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ODOR SPECIFICITIES OF THE FROG'S OLFACTORY RECEPTORS*

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A PERSISTENT obstacle to study of the vertebrate olfactory system has been the experimental difficulty of finding out about the properties of the receptor cells. Their relatively small cell bodies, sheathed distal processes, and thin, unmyelinated axons have limited most of the experimental electrophysiological investigations to recording a large number of units at a time. Adrian's categories of some major types of odor response from the olfactory bulb (Adrian 1953) and Ottoson's studies of differences in slow potentials resulting from different stimuli (Ottoson, 1958) are the best available data. Neither allows the properties of the receptors to be described in enough detail to account for the sensitivity and selectivity of the nose. Beidler and Tucker have described a method of recording from a small bundle of axons of the olfactory nerve (Beidler and Tucker, 1955). They have not yet published the results of their experiments. Zwaardemaker's early study of cross inhibition (Zwaardemaker, 1895) provided as good a set of categories to describe psychophysical odor properties as any, but it cannot lead to a unique description of the receptor mechanism. Action potentials recorded from the second-order olfactory units located in the bulb do not show unique responses to different stimuli; this may mean that odor specificity information is coded as patterns resulting from simultaneous activity of many second-order units.

This paper describes a method of recording the action potentials of olfactory receptors by using low-impedance extracellular metal microelectrodes (Gesteland, 1961). Some of the odor-specific properties of the receptors will be described.

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METHODS

All of our experiments were done on the frog *Rana pipiens*. We used either Ottoson's preparation (Ottoson, 1956) (a decorporate frog with the olfactory mucosa exposed by removing the dorsal surface of the nasal cavity), or a curarized frog with the same exposure. Responses of cells appear to be the same with either preparation. However, the curarized preparation with intact circulation is not as sensitive to overstimulation and recovered from block caused by overstimulation more readily. Furthermore, there is a curious transition in the responses of olfactory receptors caused by the successive presentation of many odors. Most of the cells lose their specificity and become responsive to all stimuli or block and respond to none. This phenomenon does not occur as soon when the animal has intact circulation. Some of the frogs had either the first nerve or the ophthalmic branch of the fifth nerve, or both, sectioned on one side.

The animal was in a plastic box with a 1 cm $\times$ 2 cm hole for the electrodes and the stimulator in the top directly over the exposed mucosa. The frog was pinned to a cork block with a silver–silver chloride plate under his head. Deodorized moist air flowed continuously through the chamber. The box and cork were thoroughly washed and left exposed to laboratory air between experiments and had no noticeable odor.

The stimuli were small puffs of odorized air from 1 ml syringes, the plungers of which were dipped in mineral oil or ethyl alcohol solutions of reagent-grade (when available) organic chemicals. Odorized air blew directly from the syringe onto the mucosa. There was no tubing as a common path for the stimuli, as we found that it very rapidly adsorbed odors and mixed them with successive ones. The odors of the stimulating chemicals were easily recognizable, and no attempt was made to achieve such purity that we could be sure that the odor was not due to impurities. (We note the recent report that zone-refined skatole is odorless (Beynor and Saunders, 1960)). It is important to stress the significance of using very low stimulus intensities. A puff of 0.2 ml of odorized air lasting 1 sec with the syringe tip 3 cm from the mucosa will typically evoke a larger response from a unit that is sensitive to the particular odor. When stimulus strengths are so large that two successive puffs cause a decrement in the amplitude of the slow potential, the receptors will certainly be in either a generally irritable or a blocked state, and no longer odor-selective.

The EOG or Ottoson potential was monitored by a micropipette filled with 3m KCl touching the surface of the mucosa, usually at the top of the eminentia olfactoria. It indicates the arrival of the stimulus at the mucosa. The maximum sensitivity of our recording system for the slow potentials is approximately 0.2 mV for a noticeable deflection of the cathode-ray tube beam. The negative-going Ottoson potential is preceded by a small

positivity for certain stimuli. The magnitude of the slow potential depends upon the nature and the strength of the stimulus.

Action potentials were measured with a platinum-black plated, metal-filled microelectrode, coupled capacitively to a cathode follower with 30 MΩ input resistance. The indifferent electrode for both microelectrodes was the chlorided silver plate. We found that some slight variations in the procedure for preparing the metal microelectrode, which we have described previously (Gesteland *et al.*, 1959) greatly improved its ability to pick up the extracellular olfactory action potentials. We break off the tip of a glass micropipette so that it is from 2 to 5μ in diameter. Next, we extrude Cerrelow 136 alloy down the pipette to fill it to the end. If responses from the region of the axon hillock are desired, the tip is next plated with platinum black from a solution of chloroplatinic acid with a little added agar. The platinum black is first deposited slowly, then rapidly enough to cause bubbling, until a large, bushy glob is formed at the tip. The agar in the plating bath is most important. It reduces the impedance of the resulting electrode in tissue, as compared with an electrode plated from a solution without agar. If responses from the cell body or distal process are desired, it is best either to grind the tip of the electrode before plating so that it is beveled like the tip of a hypodermic needle, or to break off the tip so that the glass is jagged. Then a little alloy is dissolved out of the tip with sulfuric acid and hydrogen peroxide. Platinum black is plated to fill the hollow left by alloy dissolution. This results in a low-impedance metal electrode with a glass cutting edge to lead the way into tissue. The big, bushy ball-tip electrode was used for most of the experiments described in this paper. It will easily record from receptors singly or a few at a time, and on one occasion recorded for a few moments action potentials from a single fiber in the first nerve far from the mucosa.

A heavy micromanipulator was necessary in order to stay with units for long periods of time (2 or 3 hr sometimes).

We insert the metal electrode into the mucosa in such a way that its path is very nearly tangent to the mucosa surface. The electrode will also pick up units if it is normal to the surface, but the probability of recording is greatly diminished, no doubt because of both a reduced likelihood of contacting a unit in the optimal way and because the entering electrode is much more likely to damage the receptor terminal structures and block the sensitive area on the way in. Furthermore, the electrode irritates the mucosa in the place where it penetrates, and there is movement of the cilia and mucus as the animal tries to wash away the irritant. This may well block activity of the receptors in that area.

The metal microelectrode sees spike amplitudes ranging from the noise level of 20 μV up to 2 mV. The spikes may be monophasic, diphasic or triphasic and of either initial polarity, the local boundary conditions for

current flow set by the electrode being the determining factor. The duration of the three phases of the action potential of olfactory receptors, extracellularly recorded, is from 3 to 5 msec if the electrode is deep, that is, near the axon hillock or axon. Figure 1 shows three sweeps of resting

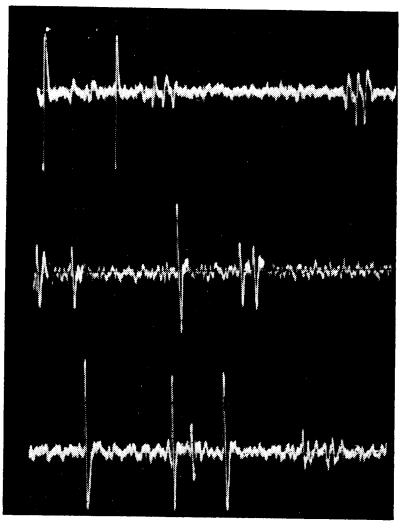

FIG. 1. Resting discharge of olfactory receptor cell axons. The lengths of the sweeps are 0.5 sec and the largest spikes are 0.4 mV peak-to-peak amplitude.

activity of units recorded in this position. The spike duration is longer, between 5 and 7 msec, when the electrode tip is on the mucosa side of the basement membrane near the cell bodies, or very near the surface of the mucosa. Action potentials recorded from fifth-nerve axons and fifth-nerve endings in the mucosa are much shorter, approximately 1–1.5 msec, typical of myelinated axon activity. For olfactory action potentials, optimal amplifier frequency response is roughly from 8 c/s to 1 kc/s. The maximum repetition rate of olfactory spikes is 20 per sec, and this is seen only rarely when a particularly appropriate stimulus is presented to a cell. A common rate for a responding cell is from 1 to 5 spikes per sec. The resting rate (which may be low-level activity of the receptors caused by room odors) is usually a few spikes in 10 sec.

Our display system operated in the following way. The vertical signal output of a monitoring oscilloscope was used to open a gate that passed the spike on to a second oscilloscope. The gate could be set to open only for spikes exceeding a preset amplitude. The Ottoson potential was added on the second oscilloscope, deflecting the base line to show the time of arrival of the stimulus at the mucosa and its relative strength. Since two or more units are often picked up by the microelectrode, we arranged a sweep expansion saw tooth to be triggered by the gate so that we could

examine the shape of the spikes. Even if two units had approximately the same amplitude, their shape was almost always characteristic, and while watching the expanded spikes, the electrode was moved slightly until different units had clearly distinguishable amplitudes. Thus it was possible to watch simultaneously the response of two or three cells to each stimulus. Figure 2 shows the resting discharge and an expanded sweep and a normal

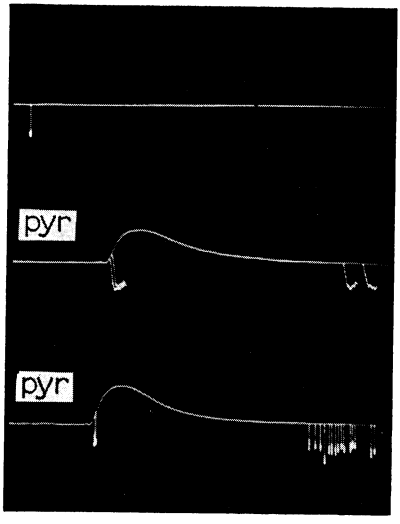

FIG. 2. Resting discharge and two responses to pyridine. Center sweep shows expanded spikes. Each expansion has a 2.5 msec duration. All sweep lengths, 10 sec.

sweep of the response of two units to pyridine. Note the different shapes of the action potentials of the two active units. The gate also was used to provide relative brightening of the spikes compared with the base line, in order to maintain more nearly uniform photographic exposure.

ODOR RESPONSES AND SPECIFICITY

A responsive unit will generally produce a burst of spikes from 1 to 4 sec in duration. This is usually followed by a quiet period about as long. For a much longer time after this there is a refractory period during which the threshold of the unit is increased. The amount of increase and the duration depend upon the strength of the preceding stimulus and its constitution. For instance, Fig. 3 shows a unit stimulated with butyric acid twice within 1 min. The second stimulus causes fewer action potentials than the first. The stimulus strengths were about equal as is shown by the Ottoson potentials. The time between the arrival of the stimulus at the mucosa and the response also depend on the particular stimulus, but not on the

strength. The usual effect of changing the strength of the stimulus is shown in Fig. 4. (We define stimulus strength as the amount of odorous

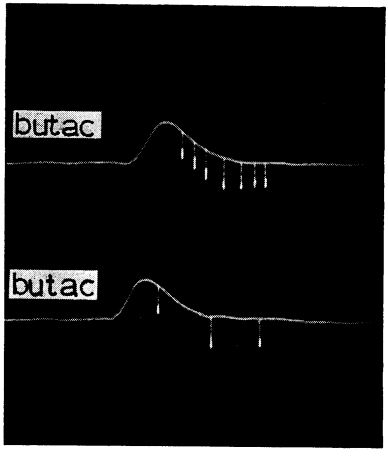

FIG. 3. Responses to two puffs of butyric acid. The lower trace was taken less than 1 min. after the upper. Sweep length, 10 sec.

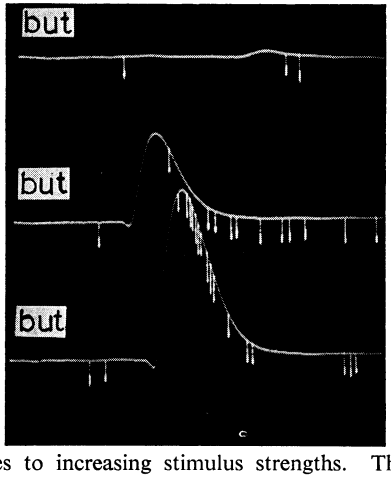

FIG. 4. Responses to increasing stimulus strengths. The top trace is the smallest; the lower, the largest puff of *n*-butanol. Sweep length, 10 sec.

substance arriving per unit time.) It shows a unit responding to three puffs of *n*-butanol. The smallest is at the top. The three records were taken far enough apart in time so that there is minimal effect of reduced sensitivity because of the preceding stimulus. The pattern of the response is strikingly similar in all three cases, even though the number of spikes increases with stimulus strength. There is always a threshold effect, and for a unit that has not had its threshold raised by multiple preceding

stimuli, the threshold is below the level at which an Ottoson potential can be distinguished. Overstimulation causes extended high thresholds. If the unit is overstimulated by several different stimuli, it will often discharge at a very high rate for many seconds, and then go into a state with prolonged high threshold. It is not a dead receptor, however, and will respond with a few spikes to an appropriate stimulus. After a long period of stimulation, even when the stimuli strengths have been kept low enough to avoid these effects, many of the units that we record are generally irritable, responding strongly to all successive stimuli. The mucosa does not return to its initial or normal state after this effect has set in. An interesting phenomenon is seen if a puff of cigarette smoke is blown at the exposed mucosa with the decorporate preparation. There is a big increase in background activity (activity of cells too far from the electrode tip to be distinguished from noise) which suddenly becomes an oscillation of 5–10 c/s. This oscillation lasts for a few seconds and is apparently phasic activity of many receptors. The receptors do not show much activity or selectivity after such an oscillation has occurred. However, it can be obtained repeatedly, and the frequency of the oscillations changes somewhat with composition of the smoke. We do not get the oscillations when circulation is intact. The dc potential of the mucosa becomes very erratic sometime after single units show the effects of massive stimulation.

When we section the olfactory nerve and let degeneration take place for a week or more, the effects on the Ottoson potentials and single-unit responses are very apparent. The Ottoson potential is reduced to approximately 50 per cent of the amplitude recorded from the mucosa with an intact nerve, and the frequency with which one can find a unit with an electrode is markedly reduced. With careful exploring, spikes can be found and they are odor-specific in their responses. This agrees with Le Gros Clark's histological studies on degeneration following first-nerve section in the rabbit where he found at least half of the olfactory receptor cells to have degenerated (Le Gros Clark, 1957). Section of the fifth nerve, on the other hand, has no obvious effect on the Ottoson potential, amplitude, and the number of active single units or their response properties. On a few occasions with a preparation with intact fifth nerve we have encountered a single unit in the mucosa with an action potential that is short compared with olfactory units, approximately 1–1.5 msec. Figure 5 shows such a unit. The large spike has a short duration, and the small one is more than twice as long. The top trace shows the resting rate, the middle trace shows the large unit responding to butyric acid, and the bottom trace, a weak response of the small unit to musk xylene. The large unit showed some response to camphor and mercaptoacetic acid, but to nothing else, even if very strong puffs were used. The small unit responded to a larger group of odors. It seems most likely that the large unit is a fifth-nerve ending.

A few times, we have recorded from a metal microelectrode inserted in the ophthalmic branch of the fifth nerve. Here also the spikes are of short duration, and the units responded to the onset of heat with a decrease in rate, and to turnoff of heat with an increase in rate compared with the

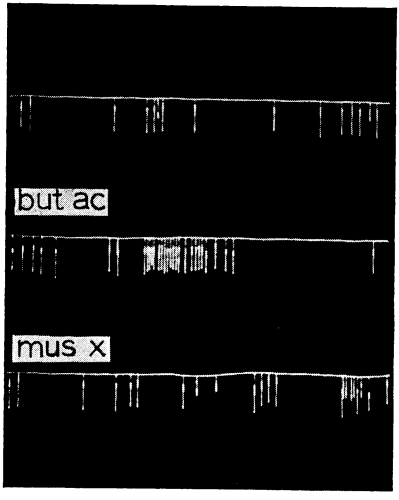

FIG. 5. Resting discharge and responses to butyric acid and musk xylene. The large spikes have a short duration. The small spikes have the usual longer duration that is typical of olfactory receptors. Slow potentials are hardly visible because of low stimulus strength. Sweep lengths, 10 sec.

resting rate. The units responded to touch with a rate increase. We did not get responses to irritating chemicals but we have not tried often to find such responses. Olfactory units recorded with the electrode as in the usual preparation do not respond to small variations in temperature and probably not to touch.

The olfactory receptors are all odor-selective, that is, each one responds to certain of the odors to which it is exposed and does not respond to others. Most show a strong response to at least one of the twenty-five odors that we have used and a weaker response to many more of them. Figures 6-14 are examples of odor-specific responses of some of the cells that we recorded.

From these records and many more, we can suggest that there are some patterns that are present in the responses of different cells, and we can begin a list of the different groups of receptors. Our list is characterized by extensive overlap, as if chemical names were not a good way to characterize these types. However, odor properties do not seem to be any better. One group responds vigorously to limonene, camphor, pinene, and somewhat less to carbon disulfide. A second responds to coumarin and musk. Group three responds to butyric acid, valeric acid, mercaptoacetic acid,

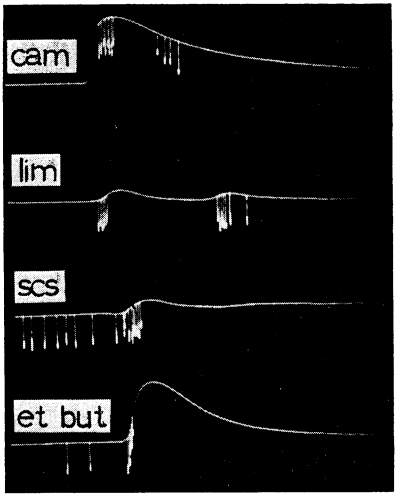

FIG. 6. A unit that responds strongly to camphor, two puffs of limonene, carbon disulfide, and slightly to ethyl butyrate. Sweep lengths, 10 sec. Note early response to carbon disulfide because of odor on the outside of the syringe as it was brought into position. A lower amplitude spike also responds to camphor in the top trace.

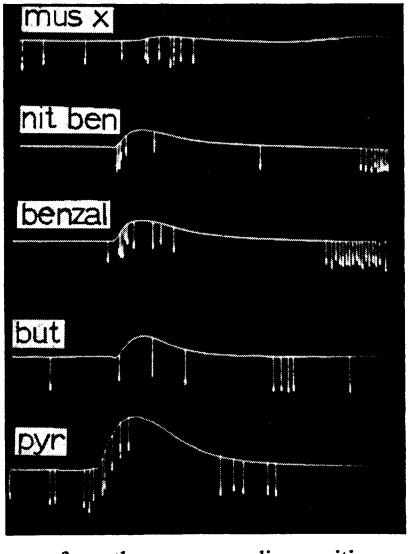

FIG. 7. More responses from the same recording position as Fig. 6. Top trace shows a weak response of the two units to musk xylene. Below it, the one unit shows long-delayed responses to nitrobenzene and benzaldehyde. The two bottom traces show a larger unit, which was not responsive to the preceding stimuli, slightly responsive to *n*-butanol, and more so to pyridine. Sweep lengths, 10 sec.

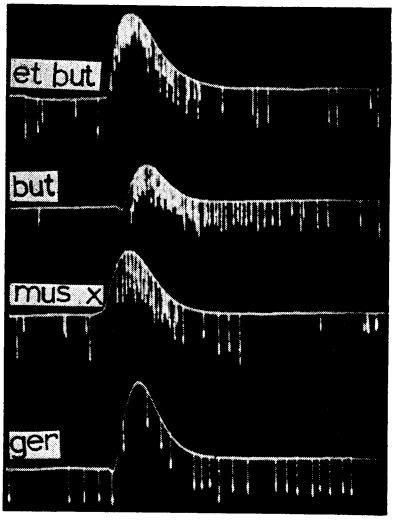

FIG. 8. Two units show vigorous responses to ethyl butyrate and *n*-butanol. Vigorous response of a different larger unit and one of the smaller units to musk xylene. Weak response of the larger unit to geraniol. Sweep lengths, 10 sec.

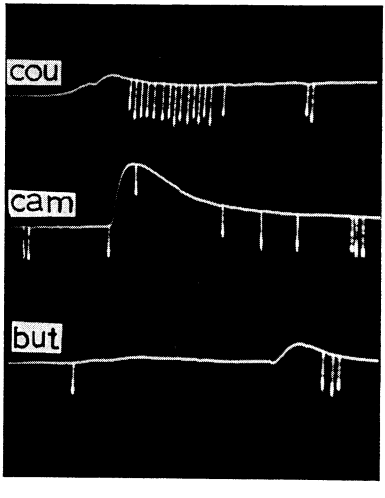

FIG. 9. Two units of different amplitude are active in these three traces. One responds to none of the three stimuli. The other responds vigorously to coumarin, and weakly or not at all to camphor and *n*-butanol. There is a continuous difficulty of separating a weak response from the usual highly erratic resting discharge rate. Sweep lengths, 10 sec.

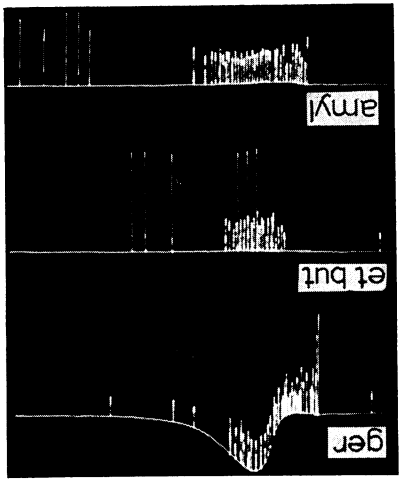

FIG. 10. Several units with clearly different amplitudes can be seen in all the traces shown here and in Fig. 11. A small unit responds vigorously to geraniol, ethyl butyrate, and amyl alcohol. A smaller unit is active as well for geraniol. A large unit responds slightly to ethyl butyrate. This unit also responds to amyl alcohol slightly, as does another slightly smaller unit. All sweep lengths, 10 sec. Except for geraniol the stimuli are too small to discriminate the slow potential.

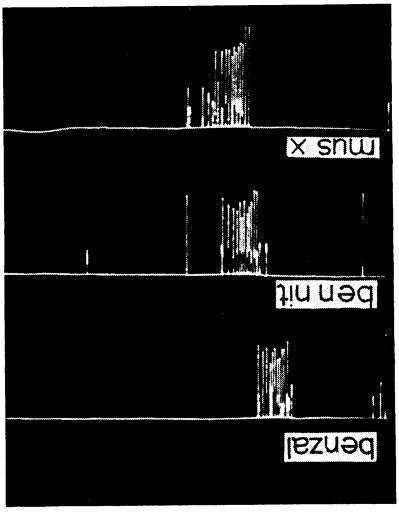

FIG. 11. From the same position as the traces shown in Fig. 10, the two large units respond to benzaldehyde, benzonitrite, and musk xylene. There is little or no response of the small units to these stimuli. Same sweep length as in Fig. 10.

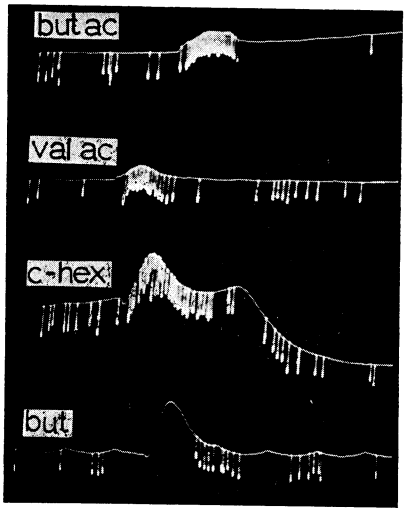

FIG. 12. A unit is shown in these four traces which responds strongly to butyric acid, valeric acid, and cyclo-hexanol. There is a weak response to *n*-butanol. Base-line drift is caused by movement of the micropipette electrode. Sweep lengths, 10 sec.

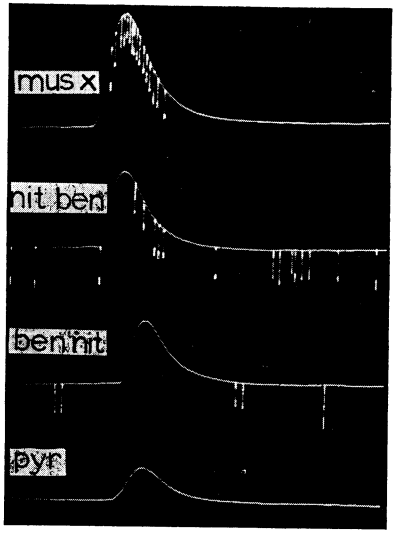

FIG. 13. A unit that responds to musk xylene, slightly to nitrobenzene, less so to benzonitrite, and not at all to pyridine. Sweep lengths, 10 sec.

and cyclo-hexanol. Group four responds to benzaldehyde, nitrobenzene, benzonitrite, musk, and amyl alcohol. Group five responds to pyridine, musk, cinnamaldehyde, and *n*-butanol. Musk is the strongest stimulus for a sixth type that does not show much response to benzaldehyde or nitrobenzene. A seventh type responds to pyridine more strongly than to most of our other stimuli. The eighth group, which is very common, responds to butanol, ethyl butyrate, amyl alcohol, and geraniol. There seem to be other types but we have not seen them often enough to be able to characterize them at all. Furthermore, it is possible and likely that these types may be condensed into fewer groups or expanded into more. If we have not used stimuli that are especially effective and, instead, are seeing responses to some of the large number of odors that weakly affect a type, it would account for much of our uncertainty and for the fact that no two units seem to be completely alike.

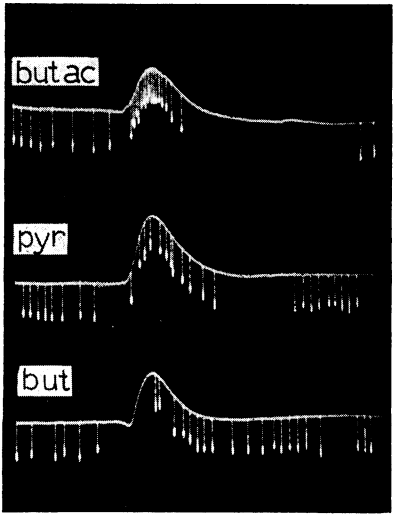

FIG. 14. A unit that shows a strong response to butyric acid, and weak response to pyridine and *n*-butanol. It could also be interpreted as showing inhibition for *n*-butanol. Sweep lengths, 10 sec.

To give some indication of the complexity that we face, we have compiled the table shown in Fig. 15. It is a list of all of the stimuli that we have used in many experiments. The second column lists the number of cells that we attempted to stimulate with each odor. The third column is the number of cells that showed a repeatable response measured as a transient increase in discharge rate. Most units respond to many things. Each cell shows such individuality in its weaker responses that, in spite of a rather large number of attempts, we have not been able to discover a unique set

of odors that accurately describes the selectivity of a limited number of receptor types. However, judging only on the basis of strong responses, it appears to us that there is a limited number of types of receptors.

Stimulus	Cells sampled	Cells responding
n-Amyl Alcohol	25	14
Musk Xylene	38	20
Benzaldehyde	36	19
Benzyl Acetate	12	6
Geraniol	18	9
Benzonitrite	22	11
Pyridine	32	15
Indole	19	9
Camphor	32	14
Methyl Salicylate	18	8
Butyric Acid	30	13
Linalool	14	6
Pinene	7	3
n-Butanol	47	19
c-Hexanol	20	8
Nitrobenzene	42	16
Triethylamine	14	5
Ethyl Butyrate	31	10
Mercaptoacetic Acid	19	6
Valeric Acid	13	4
Limonene	17	5
Coumarin	22	6
Carbon Disulfide	28	7
Cinnamaldehyde	25	6
Methyl Anthranilate	6	1
Salicylaldehyde	21	3

FIG. 15. The stimuli used in various experiments, the number of cells on which each was tried, and the number of those which responded with an increase in discharge rate are tabulated here. Weak and strong responses are lumped together.

DISCUSSION

The chemical selectivity of the olfactory receptors in the frog's mucosa which we see is a curious and unsatisfying kind of selectivity. It is analogous to a collection of poorly constructed optical filters. We could describe the optical filters as follows. No two are quite alike. The response spectrum changes with repeated use and environmental conditions. The transmission band has many notches in it and the sides of the transmission band fall off slowly and irregularly. Yet, the yellow ones are clearly a group apart from the red ones. We do not suggest that nature

has in fact given the frog such an inferior set of analyzers for odor. However, our measurements are apparently made in such a way that we cannot discern the unique properties for which we are searching. The categories that we might construct do not fall into order on the basis of simple chemical properties, or on any psychological odor groupings. There are no data on cross inhibition either at the behavioral or physiological levels for the frog, and data from other vertebrates do not help in achieving this order either. In our earlier experiments in which we used only a few odors, one from each of Zwaardemaker's major groups, it appeared that many units were uniquely sensitive to only one odor of our set. However, when we expanded the collection of stimuli, the exceptions were much more common than were the ones that responded according to our supposition.

If we assume (as appears reasonable from our data) that the resting discharge rate for the receptors is either small or zero, and therefore that inhibition is not a major part of the code for describing an odor, we can consider several different possible types of receptor mechanisms and see if any are preferred by reason of being consistent with our measurements.

1. All of the receptors are identical. In this case the number of active cells would indicate the intensity of the stimulus and the pattern of the discharge or the topographical position of the receptor on the mucosa or both would indicate the quality of the odor. Under these assumptions, we should see very similar responses to the same odor from one cell to the next, at least when the electrode stays within a restricted area of the mucosa. In fact, of course, the receptors that we record do not behave in this way at all. Differential selectivity is most obvious when the electrode is recording simultaneously from different units with clearly discriminative amplitudes.

2. There are several different species of receptor cells, each species of which has particular selectivity properties. If the receptors are like this, and if our recording method does not seriously disturb the properties of the units, we ought to see, at least occasionally, two cells that respond in the same way to our entire collection of stimulants. In fact, there is a difference in the response of any two cells with respect to some odors. It is possible that we have seen some identical cells but have disturbed their identity by our manipulations. More sophisticated experimentation might prove this to be the correct description.

3. There are a great many different receptor types, possibly one for each odor or combination of odors. This seems unlikely, as we see a strong tendency for the receptors to form at least vague odor groups.

4. There are different receptor site types that are distributed over each cell. One cell can differ from the next in having different ratios of the receptor sites. In this case the variability that we see is most reasonable, in

that whether a receptor responds or not depends on the relative occupation of the different sites, or on occupation of a minimum number of sites of more than one type. Some sites could be inhibitory, but it is not necessary to postulate this in order to have a useful code without great numbers of different cell species.

At present, the fourth receptor model seems the most compatible with our data, but we are in no position to settle the question yet.

SUMMARY

1. A new recording technique that allows the action potentials of single primary olfactory receptors in the frog to be recorded extracellularly has been described.

2. The general response patterns of the olfactory receptors have been described and correlated with the observations on the slow potentials of the mucosa.

3. Some of the odor-specific properties of the receptors have been described.

4. Possible receptor mechanisms have been discussed in the light of the new data on odor-specific responses.

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