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CHEMICAL TRANSMISSION IN THE NOSE OF THE FROG

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The first order olfactory system of a frog is of the common vertebrate plan. Sensory nerve cells are congregated in a patch of epithelium along the lining of the nose. Each neurone has an olfactory rod that extrudes terminal cilium-like processes that are washed by the mucus flowing over the nasal lining. These rods and cell bodies are wrapped in supporting cells. Each neurone also gives off a non-myelinated axon that penetrates the basement membrane and joins with others to make the bundles out of which the olfactory nerve is formed. No efferent fibres to the sensory mucosa are known, and the nerve cells there have no other processes. This ordered array of nervously independent elements produces several kinds of electrical signals. One is a slow change in the potential difference measured across the mucosa. Another is activity in the olfactory nerve. Our aim is to find what relation there is between these signals, where we may not appeal to corruption by other nervous tissue as in the vertebrate eye, by efferent processes as in the cephalopod eye, or by mutual interconnexions of the cells as in the frog's ear.

The slow potential was discovered by Ottoson (1956) and named by him the electro-olfactogram (EOG). Additional slow-potential phenomena have been described by Takagi & Shibuya (1960*a*, *b*), and Takagi, Shibuya, Higashino & Arai (1960). The activity in the nerve has been observed by Beidler & Tucker (1955) primarily in small bundles consisting of many fibres. Takagi & Omura (1963), and Shibuya & Shibuya (1963) were able to record from single units but did not report enough experiments to make any quantitative account of how activity was related to stimuli. Attempts to relate some properties of the slow potential to stimuli have been published by Ottoson (1956), Takagi *et al.* (1960) and Byzov & Flerova (1964). Higashino & Takagi (1964) have studied the slow potential as it is affected by externally applied electrical currents.

We have published earlier some observations on the activity of single fibres (Gesteland, 1961; Gesteland, Lettvin, Pitts & Rojas, 1963) as well

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as an account of some non-linear interactions of slow potentials (Gesteland, 1964). This paper is an extension of that work, modified by further experiments, and we now suggest a definite model to account for our observations. The model is similar in some respects to that proposed by Higashino & Takagi (1964). We have seen that an odour, if it affects an olfactory cell at all, can either excite or inhibit it; that every cell is affected by many odours, some excitatory for it, some inhibitory for it; and finally, that there are many kinds of cells when classified by a spectrum of odours as Schneider, Lacher & Kairsling (1964) found in insects. The processes of excitation and inhibition, furthermore, have some of the qualities described for synaptic events.

METHODS

Animals. The frogs (*R. pipiens*) used in these experiments were often pithed upward and downward so as to destroy entirely the neuraxis. Keeping a good circulation in these animals is a problem. Occasionally we would leave the cord intact to preserve blood pressure. If the pithing upward was done from a level between the posterior borders of the tympani so as to spare the medulla, the animal suffered an extremely high blood pressure and operation was difficult; hence we generally destroyed the medulla as well. In our good preparations the circulation was brisk and remained so for at least 4 or 5 hr. The pithed frog was pinned to a cork board and covered with moist gauze. The roof of one nasal cavity was removed carefully so that no blood touched the lining of the nose. The olfactory eminence could then be seen. Both the character of the mucus and its flow over the eminence were observed under the microscope. We found that too watery a mucus and too rapid a flow was pathological, and indicated some local irritation. We could produce this condition by touching the mucus surface in a few places with a glass rod, and sometimes our electrodes would do the same. Alternatively, if the mucus seemed ropy or streaky, this, too, augured ill, for while the slow potentials could be evoked, it was difficult to record from single units. (We did not work with a decorporate preparation as others do. The uncirculated olfactory mucosa will deliver slow potentials for a very long time just as the avulsed eye will yield electroretinograms. However, the nervous activity undergoes a steady and rapid degeneration after the circulation stops. The fibres begin to lose specificity and respond to almost anything that produces the slow potential, almost as if they are measuring in part an attendant current flow due to the slow potential. Finally they cease entirely. At the same time, while the olfactogram superficially appears to be large and smooth, it also undergoes a change that can be picked up most easily in experiments where two stimuli interact.)

Apparatus. The prepared frog was then put in a small Teflon box and earthed through its mouth by an Ag/AgCl electrode covered with gauze wetted in 0·9 % saline solution. A constant stream of washed moist air was directed over the exposed nasal mucosa.

Here, too, we diverge from the methods of most other workers. Ottoson at one time thought that a certain transient he observed was due to water vapour. Since then most workers have played dry, clean air on the mucosa. This inspissates the surface of the mucus quickly, a process which can be seen occurring under the microscope. If one is dealing with slow potentials, the effect of drying is not obvious; however, the effect on nervous activity is certainly bad. *In vivo* the vapour pressure in the nasal cavity must be high, especially in the rebreathing system of the frog, and so it is hard to justify a dry stream of air as a natural condition.

The nozzle for delivering the moist air was rifled, so as to ensure a laminar vortex flow with least spread and fluctuation in the issuing jet. The flow was kept constant at a variety of rates. Small holes were drilled along the rifled barrel, and through them pulses of odour

were injected at a maximum flow rate of about one-tenth that of the clean air. These pulses did not appreciably disturb the pattern of flow, and had sharp leading and trailing edges in the issuing stream. The material that was not absorbed by the mucus during a stimulus was washed away quickly by the constantly flowing air. The nozzle and the tubes leading to it from the various vials were constructed of Teflon and glass and kept clean. Further details can be seen in Fig. 1.

Electrodes. One kind was a pipette, 4–50μ o.d. It was filled with a stiff gelatin made up in 0·9% NaCl solution and connected through an Ag/AgCl electrode to a conventional d.c. amplifier of high input resistance. The other was a metal-filled glass pipette, 3–5 μ o.d.

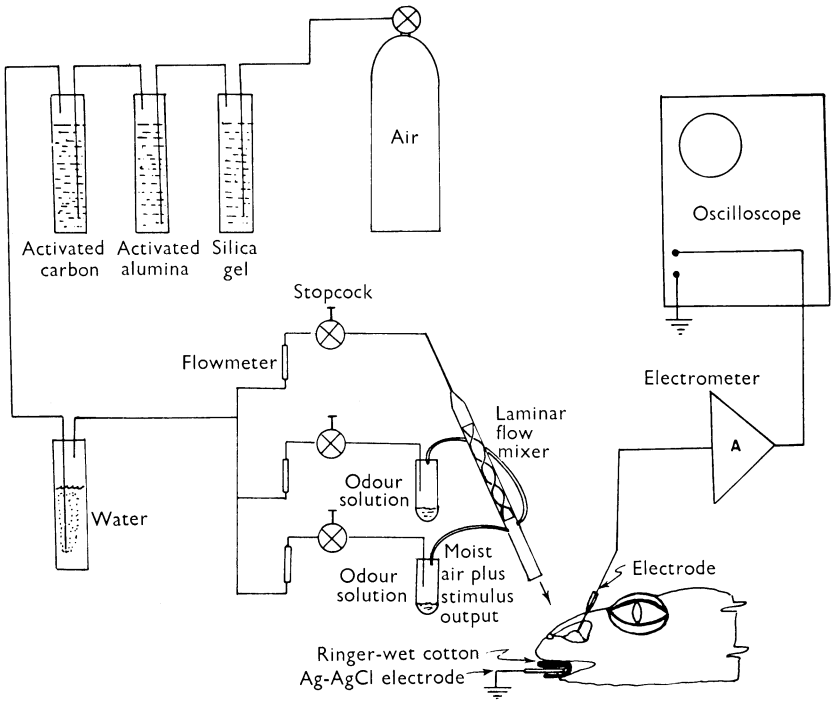

Fig. 1. The apparatus for stimulating and recording from the olfactory mucosa of the frog. The top stopcock stays open continuously, allowing a stream of moist, odourless air to play on the mucosa. Odours are turned on and off with the lower two stopcocks. A needle valve, integral with the stopcock, determines the flow rate of the odorized air. Salt-bridge electrodes of 0·9% NaCl solution are used for d.c. recording and impedance measurements. Metal micro-electrodes are used for single unit studies.

It was plated at the tip with a small and very adherent ball of gold (Dalic alkaline gold plating solution 3020, Sifco Metachemicals Inc., Cleveland, Ohio) and further plated with a thin film of platinum black. It was led to a conventional high-gain a.c. amplifier of band-pass 80–10,000 c/s and input resistance of 20 MΩ. The first type of electrode was used for recording the slow potentails or measuring impedance and the second for picking up the unit activity of olfactory neurones.

Experimental procedure. The saline electrode was placed gently on the surface of the mucus slightly to one side of the apex of the olfactory eminence. Some care had to be taken that it did not press into the mucus. It occasionally served as a local irritant and caused an

increased flow of mucus which, if it got too watery, crept up the glass and literally sucked the mucosa along with it thereby attenuating the slow potentials because the electrode had penetrated the mucosa. Alternatively, over a long experiment, the mucosa shrank slightly so that connexion was maintained by a mucous bridge that, as it thinned, caused the d.c. measurements to drift. Frequent monitoring, however, allowed us to hold the electrode in optimum position. The metal electrode was then inserted very gently and slowly into the mucus a slight distance from the other but well out the zone of local irritation. The mucus is thixotropic and resists rapid penetration; nervous elements are seriously hurt over a wide area if any local dimpling occurs on the surface. Thus the quality of mucus and speed of electrode advance are both important. We picked up nervous impulses only with those electrodes whose average noise at the mucous surface was equivalent at most to that of a 500 KΩ resistor over the same frequency band width. At the very surface some low level activity was seen, no more than about 50–100 μV in height and of varying but rather long duration, 5–15 msec. As we penetrated we would first observe externally recorded spikes lasting about 10 msec and diphasic with the initial phase negative and far larger than the second. These we attributed to the cell bodies for two reasons: first, because they varied in frequency significantly upon small advances of the electrode, as if the units were being affected in rate by the pressure, and, secondly, because they were diphasic and occurred at a small distance below the surface. However tempting it was to record from these units we seldom used them, not only because we could not assess how much the resting rate was due to electrode interference, but also because the height of the spikes varied much with frequency. (If we used conventional fine fluid-filled micropipettes we could occasionally see activity from these units at the same depth, but we could never maintain them long.) Directly beneath these units we would come up against a region of increased resistivity, and we naturally assumed this to be the basement membrane. Pressing on, we would record simultaneously several spikes of varying height and shape. Most of these would be triphasic, and, in the process of maximizing the amplitude of one or another by fine adjustment of the depth of the micro-electrode, most often we would see no fluctuations in average background rate associated with the manipulation. These spikes we took to arise from the fibres in a small bundle emerging a slight distance away on the mucosa, for such bundles course directly beneath the basement membrane. Sometimes we could see pressure effects on frequency, as if the cells were near and being tugged by the axons, and, in such a case, would find another bundle or else reposition the electrode. That we were recording from such a small bundle could be checked by going well past it so that the resistivity around the electrode dropped (i.e. we had penetrated into the submucosal space). We would then discover, on pulling back, that the spikes reappeared only when the resistivity increased, indicating that we had come up to the basement membrane from the other side. These nerve spikes were significantly shorter than those recorded from what we took to be cell bodies; generally the three phases occurred in 3–5 msec. Our best recordings were made with tri-phasic spikes, for these seem to endure the longest and were least variable in height with frequency. We attributed diphasic spikes recorded in a bundle to the local blocking action of the electrode. They are inverted in sign in comparison with what we recorded at the cell bodies, as would be expected.

These nerve impulses were 200–300 μV peak-to-peak. A good example is shown in Fig. 2*a*. Seldom could we record a single fibre in isolation. Various methods of passive filtering did not help us much in discriminating one impulse from another. Although, when spread out, the various wave shapes and heights could be told well apart from each other by eye, this information was not available in the collapsed records required by the slowness of olfactory events. Accordingly we used several crude expedients. One was a window filter, an amplitude selector which would gate in the record for a few milliseconds when the spikes exceeded a certain height. Occasionally we would use a sweep expander during those few milliseconds if we meant to discriminate the shapes of two spikes of the same height (Fig. 2*b*). Most often we would satisfy ourselves that we were seeing, through

the window filter, only two or three units, and thereafter use their comparative heights only. This, of course, is not an ideal way of doing things in the presence of so low a signal-to-noise ratio; but we were not yet concerned with those strict quantitative measures for which much finer discriminations would be needed.

The output of the window filter, the gated spikes, could be added on to the output of the d.c. channel and the gains independently adjusted for optimal display. The apparently noise-free nervous signals riding above the slow potentials have been so processed; the noise that has been excluded most of the time can still be seen in the fluctuations in height of the spikes.

In our measurements of impedance we used two of the gelatin salt-bridge pipettes touching the mucous surface a short distance apart. Through one we injected a sine wave of current, and with the other recorded the resulting wave form as it changed through the course of a slow potential. For this purpose we used a lock-in amplifier as the detector

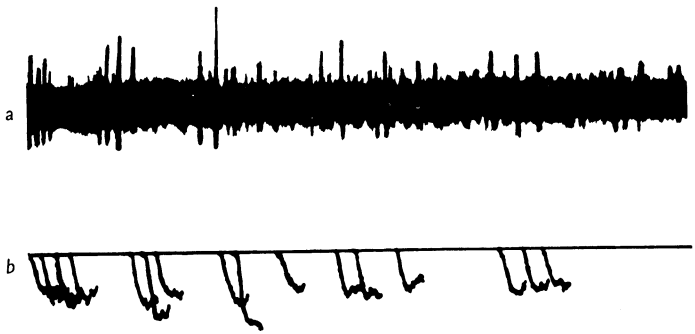

Fig. 2. Typical resting discharge recorded by a metal micro-electrode from cells of the olfactory mucosa. *a*, an unprocessed record displaying activity from a single unit close to the electrode (large spike) and other units farther away. *b*, the same signal after passing through a window filter, which opens for signals whose initial phase exceeds a preset amplitude, and recorded with the aid of a sweep expander which causes each signal occurring during the window opening to be expanded on the time base. The total sweep time is 10 sec. The superposed expansion sweep lasts 4 msec. Of particular interest in the expanded record are signals from two units with nearly identical amplitudes but clearly different shapes. In these cases the electrode was subsequently repositioned so that different cells signalled with clearly different amplitudes. Activity from four different cells can be seen in *b*. The window circuit displays the falling phase of the spike whose rising phase triggered the window. Diode restoration sets the base line.

in an active bridge circuit, whereby we could track variations in amplitude or phase. The procedure is difficult since the injected signal must be small, in the order of millivolts at the mucosal surface, while at the same time the noise is fairly high, and the important frequencies are low. The matter will be discussed later in the text together with the rationale behind the measurement.

Stimulation methods. Very early in the research we discovered that it did not pay to give either a strong puff of any odour, or to give an odour repetitively. Under both circumstances the tissue changed its physiological properties, and became less responsive, often for half an hour or more. Certain stimuli, such as tobacco smoke, would cause irreversible changes in the response of the mucosa. Our criteria for the over-stimulated state were that the slow potentials became smaller and not easily evoked repetitively, and that the fibres became either unresponsive or responsive to almost everything. With such a tissue as this

it is really impossible to say what the normal state is, and in the end we must rely on those two unsatisfactory standards, experience and intuition. However, one may suppose that a freshly opened preparation is, in a sense, in the most normal condition; the diversity of response, and the briskness and repeatability of response were most marked in the early parts of any experiment. When these qualities were lost we would find either that circulation had stopped, that the mucus had become either very gummy or very watery, or that we had unwittingly insulted the tissue with an odour that had long-term effects. Butanol and indole were almost as bad as cigarette smoke in this respect, and had to be used very sparingly. Finally, the experiments were made tedious by our finding that stimulation by any set of odours, when done too frequently, switched the nose into the same poor state. We restricted ourselves to giving one puff or one pair of puffs every minute, this being the least time between stimuli that would allow us a long run. Often a natural impatience impelled us to stimulate once every half minute, but then we could seldom maintain the mucosa diversely reactive for over half an hour. The odorous puffs were of a strength capable of producing slow potentials on the scale we were using. For many odours this is a medium-high strength. However, the interactions shown are not due to saturation effects, for the slow potentials were only a fraction of the height they could be made to assume with truly strong stimuli.

RESULTS

Slow potentials

When a puff of odour hits the olfactory eminence, the surface generally goes negative from its resting potential (measured with respect to another part of the frog) and then recovers. If the puff is prolonged, the return to the base line is much slowed until the puff ceases; then it returns more rapidly. This was first found by Hosoya & Yoshida (1937) and by Ottoson (1956) and is illustrated in curve *A* of Fig. 4, Fig. 6, and curve *A* of Fig. 7. Ottoson also observed the initial positive transient seen in curve *B* of Fig. 7 for *n*-butanol; but he did not find the purely positive transient that we found occurs with methanol (curve *A* of Fig. 9) and that Takagi *et al.* (1960) found with ethers and chloroform. Ottoson felt that the initial positive transient for *n*-butanol was artifactual, due either to water vapour or to some other hidden cause. He also did not see the negative swing produced by turning off a puff of odour, which occurs with pyrrole in our studies (Fig. 12) and was first described by Takagi & Shibuya (1959).

One can systematically increase the size of the initial positive transient by manipulating the temperature of the stimulus stream (Ottoson, 1956), by applying current (Higashino & Takagi, 1964), and by repeated stimulation. In Fig. 3 repeated puffs of anisole were given, each before the negative swing from the previous puff had subsided. The size of the positive-going initial transient seems determined by the height of the negative potential from which it takes off, as if the onset of a new puff tended to pull the slow potential back toward some base line. This is a very drastic treatment of the mucosa, which is made less sensitive for about half an hour afterward. Furthermore, the positive swing can be

reversed in sign by a pretreatment as with pyrrole (Fig. 14, open arrow on curve *B*). Finally, the recovery of the negative potential frequently overshoots the base line, and there is a low slow positive swing as in Fig. 4, curve *B* (open arrow).

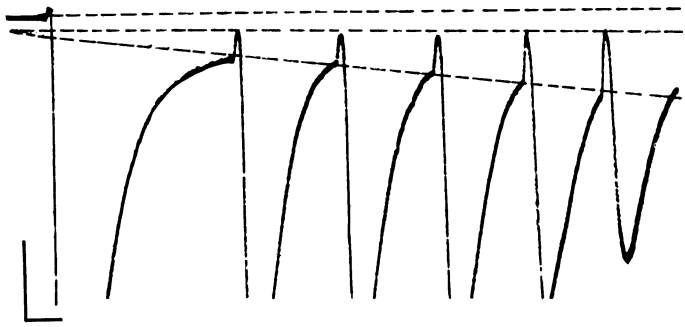

Fig. 3. These are slow potentials evoked by six successive short puffs of anisole. The scale shows 1 mV and 1 sec. Positive polarity is upward. Sweep time is 20 sec. The onset of a puff of odour causes first, a positive-going wave (top interrupted line is the base line) before the negative-going wave begins. With subsequent puffs the positive wave is larger as its initial voltage is farther away from the resting voltage, as long as it is occurring during the declining phase of a preceding negative swing.

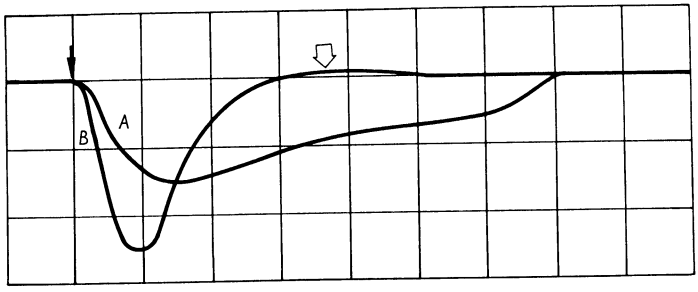

Fig. 4. These are responses of the mucosa (A), to a 7 sec low flow-rate puff of anisole and (*B*), to a 1 sec, high flow-rate puff of the same substance. The solid arrow indicates the onset of the puffs. The open arrow indicates the positive overshoot following the response. Sweep time is 10 sec; vertical scale is 1 mV per division, positive upward.

These phenomena led us to study the interactions of two slow potentials, generated either by repetition of the same odour, or by a succession of two odours. The results are seen from Fig. 4 to Fig. 17. Let us start with the anisole response in Fig. 4. Curve *A* is the response to a long weak puff and *B* the response to a short stronger puff, the puffs being delivered separately. The two puffs could be delivered simultaneously or with one lagging behind the other. The resulting slow potentials from such combined stimuli are shown in Fig. 5, where the long slow puffs were always

given at the same time on the sweep (first arrow) and the short strong puff was given simultaneously with the onset of the long puff (curve *A*) and at two successively later points on the sweep (curves *B* and *C*, later arrows). The shapes of the slow potential curves are not due to aerodynamic distortions of the stream, for if, instead of the short strong puff of anisole we deliver a short strong puff of air through the same channel, we record merely the response shown as curve *A* in Fig. 4. In Fig. 5 when the two puffs are delivered together, producing the response shown as curve *A*, the peak value of the recorded voltage is less than the sum of the peaks of the components, i.e. less than the sum of curves *A* and *B* of Fig. 4. Furthermore, the third second after the onset of the simultaneous puffs (curve *A*, Fig. 5) the potential has declined further than for the

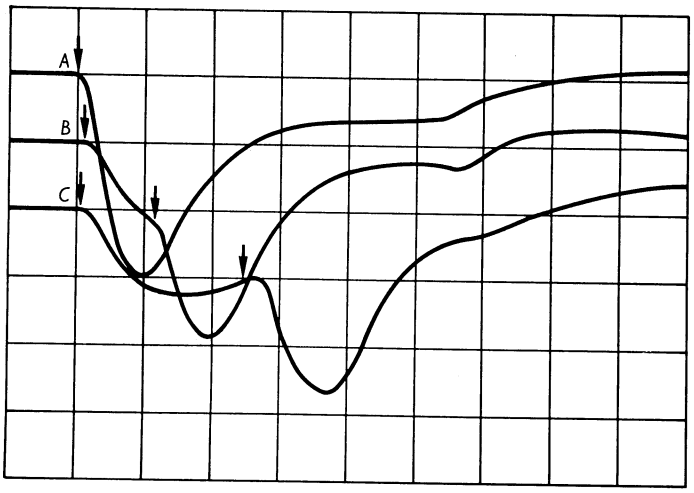

Fig. 5. These slow potentials occur when the two puffs of anisole whose responses are shown in Fig. 4 are delivered during the same sweep. *A* shows the result of both delivered simultaneously at the arrow. In *B* and *C*, the long, weaker puff was started at the first arrow and the strong, short puff was started at the second arrow. The scale is as in Fig. 4. The responses are essentially occlusive, i.e. the maximum amplitude is the same as the maximum amplitude of the short puff alone. The combination of the two puffs produces a quicker fall and larger overshoot at the end of the long puff than for the long puff delivered alone.

single long puff (curve *A*, Fig. 4). Both the occlusion of the negative swings and the increase of the following relative overshoot are shown more markedly in curve *B* of Fig. 5 where the short puff is delivered 1 sec after the onset of the long puff. The responses to anisole for a simple increase in puff duration with constant flow rate are shown in Fig. 6.

The situation changes, however, when instead of giving a short puff of anisole after a long puff we give a short puff of *n*-butanol after a long puff

of anisole. Figure 7 shows the response to each separately. Figure 8 shows responses to paired puffs with successively increasing delay of the onset of the butanol puff. The occlusion of the negative potential is much less, but the initial positive swing for butanol is enhanced in proportion as the potential at which it occurs is more negative, provided that only the

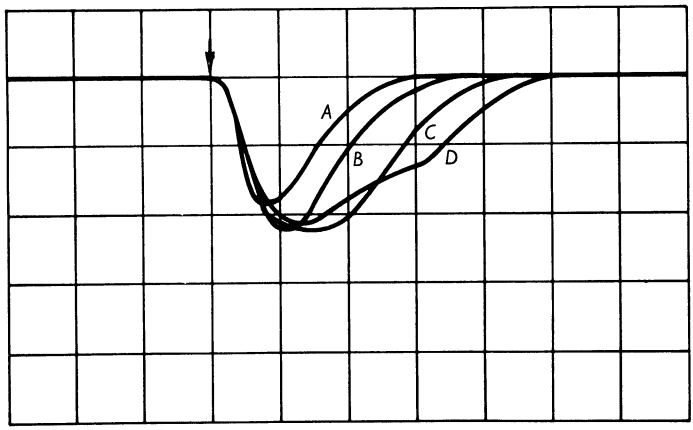

Fig. 6. These are responses of the mucosa to puffs of anisole of increasing duration and constant flow rate. Scale is as in Fig. 4. Four responses are shown superposed. The slope of the rising phase decreases as the puffs become longer, a sign of an *off* response. The arrow indicates the stimulus onset. The shortest puff lasted about 0·5 sec and the longest about 3 sec.

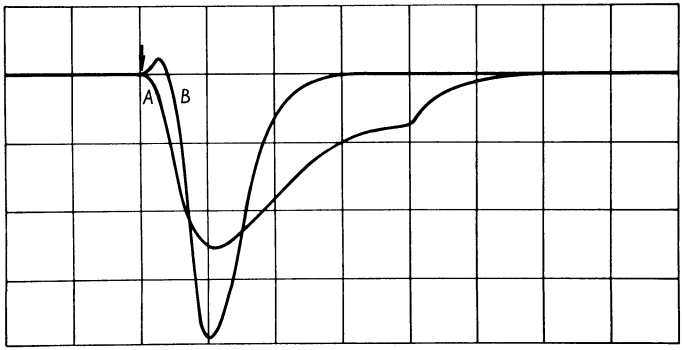

Fig. 7. On the same scale as the preceding figures are shown the response to a long, moderate strength anisole stimulus (*A*), and the response to a short, stronger *n*-butanol stimulus (*B*). The arrow indicates the stimulus of onset.

falling phase of the negative wave is considered. It is not as markedly so for an initial positive potential occurring during the rising phase, i.e. the positive transient of curve *A* of Fig. 8 is much smaller than for curve *C* (open arrows).

When butanol interacts with methanol some of the resulting curves are bewildering. The two stimuli, given separately, produce the responses shown in Fig. 9: notice that the potential from butanol is not a simple diphasic curve; but that the rising of the negative phase shows a place where the rise is accelerated (open arrow). This is not an artifact, as will be seen better in the case of pyrrole; instead it represents something like an *off* response to the sharp stop of the short stimulus. It is not proper

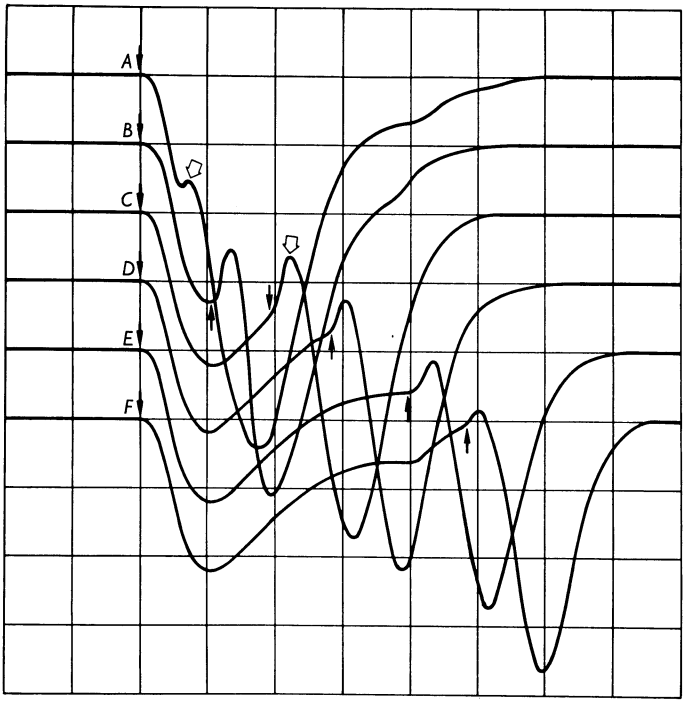

Fig. 8. Responses to the combinations of the two stimuli whose separate responses are shown in Fig. 7. The first arrow on each trace indicates the onset of the puff of anisole. The second arrow indicates the onset of *n*-butanol. The scale is the same in the preceding figures. The open arrow on curve *A* indicates the initial positive-going swing due to *n*-butanol, which is small when it occurs during the rising negative anisole response. The open arrow on curve *C* indicates a strongly enhanced initial positive swing due to *n*-butanol. The enhancement is larger, the farther the starting voltage is away from the base line providing that it occurs after the anisole puff has ceased.

yet to speculate on the variety of interactions here, except to assure the reader that none of them are aerodynamic. Two prominent features, however, must be noted. First, the response shown as curve *A* of Fig. 10 occurs when butanol follows methanol by a short time so that the positive swing of butanol ought to lie on the top of the positive swing to methanol.

In fact the initial positive swing to butanol is either flattened as in curve *A* or inverted in sign as in curve *B* (open arrows). This does not happen when the butanol onset is much later than the time that the peak of the methanol response occurs. Secondly, the negative phase of the butanol response is sharply truncated in curves *D* and *E* of Fig. 11 (open arrows) and this interaction occurs only during the rising phase of the methanol response.

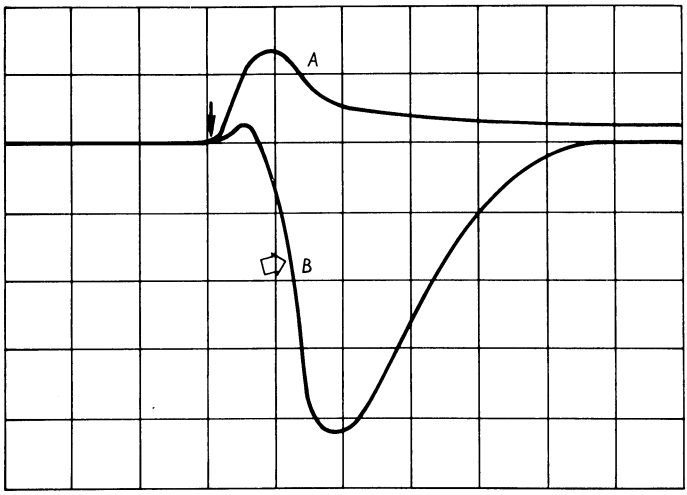

Fig. 9. Shown here are the responses, *A*, to methanol, and *B*, to *n*-butanol delivered at the times indicated by the solid arrow. The scale is the same as in preceding figures. An inflexion point in the negative rise is indicated by the open arrow.

From these interactions we move to the action of pyrrole. Figure 12 shows the responses to progressively longer puffs of odour of the same strength at a constant flow rate. Most salient is the response to turning off the stimulus, as if a new odour had been introduced. It can be seen even in the response to the shortest puff (curve *A*). It also appears, but is less marked, with anisole (Fig. 6). When butanol interacts with pyrrole, as in Fig. 14, there too in response *B* we see the inversion of the initial positive swing of butanol when it rides on top of the initial positive response to pyrrole (open arrow). The responses to butanol and pyrrole delivered separately are shown in Fig. 13.

Finally, there are the responses to two alcohols, methanol and ethanol. Ethanol most often produces a complex curve, strongly dependent in its details upon flow rate, dilution, duration and previous stimulation. Responses to stimuli of successively decreasing strength (flow rate) stimuli are shown in Fig. 15. There is an *off* response to ethanol that in part resembles the response to pyrrole. Methanol, on the other hand,

usually produces a clean positive potential as in the top group of Fig. 16 where three short puffs at different odour flow rates have been given. The middle group shows the response to equally short puffs of ethanol at three different flow rates. Here there is a positive-going *off* response occurring near the peak of the positive on response. (This can also be seen in curves C' and D' in the middle group of Fig. 17.) The bottom group shows the results of pairing these short ethanol and methanol puffs.

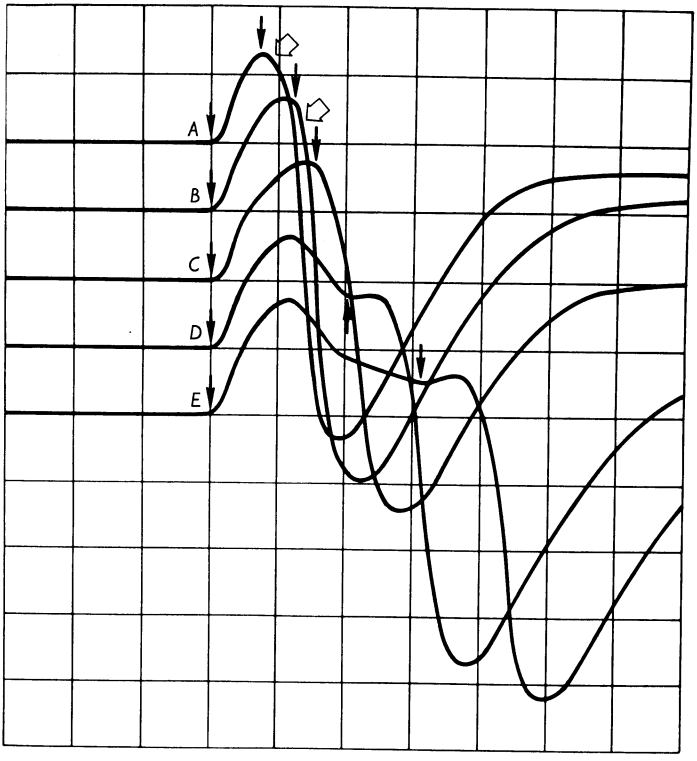

Fig. 10. These responses are evoked by combinations of the two stimuli which, separately, evoked the responses shown in Fig. 9. Methanol was delivered in each case at the time indicated by the first solid arrow. *n*-Butanol was delivered at successively later times as indicated by the second solid arrow on each curve. The open arrows indicate where the initial positive swing of *n*-butanol is expected. In fact, the initial positive swing is either not there or inverted in direction. The scale is the same as in preceding figures.

Figure 17 is the result of an experiment similar to that represented in Fig. 16 except that longer puffs of odour were used.

It is unnecessary to prolong this catalogue for our point. The slow potentials show linear and non-linear interactions. The superposition of the response to one odour on to a response to the same or another odour may yield a result in which the potentials simply add, or in which they

combine in very complex ways. The initial positive swing of some responses is enhanced by being evoked on top of the decline of a negative potential and can be reversed in direction when evoked on the rise or peak of a positive potential. There are negative *off* responses and positive *off* responses.

Yet all of this complex action occurs for a sheet of sense-organs that are uniformly oriented and anatomically independent, in which there are no synapses, and in which the connective tissue is so arranged as to suggest electrical isolation of each element from its neighbours.

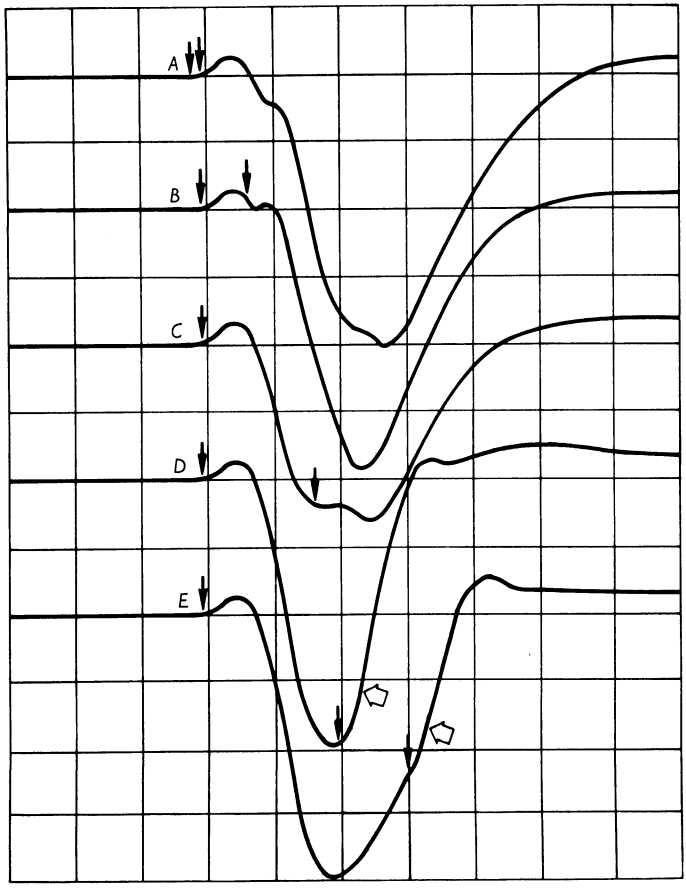

Fig. 11. Responses to combinations reversed in timing to those shown in Fig. 10. In this case *n*-butanol was delivered at the instant indicated by the first solid arrow and methanol was delivered at the time indicated by the second solid arrow. The responses are essentially additive of the separate responses shown in Fig. 9, except for curves *D* and *E* where the open arrows indicate a strong acceleration of the falling phase for *n*-butanol caused by the rising phase of the methanol response. The scale is the same as in preceding figures.

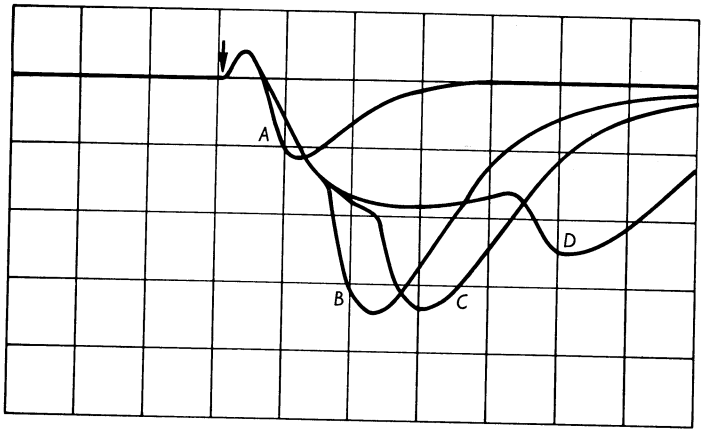

Fig. 12. Superposed responses to four puffs of pyrrole vapour of increasing duration and constant flow rate. Turning off the stimulus puff causes an increased negative swing as if a second stimulus had been delivered. This is clearly seen as the second negative-going wave in curves *B*, *C*, and *D*, and as the steeper slope of the negative swing of curve *A*. As in the previous figures, the horizontal scale is 1 sec per division and the vertical scale is 1 mV per division.

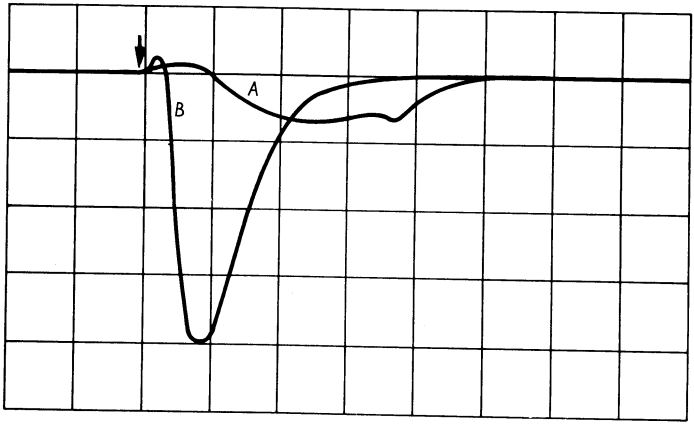

Fig. 13. Curve *A* is the response to a long, weak puff of pyrrole; curve *B* is the response to a short, strong puff of *n*-butanol. The responses of separate experiments are superposed. Stimuli were delivered at the time indicated by the solid arrow. Scale the same as in previous figures.

Nerve fibre responses

Many axons show a low rate of discharge at rest. This rate is almost rhythmic in some cases, and seems almost completely irregular in others. The effects of various odours on various fibres is shown in Fig. 18 to Fig. 27. There are several evident features. First, the response is not

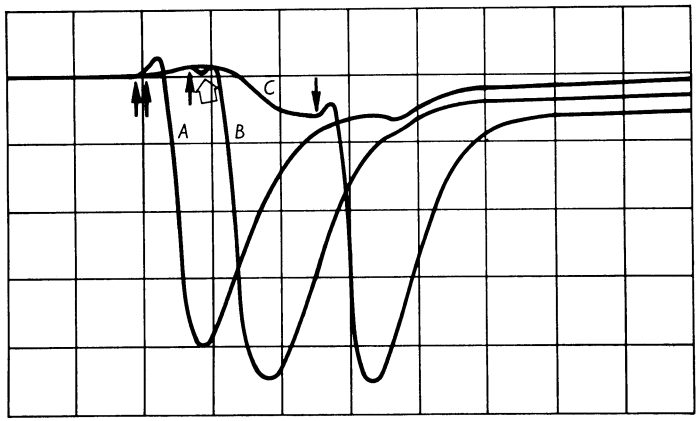

Fig. 14. Superposed responses to puffs of pyrrole and *n*-butanol delivered during the same sweep. The onset of pyrrole for all three sweeps is indicated by the first solid arrow. The other arrows indicate the onset time for the butanol puffs. Of particular interest is the inversion of the initial positive swing due to *n*-butanol when it occurs at the peak of the initial positive wave due to pyrrole (open arrow). Scale as in previous figures.

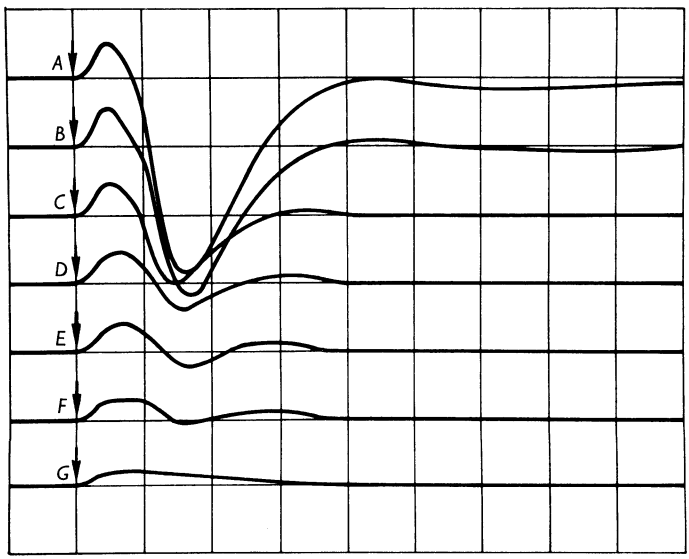

Fig. 15. Curves *A* to *G* show responses evoked by puffs of ethanol of equal duration but decreasing in strength (flow rate). The duration of the puff was slightly less than 1 sec. The scale is the same as previous figures.

determined by the slow potential (Figs. 18, 22 and 24). The individual axon gives far more discrimination than the slow potentials, and this is what we might expect. Secondly, there are three ways in which an odour may affect any fibre; it may excite it, inhibit it, or not affect it at all. The last or null-effect needs to be noted since these elements combine their information further on in the nervous system, and the lack of a response is contextually a signal. Figure 19 is the record of three units responding

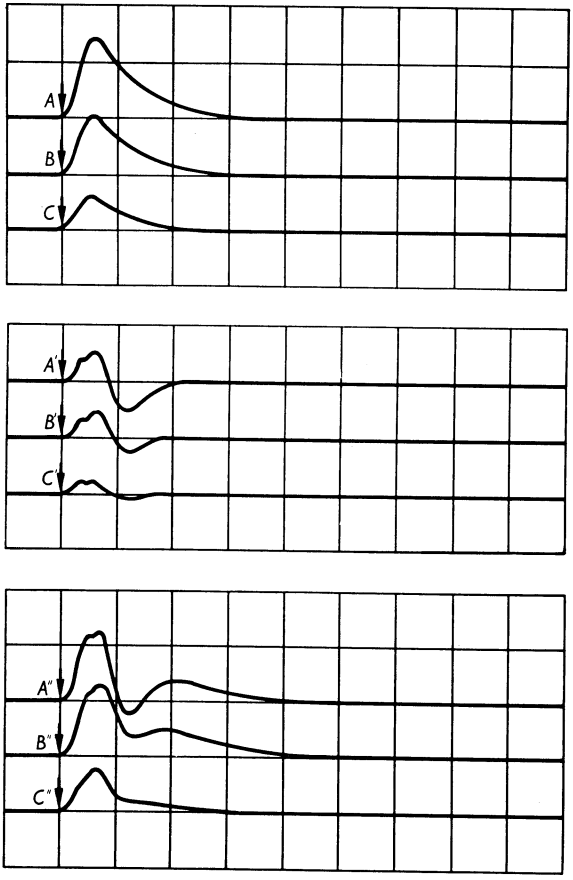

Fig. 16. The three responses at the top are due to puffs of methanol at decreasing flow rate but constant duration (less than $\frac{1}{2}$ sec). The three curves in the middle are due to ethanol puffs of decreasing flow rate but constant duration. The bottom group of curves is the result of paired puffs of methanol and ethanol. Curve A'' at the bottom occurs when the methanol puff which produced curve A at the top is delivered simultaneously with the ethanol puff which produced curve A' in the middle group. Similarly, B'' and C'' at the bottom result from combinations of B and B' and C and C'. Note the positive-going *off* response occurring at the peak of the positive-going *on* response in the middle group. Scales are the same as in previous figures.

to methanol. The largest spike is inhibited, the medium-amplitude spike is excited, and the smallest spike is not affected. It is very hard to see excitation in a rapidly firing element, and it is equally hard to see inhibition in a relatively silent one. For this introductory study we did not attempt to make a statistical analysis of our data to prove that we saw

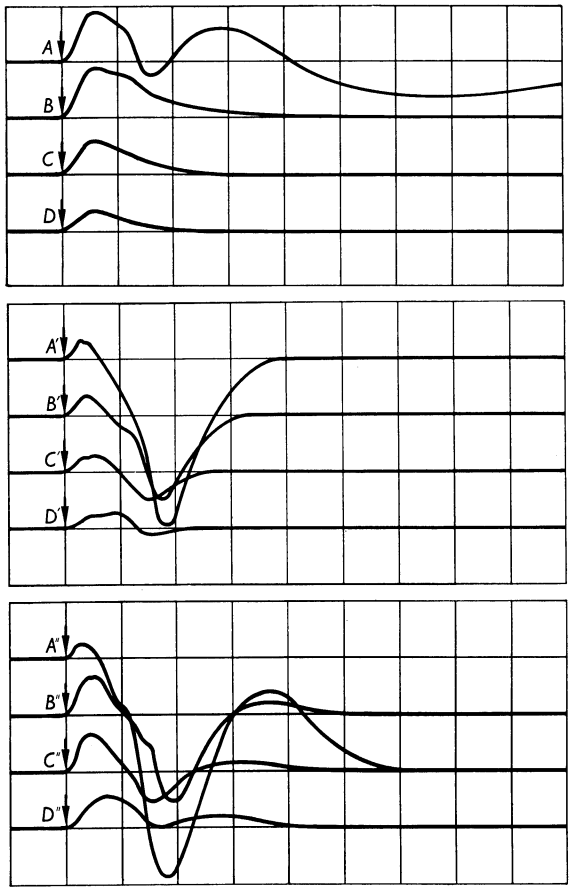

Fig. 17. As in Fig. 16, the curves in the top group are responses evoked by methanol, in the middle group by ethanol, and the bottom group by methanol and ethanol delivered simultaneously. Here the puffs were longer, approximately 1 sec. The scale is the same as in Fig. 16. The responses to single puffs and to pairs are extremely complex.

inhibition in rarely firing axons, although we are quite sure that it is the case. What is more, both excitation and inhibition are graded; that is to say we need a very strong puff of a mildly-exciting odour to imitate in part the effect of a very gentle puff of a violently exciting odour. Often

the response to an odour does not occur during the slow potential, but long afterward. The responses to three different odours that excite a unit to different degrees are shown in Fig. 20. Figure 21 shows the responses recorded from a different bundle including a unit that is weakly inhibited by one odour, more completely inhibited by a weaker puff of another odour, inhibited well after the puff for a third odour, and excited well

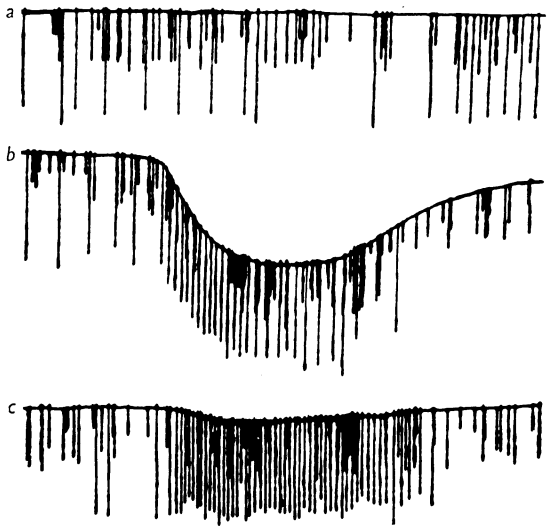

Fig. 18. These are micro-electrode recordings of unit activity. The top trace (*a*) shows the resting rate. Three units are active, one producing the large spikes of about 200 μV amplitude, another with about half this amplitude and a third with smaller spikes. The middle trace (*b*) is the result of stimulation with tetraethyl tin. The single-unit activity recorded by a metal micro-electrode is added to the slow potential recorded by a salt-bridge electrode. The bottom trace (*c*) shows the response to a weak puff of phenylethyl alcohol. It is clear that the activity of the cells is not predictable from the amplitude of the slow potential. Sweep duration is 10 sec.

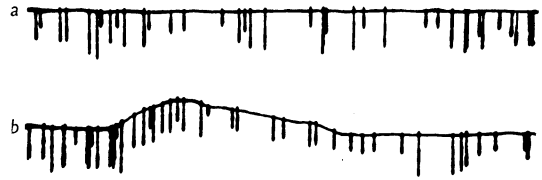

Fig. 19. Three units respond differently to the same stimulus, in this case a puff of methanol. *a* is the resting activity recorded by the electrode. In *b* the unit signalled by the large spike is inhibited by the odour puff, the unit signalled by the next-to-largest spike is excited by the odour, and the unit signalled by the smaller spike is little affected initially during the odour but excited later. Sweeps are 10 sec long.

after the puff for a fourth odour. And finally, the puff may not excite directly an increase or decrease in frequency, but rather an increased tendency toward rhythmic discharge which also is often seen after an obvious change in rate and tends to persist for tens of seconds at the strengths of odour we are using.

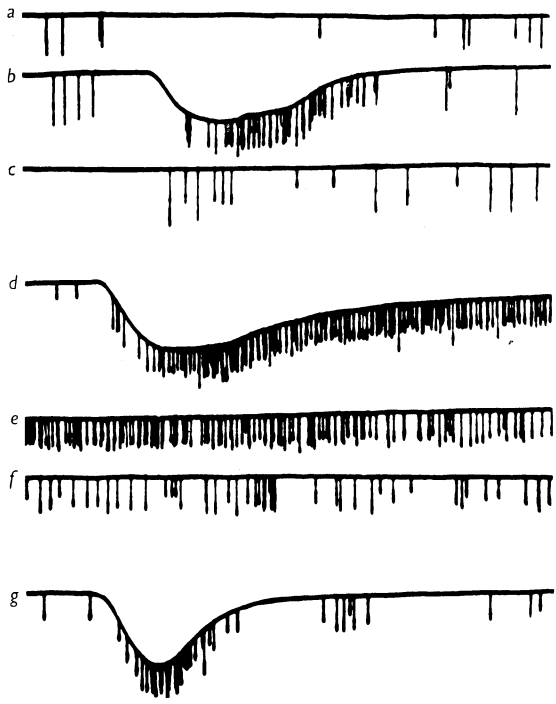

Fig. 20. These are responses of units showing graded excitation. The top trace, *a*, is the resting rate. Below it, *b*, is a burst of activity of the medium-amplitude spike in response to stimulation with diethylaminoethanol, followed, *c*, by a sweep showing the return to the resting discharge pattern. *d*, *e*, and *f* show a very strong excitatory response with a long-lasting excitatory after-effect due to tetraethyl tin, and *g* shows a weak excitatory response resulting from stimulation with geraniol. Sweeps are 10 sec long.

If we take the resting rate as the quality we study, the principal conclusions that we can draw from the records are these:

(*a*) Some units that are excited by some odours are inhibited by other odours.

(*b*) If two odours excite one unit equally, they need not have the same action on another unit; and this is the case, also, for inhibition.

(*c*) Stopping an odour may have an effect, or else an odour may produce a delayed response, and the effect may be either excitatory or inhibitory, or simply a more rhythmic firing pattern or entrainment.

Whenever we have taken two odours that seem much alike to us, such as menthol and menthone, or nitrobenzene and benzonitrile, and have found that the first units we observe respond similarly to both, a short search discovers a unit that discriminates between the two. Figure 22 illustrates this. Figures 23 to 27 show some additional details and are self-explanatory. We have printed the pictures with high contrast but have taken no further measures to make all spikes clearly distinct in height for easy tracking. The fluctuations in height of a single-unit spike are due to the noise level, and although the data can be processed to rid us of ambiguities, we are not yet prepared to undertake the fine analysis that warrants that additional apparatus. We have not been able to use a wide

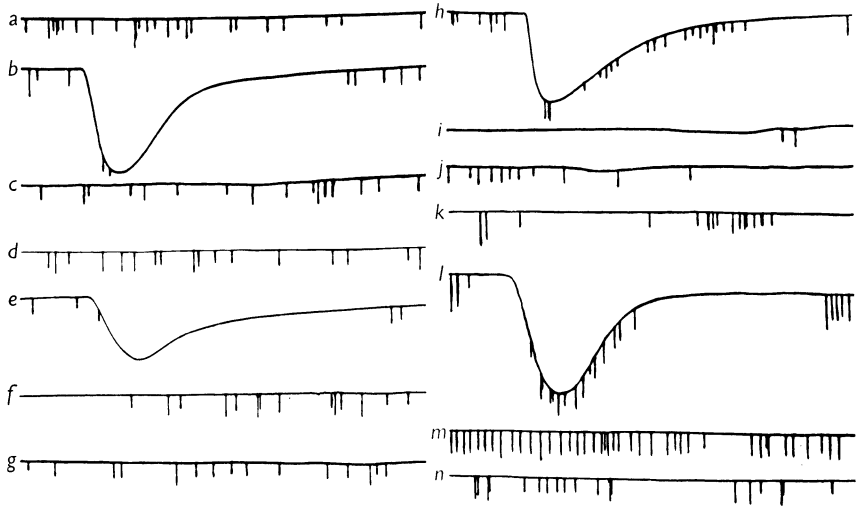

Fig. 21. Responses of units demonstrating graded inhibition. *a* is the resting discharge pattern; *b* shows weak inhibition of all the spikes resulting from strong limonene stimulation; *c* shows the return to resting level; *d* is the resting rate again, and *e* and *f* show a more complete and longer lasting inhibition of the small spike resulting from a short, weak puff of tetraethyl tin; *g* shows the resting rate again; and *h* shows the slight initial excitation followed by long-lasting inhibition produced by camphor; *i*, *j*, and *k* show the return to the resting rate. *l* shows the response to diethylaminoethanol, a late inhibition which is long-lasting for the small unit and strong, late excitation of a larger unit *m*. *n* shows the return to the characteristic resting activity.

variety of odours, for the experiments are slow to do, and our puff rate of 1/min does not allow much search in the course of an hour, for we must recheck the responses of a fibre to make sure that it has not gone into a different state, and must also adjust the strength of a stimulus over several puffs. Nevertheless, we can say that the olfactory classifications that have been suggested, e.g. the very attractive steric hypothesis of

Amoore (1964), do not seem to give us high predictability yet for the action of one odour from the action of another. It is probable that the groups can be shown by a more extensive search and a statistical analysis on far more data than we have. In any event, our study was not directed at a chemical basis for a theory of smell, but only the physiology of the receptors, in so far as it provides a mechanism whereby chemical information is transformed to nervous signals.

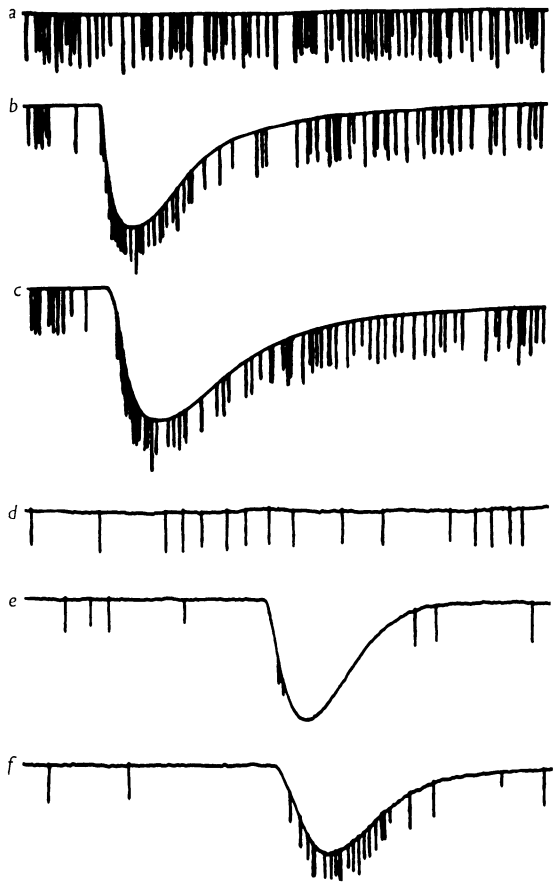

Fig. 22. The top trace, *a*, shows the resting rate of a unit which responded similarly (the spike slightly smaller than the largest in the group shown) to menthone, *b*, and menthol, *c*. Both odours which, to man, smell very similar, mildly excited the unit. *d* shows the resting rate of another cell which sharply separated the two odours. *e* shows its response to menthone and *f* its response to menthol. Sweeps are 10 sec long.

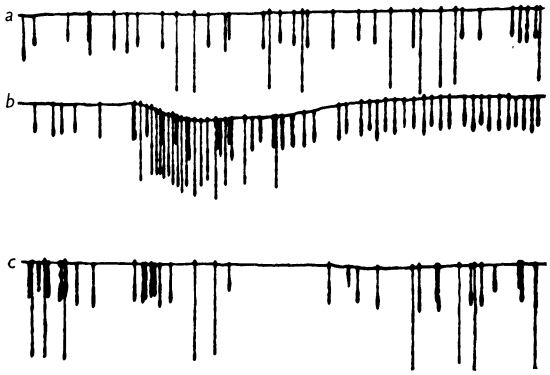

Fig. 23. Different time courses of excitatory response to the same odour are illustrated for the two units whose resting rates are shown in trace *a*. On stimulation with a weak puff of diethylaminoethanol *b*, the unit which is signalled by the large spikes fires rapidly at the onset of the stimulus. The unit signalled by the small spikes is excited following cessation of the stimulus. *c* shows alternating periods of activity and silence that are often seen following a stimulus as the units approach their low and irregular resting activity pattern.

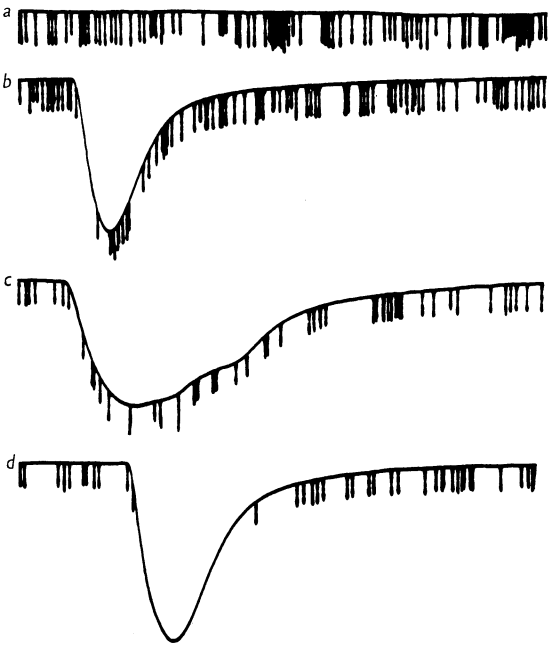

Fig. 24. A particular unit may respond in different ways to the same stimulus as strength and duration are varied. Trace *a* shows the resting rate. *b* is the response to a very short puff of limonene of medium strength. *c* is the response to a weaker, much longer puff of the same substance, and *d* is the response to a very short, very strong stimulus. Controls between stimulations were as in *a*. Increasing strength means an increased rate of flow through the stimulus bottle. Sweeps are 10 sec long.

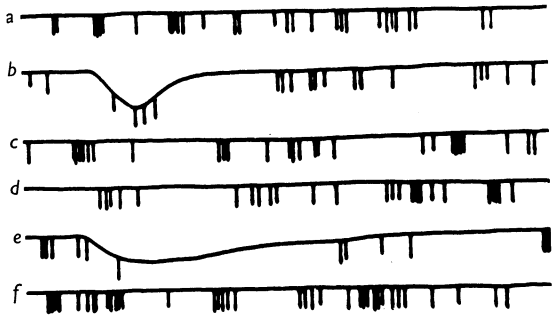

Fig. 25. Responses of a single unit to a short, weak puff of ethanol, *b*, and a long, weak puff of the same substance, *e*, showing graded inhibition. *a* is the background rate and *c*, *d*, and *f* show the after-effects of the stimuli. Sweeps are 10 sec long.

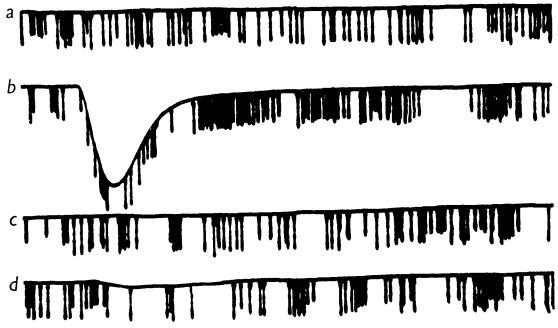

Fig. 26. Excitatory, bursting discharge patterns characterize responses of some cells to some odours. *b* shows the unit signalled by the smaller spikes being slightly excited by benzonitrile and the unit signalled by the larger spikes firing in bursts of very many spikes following the stimulus. *a* is the resting rate and *c* and *d* show the return to it. Sweeps are 10 sec long.

Impedance changes

As will appear in the discussion, it is difficult to conceive a single process generating the slow potential changes. Not only are there two polarities of response, but also linear and non-linear interactions between responses. The slow potentials probably arise as a consequence of changing generator currents, for in our experiments not only can we rule out a poisoned electrode potential, since the contact is with a saline bridge, but also we can draw current from the responses. If these currents arise as a result of changing ionic permeability of the membrane somewhere on the receptors there ought to be an associated change of impedance across the tissue. The receptors are not so densely packed as in other sensitive epithelia,

such as the retina; but if the interstitial supporting cells, wrapping the olfactory rods, are not reactive to odours, some part of the current applied to the mucosa must flow through the rods and associated structures. Furthermore, since this tissue is bounded by air there would be less shunt than in such tissues as retina. Finally, if there were more than one process involved in the generator current, they might be distinguished by differences in the changes of impedance. What we were searching for was not so much a measure of what was happening during the slow potential as a way of distinguishing between generator processes. To that limited extent we succeeded.

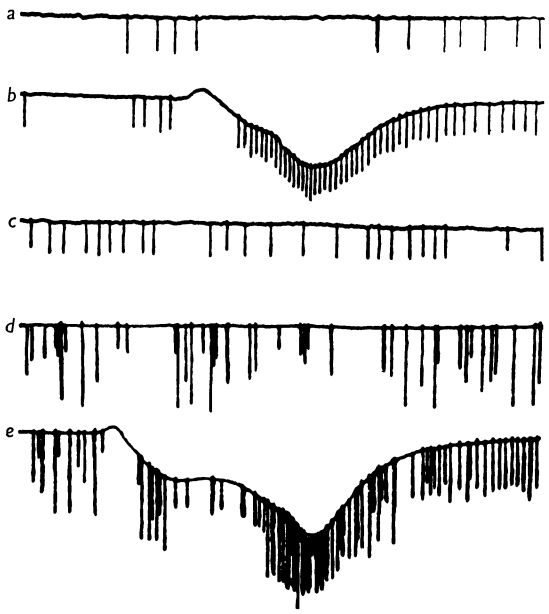

Fig. 27. Responses of different units to the same stimulus can be very different. *b* shows a single unit strongly and simply excited just before the *off* of a short pyrrole puff. *a* is the resting rate and *c* is the activity about 1 min after the stimulus. *d* shows the resting rate of several cells recorded from another location. *f* shows the responses to pyrrole, excitatory activity of the large unit during the ascending phase of the *on* response and during the *off* response, little change in the activity of the smallest unit, and weaker but continuous excitation of the medium-amplitude unit after the *off* response.

Consider what will happen in the vicinity of an a.c. current source applied between earth and a point on the surface of the mucus over the olfactory epithelium. Some of the current will flow to earth through the tissue directly beneath the electrode, and some through the tissue in the surround, the less the further away from the source. If the transverse

impedance of the tissue lessens, the current flow locally will be greater and therefore less will flow laterally.

Accordingly, local fluctuations in impedance will be reflected in changes of a.c. voltage recorded elsewhere on the surface of the mucus. Since we are working with a complex transmission sheet rather than a transmission line we cannot expect to make better than a qualitative observation.

When we used two 0·9 % saline electrodes about 0·3 mm apart in the region of the eminence and applied a 40 c/s current between one and earth through a blocking capacitor, we were able to record both the sine wave and the slow olfactory potentials with the other. In the recording system the sine wave could be balanced out differentially without affecting the slow potential, and so we could detect fluctuations in the a.c. signal amplitude. Profound changes could then be seen during the slow potential. However, two caveats were quickly apparent. First, if we applied currents in such a way that the peak-to-peak amplitude recorded at the junction of the current source and the mucus was as large as but no greater than the negative-going slow potential, the negative half-waves shifted in phase and became irregular and ragged at the peaks. This turned out to be due to the firing of subjacent neurones. It suggested to us either an extreme sensitivity of these cells, or that an appreciable fraction of the current must be passing through them. The situation was made very clear when we made a Lissajou figure of the applied current versus the surface potential at the junction of source electrode and mucus. Using the Lissajou figure as a monitor we finally determined that the maximum permissible a.c. voltage (yielding an elliptical figure) at the junction was about 2–3 mV in a freshly opened preparation. Secondly, it was apparent that we were getting a violent phase shift as well as amplitude change, and that the two could not be separated at frequencies above 60 c/s. We used a lock-in amplifier as a detector for the unbalance signal from the bridge circuit. At a penalty of some loss of time resolution (about 0·2 sec) we achieved a large enhancement of signal-to-noise ratio since the instrument responded only to signals with a fixed phase relation to the measuring signal. Further, since the reference signal can be shifted in phase, the instrument can be tuned to read magnitude changes or phase changes in the signal. We found that maximum separation of amplitude and phase-shift occurred between frequency limits of 28 and 40 c/s. This low frequency put serious constraints on time resolution of the events occurring during a puff of odour. If we used a sharp, narrow band-pass filter the Q was so great as to cause the amplifier to ring, whereas with a long integration time to smooth the signal we could not resolve events during the short early positive phase of the slow potential.

The specific operation of the lock-in amplifier is as follows. The recorded sine-wave signal at, say, 40 c/s is balanced with a signal of the same frequency fed through an attenuator and phase shifter. Then the amplitude is put slightly out of balance to provide a base-line signal. This in turn is fed through a tuned-amplifier and thence to an electronic switch that inverts the signal every 180°. When the switch is set to the zero crossings of the sine wave, the result is a full-wave rectification. Variations in the averaged magnitude of this signal give a measure of the change in amplitude of the input signal, since the output is relatively insensitive to phase changes. When the switch is set for the maxima and minima of the sine wave, the symmetry of the resulting waves around the zero-crossing

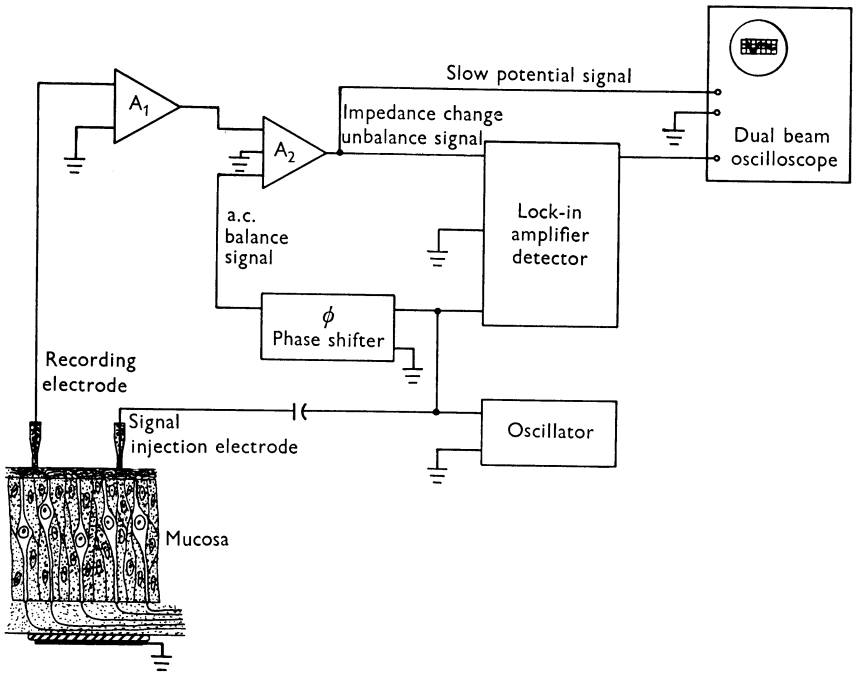

Fig. 28. Schematic diagram of the apparatus used for measuring impedance change. The lock-in amplifier is a Princeton Applied Research Type JB-5. The slow potential is displayed on the upper beam of the oscilloscope and either the magnitude change or the phase change of the balanced a.c. signal on the lower beam. The amplifier A1 on the left is a high-input impedance, low-noise unity-gain electrometer followed by a Tektronix Type D High-Gain d.c. Amplifier in a Type 536 Oscilloscope. The other amplifier, A2, is a Tektronix Type 3A1 Dual Trace Amplifier in a Type 565 Oscilloscope used in the differential mode to subtract the a.c. balance signal from the a.c. signal recorded by the micro-electrode. A section of the mucosa under the electrodes is drawn to give some indication of the anatomical relations. The preparation consisted of a pithed frog with strong circulation, not an isolated piece of olfactory epithelium.

yields an average amplitude of zero if there is no shift in phase but only an amplitude shift. When a phase-shift occurs it sets up an asymmetry between the part of the sine wave below the zero-crossing and that above, and so gives a change in averaged magnitude which is relatively more sensitive to phase shifts of the input signal than to amplitude shifts. Thus, by first balancing the detector for zero-crossings and then delivering an odour puff we detect essentially a change in the magnitude of the impedance seen by the electrode. Then, by rebalancing the detector for null output and delivering another puff of odour, we detect changes in the signal which principally measure changes in phase angle of the impedance seen by the electrode. Figure 28 is a block diagram of the apparatus.

Figure 29 shows impedance magnitude and phase angle changes which

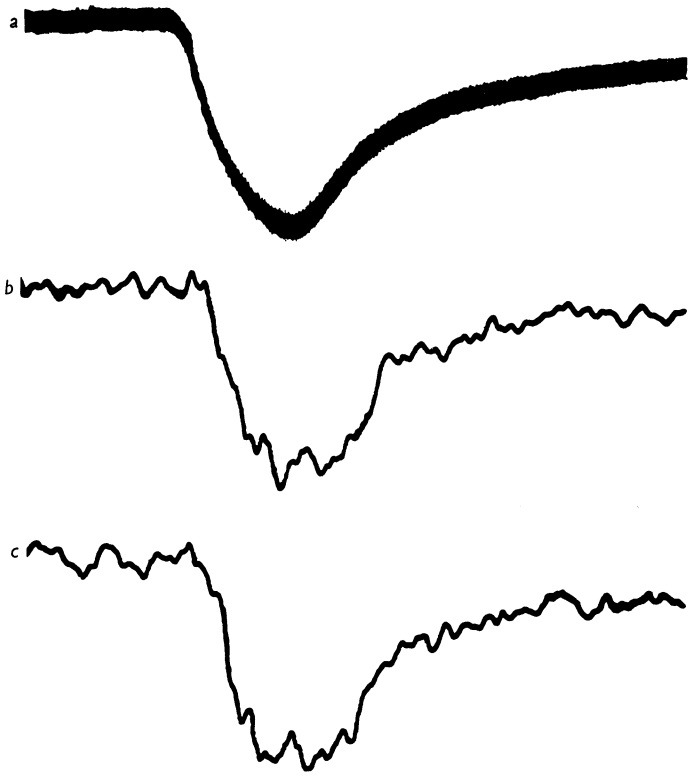

Fig. 29. Stimulation with a puff of tetraethyl tin produces the slow potential, *a*. The width of the line is due to the incompletely cancelled 40 c/s a.c. impedance measuring signal. *b* shows the variation in magnitude and *c* shows the variation in phase angle occurring concomitantly with the slow potential. *b* and *c* follow essentially parallel time courses. These changes are quite small, of the order of 1–5% variation in the magnitude and phase angle of the recorded signal. The amount of change depends strongly on the distance between the two electrodes. Sweep time is 10 sec.

occur during a puff of tetraethyl tin. In this case the two components change together. Figure 30 shows the results of the same experiment with pyrrole as the stimulus. Here the change in magnitude of impedance follows a quite different time course from the change in phase angle. Both measurements were made at a frequency of 40 c/s. If we measure at 70 c/s the result is shown in Fig. 31. The random noise components of the detected signal were not smoothed.

Fig. 30. The slow potential for a puff of pyrrole is seen in *a*. The magnitude change, *b*, and phase angle change, *c*, are taken at 40 c/s during the slow potential. With pyrrole, the magnitude change is long lasting while the phase-angle change is abruptly truncated at the cessation of the stimulus. Sweep time is 10 sec.

DISCUSSION

The resting firing rate of single fibres in the olfactory nerve is exalted by some odours and depressed by others. Since there are no synapses involved, these effects must be due to the direct action of the receptor mechanism on the spike generation. Thus, odours may be considered to act on the olfactory neurones as chemical transmitters are said to act post-synaptically at junctions elsewhere in the nervous system. A single

receptor must have at least two kinds of site (or active states of the same site) with respect to generator action. Each kind of site on one receptor cell may be activated by a variety of chemicals. We feel that every olfactory receptor is of this nature, but our limited supply of odours did not permit us to find the chemical signature of many of the elements we saw. Often we could find nothing in our array that would strongly alter the firing of a unit. Sometimes we could get a combination of two odours,

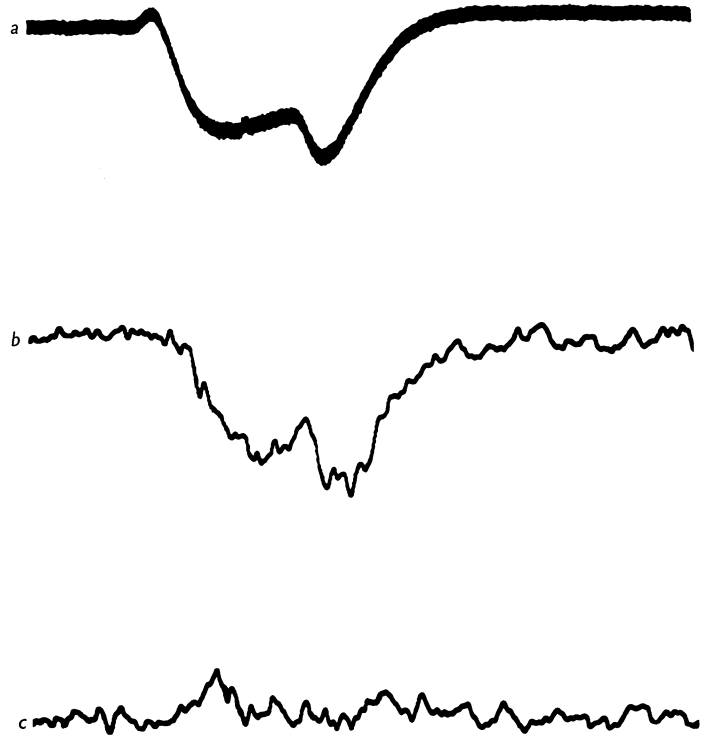

Fig. 31. These are the results of making the same measurement for a puff of pyrrole as in the preceding figure, but using a higher frequency a.c. signal for monitoring impedance, in this case 70 c/s. At this measuring frequency, the phase-angle change *c* shows little variation because it is swamped by the magnitude change *b*. Trace *a* is the slow potential. Sweep time is 10 sec.

say a musk and an alcohol, that together excited a fibre where neither compound alone had an appreciable effect. On this view, the physiological problem at the receptor divides naturally into two parts. First, is the question of what mechanism is involved in the activation of a site by a chemical, what the binding forces are, and what constitutes chemical similarity for a site. Secondly is the question of how affected sites pass on information, and how that information is represented in the nerve. This paper is concerned primarily with the second question.

We are inclined to think that the distal, chemically sensitive, part of the receptor communicates with the proximal soma axon by means of the electric current passing between them flowing one way externally through the surrounding tissue and the other way internally through the rod. Since there is a well-defined and large current flow between the surface of the mucus and the depths during stimulation, any appeal to diffusion of special materials down the axoplasm of the rod to the soma, or to some other intercalated event, is unnecessary, at least with the data we have now. If the slow potentials are generated by current flow from the sensitive ends of the receptors, providing many shunts everywhere across the mucosa will seriously attenuate the external signs of the generator currents in those cells that remain working but will not alter the nature of those currents. (A similar situation occurs with the retinogram in cases of retinitis pigmentosa, or other affections of the retina that provide many local degenerations.)

If the signal that governs the rate of axon firing is the current passing out through the soma-axon membrane, then we would expect excitation to occur by means of increasing that flow. This would be consonant with our measuring that the distal ends of the receptors go negative with respect to the soma axon in the external medium as measured by the slow potential (which diminishes as one penetrates the mucosa and becomes zero at the internal limiting membrane). Inhibition, however, can occur in a variety of ways. The chief mechanisms that occur to us are these: a counter-current is generated by inhibitory sites, or the excitatory currents are locally shunted out in the distal part of the receptor and so less current flows through the soma axon, or the excitatory generators are turned off. These various possibilities can be assessed by studying the slow potential, since it ought to reflect what is occurring in many elements simultaneously. The splendid anatomical isolation of the separate receptors and their orderly arrangement suggest we try a direct interpretation. The records show, in general, that positive swings of the slow potential are usually much smaller than negative swings; that the height of a positive swing is often enhanced by its being produced above the falling phase of a negative swing; and that some positive swings are inverted in sign when produced above the other positive swings. Indeed, the impression from the figures is that positive swings and their inversions tend to return the slow potential to a certain level by an amount that is related to the displacement of the potential from that level. Finally, we must say that for single unit studies, inhibition occurs most generally in many elements during a positive phase of the crudely recorded slow potential, and excitation during the negative phase.

These records bring to mind the sort of inhibitory shunt first observed

by Kuffler & Eyzaguirre (1955). The results make unlikely the notion that the positive-going transients are due to generators of counter-current. Consider such a generator site that on receipt of the proper olfactant causes current to flow outward, say by an ionic pump action. The pump would also have to be sensitive to membrane potential as determined by currents from other sites, and truly, this could be arranged to give greater current, the greater, say, the depolarization. But then, how does one explain the reversal in sign of transient on polarizing the membrane? Of course, except by direct experiment, one can never exclude this sort of mechanism which needs so much *ad hoc* modification. But there is no need to postulate any mechanism more complex than is needed to explain the data.

Thus, the only distinction we propose to make is between two restorative processes: (*a*) turning off sites that on stimulation pass current in either direction, and (*b*) locally shunting the currents from those sites so that less current passes through the soma axon. Here impedance measurements make a clear distinction. We cannot say what the processes are whose effect we are measuring when we do the experiment as described. But whatever causes the signal to fluctuate can be analysed into two components, in this case an amplitude change and a phase shift. If both always change together, or if only one changes, then, with respect to the measure we could not say whether we are seeing more than one process. But if both change together sometimes, and differently at other times, we must be detecting at least two processes. Suppose that we suspect two processes. Our strategy for determining that this is so is direct, since we are dealing here with changes of permeability to one ion or another. Such a change is a non-linear process since the ionic milieu is constant, and it is unlikely that the non-linearities are the same for the two ionic species. Since we know that a phase-shift can occur, we can find some frequency at which it is best separated from amplitude changes. If we find at some frequency that the phase shift varies differently from the amplitude change and in such a way as accounts qualitatively for the evidence on which we guessed at the two processes, then we feel reasonably justified in saying we have detected those two.

The *off* response in the slow potential to pyrrole is most directly interpreted as the resultant of a positive driving process that falls quickly when the stimulus stops and a negative-driving process that falls slowly when the stimulus stops. The *off* response would then represent the release of the second from the constraint of the first. The impedance records taken with a pyrrole stimulus (Fig. 30) show two comparable functions, with the magnitude change having the characteristic we have assumed for the negative-driving process and the phase change having the charac-

teristic of the positive driving event. Tetraethyl tin, on the other hand (Fig. 29), does not show so marked an *off* response and, as we would expect, the impedance magnitude and phase changes follow essentially parallel time courses. Both stimuli cause magnitude and phase changes in impedance that are not coincident in time at the onset of the stimulus and such is generally the case. This implies either a third potential-generating process or that the two processes which we describe have a complex relation to the potential. We have not been able to get much information about the events occurring early in the activation process.

What is most important in the impedance figures for pyrrole is that the magnitude shows no decrease at the time of the *off* response but instead continues its slow, orderly return to the base line. The low value of the negative slow potential during the puff cannot then be due to a diminution of the negative driving process. Thus we are able to exclude turning-off ion transport sites as the positive-driving and, possibly, inhibitory process.

We are then left, as the best guess now, with the Kuffler & Eyzaguirre mechanism. They recorded intracellularly, however, and so could suggest that the shunt was such as to drive the membrane potential to the equilibrium potential of some ion, say K^+ or Cl^-. We are recording only external currents and this is an indirect measure that permits us to say very little about what ions could be involved. Even the reversal in sign of the positive transient, when occurring on another positive wave, does not allow us to adduce a three-ion mechanism, since it could well be that the reversed sign was because that transient represented a particular ratio of increased Na^+ and K^+ permeability, whereas the wave on which it rode represented a different ratio (if we take these ions to act here as they do elsewhere in nerve). It would be idle to speculate further.

The mechanism whereby the chemically sensitive sites communicate with the axon seems well modelled by the present view of how postsynaptic patches communicate with the axon in central neurones. The olfactory neurones are combinatory of chemical information in the same non-linear way that a stretch receptor in the lobster is combinatory of information coming to it.

But still there are loose ends. One startling result of our impedance studies was that the application of a current source to the mucus, producing at that mucous junction a voltage to earth of the same order as that seen in large slow potentials, also sets off a firing in the subjacent neurones. The oscillations found by Adrian (1957) in the tract, and by Ottoson and others in the slow potentials of mucosa on prolonged or strong stimulation, are very similar to those seen in other overstimulated or poisoned nervous tissue. One case that immediately comes to mind is

the steady oscillation of the retina of the rat, and the entrainment and synchrony of nervous firing in the optic nerve after treatment with barbiturates (Brown, 1963). In such phenomena there has always been the hint that we are dealing with coupled oscillators for which the couplings are weak leakage currents. Associated phenomena are seen in peripheral nerve where the slightly staggered impulses in fibres of the same conduction velocity tend to pull into phase by virtue of the currents passing between them. It would cease to be a matter of surprise, then, that the olfactory mucosa, deprived of circulation, or injured chemically, goes into an hyper-excitable state, where all the single units respond with an increase in frequency to any large, negative slow potential and a decrease to every positive potential, for they are being entrained. It is not that the excitatory sites have all become of the same kind, but that any sites that are triggered set up a chain reaction, if they are dense enough, in neighbouring elements that have been made more sensitive to current. But whereas in other tissue, as in the spinal cord with the oscillatory dorsal root reflex, or in the retina with the barbiturate spindles, the phenomena are purported to be explained by complex nervous connexions, here there can be no appeal to such evasions. There just are no synapses. If we must be as cautious of such entrainment as our experience indicates, then nothing of consequence can be learned from decorporate heads and uncirculated mucosae with respect to how the olfactory system works, nor can we use puffs of smell that set up large slow potentials. This last caveat applies as well to the work presented here, for we must admit that for the sake of making our points about the mechanism of slow potentials we used stimuli that were larger than those we would use if we were studying the nature of chemical sensitivity.

The question of how olfactory fibres discriminate, if known in enough detail, would give valuable hints toward understanding the mode of transduction. We cannot claim to have enough information about odour discriminations. Yet there is a definite indication of what kind of a story we will find. It was pointed out that if we choose pairs of odours which smell similar to each other or which are sterically similar by Amoore's (1964) categories, we can often find a single fibre clearly discriminating between them without too much searching. Let us suppose in the very limit, that we have utter chaos; that every fibre is sensitive, more or less, to every odour from being greatly inhibited to being highly excited, and that the fibres are not like each other in their response to any group of odours. The universe of odours would then be seen by every fibre from a different point of view. It would be possible then, by simply having very many fibres, to distinguish very many compounds, for we would treat the characteristic of every fibre as a separate dimension. More

restricted transformations of this kind are used in other fields, e.g. the optical hologram or Fourier transformation of an image where every point in the transformation carries information about the phase and amplitude of every point in the initial image, and the initial image can be reconstructed from any small area in the final hologram. All that increases as one uses more area of the hologram is the resolution in the reconstructed image. But while such a mechanism is possible in the chaotic limit, it is unlikely to be so extreme for one reason: people tend to judge compounds as similar and dissimilar in much the same way. While no successful odour space similar to colour space has ever been constructed, it is undeniable that the neighbourhood of any odour shows a definite spatial organization. Metamerism of odours occurs between mixtures, and pure compounds can smell like mixtures. That metamers can be adjusted continuously until they smell alike tells us that the immediate neighborhood of an odour is continuous. But while we do expect to come up with a notion of similarity in smell space based on similarity in site affection, and in turn based on chemical similarity (Pauling once remarked that anything that looks like camphor on the molecular level ought to smell like camphor, a notion also pursued by Amoore), yet we also expect that the transformation from compound space to smell space partakes in part of the sort of integral operation we have considered in the limit.

SUMMARY

1. Fibres of the olfactory nerve often have a continuous, but noisy, activity. They are inhibited by some odours, excited by others, unaffected by yet others, and sometimes show a response in which noisy base-line activity becomes more rhythmic.

2. For any pair of compounds that excite or inhibit one fibre in the same way, with very little search one can find a fibre that is affected differently by the two. This observation applies so far only to the limited variety of compounds we used.

3. The slow potentials recorded grossly from the mucous surface over the olfactory epithelium are compounded of at least two processes that oppose each other. One kind of process tends to return the potential to a certain level or drives it positive; another kind displaces the potential in the negative direction.

4. These two processes can be distinguished not only by linear and non-linear interactions between slow potentials but by a crude measurement of impedance that, at a low frequency, identifies the positive-driving process with a change in phase, and the negative-driving process with a change in magnitude.

5. The positive-driving, or resting-level-seeking process, is tentatively identified with an inhibitory action of active sites in the receptors, and the other is identified with excitatory action of active sites.

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