 15-s stimuli. (E) Concentration/time profile for 10- and 20-s stimuli. (F) Response of unit 6 to $10^{-4}$ M glycine. 10- and 20-s stimuli. The 20-s stimulus starts to fall in concentration early because of the limited volume of the stimulus loop.

**Duration**

When stimuli of two nominal durations were given, responses were modified in one of three ways. (a) Features of the pattern coinciding with the rising phase and with the falling phase of a stimulation were unchanged but shifted in time to match the rising and falling phases of the different duration stimuli. In Fig. 7B the pattern of firing rate during the falling phase of the different duration stimuli is almost identical but is shifted to the right for the longer duration stimulus. Firing rate during the third (plateau) period of the 10-s stimulus is not significantly different from that during the second period when the concentration first reaches its maximum value. 7 out of 14 responses in which different
durations were tested were of this type. (b) The response pattern was unchanged by a change in duration, and firing rate was more consistently related to time after stimulus onset than to the changing stimulus concentration. Only two response sets showed this relationship. Fig. 7D shows one of these where the response at shorter duration was a simple suppression (response in the other set was a complex excitation). In Fig. 7D, firing rate had returned to the spontaneous level by the fourth period for each duration although the concentration at this point varied greatly between stimuli of different duration. During 15-s stimuli the concentration was still at its plateau level, but during 5-s stimuli it was almost back to zero. (c) The remaining response sets showed a combination of these events. Some features of the response pattern remained unchanged despite changes in instantaneous concentration, and other features were shifted to coincide with the falling phase of different duration stimuli. Fig. 7F shows a unit which was initially suppressed but gave an excitatory burst on the falling phase of a 10-s stimulus. A 20-s stimulus demonstrates that this was not an "off" effect, inasmuch as it appeared at the same point despite maintained concentration. Firing rate change during the later falling phases of both stimulus durations was similar, but the pattern was displaced in time with the longer duration.

Rise Time

In the olfactory system, the effect of rate of rise of stimulus concentration has not previously been tested independently of a change in peak concentration. Responses to pairs of stimuli with different rising phases but identical peak concentrations and falling phases are shown in Fig. 8 B–D for one unit and in Fig. 8E for another unit. Fig. 8B shows the identical falling phase response to slow-rise and normal-rise stimuli at 10^{-4} M concentration. The firing rate during the rising phase follows the change of concentration in each case, increasing more slowly with the slow-rise stimulus profile. Fig. 8C shows the same relationship at 10^{-6} M. The response at 10^{-2} M, on the other hand, shows a slight excitation during the slowly rising stimulus which is not seen in the response to the normal-rise stimulus. This is followed by a decrease towards, but not reaching, the suppressed firing rate which was observed with the standard normal-rise stimulus. Another unit excited by low and suppressed by higher concentration (Fig. 8E) shows a similar transitory excitation during the slow rise to high concentration (broken line). The suppression with normal-rise, high concentration stimuli and the excitation with normal-rise, low concentration stimuli are shown by the solid line and the dotted line respectively.

Although, in some units, there are changes other than a simple stretching out of the response pattern during the slow-rise stimuli, these cannot necessarily be interpreted as a sensitivity to rate of change per se. Because excitation is characteristic, in these units, of response to lower concentrations, it might be expected to precede the suppression at higher concentration if the rate of rise is slowed sufficiently to allow the unit to respond to submaximal concentration.

Changes in Nominal Concentration

In approximately half of the tests the type of response given to a particular substance was similar at all nominal concentrations. The changes in response as
stimuli of higher concentration were delivered involved only an enhanced excitation or suppression with perhaps an enhanced rebound. For the other half of the data, changes in response, as nominal concentration was changed, were not so predictable. Fig. 9 includes examples of three different ways in which response may reflect changed concentration. The first unit (Fig. 9 B) had an increased firing rate during the period of maximum concentration for each of three concentrations of glycine. The excitatory response at $10^{-6}$ M, although consistent, was not statistically significant. The pattern of response, however, was similar at all three concentrations, especially so for the two that were well above threshold.

The pattern of response for the second unit (Fig. 9 C) was also basically the same at the three concentrations. The suppression of firing rate was slightly greater during the peak concentration period for each of the successive concentration steps. The third sequence of response patterns (Fig. 9 E) was more complicated. The initial excitation increased from $10^{-6}$ M to $10^{-4}$ M, but a suppressive epoch appeared during the peak concentration at $10^{-3}$ M. At $10^{-2}$ M this suppression had expanded to include all SPH periods.

The distribution of response classes within and between concentrations can be seen in Table II J. When data for all substances were combined, there were no significant differences in the distribution of excitatory, suppressive, or null response at the three concentration levels ($3 \times 3 \chi^2$ test). These proportions of each response class at different concentrations do not reflect the large change in response which may occur in individual units when the same substance is delivered at different concentrations.
Changes in response class observed with a two-log unit change in nominal concentration are tabulated in Table III. Responses are condensed into four major classes: simple excitation (E); simple suppression (S); complex excitation (Ec); and complex suppression (So). Table III shows the distribution of response classes at the higher of two stimulus concentrations (columns) for units which gave a particular response at the lower concentration of the same substance (rows). This table can be read either from left to right or from top to bottom.

Simple excitation is the response most frequently found, and this response type is generally unchanged with a two-log unit concentration change. Units giving simple suppression at the lower concentration are also generally unchanged in response to a two-log unit increase in concentration. Units giving complex responses (excitatory or suppressive) at the lower concentration how-
### Table II

**Response Classification**

<table>
<thead>
<tr>
<th></th>
<th>A. Alanine</th>
<th>B. Glycine</th>
<th>C. β-Alanine</th>
<th>D. Arginine</th>
<th>E. Serine</th>
</tr>
</thead>
<tbody>
<tr>
<td>N%</td>
<td>21 14</td>
<td>16 10 20 50 90 19</td>
<td>33 29 27 5 11 25 8</td>
<td>18 27 23</td>
<td>3 26</td>
</tr>
<tr>
<td>U2</td>
<td>5 10 17 7</td>
<td>3 - 2 4 6</td>
<td>- 17 7 2</td>
<td>- 7 2</td>
<td>- 1</td>
</tr>
<tr>
<td>E&lt; + E1</td>
<td>34 45 50 55 55 50 58</td>
<td>33 38 35</td>
<td>51 39 29 26</td>
<td>29 30 33</td>
<td>33</td>
</tr>
<tr>
<td>E1</td>
<td>14 - 33 11 20 10 - 13</td>
<td>8 15 - 12</td>
<td>11 11 25 13</td>
<td>12 57 7</td>
<td>7</td>
</tr>
<tr>
<td>E2</td>
<td>7 - 17 11 35 5 - 2</td>
<td>4 - - -</td>
<td>3 - 13 4</td>
<td>- - -</td>
<td>- -</td>
</tr>
<tr>
<td>E total</td>
<td>55 45 83 54 60 45 50 52 42 54 50 43 43 41</td>
<td>41 41</td>
<td>41 41 41</td>
<td>41 41 41</td>
<td>41 41 41</td>
</tr>
<tr>
<td>S&lt; + S1</td>
<td>17 10 - 15 20 10 - 13</td>
<td>25 15 - 19</td>
<td>11 29 13 28</td>
<td>18 22 19</td>
<td>19</td>
</tr>
<tr>
<td>S1</td>
<td>3 14 - 7 10 20 15 15 - 13</td>
<td>8 - 4</td>
<td>19 4 13 13</td>
<td>24 9 14</td>
<td>14</td>
</tr>
<tr>
<td>S&lt; + S4</td>
<td>10 13 - 10 5 - 2 - 17 11 20 15 15 - 13</td>
<td>8 - 4</td>
<td>19 4 13 13</td>
<td>24 9 14</td>
<td>14</td>
</tr>
<tr>
<td>S total</td>
<td>21 55 63 50 50 45 50 50 42 54 50 43 43 41</td>
<td>41 41 41</td>
<td>41 41 41</td>
<td>41 41 41</td>
<td>41 41 41</td>
</tr>
<tr>
<td>n</td>
<td>29 21 6 56 20 20 8 48 12 13 1 26</td>
<td>56 28 8 72 17 25 3</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. Phenylalanine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. Taurine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. Tank water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. Arginine HCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J. Goss</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K. Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N%</td>
<td>21 14 11 17 10 18 14 6 5 15 14 7 11 25 20 33 10 16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U2</td>
<td>3 - 2 4 6</td>
<td>3 - 2 4</td>
<td>3 - 2 4</td>
<td>3 - 2 4</td>
<td>3 - 2 4</td>
</tr>
<tr>
<td>E1</td>
<td>11 13 16 11 20 15 15 11 - 18 5 43 - 14 13 11 21 64 13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>7 - 17 11 35 5 - 2</td>
<td>4 - - -</td>
<td>3 - 13 4</td>
<td>- - -</td>
<td>- -</td>
</tr>
<tr>
<td>E total</td>
<td>78 50 7 63 64 55 61 58 - 46 50 55 71 - 61 55 47 56 173 51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S&lt; + S1</td>
<td>25 - 16 - 18 39 - 41 20 - 14 21 19 6 64 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>11 - 5 21 9 - 14 6 - 5 5 14 - 7 11 10 6 55 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S&lt; + S4</td>
<td>11 - 5 - - - - - - - - - 1 4 5 8 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S total</td>
<td>22 25 7 26 21 27 21 44 - 45 25 14 - 21 54 34 15 107 51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| n      | 9 8 2 19 14 11 5 28 18 2 2 22 20 7 1 26 175 152 54 34 | 100%

Classification of all responses according to the temporal pattern of response as measured by SPH. Each substance has a separate subtable (A-I). In each subtable the concentration columns show percent of units tested with that concentration which gave each class of response. The bottom line shows the number of units tested with that concentration. The total column for each substance gives percent of all tests with that substance which gave responses belonging to each class. The number of responses is shown in the bottom line. Concentration columns are identified by log M concn. Data for 10⁻⁴ M are included with those for 10⁻³ M (3-4). Tank water was used undiluted (un) and at two dilutions. The concentration table (K) shows the percent of each response class at each concentration when data for all substances are pooled. The totals columns (K) include all the data and show numbers and percentages of each response class. The bottom line shows the total number of independent tests of response. Including repetitions, the total number of stimulus presentations was 1501. Response classes are defined according to Fig. 6.

- N% = Null response. Included are all responses which were not significant departures from spontaneous variation. Units failing to respond to any stimulus are excluded from the table.
- S< + S1 = Single response of the indicated class. Percentages not calculated for numbers <6.
- E< = Unclassifiable. Included are significant responses whose pattern was not clearly defined.
ever, are significantly more likely to change their fundamental response type
(excitation to suppression or vice versa) when concentration is increased by two
log units than is the case for units giving simple responses at the lower
concentration ($\chi^2$ test, $P < 0.01$).

Simple suppressive responses at the lower concentration are less often
changed in fundamental response type with the increased concentration than is
the case for other response classes ($\chi^2$ test, $P < 0.05$). Units giving simple
suppressive responses at higher concentration, however, are more likely to be
changed in fundamental response type with a concentration decrease than is the
case for other responses ($\chi^2$ test, $P < 0.05$). Expressed in another way, once
simple suppression is established, it tends to be maintained at higher concentra-

**Table III**

<table>
<thead>
<tr>
<th></th>
<th>$E_x$</th>
<th>$E_c$</th>
<th>$S_s$</th>
<th>$S_c$</th>
<th>$N$</th>
<th>$U$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower concentration</td>
<td>16</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>$E_x$</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>$E_c$</td>
<td>0</td>
<td>1</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$S_s$</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>$S_c$</td>
<td>10</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$N$</td>
<td>34</td>
<td>17</td>
<td>20</td>
<td>10</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Responses to the same stimuli at two concentrations 2 log units apart (either $10^{-4}$ and $10^{-6}$ M or $10^{-2}$
and $10^{-4}$ M) are entered in the table. Rows show the distribution of response classes at the higher
concentration when the response at the lower concentration in the same set of units was as indicated
at the left. Columns show the distribution at the lower concentration when the response at higher
concentration was as indicated along the top of the table. $E_x =$ simple excitation; $E_c =$ complex
excitation; $S_s =$ simple suppression; $S_c =$ complex suppression; $N =$ null response; $U =$
unclassifiable response. Units which did not respond at any concentration are not included.

Changes in response which occur with changes in nominal concentration are
difficult to illustrate with conventional concentration-response curves because
the response at any given concentration cannot be adequately represented by a
single number. It is clear, however, that concentration-response relations differ
considerably from unit to unit and from substance to substance and that some
concentration-response functions must be nonmonotonic. This is illustrated in
Fig. 10 for the response of 11 units to glycine.

**Discussion**

This study has demonstrated that there are consistent and stimulus-characteris-
tic temporal patterns of response in goldfish olfactory bulb neurones. We
suggest below that these patterns are produced mainly, though not entirely, by
two factors: (a) the rise and fall of concentration in the olfactory sac which
occurs during the stimulus pulse; and (b) the idiosyncratic relationship between
concentration in the sac and impulse frequency in the olfactory bulb cells. This relationship appears to vary from cell to cell and from stimulus to stimulus, thus accounting for the range of different patterns produced. The relationship may also be a nonmonotonic function and thus produce complex temporal patterns as the concentration of the stimulus passes through certain ranges. Previous researchers have suggested that odor quality may be coded by spatial patterns of activity, by temporal patterns, or by both (Adrian, 1953; Mozell, 1958; Moulton, 1967). We suggest that the temporal patterns of activity, developed simultaneously by many neurones, may code both odor quality and odor intensity.

![Figure 10](image-url)

**Figure 10.** Concentration-response relations. Responses of 11 units to glycine. Magnitude of response, measured as the normalized average firing rate for the rise and plateau phases of the stimulus, is plotted on the ordinate. Three widely separated concentrations were used for most units; some could only be held long enough to test two concentrations.

The importance of the stimulus time-course in determining the pattern of response suggests that the control of stimulus parameters such as concentration, rise time, and duration is particularly important in experiments where odor responses are assessed.

**Magnitude of Response vs. Pattern of Response**

Most investigators have approached the problem of olfactory coding by characterizing response in terms of response magnitude rather than temporal pattern. However, many researchers have observed temporally patterned responses to odors in many species (Mancia et al., 1962; Døving, 1964, 1965, 1966a, b, c; Mathews, 1972b). In fish, Nanba et al. (1966) and Døving (1966a) commented that the patterning appeared to be a candidate code for odor quality identification. Kauer (1974) by using more controlled stimuli in the salamander related the patterns to phases of the concentration-time profile of the stimulus and showed that this relationship changed with concentration. However, no worker appears to have made a direct comparison of the effectiveness of different measures of response as we have attempted to do here.
The features of the response that are most consistent when the same stimulus is repeated, but most varied when different stimuli are delivered, can be considered reasonable candidates for stimulus quality coding. As shown in Table I, the temporal pattern of response as measured by SPH and \( \text{Av} \, \tau_p \), is more efficient in characterizing responses than three measures of response magnitude. This finding also suggests that temporal patterns may be important in odor quality coding and confirms that the statistics \( \tau_s \) and SPH are suitable for use in further analyses.

The detailed investigation of temporal patterns planned here required a more rigorous definition of the response and this was obtained by averaging responses over several repetitions of the same stimulus. The 2-min interstimulus interval required to prevent interactions between successive stimuli made it impossible to accumulate large numbers of repetitions as well as testing different stimuli. Considerable variance remained when the responses were displayed as a peristimulus-time histogram with a 1-s bin-width, but some of the finer details of the pattern appeared to be consistent. Fast-rising excitatory responses, for instance, often had a consistent double peak occurring within a single stimulus period. Some information is probably lost in the further abstraction of the data into stimulus period histograms, but the main features of the response are preserved, and their relation to the time-course of the stimulus is emphasized.

**Factors Contributing to Temporally Patterned Responses**

Three main sources of input to the second order cells of the olfactory bulb have been demonstrated (in mammals, see Shepherd, 1972). These are (a) the excitatory input from receptor cell axons in the glomeruli, which, indirectly, may also inhibit other second order cells, via; (b) the intrabulbar inhibitory input from other second order cells; (c) centrifugal influences from higher brain centers, which reach the second order cells via bulbar interneurones.

In fish, the synaptic organization (Andres, 1970; Ichikawa, 1976) and bulbar function (Macleod and Lowe, 1976) appear to be similar to those of mammals. The centrifugal fibers from the forebrain may exert considerable influence on second order cells in fish (Døving and Hyvarinen, 1969) as in mammals (Moulton and Tucker, 1964; Macrides, 1976). In the experiments reported here, the olfactory crus carrying these fibers was transected. This procedure probably does not entirely isolate the bulb from the anterior olfactory nucleus, portions of which may be found in posterior central regions of the bulb itself (Sheldon, 1912). It does prevent modulation of second order activity, via other sensory pathways (Døving, 1966d) or higher order feedback circuits (Price and Powell, 1970). Because the olfactory crus is cut in these experiments, the temporal patterns observed can be attributed predominantly to the temporal characteristics of three factors: the primary receptor input; the neuronal circuits of the olfactory bulb; and the stimulus profile itself.

**TEMPORALLY PATTERNED STIMULUS** Where stimuli are delivered in a flowing stream, the beginning and end of the stimulus pulse cannot be sharp because of diffusion and mixing as the pulse moves down the delivery tube to the animal. In the present experiments we did not attempt to sharpen the edges
of the stimulus pulse because more information appeared to be obtainable about the relation between firing rate and concentration level with the slower rising and falling stimuli. In addition, this mode of delivery is probably closer to that occurring naturally.

Inasmuch as the stimulus concentration is itself varying over time, this must contribute to some aspects of the temporal pattern in all units. Several cells do show, for some substances, a time-course of response closely correlated with the time-course of the stimulus (either positively: Figs. 5 A and 9 B; or negatively: Figs. 5 B and 9 C). For these units the impulse frequency at each point can be explained as a simple function of the concentration present at the time. Other units, however, generate consistent temporal patterns which bear no simple relation to the stimulus profile. Complex patterns are not restricted to certain units, however, but can also occur when units giving simple patterns are stimulated with a different substance, or even with the same substance at a different nominal concentration. This is the case even when the stimulus time-course, in terms of rise time, plateau duration, and fall time, is kept constant. Three main possibilities can be envisaged to explain the more complex patterns: (a) the impulse frequency of the cell is a fixed but complex function of the concentration level in the olfactory sac; (b) impulse frequency is a function of the rate of change of concentration at each point in the response; (c) there are delays in the bulbar circuits such that the impulse frequency is a function of the concentration (or rate of change of concentration) occurring at some previous point in time.

Kauer (1974), in his concept of concentration tuning, has already suggested the first possibility: that cell output can be a complex, nonmonotonic function of concentration. Fig. 9 E shows a cell which behaved in a concentration-tuned way. It is excited by low concentrations (10^{-8} M, 10^{-4} M) and suppressed by high concentration (10^{-2} M). At an intermediate nominal concentration, the low levels of the stimulus substance present at the beginning and end of the profile are associated with excitation whereas the higher concentration during the plateau is associated with suppression. This produces a response of the E_3 class.

Further support for the first hypothesis comes from the tests with stimuli of different rise time. Although the number of cells tested has been quite small, there is, so far, no evidence for a sensitivity to rate of rise per se, such as can be shown in stretch receptors (Ottoson and Shepherd, 1969). The results cannot be explained by the second hypothesis alone, but the patterns produced can be explained on the basis of the first hypothesis, if an additional assumption is made about units that are excited by low and suppressed by high concentrations: that, when faster rising stimuli of high concentration are delivered, the concentration may pass through the excitatory range too fast to elicit any spikes. This explanation accounts for the observed response to rising concentrations, but not all features of the response patterns fit the hypothesis so well. This is especially true during falling concentrations.

If the hypothesis is correct, all temporal patterns could be attributed to the temporal change in the stimulus itself. Features of the response pattern (peaks and valleys of the SPH) could always be correlated with the concurrent behavior
of the stimulus. The use of stimuli of different durations shows that some units follow this rule when the stimulus time-course is altered to delay the falling phase (Fig. 7 B). In other units, however, some features of the response are unaffected by such changes in time-course. Some of these responses may simply be produced by a phasic input from the receptors (e.g., Fig. 7 D) but others clearly could not be. In Fig. 7 E, for example, the intermediate phase of the response appears independent of the concurrent behavior of the stimulus but later phases appear to be dependent on it. This relationship was also shown by Døving (1964) for some units. Such an independent phase would be very important in olfactory coding, even if it were only a transient component of a longer response, inasmuch as it outlasts reasonable behavioral decision times. Although apparently independent of the concurrent concentration, such features of the response maintain a constant temporal relation to the initial rise of concentration. They may be determined by some neural process or circuit of long (seconds) time constant (hypothesis c above). Since the bulb and higher neural centers are disconnected in these experiments, a neural circuit with this property would have to be intrabulbar. Non-bulbar possibilities include the more peripheral processes, such as the transduction itself or the generation of spikes by receptors which might have long time constants. These are considered below with the general question of receptor cell patterns.

The experiments using different rise times and durations clearly indicate that the temporal change of stimulus concentration is a major determinant of the pattern of response in most cases. Experiments with different nominal concentrations demonstrate the nonmonotonic relationships between stimulus concentration and impulse frequency during the plateau phase of the stimulus which exist for many units and substances. These relationships modified by the delay factor discussed above could account for the temporal patterns produced during the rise and fall of stimulus concentration.

Factors contributing to the idiosyncratic relationship between concentration and bulbar spike frequency could include the following: the distribution of various receptor site types and their affinity for the stimulus; the processes leading to receptor impulse generation; the distribution of receptor axons to different regions of the bulb and the internal circuits of the bulb itself. The relative influence of these factors can not be estimated from bulbar recordings but the synaptic organization of the bulb appears to be sufficiently complex, so that all the properties discussed in the previous section could reasonably result from the operation of bulbar circuits.

**RECEPTOR CELL TEMPORAL PATTERN**

There have been some reports of temporal patterns in the receptor cells of various species (Shibuya and Tucker, 1967; Mathews, 1972 b; Holley et al., 1974) but these seem to be rare. Most cells in air breathing species appear to have simple tonic or phasic-tonic responses (O'Connell and Mozell, 1969; Getchell, 1974; Baylin, 1975). As far as can be determined from multi-unit studies (Suzuki and Tucker, 1971; Sutterlin and Sutterlin, 1971), this is also true of fish.

A phenomenon which may be important in this context is the decline in spike amplitude at high firing frequency which is often observed. If the spikes decline into the base line this may account for some of the cases of apparent temporal
patterning (Holley et al., 1974). In such cases it is unclear whether the spike generation mechanism is inactivated or whether the site of spike generation moves further away from the depolarized cell body so that spikes can no longer be recorded. In the first case, the information transferred to the bulb would be affected. Spike inactivation is one of the few processes, at the peripheral level, which might have a time constant consistent with the time-locked features of the second order response mentioned above. It seems unlikely, for instance, that the transduction process has time constants long enough to contribute to the time-course of any of the temporal patterns described here.

A further contributor to second order cell patterns could be the sequential activation of receptors as the stimulus front passes through the olfactory sac. For most of the stimulus time-course, however, the delay between incurrent and excurrent nostril profiles is ~ 1 s. This might contribute to the fine structure of the temporal pattern but should have little effect on the main features considered here.

**The Significance of Suppressive Responses**

As the nominal concentration was increased, excitation at all concentrations was the most common sequence of responses observed in the goldfish. However, as Table III shows, many cells were excited by low and suppressed by higher concentration, a sequence which has been observed in several species (Døving, 1964; Kauer, 1974). It has been suggested that suppression only occurs at nonphysiological concentrations (Higashino et al., 1969) although it is not clear how a physiological concentration can be defined, especially for chemicals which do not occur in the natural environment of the animal. Certainly suppression does occur in some units at concentrations which appear to be below threshold for most units. Some of these units are excited by other substances but give suppressive responses to all concentrations of some substances. Such responses usually belong to the simple suppressive classes (S₀ and S₁) and could arise if the unit were located in an inhibitory surround close to a region of excited second order cells. In the salamander, Kauer (1974) found that units giving simple suppressive responses did not change their response patterns when stimuli of higher concentrations were used. This is generally true in the goldfish as well, where only one case was observed of a conversion from a simple suppression at lower concentration to excitation at higher concentration. When the nominal concentration was reduced however, excitation was found in 8 out of 20 units which gave simple suppression at higher concentration (Table III). In such cases examination of the filmed records does not reveal any evidence for an initial burst of activity before the suppression, which might have suggested that the response should properly have been classified as an excitation. In the goldfish, therefore, unlike the salamander, simple suppression cannot be segregated from other classes of response.

**Coding**

It appears that some form of ensemble code is the most likely mode for the representation of odor quality information in goldfish olfactory bulb. The variation in the sequence of interspike intervals in successive presentations of
the same stimulus suggests that pulse interval coding (Perkel and Bullock, 1968) is unlikely. Døving and Hyvarinen (1969) reached the same conclusion after a rigorous analysis of activity in burbot olfactory bulb cells. Different cells are not specifically sensitive to different substances, and neither are particular temporal patterns unique to particular substances (Table II).

A similar lack of specificity in taste neurones led to the proposal of an ensemble code consisting of a comparison of simultaneous firing rates in a large number of cells (Erickson, 1963). Inasmuch as the temporal pattern of response observed here carries more information than an instantaneous or averaged firing rate, a comparison of temporal patterns could provide more information about odor quality than a simple firing rate comparison. This comparison of temporal patterns across neurones can also be thought of as a monitoring of the temporal change in an across-neurone pattern. A stimulus concentration which rises and falls with a time constant in the order of 1 s or longer will develop successively the across-neurone patterns characteristic of each concentration level traversed. Such stimuli are presumably common in the natural chemical environment and would be sampled continuously by the natural odor delivery mechanism of the goldfish. If each unit had the same concentration-response function for all substances, only concentration information could be encoded in the temporal variation of across-neurone pattern produced by such stimulation. Functions differ significantly, however, and the changing pattern could thus resolve ambiguities in the information that stationary patterns provide about both stimulus quality and concentration.

In the present studies the similarity coefficients for each odor pair comparison were averaged across all units and the resulting average $r_s$ values showed a wide range. Glycine and alanine, for example, have a high $Av r_s$, whereas glycine and taurine have a very low $Av r_s$. Since $r_s$ is a measure of similarity of temporal pattern this indicates that patterns are sufficiently different on average to play a role in odor quality coding.

**Implications for Odor Similarity Studies**

Odor similarity as judged by the response similarity in individual units clearly changes with concentration in many of the bulbar units we observed in the goldfish. Because different units have different thresholds and different concentration-response functions, each unit cannot be sampled at the same point in its dynamic range for all substances, when odors are delivered at only one concentration. Previous response similarity studies have attempted to control odor intensity (as distinct from odor concentration) by matching the odors on some criterion such as EOG amplitude (Døving, 1966b, c; Higashino et al., 1969), but the pattern of activity across units could still be expected to change with concentration.

It is conceivable that the overall similarity of response might not change although each unit response was changed, but this could only be tested if similarity measurements were made for a reasonable number of stimuli at two or more concentrations. Such measurements have been made using data from units described in this paper and are reported elsewhere (Meredith, 1974, and in preparation). It appears that the response similarity for some of the
substances remains the same between $10^{-4}$ and $10^{-2}$ M concentrations but that this is not the case for all comparisons. This was particularly true of comparisons, one of whose members was either arginine or $\beta$-alanine.

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