

Electrophysiological Studies of a Water Receptor Associated With the Taste Sensilla of the Blowfly

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ABSTRACT Electrophysiological evidence is given that water is the specific stimulus for a fourth sensory cell associated with the taste sensilla of the blowfly. Water elicited impulses from a single cell which responded in two distinct phases: an initial rapid rate of discharge followed by a lesser, sustained steady rate. The latter, in the case of sucrose solutions, was inhibited in direct proportion to the log of the osmotic pressure over a 10^4 range of pressures. Other non-electrolytes inhibited, but the effect could not be simply correlated with parameters of the solutions. Electrolytes inhibited the water response more sharply and at lower concentrations. The inhibition in all cases was not dependent on impulses in the other sensory cells of the taste sensillum.

The taste sensilla of the blowfly are long hairs and associated sensory cells from which the electrical response of the primary taste receptors can be readily recorded (Hodgson and Roeder, 1956). Histological studies have revealed three sensory cells associated with these hairs. They are bipolar neurons with their cell bodies and nuclei located beneath the socket of the hair. The distal processes of two of the cells were believed to enter one lumen of the two in a hair and proceed to the tip. The distal process of the third cell appeared to terminate in association with the hair socket, suggesting a mechanosensory function (Dethier, 1955). Electrophysiological studies gradually showed the function of the three cells to be receptors for which adequate stimuli are salt, sugar, and motion, respectively (*cf.* Wolbarsht and Dethier, 1958).

Stürckow (1960) has reported that up to five sensory cells can be seen in histological studies of the chemosensory hairs of the blowfly, *Calliphora erythrocephala*. In addition, she observed three kinds of impulses in recordings from these receptors during stimulation with chemicals and states that they are from three chemosensory cells. However, she was unaware that Wolbarsht and Dethier (1958) and Wolbarsht (1960) had demonstrated the existence of

a mechanosensory cell associated with blowfly taste receptors, so that the third type of impulse could have been from the mechanoreceptor cell.

Recent physiological studies of "hunger" and "thirst" made it apparent that ingestion of water by the fly was controlled independently of ingestion of sugars (Evans, 1961; Dethier and Evans, 1961). Together with other evidence, this made it necessary to postulate the existence of an independent

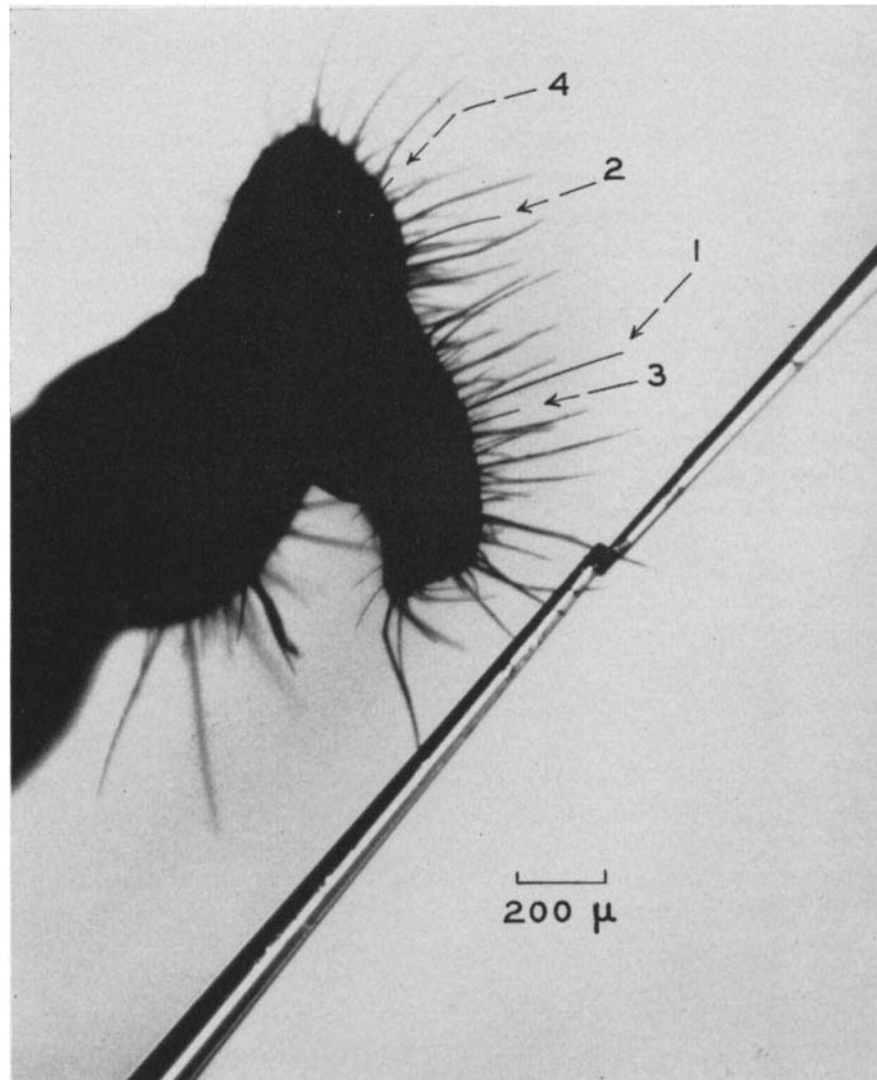


FIGURE 1. A lateral view of the blowfly labellum. The four characteristic sizes of the most peripheral hairs are numbered in order of decreasing size. One of the largest is clamped between two opposing glass capillaries, one of which is the fluid-filled recording electrode. (Photo by John Spurbeck—the numbered hairs were retouched.)

receptor for water. Accordingly, we sought electrophysiological evidence for such a receptor. A preliminary report of this work has been published (Mellon and Evans, 1961).

MATERIALS AND METHODS

The electrical response of the neurons in the chemosensory hairs on the labellum of the blowfly, *Phormia regina* Meig., was recorded by the method of Morita (1959). The method will be described in some detail since this has not been done previously. A photograph was obtained of the recording situation (Fig. 1). In it the labellum and part of the haustellum of the proboscis are shown in a lateral view. Fringing the labellum are several sizes of hairs, all of which contain chemosensory neurons. Between the opposing glass capillaries is a hair of the largest type (No. 1); all the following results were obtained from this type. Three other characteristic sizes are distinguished in the photograph. Unpublished observations by ourselves and others (Wolbarsht, personal communication) indicated differentiation among the hair types; the different sizes of hairs and even perhaps similar sizes in different positions on the labellum may differ in their relative sensitivity to various stimuli. While there is no evidence as yet, it may be that the four types of receptor cells described below are not found in all the hairs. The largest hairs apparently always respond to all four of the adequate stimuli.

The indifferent electrode, a saline-filled capillary, was used to hold the isolated proboscis; it was inserted into the cavity of the proboscis. The recording electrode is one of the pair of opposing capillaries in Fig. 1. Its electrolyte content can influence the receptors (see below); generally 1 molal choline chloride was used. By means of micromanipulators, the hair wall was gently cracked or crushed between the recording electrode and the opposing capillary (which merely serves to hold the hair in place) until the recorded noise level showed that the electrode was in effective electrical contact with the interior of the hair. Normally, the wax and chitin of the hair have a very high electrical resistance everywhere but at the hair tip. Stimulating solutions in a third glass capillary were now applied to the tip of the hair.

The signal was led from silver chloride-coated wires in contact with the electrolyte in the two electrodes into a high impedance preamplifier (MacNichol and Wagner, 1954), amplified further, and displayed on a cathode ray oscilloscope as described by Wolbarsht and Dethier (1958).

The method of stimulation by chemicals greatly influences the results. Evaporation from the capillary containing the stimulating solution rapidly concentrates the solution near the air-liquid interface (*cf.* Wolbarsht and Dethier, 1958). To prevent spurious results due to this factor, the solution in the stimulating pipette was blown out a second or less before contacting a hair, replacing the solution at the capillary tip with solution from a large reservoir connected to it. Because of evaporation and probably other factors, reproducible quantitative data have not been obtainable when stimuli were applied for prolonged periods. If, however, stimulation was restricted to a second or less, repeated tests gave similar results; all the present results refer to rates of response during the first second or less of stimulation.

In analyzing the time course of response to a constant stimulus, the method of Hartline and Graham (1932) was used. The duration of the intervals between impulses is the measure of frequency. To eliminate some of the random variability, frequencies are calculated from the mean of successive groups of four intervals; that mean frequency was arbitrarily assigned the time after application of the stimulus at which the second impulse in the group occurred.

Concentrations are expressed in molality units (M) to facilitate the conversion to activity and osmotic pressure that was necessitated by the high concentrations sometimes tested.

All of the quantitative data below were obtained under certain strict conditions: periods of stimulation no longer than 1 second, maintenance of a constant temperature (24°C), gentle handling of the preparation, and recovery periods of 4 to 5 minutes between successive tests.

RESULTS AND DISCUSSION

Spontaneous Activity

The question of activity in any of the sense cells in the absence of apparent stimuli has been considered by several investigators; there is reported to be little (Tateda and Morita, 1959, Fig. 3 and 4) or none (Wolbarsht and Dethier, 1958). Since a small amount of activity in each of the many receptors of a fly could be of considerable behavioral significance through spatial and temporal summation, the point has been reexamined.

The recording method of Morita (1959) that was used here is the only one available that can be used to directly observe the unstimulated state, but it contains several possible unapparent sources of stimulation. Since the preparation has a high impedance and high electrical sensitivity (Wolbarsht, 1958), the equipment used must have a suitably low grid current ($< 10^{-11}$ amps). Morita (1959) states that his equipment met this standard, and the MacNichol-Wagner preamplifier used here has a grid current below 10^{-12} amps (Wolbarsht and Dethier, 1958). A second possible source of stimulation is pressure of the opposing capillaries on the sensory cells. By gradually increasing the force between the capillaries, it can be observed that pressure does elicit impulses, resulting eventually in a short, very rapid burst of impulses after which no further response can be obtained to chemical stimuli at the hair tip. The neurons involved have not been identified. Similar consequences of injury by electrodes to retinal neurons were noted by Hartline *et al.* (1952). Finally, as Morita recognized, electrolyte from the recording electrode can stimulate the salt receptor. Wolbarsht (1958) has shown that regions of the distal processes of the chemoreceptors other than at the hair tip are differentially sensitive to chemicals when made accessible to the chemicals. When a chemical that stimulates the salt receptor (*e.g.* 0.1 M

NaCl) is used in the recording electrode, a low frequency discharge of a receptor is often observed, probably the salt receptor. By using a non-stimulating electrolyte (1 M choline chloride) in the recording electrode and controlled crushing of the hair, we have found numerous preparations in which there was no spike activity over periods of many minutes in the absence of intentional chemical and mechanical stimuli. While, of course, this does not prove the absence of spontaneous activity in these hairs, it does suggest that any such activity generally is extremely low when possible sources of stimulation are minimized. The "spontaneous" rate of a few impulses per second observed by Tateda and Morita (1959, Fig. 3 and 4), it is felt, could result from pressure or from NaCl ($\frac{1}{8}$ M) in their recording electrode.

Evidence for a Distinct Water Receptor

Stimulation with pure water (including three times glass-distilled water) elicited a rapid train of impulses of small amplitude (Fig. 2 *a* and 3 *a*). In long continued recordings, the spike amplitude was constant, apart from random fluctuations in the noise level; no electrical summations occurred; and no pair of impulses occurred close enough in sequence for one to be in the refractory period of the other. On the basis of the foregoing criteria then, water ordinarily activates a single neuron. The record of Fig. 2 *a* contains one impulse of another cell; in this it is atypical, but was included because subsequent records from that preparation show features that can rarely be demonstrated.

The question now arose whether it was one of the three known sensory cells associated with the hair or another cell that responded to water. Specific stimuli for the three known cells were shown by Wolbarsht and Dethier (1958) to be NaCl, fructose, and motion of the hair. We examined the question by testing sequentially water and water paired with each of the other stimuli; in each case either one or two spike types should appear in the recording. An aqueous solution of a stimulating sugar (*e.g.* sucrose or fructose) activated the sugar receptor as well as the water receptor (Figs. 2 *b* and 3 *d*). Likewise, at some concentrations of salt in water, the responses of both the salt and water receptors were observed (Figs. 2 *c* and 3 *e*). Typically, when recording in this manner, the amplitude of the salt and sugar receptor spikes is nearly identical (Figs. 3 *d* and *e*). In a rare preparation they are not, and the three impulses of the chemosensory cells are distinguishable during simultaneous stimulation by salt, sugar, and water (Fig. 2 *d* and *e*).

Exciting the mechanoreceptor without disturbing the recording conditions was a matter of chance. While water was applied to the hair tip, the recording electrode and its opposing capillary were quickly moved toward the base of

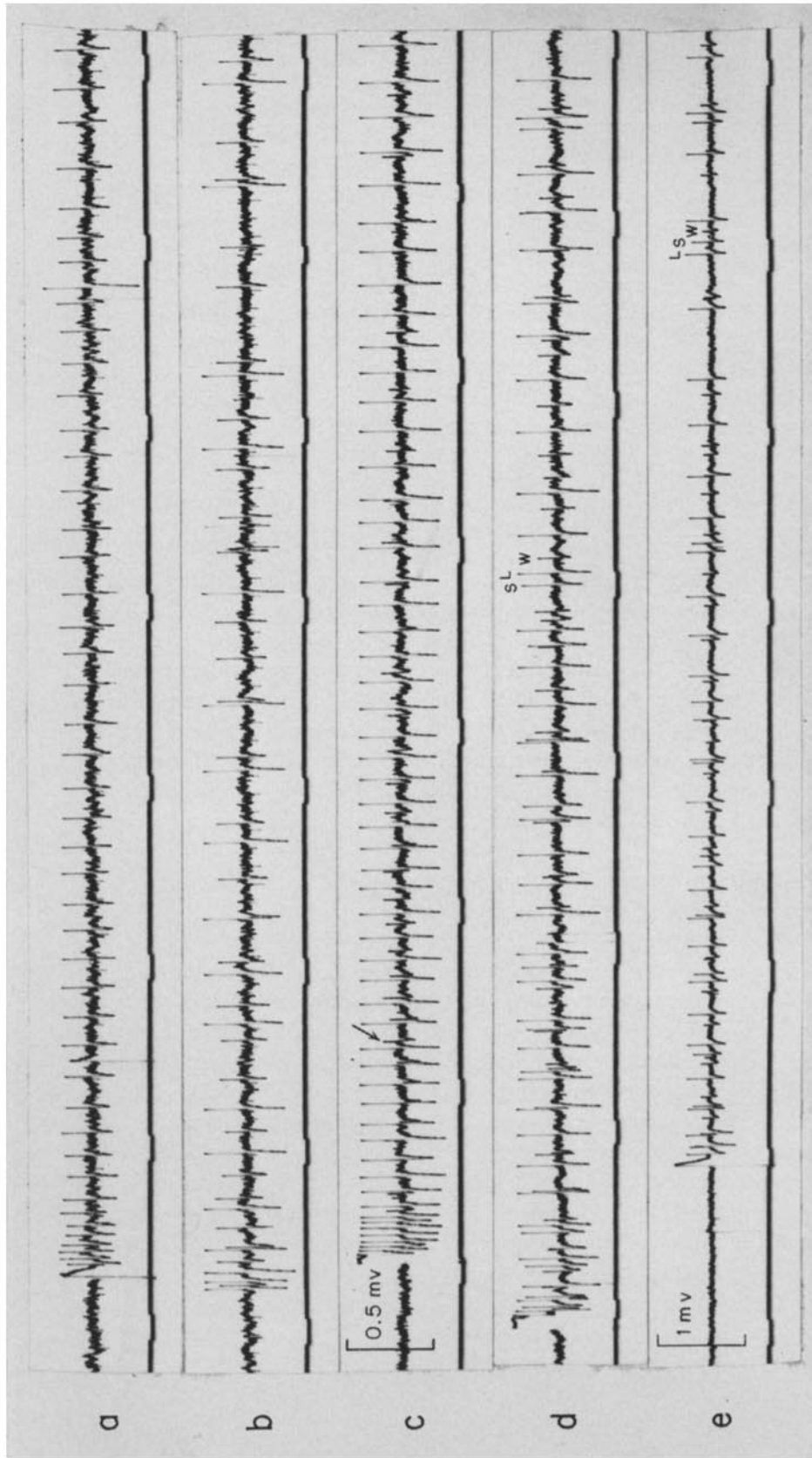


FIGURE 2. Electrical recordings of the response of a single taste sensillum to successive tests of several stimuli. In each case an artifact indicates the time of application of the stimulus. The voltage calibration in *c* applies to records *a-d*. Time base, a square pulse every 0.14 sec. (*a*) water alone (a single salt or sugar spike appears near the end of the record); (*b*) 2 M fructose; (*c*) 1 M NaCl (arrow indicates a water spike); (*d* and *e*) stimulating solution 2 M with respect to fructose and 1 M with respect to NaCl. L, S, and W indicate salt, sugar, and water spikes respectively. Occasional electrical artifacts occur (e.g. one before the application of the stimulus in record *e*).

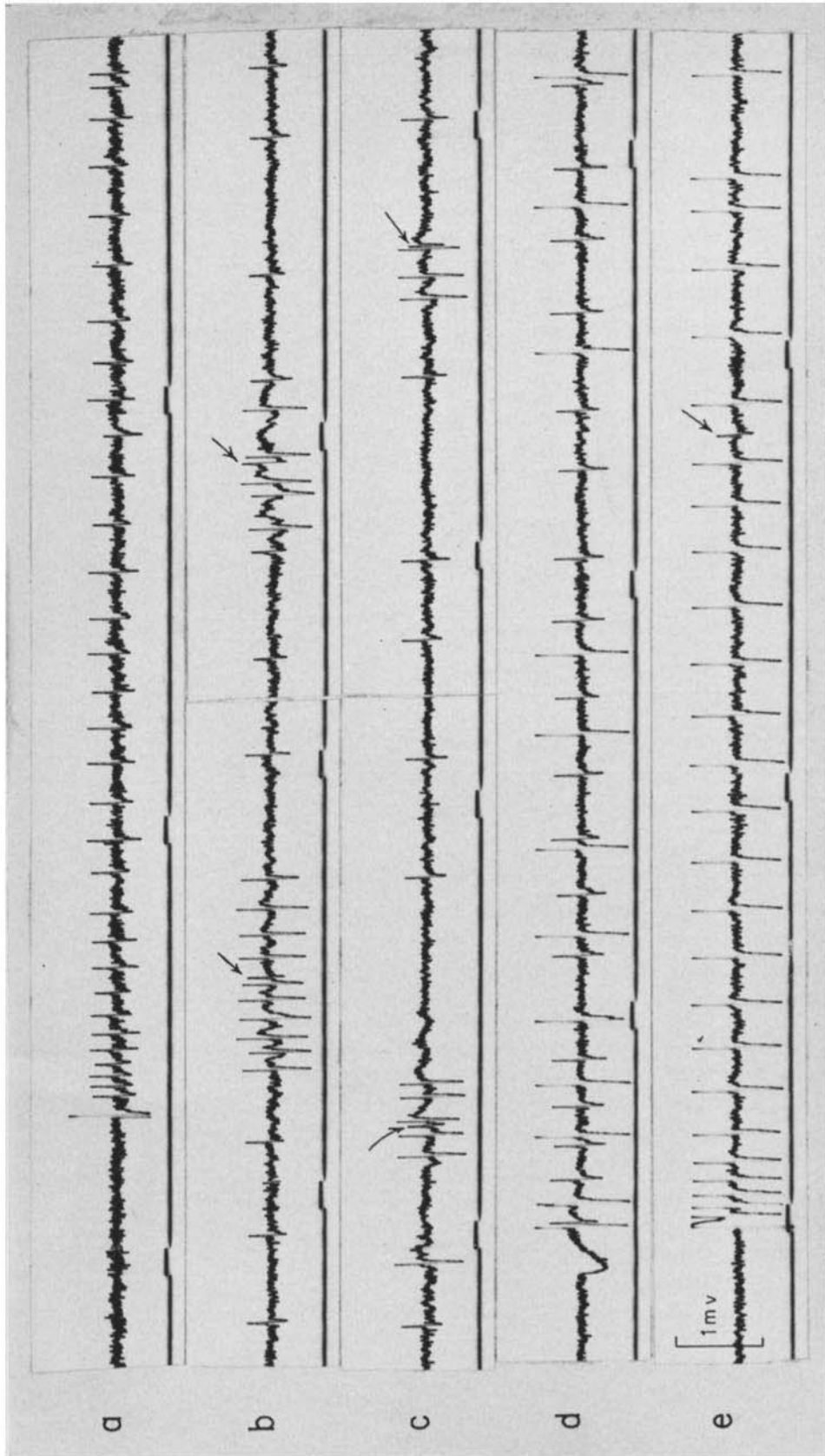


FIGURE 3. Electrical recordings of the response of a single taste sensillum to water and successive tests of three other stimuli paired with water. The burst of motion spikes (as indicated by the arrow) as well as before and after the bending. The responses to approximately equal mechanical stimuli show progressive adaptation. (d) 2 M fructose; (e) 1 M NaCl (an arrow indicates a water spike).

the hair, bowing the proximal hair shaft and stimulating the mechanoreceptor. In a fortunate preparation (Fig. 3 *b* and *c*), the contact of the electrode was not broken by the movement. The water impulses (arrows in Fig. 3 *b* and *c*) were smaller than the motion impulses and occasionally were observed to occur unaltered amidst a burst of motion impulses. These data are taken as direct evidence that water activates a fourth cell associated with the hair.

These results are in substantial agreement with the report of Wolbarsht (1957) that water alone evoked discharge of a cell in the hair and that addition of sugar sometimes stimulated another neuron.

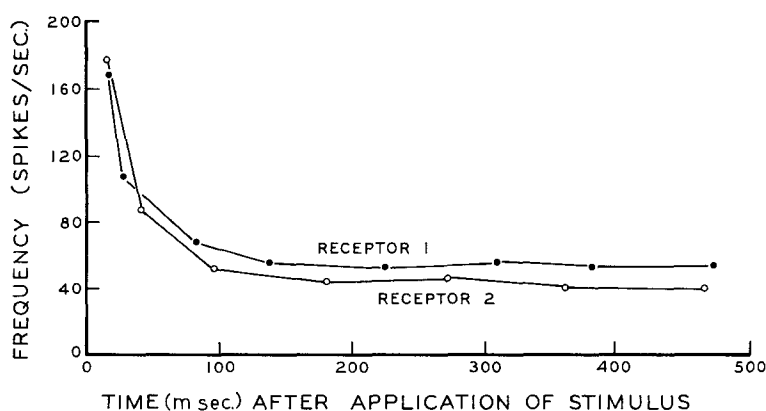


FIGURE 4. The time course of response to a water stimulus of two receptors. An initial rapid phase is followed by a lower, constant rate of discharge.

The Influence of Solutes on the Water Response

Water as a stimulus for a receptor poses an interesting problem of mechanism. We have obtained some evidence on the effect of solutes on the water response which has shown an interaction between stimuli for the other chemoreceptor cells and stimulation of the water receptor.

NON-ELECTROLYTES When an ascending concentration series of aqueous sucrose solutions was assayed on individual taste sensilla, the water response was found to decrease with increasing sucrose concentration. Since sucrose is among the most effective stimuli for the sugar receptor, either electrical activity in that receptor or the physicochemical properties of the solutions or both might be responsible for the depression of the water response. The quantitative relationships between rate of discharge of the sugar receptor, stimulus intensity, and time after application of the stimulus have not yet been adequately studied. However, two non-stimulating non-electrolytes, mannose and glycerol, also were found to inhibit the water receptor, so that

spike activity in other receptor cells was not necessary for the inhibition by non-electrolytes.

The first impulse after application of pure water to the hair tip occurred as soon as 6 msec. Solutes that inhibited the water response increased the latency.

The discharge of the water receptor occurred in two phases: an initial rapid rate of firing which quickly (100 msec. or less) decreased to a fairly constant level which was maintained for 500 msec. or more (Figs. 2 *a*, 3 *a*,

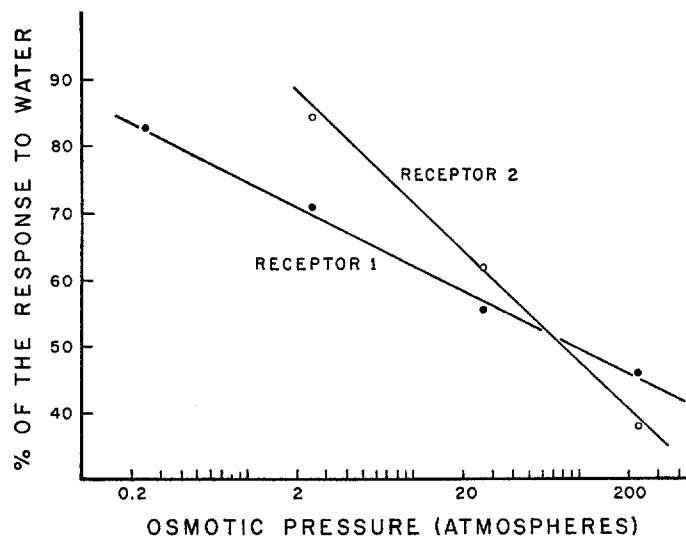


FIGURE 5. The response of two water receptors to a series of sucrose solutions as a function of the log of their osmotic pressure. The rate of response is the steady rate with reference to the steady rate evoked by water alone.

and 4). Inhibition by sucrose reduced the initial water response more than the steady rate; but a brief amplifier blockage upon contact of the stimulus with the preparation prevented accurate quantification of the initial response, and so the steady rate is used in the following analysis.

Of the solutes tested, only in the inhibition by sucrose was a uniform relationship found between a parameter of the solutions and the degrees of inhibition. Over a range of at least four log units, the inhibition of the steady water response was linearly correlated with the log of osmotic pressure (Fig. 5; experimental osmotic pressure values from Glasstone, 1946). By extrapolation it was found that the two receptors illustrated would first be inhibited when the osmotic pressure was 0.01 and 0.06 atmosphere or at about 10^{-4} and 10^{-3} M sucrose. Because of the small degree of inhibition indicated, experimental data were not sought below 0.01 M sucrose; and, therefore, the

possibility of a threshold for the effect above the calculated values has not been excluded.

While it is tempting to consider the water receptor an osmoreceptor, data on other non-electrolytes suggest the relationship may be merely fortuitous; even so it adequately describes the inhibition by sucrose. Glycerol solutions were tested in the same way; they did not evoke the response of any receptor cell other than water, but did inhibit the latter. However, no simple relationship was found between parameters of the solution and the

TABLE I
INHIBITION OF THE WATER RESPONSE
BY GLYCEROL AND MANNOSE

The relative frequencies for glycerol are the mean values from nine receptors and those for mannose are individual values from two receptors. In each case the frequencies are the steady rates between 100 and 500 msec. after application of the stimulus.

$\mu \phi$ *	Glycerol Response to water	M	Mannose Response to water
	<i>per cent</i>		<i>per cent</i>
0.1	92	0.1	104, —
0.5	86	1.0	93, 88
1.0	79	3.0	41, 48
2.0	60	5.0	36, 8
3.1	45		
5.25	31		
10.7	12		

* The molality times the osmotic coefficient (from Scatchard *et al.*, 1938).

degree of inhibition. The inhibition by glycerol was less below 1 M than in the case of sucrose, but above this concentration became much greater, until the response was nearly absent at 10 M (Table I). Since glycerol is a considerably smaller molecule than sucrose, a larger, non-stimulating carbohydrate (mannose) was tested. Unfortunately osmotic coefficients for mannose apparently have not been determined. At 0.1 M where the coefficient must be nearly unity, the inhibition resembled that by glycerol; it was less than that by sucrose; and like that by glycerol, the inhibition becomes much greater above 1 M (Table I).

Regarding the mechanism of the inhibition then, we can say only that it is not dependent on spike activity in other sense cells and that it is related to the osmotic pressure in the case of sucrose solutions.

ELECTROLYTES The only known chemical stimuli for the salt receptor cell are a few monovalent salts (Na, K, and Rb chlorides and nitrates are the most effective; Evans and Mellon, unpublished). Hodgson and Roeder (1956)

and subsequent investigators have observed two spike types during stimulation by dilute salt solutions; the one that disappeared when concentrated solutions were tested was attributed to the sugar receptor. Instead, the following evidence shows it to be the formerly unsuspected impulse of the water receptor cell. A series of NaCl concentrations and pure water were tested for their effect on the water response. In contrast to inhibition by non-electrolytes, the inhibition by NaCl had a sudden onset and rapidly became complete. In Fig. 6 is plotted the water response of a single receptor at

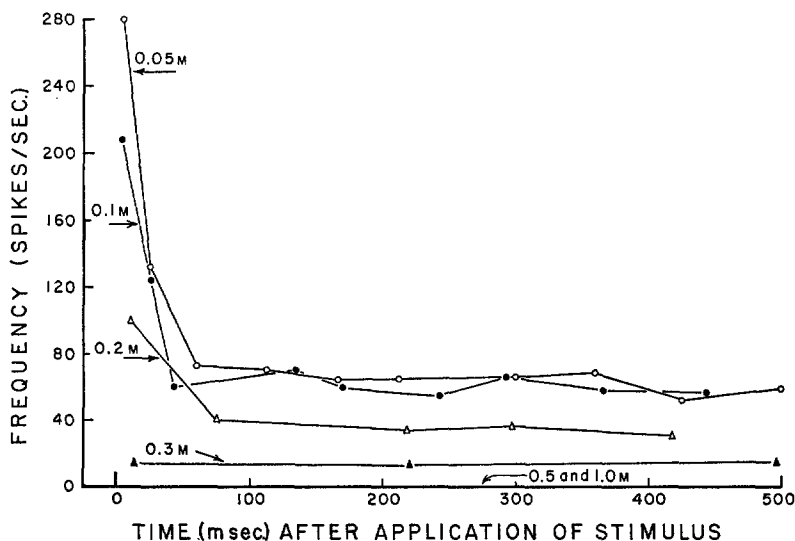


FIGURE 6. The time course of the response of a water receptor as influenced by NaCl at several concentrations. The two highest concentrations contained too few water impulses to count reliably.

several concentrations of NaCl. As before, there are two response patterns; an initial, rapidly declining phase and a steady rate of discharge. Both are essentially the same at 0.05 and 0.1 M NaCl. Between 0.1 and 0.3 M, the steady water response is reduced to less than a third of that to pure water. At higher concentrations, the steady rate is below countable levels. This inhibition is characterized by its sudden onset and rapid increase with NaCl concentration. As before the initial response was more markedly inhibited than was the steady response.

Again the effect was not dependent on spike activity of the other receptors. CaCl_2 , which does not stimulate any receptor at any concentration (Evans, 1958, and unpublished observations) abolished the water response at 0.01 M. High concentrations of LiCl and choline Cl did not elicit appreciable activity of any receptor, but the water response was absent.

Under the standard conditions described above, in all more than twenty-five

preparations were tested for inhibition of the water response by NaCl and sucrose. The results from all were quantitatively similar to those illustrated in Figs. 5 and 6. Under very different conditions, similar results were sometimes obtained (Fig. 3), but occasionally the inhibition was much less than usual (Fig. 2). The reason for the discrepancy is not known, but probably lies in the conditions of the experiment rather than in variation in the properties of different receptors.

Behavioral Significance

The blowfly reflexively initiates feeding when the taste receptors are stimulated with sugars and water, and salts block the response to these. Since only two cells appeared histologically to enter a taste sensillum, it was thought that sugars and water stimulated one of these, and salts the other, the input of the two fibers underlying the taste qualities acceptance and rejection (Dethier, 1955). The present evidence shows that water stimulates a third chemosensory cell and accounts for recent observations that the fly can distinguish water and sugars (Evans, 1961; Dethier and Evans, 1961). The observed interaction between the stimuli for the sugar and salt receptors and the water receptor results in a complex input pattern. Since NaCl is rejected and water accepted, the sharp inhibition of the water response by NaCl provides an "enhancement of contrast" between different taste qualities. The analogy with the similar phenomenon observed in the *Limulus* eye (Hartline and Ratliff, 1958), however, is fitting with regard only to the effects and not the mechanism, since the inhibition here appears to be a direct action of salts on the water response and not an interaction between peripheral units as in *Limulus*. The inhibition of the water response by non-electrolytes is a much weaker effect, but more complex because it occurs between two acceptable stimuli or an acceptable and a tasteless (glycerol) compound.

Because the "hunger" and "thirst" controlling systems so modify the effect of the chemosensory input (Evans and Barton Browne, 1960; Dethier and Evans, 1961), it does not appear possible to make behavioral predictions based on stimulation by water and sugar apart from the observations that led to the present investigation: that the fly can distinguish water from sugar and that the ingestion of each is independently regulated (Evans, 1961; Dethier and Evans, 1961).

CONCLUSIONS

1. Water was found to stimulate a fourth sensory cell associated with the blowfly taste sensillum.

2. The response of the water receptor during brief periods of stimulation occurred in two phases: an initial rapid rate of discharge followed by a lower steady rate of discharge that was maintained for more than 500 msec.

3. Addition of solute to water invariably decreased the water response if it had any effect, so that water is the most effective stimulus for that cell that has been tested.

4. Sucrose solutions depressed the steady water response as a linear function of the log of their osmotic pressure over more than a 10,000-fold range of concentrations. Other non-electrolytes (glycerol, mannose) also depressed the water response, but the effect could not be correlated in any simple way with the properties of the solutions.

5. Electrolytes depress the water response completely over a very narrow range of concentrations. Between 0.1 and 0.5 M, NaCl reduces the steady water response from maximal to near zero. CaCl₂ abolishes the response to water at 0.01 M.

6. The inhibition in all cases is attributable primarily to effects of the solutes on stimulation of the receptor cell by water and not interactions among the four receptor cells, since solutes inhibited that did not elicit impulses from any cell. The possibility of electrical interactions superimposed on this has not, however, been excluded.

7. In all cases, the initial response of the water receptor was more markedly inhibited by solutes than was the steady response.

8. The specificity of the four sensory cells associated with the taste sensillum is shown to be narrower than formerly suspected; adequate stimuli, so far as is known, are only water, certain sugars, some monovalent salts, and motion respectively.

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