Physical Variables in the Olfactory Stimulation Process

DON TUCKER

From the Department of Biological Sciences, The Florida State University, Tallahassee

ABSTRACT Electrical recording from small twigs of nerve in a tortoise showed that olfactory, vomeronasal, and trigeminal receptors in the nose are responsive to various odorants. No one kind of receptor was most sensitive to all odorants. For controlled stimulation, odorant was caused to appear in a stream of gas already flowing through the nose. Of the parameters definable at the naris, temperature, relative humidity, and nature of inert gas had little effect on olfactory responses to amyl acetate, whereas odorant species, odorant concentration, and volume flow rate effectively determined the responses of all nasal chemoreceptors. An intrinsic variable of accessibility to the receptors, particularly olfactory, was demonstrated. Flow dependence of chemoreceptor responses is thought to reflect the necessity for delivery of odorant molecules to receptor sites. Since the olfactory receptors are relatively exposed, plateauing of the response with flow rate for slightly soluble odorants suggests an approach to concentration equilibrium in the overlying mucus with that in the air entering the naris. Accordingly, data for responses to amyl acetate were fitted with Beidler's (1954) taste equation for two kinds of sites being active. The requirement for finite aqueous solubility, if true, suggests substitution of aqueous solutions for gaseous solutions. A suitable medium was found and results conformed to expectations. Olfactory receptors were insensitive to variation of ionic strength, pH, and osmotic pressure.

The results of most studies in olfaction have been interpreted in terms of olfactory receptor characteristics. But in the majority of cases no direct evidence was obtained. Instead, responses of other structures were observed for presumed activation of olfactory receptors and from such data deductions were made about the nature and response characteristics of olfactory receptors. The only method, presently available, that can possibly yield direct information is the recording of electrical concomitants of the nervous response. Essentially three distinct electrophysiological methods have been developed: depth electrode recording in the olfactory bulb; the recording of potentials led from the surface of the exposed olfactory mucosa; and the recording of action potentials from small portions of the olfactory nerve. The specialized
part of the nasal mucosa that is the olfactory organ connects by the olfactory nerve to the olfactory bulb, located within the cranial cavity. The organ contains neuroepithelial cells (the receptors), sustentacular cells, and basal cells. Associated with it are Bowman's glands, which continuously bathe the surface with their secretions. The axons of the receptor cells are intimately associated with Schwann cells in the nerve, so that compact bundles containing a few hundreds to many thousands of nerve fibers are formed (Gasser, 1956). Within the olfactory bulb, and therefore within the central nervous system, is the first synapse in the olfactory sensory pathway.

Adrian (1942, 1950) developed the method of depth electrode recording from the olfactory bulb. This method cannot yield the most direct information about the responses of the receptors, because it is believed that with natural stimulation the responses recorded are from postsynaptic cells (Adrian, 1956). One-to-one transmission of impulses is not a general characteristic of synapses and there is a high degree of convergence of the olfactory receptor nerve fibers upon the postsynaptic cells. From Allison and Warwick's (1949) figures one can calculate that in the rabbit there are about one thousand primary fibers for every postsynaptic fiber connecting with other parts of the central nervous system.

The recording of electrical potentials from the exposed olfactory organ (mucosa) has been employed by Ottoson (1954, 1956) and by Takagi and Shibuya (1959, 1960). This method has the advantage of direct contact with the receptor cells, but it necessitates interference with the natural circumstances of the organ. For an extreme example, the head is literally cut off in the typical frog preparation. The exact origin of the potentials is still not agreed upon (Mozell, 1961).

The detection of action potentials in the sensory nerve has been a powerful method in the study of the other senses. Beidler and Tucker (1955) reported that, contrary to the belief once popular, responses of primary olfactory nerve to natural stimulation of the organ can be recorded with standard electrophysiological methods. The synchronous discharge (compound action potential) in response to electrical stimulation was recorded from olfactory nerve more than a half century ago (see Gasser, 1956). Given the belief that a nerve fiber produces its effect by conducting impulses, it appears evident that the most direct measure of the olfactory receptor response is the rate of generation of the impulses that appear in its axon.

The purpose of this study was to examine in detail the nature of the effective stimulus for the olfactory receptors and to characterize the response as a function of the stimulus. Such information should be valuable for critical examination of the numerous theories of olfaction that have been proposed. Other types of nerve in the nasal epithelium were also found to respond to odorants (Beidler and Tucker, 1955, 1956, and Tucker, 1960). Therefore,
there exists a possible source of error in experiments that depend upon behavioral or subjective responses if the results are interpreted as reflecting the properties of olfactory receptors rather than the total capabilities of the organism. For this reason the scope of the study was broadened to include the investigation of olfactory, vomeronasal, and trigeminal nerves.

The vomeronasal system appears to be a specialized division of the olfactory system. Although the vomeronasal organ, or organ of Jacobson, is vestigial in the human and in some other species, it is present in most mammals, reptiles, and amphibians. The anatomical relationships of the olfactory and vomeronasal systems have been well depicted by Huber and Guild (1913) for the rabbit. Sympathetic and parasympathetic (motor) nerve fibers to the nose are distributed with branches of the trigeminal nerve (Corbin, 1940). Various sensory faculties are also mediated by the trigeminal nerve. Its fiber endings apparently are directly responsive, since no adjunctive sensory cells have been recognized.

METHODS

The terrestrial tortoise *Gopherus polyphemus* was selected after experimentation with several mammals, amphibians, and reptiles. This choice of experimental animal was based on relatively simple geometry of its nasal cavities. The animals, most of which weighed 1 to 4 kg, were generally anesthetized with ethyl urethane at an intraperitoneal dosage of 2.5 gm per kg. Some were obtained from animal dealers and the rest were collected locally. Time of year, sex, and age (weight) apparently had no effect on any results. During winters the animals had to be dug out of their burrows. Freshly caught animals were uniformly superior to those supplied by dealers, the preparations giving livelier and longer lasting responses.

A modified Ringer's solution was devised for *Gopherus* and has the following composition:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>124 mM</td>
</tr>
<tr>
<td>KCl</td>
<td>2.28</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>1.44</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>0.53</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>7.14</td>
</tr>
<tr>
<td>NaH₂PO₄</td>
<td>0.25</td>
</tr>
<tr>
<td>Water</td>
<td>Solvent</td>
</tr>
</tbody>
</table>

*Electrical Recording*  Small strands of nerve were dissected free under Ringer's solution and detached centrally so as to form peripherally directed twigs. Twigs were obtained from the intracranial portions of the olfactory and vomeronasal nerves and from trigeminal nerves coursing within the cartilaginous capsule over the dorsal part of the olfactory cavity. Each twig was placed on a pair of Pt-Ir wire electrodes spaced about 1 mm apart. Mineral oil was substituted for the Ringer's solution to
permit recording of action potentials. Each twig was connected by its pair of electrodes to the cathode follower input of either an AC or a DC amplifier (Grass models P5 and P6). The differential mode of recording was always employed.

If a twig is suspended from one electrode and the other electrode is placed on the bulk of the nerve or anywhere on the head, or if the single-ended mode of recording is used, potential variations from the olfactory bulb are almost always recorded in addition to the desired signals from the nerve twig.

Preamplifier output signals were displayed on a dual-beam oscilloscope for visual monitoring and photography. They also were conducted to a short time averaging circuit, Beidler’s (1953) “integrator,” which gives an indication of response that is proportional to the number of nerve impulses per unit time appearing at the recording electrodes. Output signals from the integrators were displayed on a Sanborn multichannel recorder. Many different recording arrangements were used, e.g., AC and DC preamplifiers driven by the same signal from a cathode follower common to both.

**Stimulus Control**  The stimulus generally can be precisely specified only at the naris. Pertinent variables are the kinds of odorant and their concentrations, the composition of the carrier gas, the temperature, the volume flow rate of odorous medium into the naris, and the time course of that flow.

It is assumed that the flow rate in the naris equals the flow rate out of the choana (the choanae are separate in *Gopherus*). In all the experiments described here the animals were tracheotomized so as to avoid interference from respiratory flows. The pulse does cause a small pumping effect in the nasal cavity.

Often it was convenient to test a preparation without recourse to the complete apparatus used for stimulus control. For this purpose small quantities of odorants were kept in polyethylene wash bottles and from any one a brief puff of odorous air could be directed into the naris. Intensity of stimulation was varied by controlling the strength of squeezing and the distance between the nozzle and the naris. This method will be referred to as the puff technique of stimulation.

More precise control of the stimulus was effected with the apparatus schematically diagrammed in Fig. 1. As indicated, the air was cleaned with silica gel and activated charcoal. These adsorbents were routinely replaced with factory fresh material. Nearly all the parts were made with glass. Connections with lines of glass tubing were made with spherical ground glass joints. Critical joints were gasketed with teflon film pressed into shape while under tension. The breathing chamber was a circular glass cylinder with a port in the side into which the animal’s nose fitted. The breathing chamber exited freely to atmospheric pressure and air was drawn through the nose by the suction apparatus connected to the cannula in the choana.

The olfactometer is a continuous flow dilution system; the banks of rotameters and needle valves incorporated in glass teflon stopcocks permit flow ranges of 0.1 to 100

---

**Figure 1 opposite.** Diagram of apparatus for stimulus control. Stopcock positions are shown for condition of operation: Wash is being conducted to the breathing chamber, from whence a portion is being drawn through the nose. Odor B is diluted with clean air in the mixing manifold for switching to the breathing chamber. Odor channel A is on stand-by.
Its output to the breathing chamber is controlled by the flow switch (Fig. 1), which consists of two glass teflon stopcocks ganged together and modified to transpose two lines by making the cross-connections before interrupting the prior connections (make before break in analogy with electrical circuitry). The action of switching effectively controls the appearance of odorant molecules in the air of the breathing chamber, into which a constant flow rate of 100 cc/sec. is maintained.

Since an odorous air stream was saturated with the odorant at 1.05 atm pressure while the diluted odor stream was delivered at 1.00 atm, a correction factor was introduced in the dilution ratios for the pressure excess in order to express the stimulus concentration in terms of a standard state specified by 1.00 atm pressure and 20°C temperature. The concentration range available was $10^{-4}$ to unity ($10^{-0.05}$) of saturation, with the accuracy being determined by the rotameters. Individual meters were found to deviate up to 20 per cent from values on the curves that were supplied. Recalibration was done with a wet test gas meter and a clock. The equivalent of the fraction of air saturated with odorant may be taken equal to the mole fraction of odorant in an imaginary solution of idealized properties. Converting from the pure substance to the saturated vapor pressure (20°C) for the standard state, multiplication by the dimensionless fraction yields the partial pressure. Solving the ideal gas law equation for the ratio of number of moles to volume gives the concentration in units of molarity, which is temperature- and pressure-dependent. Such calculations are valid to the extent that the ideal gas law holds for an odorant in question. Expression of the stimulus odorant concentration in terms of concentration at vapor saturation was chosen because it accurately reflects the experimental manipulation by which it was obtained.¹

When the air streams reached the rotameters they had already attained the ambient temperature of 24°-25°C. Thus the stimulus and the preparation were at the same temperature. In order to investigate the effect of temperature variation the room temperature was varied over the range of 20°-30°C. To extend the range the animal was isolated in a box through which hot or cold air flowed. Temperature equilibration of the head was assured by bringing the stomach to equilibrium, which required at least 2 hours even when forced by initially overshooting the desired temperature. Dry air was used as the diluting gas in some experiments and wet air in others. In the latter instances at temperatures below 20°C, partial dilution with dry air was necessary to prevent condensation of water in the glass heat exchanging coil introduced before the breathing chamber. A second clean air stream was made available for dilution purposes by omitting odorant from the channel marked odor B in Fig. 1.

The composition of the carrier gas was altered by introducing various gases into the odorant and diluting flow channels of the olfactometer. The wash channel always

¹ Comparison with other studies may be facilitated by collecting numerical values for some of the odorants used. The vapor pressures of n-amyl acetate, butyric acid, and benzyl amine at 20°C are 2.95, 0.75, and 0.52 mm Hg, respectively; as calculated from data in Jordan's (1954) handbook. A long extrapolation for geraniol gives a value one-hundredth of that for amyl acetate. Most of the compounds used were the best grades available from Eastman Organic Chemical Department, White Plains, New York. Essences and essential oils were obtained from American Aromatics, Inc., New York.
delivered wet air, so that a standard reference environment was available. Bottled gases (Matheson Co.) included air, 5 per cent carbon dioxide in air, oxygen, nitrogen, argon, and helium. By use of one gas in an odorant channel with omission of the odorant and a different gas for the other odorant and diluting flow channels, the composition of the carrier gas was varied independently of the odorant concentration. Three odorants were used in the olfactometer; amyl acetate, butyric acid, and benzyl amine. Nitrogen was used in the channel containing benzyl amine to avoid oxidation of the amine.

Facilities for holding and positioning the electrodes were not designed for simultaneous recording from the three kinds of nerve investigated; therefore, simultaneous recording from pairs of twigs was done in different combinations. In general, any particular type of experiment was replicated with three different animals. In some instances many more replications were made.

RESULTS

For preliminary testing of preparations it was convenient to puff odorous air into the nose from any one of polyethylene wash bottles containing small quantities of odorants. All three kinds of nerve; olfactory, vomeronasal, and trigeminal, responded to effective stimuli with bursts of asynchronous activity.

Trigeminal responses are characterized by easily discernible action potential spikes if the intensity of stimulation is not too great. However, individual spikes can be recognized in responses from olfactory and vomero-
nasal preparations only if the twigs are very small, approaching an estimated
diameter of 10 microns. Such a twig could contain a maximum of 2500
fibers, uniformly 0.2 micron in diameter. Simultaneous AC and DC recording
from the same twig reveals that the high frequency components of the action
potential are superposed on a slow DC displacement, as in Fig. 2. The records
in the figure also illustrate the necessity for careful interpretation of records of
slow potentials obtained with condenser-coupled amplifiers. The chemical
stimulus can be made so impulsive as to cause the initial part of the response
to approach that obtained with an electrical pulse, but the chemical stimulus
is not dissipated nearly so rapidly as is the electrical stimulus.

Multidolorant Testing of Preparations. The puff technique of stimulation,
although difficult to control reproducibly, was favorable for testing with many
different odorants on the same preparation. Most experiments involved
simultaneous recording from olfactory and vomeronasal twigs. Intensity of
stimulation was adjusted for moderate size of either one, or both, of the
responses. Comparison of the two responses to what was effectively one stimu-
lus at the level of the naris is the basis for describing the results. In brief,
aldehydes and nitrogen-containing compounds tended to be effective stimuli
for both olfactory and vomeronasal receptors. But the olfactory receptors
responded well to more of the compounds tested than did the vomeronasal
receptors. Within a homologous series there appeared to be a smooth transi-
tion from one type of response to the other. Thus the vomeronasal receptors
responded better to the lower molecular weight members of a series. The
vomeronasal response predominated for the two smallest aliphatic alcohols
and in the fatty acid series the cross-over to olfactory response predominance
occurred in going from iso-valeric (after n-butyric) to n-valeric acid. The
olfactory response was considerably the greater one for all members of the
acetate ester series. One vomeronasal preparation was found that exhibited
atypical behavior to alcohols; its response peaked at n-butanol and was
unusually great for n-pentanol, but it was quite insensitive to the correspond-
ing iso-alcohols. A few experiments with trigeminal twigs paired with olfac-
tory or vomeronasal twigs indicated trigeminal response behavior more like
the typical vomeronasal receptors than like the olfactory receptors.

A stimulus defined at the level of the naris was not equally effective for two
small groups of receptors belonging to the same kind of nerve, olfactory or
vomeronasal. Although in general the two responses were qualitatively similar
to one another for variation of the kind of odorant, a stronger puff was re-
quired to make both responses approach their limiting, maximal values than
was necessary for the more sensitive twig. The differences in response were
best interpreted as reflecting differences in accessibility of the stimulating
odorant to the two small populations of receptors. Twigs from the medial
part of the olfactory nerve project to the septal mucosa. A window to the olfactory cavity was made through the top of the nose and the areal distribution of an olfactory twig was mapped. The mucosa was stimulated electrically, mechanically, and with small-orifice air streams, and responses were detected at the usual recording site; and conversely the intracranially situated twig was stimulated electrically while the mucosa was explored with an electrode to record the antidromic compound action potential (which was largely monophasic with DC recording). Most small olfactory twigs projected to roughly oval areas of less than 1 mm².

A similar analysis of the vomeronasal system was complicated by its anatomy. The organ of Jacobson has a small pore-like opening within the nasal cavity. Although the opening is directed toward the naris, how can odorant molecules penetrate to the sensory epithelium lining the organ as readily as to the olfactory mucosa lining the much more accessible olfactory cavity? The vomeronasal organ is housed in a cartilaginous extension from the nasal septum and the extension has the appearance of a baffle at the entrance to the olfactory cavity. Thus the respiratory passageway turns laterally; subsequently it courses ventrally and then medially to the choana in the roof of the mouth. Inconclusive evidence from mapping experiments suggested that some of the fibers in the vomeronasal nerve may innervate an epithelial area in the respiratory passageway, an area apposed to the vomeronasal organ lying within the tissue.

A by-product of the electrical stimulation experiments was a determination of maximal conduction velocities, which were found to be 18 to 20 cm/sec. for both olfactory and vomeronasal nerve. Gasser (1956) found a similar value for the pike olfactory nerve. The pronounced inhomogeneity of the trigeminal nerve prevented a similar determination for the fibers that respond to odorous stimulation of the nose, but they appear to belong to the most slowly conducting afferent group.

Variable Parameters That Had Little Effect on the Olfactory Response to Amyl Acetate

The stimulus control apparatus of Fig. 1 was used. The output of the olfactometer to the breathing chamber was switched between wash and odor while a constant flow through the nose was maintained. Neural activity appearing at the output of the AC amplifier was passed through the integrator, which was adjusted for a discharge time-constant of about 1.5 sec. Thus a running average of the olfactory response was obtained and recorded for later measurements. Fig. 3 shows the appearance of such records. Notice that the highest response levels are practically equal for the three applications of amyl acetate at 10⁻³ of saturation which were preceded by periods of rest. The initial response of unadapted receptors is highly reproducible. Subsequent descriptions will be facilitated by identifying the initial, high level component
of the response as the phasic response and, for sufficiently long duration of stimulation, the steady-state level as the tonic response. It will be seen that the tonic response is depressed for high stimulus strengths, even to levels below the background activity obtained with clean air flowing through the nose or with no flow. In such instances a transient increase of the response occurs when the stimulus is terminated. Takagi and Shibuya (1959) have described "on"-responses and "off"-responses recorded from the olfactory epithelium.

![Graph](image1)

**Figure 3.** Recorded display of integrated olfactory response for continuous flow through the nose at 1.0 cc/sec. and air in the breathing chamber switched from clean to odorous air for various durations. First trace; 40 sec. applications of amyl acetate at $10^{-3}$, $10^{-3/4}$, $10^{-1/2}$, and $10^{-2}$ of saturation (20°C). Second trace; amyl acetate at $10^{-3}$ of saturation on for 40 sec. followed by alternations of 5 sec. off and 5 sec. on to demonstrate the effect of adaptation.

Most of the experiments in this section involved simultaneous recording from two twigs of olfactory nerve. A few vomeronasal twigs were paired with olfactory twigs, but the trigeminal behavior was not investigated systematically. Amyl acetate concentration was varied from $10^{-8}$ to $10^{-3}$ of saturation (20°C) in $\frac{1}{2}$ log steps and nasal flow rates were of the order of 1 cc/sec. Under these conditions the phasic olfactory response (unadapted) was approximately a linear function of the logarithm of concentration of amyl acetate as shown by the integrator records in Fig. 3. The vomeronasal response was relatively small and the trigeminal response was nil. The magnitudes of both the phasic (initial) response and the tonic (steady-state) response were
used as a measure of the stimulating efficiency of an odorant in subsequent experiments.

(A) RELATIVE HUMIDITY The water content of the carrier gas, air, was varied. Odorant was omitted from the second odor channel of the olfactometer so as to make dry air available for dilution purposes. Since the dry air saturated with amyl acetate was at most 1 per cent of the diluted odor stream, the water content of the latter could be varied over the range of 0 to 99 per cent saturation at 20°C. The range of relative humidity of the medium delivered to the naris at room temperature was calculated to be 0 to 88 per cent.

No effect on the responses to amyl acetate was seen for variation of relative humidity. However, dry air accelerates the normally slow deterioration of the preparation, an effect apparent only when high nasal flow rates are used.

(B) GASEOUS MEDIUM The carrier gas, air, was mixed with another gas or else two other gases were mixed. Complete substitution of 5 per cent carbon dioxide in air, or of oxygen, nitrogen, or argon for ordinary air had no effect on the phasic olfactory response to amyl acetate. During these experiments the animal respired room air through the tracheal opening.

A slight reduction of the tonic olfactory response to amyl acetate occurred for conditions under which the spontaneous activity (odorant not applied) was reduced also. Whenever the breathing chamber was switched from air to a medium free of oxygen, the background olfactory activity began, after a latency of about 20 seconds, to decline slowly and reached a residual level in about 30 more seconds. When air was readmitted there was a burst of neural activity which overshot and then returned to the pre-existing level. Variation of the oxygen content in a mixture with A or N₂ revealed a threshold for appearance of the effect, at about 1 per cent O₂. On the other hand, whenever CO₂ appeared in the breathing chamber, the background activity, without appreciable latency, began to decrease and reached some lower level in about 30 seconds. Upon removal of CO₂ the neural activity returned slowly to the original level, taking about 30 minutes to do so. No threshold for the CO₂ phenomenon was found, but the effect was roughly proportional to the concentration of CO₂ used. At the maximum of 5 per cent CO₂ used, the residual level of neural activity was quite small, about equal to that for the complete exclusion of O₂. The absolute background of electrical noise was determined after killing the receptors in the olfactory organ by various methods; e.g., prolonged irrigation of the nose with distilled water. The effects of O₂-lack and CO₂-presence were absent or slight in vomeronasal preparations and were not determined on trigeminal receptors.

(C) TEMPERATURE The animal was isolated in a box through which hot or cold air flowed so that the preparation and the stimulus were at the
same temperature. The temporal course of the olfactory response was noticeably faster with temperatures above 30°C and slower below 20°C. A constant mole fraction of amyl acetate in air (wet or dry), sufficient for a moderate response, was delivered through the naris at a constant flow rate. No temperature dependence of the magnitude of the olfactory response to amyl acetate could be demonstrated when the ambient temperature of the room was varied over the range of 20°–30°C. When the temperature was extended down to 10°C and up to 35°C, then the magnitude of the integrated olfactory response appeared as a monotonic, slowly decreasing function of the temperature.

Occasionally, the response was abnormally large at lower temperatures. This effect was traced to a probable change in physical dimensions within the nose and is described in the section called “accessibility.” Temperature dependence of vomeronasal and trigeminal receptors was not investigated.

**Major Parameters That Determined the Responses to Odorants**

The stimulus variables were the kind of odorant, its concentration, and the nasal flow rate. Amyl acetate and butyric acid were used in one set of experiments and amyl acetate and benzyl amine in another. Vomeronasal twigs were paired with olfactory twigs and trigeminal twigs were paired with olfactory twigs in simultaneous recording experiments. The standard for comparison was therefore the olfactory response to amyl acetate. The temporal pattern of stimulation for each combination of odorant concentration and nasal flow rate was odor switched on for 5, 10, and 30 seconds at each ½ minute after the nasal flow was established. The diluting gas was wet air and all experiments were at room temperature.

(A) **STIMULUS VARIABLES: ODORANT, CONCENTRATION, AND FLOW RATE**

Integrated olfactory and vomeronasal responses to amyl acetate are shown in Fig. 4. For the control value of concentration the switching of outputs to the breathing chamber was performed, but all the stopcocks associated with the rotameters in channel A (amyl acetate—Fig. 1) were turned off. The corresponding stopcocks in channel B (butyric acid or benzyl amine) were turned off during the amyl acetate run. The flow through the choana was zero for the control value of nasal flow rate, but there was the possibility of an eddy current in the naris caused by the 100 cc/sec. flow past the tip of the nose. The influence of flow through the nose is obvious in Fig. 4 for the lower concentrations of amyl acetate. The temporal parameter of the stimulus is of sufficient influence to warrant classification as a fourth determining variable, but it is common to all the experiments. Notice also that the phasic olfactory response (“on”-response) behavior is quite regular and is independent of the temporal pattern if a response does not follow too
closely upon a preceding response. The undershoot of the response after its initial component, for stimulation of sufficient intensity and duration, may be due to refractoriness.
Integrated responses to butyric acid of the same olfactory-vomeronasal preparation are shown in Fig. 5. Notice in Figs. 4 and 5 that for the control (zero) value of concentration there was a transient olfactory response each
time the breathing chamber was switched to odor, at least for the higher nasal flow rates. This was due to a brief pulse of previously adsorbed odorant being driven off the teflon plugs of the flow switch in switching from wash to odor. These artifactual responses to the brief pulses of desorbed amyl acetate make it difficult to estimate the actual values of the phasic olfactory responses to butyric acid in Fig. 5. It is clear, however, that the phasic olfactory response to butyric acid was never very great. The still smaller tonic response was maximal early in the run and became conspicuously depressed at the higher flow rates and concentrations. Combinations of the highest values of flow rate and concentration were omitted to avoid damaging the receptors. The vomeronasal response tended to be relatively high when the olfactory response was depressed below the normal background level of activity. Otherwise, the vomeronasal response to butyric acid tended to be mostly phasic, as it was for amyl acetate. In general, there was a greater tendency for the vomeronasal nerve to develop "off"-responses.

Fig. 6 shows integrated responses recorded from an olfactory-trigeminal preparation with amyl acetate stimulation and Fig. 7 shows the response of the same preparation to benzyl amine. Comparison of the responses for the intercalated runs at $10^{-2.9}$ amyl acetate in Fig. 7 with those for the corresponding flow rate run in Fig. 6 shows that the state of accessibility was changed dramatically. The accessibility was relatively constant throughout this run with benzyl amine, whereas in most similar preparations the accessibility fluctuated during experimentation. In every instance in which the accessibility attained a stable value during a run with benzyl amine, that value was considerably greater than the one for the preceding run with amyl acetate. These phenomena seem to correlate with the greater sensitivity of the trigeminal receptors to benzyl amine (Fig. 7) whereas, on the other hand, the olfactory receptors are much more sensitive to amyl acetate (Fig. 6).

Responses of all three kinds of receptors increased with increase of nasal flow rate or with increase of concentration of any of the three odorants tested. The trigeminal response to butyric acid increased in approximately parallel fashion with the vomeronasal response to butyric acid. With increasing stimulation, the vomeronasal response to benzyl amine tended to appear before the olfactory response to benzyl amine.

The olfactory response behavior was qualitatively similar for all odorants used, but the quantitative relationships between the various components were different. Assuming an adequate rate of flow through the nose, as the concentration is varied upward the phasic on-response approaches a limiting value, the tonic response peaks and then decreases, and at the higher concentrations a phasic off-response becomes increasingly apparent. At the highest concentrations an injury discharge, increasing rapidly with time, arises from the unstable level of the tonic response if the odor is on too long and con-
### Figure 7. Responses to benzyl amine from preparation of Fig. 6. Run began 1½ hours after end of preceding figure. Notice the omission of ½ minute applications of odor in the lower right corner. The stimulus sequence was otherwise the same as in Figs. 4 to 6.

Continued application of odor kills the receptors. The vomeronasal response behaved like the olfactory response; however, the phasic components were more prominent. The trigeminal response never exhibited phasic on- and
off-components. If the odor was on long enough, the trigeminal response approached a level characteristic for the odorant concentration and nasal flow rate. When the odor was turned off, an appreciable time was required for the trigeminal neural activity to return to the background level. At lower concentrations and flow rates the rate of rise of the response was less than the rate of fall, whilst at higher concentrations and flow rates the converse was true. The phasic on- and off-components of the olfactory response were not seen if the switching between wash and odor was done so slowly that the concentration change did not approximate a step function. Consequently, a characteristic time for initiation of the response is inferred.

![Figure 8](image)

**Figure 8.** Mechanism of changes of olfactory accessibility. Photomicrographs of view inside olfactory cavity showing communication with the respiratory passageway. Electrical stimulation of the cervical sympathetic nerve produced the decrease in aperture seen in the second photograph. The maximum displacement was about 1 mm.

(b) ACCESSIBILITY The intrinsic variable of position of a receptor in the olfactory organ influenced quantitatively its response characteristics. Also, the same externally defined stimulus was not always equally effective for eliciting a response from a particular group of receptors. This was found to be due to the variability in access of the odorous stimulus to the receptors. Access could be varied experimentally by electrically stimulating the cervical sympathetic nerve in a suitable preparation, thus decreasing the aperture leading into the olfactory cavity (see Fig. 8). With odorous air at constant concentration flowing through the intact nose at constant volume-flow rate, stimulation of the sympathetic nerve caused a dramatic reduction of the response recorded from an olfactory twig projecting to the septal mucosa. Thus the accessibility was reduced.

Relative displacement of the olfactory mucosa was also detected when
amyl acetate was introduced into the naris but not when \(10^{-1.5}\) amyl acetate was used. This correlates with the trigeminal threshold for amyl acetate as may be seen in Fig. 6. Noting the trigeminal sensitivity to benzyl amine, Fig. 7, it appears that the change of accessibility can be reflexly mediated by chemical stimulation of trigeminal receptors. This seems to be true at least for the anesthetized animal. The records of Fig. 5 were undoubtedly compounded by the butyric acid stimulation of trigeminal receptors.

(c) CONTAMINATING ODORANTS AND "FLOW RESPONSE" Earlier experiments revealed what seemed to be a response of the olfactory receptors to the flow of clean air through the nose. The threshold was often less than 1 cc/sec. and the magnitude of response increased in striking proportionality with the logarithm of flow rate to values, at 100 cc/sec., even higher than the maximal response obtainable with amyl acetate. The vomeronasal receptors displayed a similar response, but it was always much smaller. The effect was found to be caused by contaminating odors derived from a 9 inch length of polyvinyl chloride plastic tubing, connecting the wash-odor flow switch to the breathing chamber. The tubing behaved as a continuous source of the unknown odorant species, delivering it at a constant rate for the steady-state condition of 100 cc/sec. flow of air through the tubing. The magnitude of the "flow response" decreased with temperature and became nil at 10°-12°C.

For the greatest success achieved in coping with this problem, the threshold for the "flow response" was about 3 cc/sec. and at 31.6 cc/sec. the response was 5 to 10 per cent of the maximal response obtainable with amyl acetate. As may be seen in Figs. 4 to 7, an appreciable "flow response" was also seen for the vomeronasal and trigeminal receptors at the highest rates of flow. It is conceivable that with extreme flow rates there might be mechanical stimulation of some of the receptors.

Interpretation of the Olfactory Data The magnitude of the phasic olfactory response to amyl acetate (data from Fig. 4) is plotted as a function of concentration and as a function of nasal flow rate in Fig. 9. It appears that the effect of increasing the nasal flow rate is to increase the odorant concentration at the group of receptors being recorded from, until ultimately the concentration can go no higher. This effect appears to be relatively independent of the input concentration at the naris. Hence it can be said that the concentration at the receptors appears to be a constant fraction of the concentration at the naris for a constant nasal flow rate. Therefore, the curves that coincide for the response vs. concentration plot represent known values for the concentration at the receptors; the limit logically being equality of chemical potential in the gaseous and liquid phases. The lower curves in the plot of response vs. concentration translated to lower values on the concentration scale should be superimposable on the upper curves. Such an operation should give the
Figure 9. Olfactory phasic responses to amyl acetate. Data are from Fig. 4.
response as a function of the concentration at the receptors below the range available from the olfactometer. The four lower curves in the left plot of Fig. 9 were translated to the left 0.50, 1.05, 1.42, and 3.50 log units and are shown in Fig. 10. Beidler's (1954) taste equation was used to describe the data. It was necessary to hypothesize the existence of two different kinds of olfactory receptor sites for amyl acetate. The response curve of each is shown in Fig. 10 as well as the composite curve that is shown to fit the data. It is not known whether the two different types of sites are found on two different olfactory receptors.

(A) THE STIMULUS AT THE RECEPTOR LEVEL  The assumption that the concentration of amyl acetate at the receptors approaches equilibrium with the concentration at the naris for high nasal flow rates in a short time relative to the response characteristic can be examined for physical reasonableness. The normal thickness of the mucous layer over the sensory epithelium is not known, but the appropriate units are microns. If we assume that the receptor sites are on the olfactory hairs (cilia) and that they are near the mucus-air interface (Davies and Taylor, 1957; Gasser, 1956; Hopkins, 1926), the problem is then to estimate the time course of the concentration of amyl acetate near the surface of the mucus. When the odorant molecules appear in the air stream, adsorption at the interface will occur; simultaneously absorption, represented by diffusion into the aqueous medium, will also occur. However, the equilibrium (highest) surface concentration integrated over a unit area will not consist of nearly so many molecules as are diffusing into the bulk of the solution under that surface. Therefore, the continuous supply of amyl acetate molecules introduced in the nasal air stream is needed to set up and maintain the lengthening concentration gradient in the mucus. Using the appropriate equation for diffusion in one dimension in a semi-infinite system (Jacobs, 1935, Equation 100), taking the boundary condition that at time \( t = 0 \) the concentration just inside the interface assumes the constant value \( C_0 \), and estimating the coefficient of diffusion for amyl acetate at \( 6 \times 10^{-6} \) cm²/sec.; values of \( C \) as a function of the depth of penetration, \( x \), into the aqueous medium were calculated and are tabulated below.

<p>| Values of ( C ) and ( x ) for ( t = 1 ) sec. |
|---------------------------------|-----------------|</p>
<table>
<thead>
<tr>
<th>( C/C_0 )</th>
<th>( x ) in microns</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>58</td>
</tr>
<tr>
<td>0.2</td>
<td>45</td>
</tr>
<tr>
<td>0.3</td>
<td>37</td>
</tr>
<tr>
<td>0.4</td>
<td>30</td>
</tr>
<tr>
<td>0.5</td>
<td>24</td>
</tr>
<tr>
<td>0.6</td>
<td>19</td>
</tr>
<tr>
<td>0.7</td>
<td>14</td>
</tr>
<tr>
<td>0.8</td>
<td>9.0</td>
</tr>
<tr>
<td>0.9</td>
<td>4.4</td>
</tr>
</tbody>
</table>
In 4 seconds the distances will have doubled for corresponding concentrations, because relative values of $C$ are constant for constancy of the ratio of $x/\sqrt{t}$. Integration of the concentration with respect to the distance gives 0.04 micromoles of amyl acetate in 1 sec. for 1 cm$^2$ and surface saturation, assuming that the solubility of amyl acetate in mucus is 14 millimolar, as it is for water. Surface saturation corresponds to the boundary condition, which is based on the assumption that adsorption equilibrium is approached in a negligibly short time. Thus 0.04 micromoles is a conservatively large estimate for the amount of amyl acetate absorbed per cm$^2$ of mucosa in 1 sec. That amount is contained in $\frac{1}{4}$ cc of air saturated at 20°C. 1 cc of odorous air contains as many molecules of amyl acetate as have diffused into the aqueous phase in 16 seconds. The nasal capacity, naris to choana, for an average sized Gopherus, is about 0.15 cc and the nasal geometry is simple compared to that for common laboratory animals. Therefore, 1 cm$^2$ is a reasonable estimate for the total mucosal area and it is likely that at flow rates of 3 to 30 cc/sec., the concentration at various depths in the mucus rapidly approaches equilibrium with the concentration in the air.

It becomes obvious that the dependence of the olfactory response (and others) on the nasal flow rate reflects the need to transport the stimulus molecules to the receptors. Confining our attention to the phasic on-response means that the quantity of interest is the effective concentration attained at the receptors in a time of the order of magnitude of one second; i.e., the characteristic time for initiation of the response.

The phasic response to amyl acetate can be described by a family of curves plotted against flow rate as in Fig. 9, which can be extrapolated to values of flow rate below the lowest shown. Benzyl amine yields a similar family of curves displaced considerably to the right, even more than would appear from Fig. 7 if the accessibility had not increased and if the flow rate could be increased sufficiently for the extrapolation in this direction. Butyric acid would give a family of curves falling between the extremes of amyl acetate and benzyl amine. Since benzyl amine is miscible with water in all proportions, aqueous solubility of the odorant appears to be the parameter that controls position of the response curves on the flow rate coordinate.

The relationships are best visualized by imagining the response plotted as a surface over the rectangular coordinate system of log concentration vs. log flow rate. Then three independent constants characterize the olfactory response of a small, homogeneous population of receptors to an odorant: (a) The maximal value of the response, characteristic of the particular odorant-receptor combination; (b) A constant, $K$, fixing the position on the concentration scale; (c) An undefined constant, involving the mucous solubility of the odorant, fixing the position on the flow rate scale. The latter is also a function of the accessibility (physical dimensions), which can be varied enough
to shift the response curves for amyl acetate over 1 log unit or more on the flow rate coordinate. The influence of the third factor is also seen in the finding that the responses from two olfactory twigs projecting to different locations in the organ are described by two similar curves displaced from one another on the flow rate coordinate. Too few data were obtained to determine whether the two kinds of receptor sites responding to amyl acetate are present in the same ratio at different places in the olfactory organ.

Although the range of concentration available from the olfactometer was severely limited, a curve was deduced for the entire range of phasic olfactory response as a function of the concentration of amyl acetate at the receptors. The extrapolation in going from Figs. 9 to 10 needs direct confirmation. The curve in Fig. 10 is a prediction of what should be obtained for a maximizing rate of nasal flow if the amyl acetate concentration at the naris is varied over the range of $10^{-8}$ to unity of vapor saturation (20°C). Odorant was omitted from the second odor channel and the olfactometer arranged to perform dilution in two steps. Data were obtained for two-stage and single-stage dilutions, with an overlap of the ranges thus obtained. The results from three olfactory preparations are pooled for Fig. 11. The mean of the responses plus or minus the standard deviation of the mean is plotted for each concentration setting (each of which was recalibrated), except for the higher values where the responses diverge systematically. The divergence was in part due to differences in procedure. At high concentrations of amyl acetate the nose has to be flushed a long time before application of the next stimulus in order to reproduce the response. Also, the effect of trigeminal stimulation (beginning at $10^{-4}$ amyl acetate) on the internal dimensions of the nose should be recalled. The curve fitted to the data represents total response from the two classes of receptor sites recognized previously. The ratio of the maximal responses for the two groups is 2.5:1 and the equilibrium constants are 274 and 17,800 reciprocal activity units.

(b) THE ASSOCIATION REACTION After Beidler's (1954) theory of taste stimulation, we may postulate that molecules of odorant $A$ plus receptor cell sites $S$ interact to form receptor-odorant complexes, or

$$\text{(1)} \quad A + S \rightleftharpoons AS.$$ 

It is assumed that the concentration of odorant, $[A]$, is maintained regardless of the amount, $a$, bound to the receptors, so that a conservation equation is written only for the receptor sites. We postulate further that the effect of odorant molecules bound to receptor sites ultimately results in a response, $r$, directly proportional to the number, $a$, of sites occupied and hence that $r$ is bounded by zero and a maximal response, $R$. That is, since $0 \leq a < n$, where
Figure 11. Olfactory phasic responses to amyl acetate vs. concentrations at the naris (olfactometer range extended) for nasal flow rate in the range in which the response plateaus.
\( n \) is clearly all of the sites, and, \( r = a a, \) then \( 0 \leq r < R. \) The equilibrium constant for the association reaction written above is

\[
K_{\text{asso}} = \frac{[A][S]}{[AA][S]} = \frac{a}{[A](n - a)} \frac{f_{AS}}{f_A f_S},
\]

where the braces \{ \} denote thermodynamic activities and the \( f_i \) denote activity coefficients.

For didactic purposes only, one may ignore the probability of deviations of the thermodynamic activity coefficients from unity. Accordingly,

\[
a = \frac{NK[A]}{1 + K[A]}
\]

or

\[
r = \frac{RK[A]}{1 + K[A]}
\]

where \( K \) is the equilibrium constant. Most investigators plot the magnitude of response, \( r, \) vs. the logarithm of the concentration, \([A]\). For this reason Equation 2 was differentiated with respect to \( \ln [A] \), evaluated, and then integrated to obtain:

\[
r = \frac{R}{2} \left( 1 + \tanh \frac{1}{2} \ln K[A] \right)
\]

Note that at \( r = R/2, \) the concentration, \([A]\), is equal to the reciprocal of the equilibrium constant, \( K. \) This equation was used to determine the two lower curves in Fig. 10.

Provided that one is actually dealing with thermodynamic equilibria (cf. both Nickerson, 1956, and Stinson and Burton, 1960), the distances of the equilibrium constants on the logarithmic concentration scale from the origin are directly proportional to the energies of binding, since the standard free energy change of the reaction per formula unit is given by \( \Delta F^\circ = -RT \ln K. \) A different standard state for \( A, \) e.g. vapor pressure of the pure substance in contrast to molarity, merely displaces the origin of the logarithmic scale.

If more than one type of receptor site is necessary to adequately describe the experimental data, then Equation 2 becomes:

\[
r = \frac{R_1K_1[A]}{1 + K_1[A]} + \frac{R_2K_2[A]}{1 + K_2[A]} + \cdots
\]

Such an equation was used to determine the top curve in Fig. 11.

**Aqueous Solutions of Odorants** According to the above, one should be able to stimulate the chemoreceptors in the nose with an aqueous solution of
an effective substance. To determine this, an aqueous medium that supplies certain essential features of the normal mucous environment of the olfactory receptors was devised for *Gopherus*. It contains 1.4 millimolar CaCl$_2$ and 0.17 molal NaCl or its osmotic equivalent of sucrose. Systematic investigation of the solution parameters showed that the olfactory receptors are insensitive to variation of ionic strength, to variation of pH over several units and to variation of osmotic pressure over a range of about ± 20 to 25 per cent. A solution free of calcium ions flowed through the olfactory cavity causes intense neural activity in the olfactory nerve, and if the flow is continued, the receptors are killed in a few minutes. Ubiquitous contaminating odors must be removed from the medium.

Aqueous medium was prepared and cleaned with activated charcoal that was neutralized and leached with the medium until equilibration with the ionic constituents was approached. The prepared medium was used to make a 10 mM solution of butyric acid and saturated solutions of amyl acetate and geraniol, from which concentration series were obtained by dilution with the cleaned medium. Solutions were flowed through the olfactory cavity from the cannula in the choana and out a hole in the top of the nose. The naris was loosely plugged with cotton. The control apparatus was arranged to simulate the action of that used for gaseous media, but the flow through the nose was interrupted during the switching between wash and odor solutions. Simultaneous recording from olfactory and vomeronasal nerve twigs was performed in most cases.

In summary, the results were strikingly similar to those obtained with odorants introduced in the gaseous phase. The maximal responses obtained with butyric acid were relatively small for the olfactory receptors and large for the vomeronasal receptors, whereas for amyl acetate the converse was true. The effects of geraniol were similar to those of amyl acetate. Responses of an olfactory preparation to geraniol and to amyl acetate in solution are shown in Fig. 12. The handbooks that were consulted list geraniol as being insoluble in water, but clearly its solubility is finite albeit small. At each concentration the flow through the nose was switched to the odorous solution twice for 5 sec. and then once for 60 sec. The regularly increasing deflections, occurring at each onset of flow of wash solution, were caused by brief pulses of the odorant which was desorbed from the choanal tubing into the column of wash solution while it was stationary 20 to 30 sec. between each stimulus sequence. The difficulties encountered because of sorption phenomena and contaminating odors cannot be exaggerated. The odors were present at sensible levels in all the solutions. The medium used for making up the amyl acetate solution in Fig. 12 was not as clean as was that used for wash purposes. A test with solution saturated with geraniol was omitted because a 2 minute application usually kills all the olfactory receptors. A 2 minute application of
Figure 12. Integrated olfactory responses to geraniol and to amyl acetate introduced in an aqueous solution medium flowed through the olfactory cavity at 1/3 cc/sec. Concentrations are indicated in terms of the saturated solutions. At each concentration the flow was switched to the odorous solution twice for 5 sec. and then once for 60 sec.
amyl acetate saturated solution or 10 mM butyric acid often incapacitates some of the receptors.

Flow rate dependence of the responses was seen for odorants introduced in aqueous solution. Important factors in controlling the transport of the stimulating molecules to the receptors for both gaseous and liquid media are the laminar nature of the flow through the nose and the diffusion of the molecules to regions of lesser chemical potential. Much higher flow rates can be attained with gaseous solutions and coefficients of diffusion are vastly greater, but the specific concentration in the liquid medium may be considerably higher than that in the other phase for equilibrium between the two. The abrupt stopping and starting of the liquid flow through the nose during switching between wash and odor did not produce any mechanical stimulation of the olfactory receptors. This was evident when all parts of the stimulus control apparatus were thoroughly cleaned and the cleaned solution medium was placed in both the wash and the odor channels.

DISCUSSION

It is curious that there is a long lived controversy about whether one can perceive an odor when it is introduced into the olfactory cleft in an aqueous saline solution. Proetz (1941, p. 366) gives directions for a version of the experiment and Moncrieff (1946, p. 74) has a historical discussion. Negative findings from such an experiment do not prove there was no stimulation of the receptors. That fact appears to be appreciated by Le Magnen (1944-45), who recognized also the importance of the flow variable in the human case. It may be surmised that the equivocal interpretations are due to the nature of the response indicator: the human, subjective sensation. Perhaps the spatio-temporal aspects of the stimulus in aqueous solution experiments are so foreign that there is little experiential basis for a recognizable sensation. The response of a small group of olfactory receptors is detected directly in the electrophysiological approach.

Attempts to simulate respiration were abandoned and control of the appearance of odorant molecules in a constant flow of air into the nose was chosen as favoring the elimination of possible mechanical artifacts. Ueki and Domino (1961) suggested a physiological role of mechanical stimulation in olfaction, but the above results obtained with aqueous and gaseous solutions do not support such a position. The confusion arises because the nasal flow rate is a first order variable in the olfactory stimulation process and because it is extremely difficult to find construction materials that do not contribute unwanted odors.

The phasic olfactory response has been emphasized relative to the tonic
response. The phasic response is especially interesting physiologically, for the olfactory receptors are stimulated intermittently during normal respiration. Various facets of the olfactory response are suggestive of the narcosis model for stimulation that Mullins (1955) proposed: the rise of the steady-state response to a maximum succeeded by the decrease to depressed levels of neural activity at high concentrations, and the occurrence of on- and off-responses. Mullins' general theory is compatible with the quantitative description found for the phasic (on) response to amyl acetate. However, the original application of the equation for describing taste data was to the steady-state response (Beidler, 1953, 1954). It may be noted that sapid substances are normally present in the mouth for a considerably longer time than the duration of an inspiration. Requirement for a good sustained response performance of the olfactory receptors may have become evolutionarily unimportant.

The presence in the nose of other sensory systems that respond to odorants should never be forgotten in olfactory experimentation that depends upon behavioral or subjective responses. The temporal behavior of the trigeminal response to odorants suggests analogy with a thermally lagged heat system, whereas the olfactory receptors respond much faster. That the temporal characteristic of the vomeronasal response is so similar to that of the olfactory response is difficult to understand. The receptors are thought to be in the sensory epithelium lining the organ of Jacobson, which has only a small duct communicating with the nasal cavity. It is therefore significant that some evidence was found for innervation of an area in the respiratory passageway by fibers of the vomeronasal nerve. It is doubtful whether the receptors within the organ proper were ever stimulated with odorants in the experiments reported. Caution should be exercised in extrapolation of these findings because of species variability of the anatomy of Jacobson's organ.

The Stimulus at the Receptor Level The implication has been made that the stimulus should be regarded as the concentration of odorant in the immediate environment of the receptor. For odorants of slight aqueous solubilities and high rates of nasal flow, equilibrium between the concentrations in the mucus and in the air should be approached rapidly, as the calculations for amyl acetate showed. Small differences in concentration at various depths in the mucus would hardly be noticeable because of the logarithmic nature of the response dependence on concentration. But the far greater solution capacity of the mucus for highly soluble odorants suggests that in this case the surface concentration may still be increasing at an appreciable rate when the phasic response has already been initiated. Thus it is interesting that Stuiver (1958) chose as the significant quantity the time rate of absorption of odorant per unit area of mucosa. For the relatively simple geometry of the human nose and the assumption that all odorant molecules striking the surface of the
mucus are trapped there, he found an approximate equation that exhibits flow rate dependence and saturation at high flow rates. However, his assumption that all odorants should be quantitatively similar in this regard is made untenable by the findings reported here. Stuiver's (1958, p. 41, Equation 5) equation does not take into account the decrease with time of the number of molecules striking the mucous surface which are effectively trapped there, but the value of his contribution is the re-emphasis of the problem of transporting the stimulus molecules to the receptors. Stuiver's assumptions are most applicable to the extreme case of highly soluble compounds, whereas Moncrieff's (1955) assumption of an adsorption equilibrium is descriptive of the opposite extreme. The olfactory threshold theory of Davies and Taylor (1957) assumes that concentration equilibria are set up between the air, the mucus, and the receptor sites. It remains to be seen how appropriate the assumption is for the more soluble odorants.

The magnitude of integrated olfactory response to amyl acetate presented at a constant mole fraction in the air entering the nose was found to be relatively insensitive to temperature variation. Since with a decrease in temperature the nerve action potential is prolonged and may be larger, it is questionable whether the response in terms of nerve impulses per unit time actually increased with reduction of temperature. However, Davies and Taylor (1959) predicted a higher threshold concentration at a higher temperature for most organic compounds and it would be reasonable to expect parallel behavior of suprathreshold responses. The comparatively low nasal flow rates at which the olfactory response to amyl acetate reaches a plateau is explicable on the basis of an approach to concentration equilibrium between the receptor medium and the odorous medium flowing through the nose. Concentrations used for the temperature experiments were in the range for linear increase of the response relative to the logarithm of concentration. If the pure substance is chosen for the standard state, then for a constant mole fraction of amyl acetate the activity increases with decrease of temperature, which accords with the small increase in the integrated olfactory response. In other words, the response appears to be relatively independent of the temperature when the odorant concentration is given in terms of partial pressure referred to the vapor pressure of the pure compound. Such behavior is not suggestive of a chemical reaction with reactants and products separated by an appreciable energy of activation. The rapidity with which the response can be reversed is notable in this connection, but the phenomenon of adaptation which dissects the response into phasic and tonic components is partially responsible.

The stimulus at the receptor level was deduced from study of the response recorded from a group of receptors in a circumscribed part of the olfactory organ, with the externally specified stimulus at the naris varied systematically. The response recorded from a twig of olfactory nerve at any instant is a sum-
formation of potentials of various sizes contributed by impulses near the active electrode in fibers lying at various distances from the electrode. Beidler's (1953) integrator circuit gives a short time average of the instantaneous values, which may be taken as a proportional indication of the number of nerve impulses per unit time traversing the nerve trunk lying over the active electrode. The prerequisites set forth by Beidler for the integrated response to be truly representational of the response of a receptor field are admirably fulfilled by olfactory and vomeronasal nerve, even for the smallest twigs that can be dissected free. The foregoing statements are based on the assumption of independent conduction in the nerve fibers. Adrian (1956), quite rightly wondered whether the contiguous packing of the fibers in bundles sheathed in Schwann cell membranes might cause interaction among the fibers. Perhaps a whole bundle conducts as a unit so that what appears as a single action potential would not manifest conduction in a single fiber. Nevertheless, even if this speculation were true, it is difficult to devise an argument that would invalidate the original interpretation of the integrated response. What may be truly ambiguous is whether the AC amplifier output after integration parallels rigorously the DC amplifier output. The supposition is that the DC record, as in Fig. 2, represents summation of action potentials from many fibers; i.e., a proportional indication of the response of a receptor field.

Stimulation of the Organ  Adrian (1951, 1956) has emphasized at length the importance of the spatial and temporal patterning of the stimulus over the olfactory organ. These features were deduced from olfactory bulb recordings (see Mozell, 1958, for discussion of several of Adrian's papers). Beidler (1957, p. 42) has suggested that the olfactory organ may analyze a complex aroma in a fashion similar to the operation of a gas chromatograph. However, the olfactory organ is of a higher order of complexity that should be emphasized. The laboratory instrument can be regarded as a lineal system with a point detector located at the output; it has an inherently long time constant. The olfactory organ is a surface, embedded with millions of detectors—a system inherently capable of spatiotemporal analysis. Add the refinement of various types of detectors, i.e. receptor differentiation, and the system has even greater analytic capabilities. Finally, the typical mammalian organ has a very complicated geometry. The feature of structural elaboration seems to correlate with the macrosmatic condition (Negus, 1957 a and b).

Trigeminal innervation seems to be particularly dense in the non-olfactory portions of the nasal mucosa, and it is well known that there are trigeminal fibers responsive to tactile stimulation, differences of temperature, etc. The responsiveness of small trigeminal fibers to odorants suggests that the whole nasal mucosa should be regarded as an organ adapted for chemoreception.
The apparent mediation by the trigeminal receptors of the reflex control of accessibility to the olfactory cavity bears analogy with the visual reflexes. That stimulation of the cervical sympathetic nerve in the rabbit increases the accessibility (Tucker and Beidler, 1956) whereas the converse is true for Gopherus should pose no problem. The smooth muscle systems that open and close the alligator naris are both innervated by the sympathetic nerve (Bellairs and Shute, 1953). The effect of electrically stimulating the nerve would simply indicate which muscular system is the stronger. Reflex control of mucus secretion, which can cause profuse flow over respiratory areas of the nasal mucosa and from the organ of Jacobson, obviously affects the transport of stimulating molecules to receptors lying under the mucous flow. Personal experience of the effects of sniffing versus shallow breathing can enhance appreciation of the variation possible in chemical stimulation of the nose that is subject to the organism's volition.

The Association Reaction

The primary justification for assuming that olfactory nerve impulses are initiated by the binding of odorant molecules to olfactory receptor sites is the success afforded by this approach for the description of certain experimental data. It might be that chemosensory receptors in general operate by a mechanism common to all. The limited experience with quantitative olfactory data is certainly parallel to the results from gustatory studies (Beidler, 1953, 1954, 1961). For generality, allowance must be made for the possibility of more than one kind of receptor site responding to a particular chemical compound. Thus, the olfactory response to amyl acetate was described by assuming two kinds of sites active. But the n-amyl acetate used for getting the data of Fig. 11 had a slightly different odor from that used for the previous experiments. The question arises as to whether a mixture of two odorants (a known plus a contaminant), interacting with a single class of receptor sites would yield data that could be interpreted as evidence for two kinds of sites. The methods used ensure that the two odorant concentrations would be related by a constant of proportionality. Assuming that Equation 2 would describe the response of one kind of receptor site to either odorant separately, with a characteristic R and K for each, then it is easy to show that the response for both odorants present in constant ratio is given by a single term as in Equation 2. Of course, the apparent R and K are functions of those for the two odorants and their ratio of concentration. The important result is that evidence for more than one kind of gustatory receptor responding to some compounds.

* Eastman Organic Chemical Department No. 2360 n-amyl acetate. A chromatographic analysis, kindly performed by Dr. William Dawson, suggested the presence of free amyl alcohol in one sample.
The Concept of Receptor Sites

It is of great interest that the pharmacologists have had to postulate at least two types of receptors for the sympathetic nervous transmitter noradrenaline (Furchgott, 1960). The organic chemist's approach to the study of the so called \( \alpha \) adrenergic receptors has yielded several tentative deductions. Belleau (1960) developed a quite detailed picture of the receptor site and of its interaction with various molecules. Further, he gave a molecular mechanism for non-competitive inhibition exhibited by one class of compounds. He conceived the receptor site as a precise molecular structure situated in an accessible surface of a protein structure. Allowance must be made for possible dispersion of the constants characterizing the interaction of a substance with the group of sites, for the microenvironments of the sites might not be identical.

By contrast, the olfactory receptor site conceived by Davies and Taylor (1957) is an elusive entity. Their theory, which leads to a description of olfactory threshold data, embodies the postulate that the receptive surface of the sensory cell is non-differentiated and that a site is a minimal area in which a critical number of odorant molecules is concentrated to initiate a response. A first step in setting up the theory is the writing of an equation for Langmuir type adsorption to describe the binding of odorant molecules to the receptive surface of the cell. Beidler (1954) noted the similarity of his taste equation to the Langmuir adsorption isotherm and the use of a similar equation to express the binding of ions by proteins. Finally, Mullins' (1955) narcosis model for olfaction implies various cell types among which the receptive surface membranes are characteristically differentiated.

Analogs of the Association Reaction

Any equation, expressing a relationship between two physical quantities, which can be put into the form of Equation 2, formally obeys the mathematics of the succeeding equations. As has been seen, this is true for any simple association reaction of definite chemical species and apparently it holds for appropriate olfactory and gustatory data. It is also true for the fundamental relationship of enzymology generally known as the Michaelis-Menten equation (Edsall and Wyman, 1958, pp. 620-623) and for the Langmuir adsorption isotherm (De Boer, 1953, p. 55). Of interest for possible computer applications is the existence of electrical circuit analogs. For example, a constant current generator loaded with two resistors in parallel sees an equivalent resistance

\[
R_p = \frac{R_1R_x}{R_1 + R_x}, \quad 0 \leq R_p < R_1,
\]

which is bounded as indicated if one of the resistors \( (Rx) \) is variable over all positive values. The "response" is the voltage \( V \) which is related to \( R_p \) by
the proportionality constant $I$ (the constant current), viz., $V = IR_1$. Since $R_1 = 1/G_1$,

$$V = \frac{V_{\text{max}}}{R_1 + R_s} = \frac{V_{\text{max}} G_1 R_s}{1 + G_1 R_s}.$$ 

The equations descriptive of the physical systems listed above are all homeomorphic. Therefore, it is clear that success in describing chemosensory receptor responses with Equation 2 is not a demonstration of odorant binding at specific sites, of non-specific adsorption at the surface of the cell, of an enzymatic mechanism nor of an electrochemical mechanism. More information is necessary in order to deduce what odorant molecules interact with, and what is the effect of this interaction, which ultimately results in a nerve action potential as the visible sign of a response. An illustrative example of argumentation in choosing among models for a particular system is furnished by Cohen and Monod's (1957) choice of the catalytic (kinetic) model over the stoichiometric (equilibrium) model for bacterial permeases. Lastly, the problem of nervous stimulation is far more general than the problem of olfaction per se (Mullins, 1959, 1961).

It is a pleasure to record the guidance and contribution of my major professor and coworker, Dr. Lloyd M. Beidler.

This work is from a dissertation submitted to the Graduate School of Florida State University in partial fulfillment of the requirements for the degree Doctor of Philosophy.

This investigation was supported by a Predoctoral Fellowship (BF-7977) from the National Institute of Neurological Diseases and Blindness, Predoctoral (2G-436), and Postdoctoral (2B-5258) Traineeships from the United States Public Health Service.

Received for publication, May 16, 1962.

REFERENCES

ADRIAN, E. D., 1942, Olfactory reactions in the brain of the hedgehog, J Physiol., 100, 459.
ALLISON, A. C., and WARWICK, R. T. T., 1949, Quantitative observations on the olfactory system of the rabbit, Brain, 72, 186.
BEIDLER, L. M., 1957, Facts and theory on the mechanism of taste and odor perception, in Chemistry of Natural Food Flavors (Symposium), Quartermaster Food and Container Institute for the Armed Forces. Chicago.


CORBIN, K. B., 1940, Observations on the peripheral distribution of fibers arising in the mesencephalic nucleus of the fifth cranial nerve, *J. Comp. Neurol.*, 73, 153.


STUIVER, M., 1958, Biophysics of the sense of smell, Doctoral thesis, Rijks University, Groningen.


