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Anesthetic stimulation of insect water receptors

(chemical sense/sensory coding/behavior/blowfly)

V. G. DETHIER* AND NANCY GOLDRICH-RACHMAN†

* Department of Zoology, University of Massachusetts, Amherst, Mass. 01002; and † Department of Biology, Princeton University, Princeton, New Jersey 08540

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ABSTRACT **Halothane, chloroform, and carbon tetrachloride, in the vapor and liquid phases, stimulate the water receptor of the blowfly *Phormia regina*. There are three successive phases of response to long-lasting stimulation by halothane: stimulation of the water receptor for the first 19 sec, narcosis for the next 80 sec, and stimulation of all receptors after 80 sec. The behavior of the fly is correlated with these phases. A thirsty fly extends its proboscis and attempts to drink during the first phase, withdraws its proboscis during the second, and extends in a manner characteristic of aversion in the third. A water-satiated fly responds only in the third phase. These results indicate that both the labeled line and the across-fiber hypothesis of sensory coding apply to the blowfly. At the level of sensory transduction the data do not rule out the possibility that streaming potentials are normally involved in stimulation of the water receptor. They are also consistent with a hypothesis that neutral narcotics stimulate the water receptor by facilitating the passage of sodium ions through the dendritic membrane.**

There is a growing body of evidence lending support to the suggestion that individual chemoreceptors of insects may be sensitive to a broader spectrum of chemicals than was formerly believed possible (1–8). This is not to say that the traditional water, sugar, and salt receptors are nonspecific; however, in the blowfly *Phormia regina* the sugar receptors on the labellum also respond to L-isoleucine, L-leucine, L-methionine, L-phenylalanine, and L-tryptophan (7) and the salt receptors to L-proline and hydroxyproline (7) and to formic acid and the glycosides, sinigrin and tropaeolin (8). Furthermore, labellar receptors respond to some vapors as well as to solutions (4). Responsiveness of gustatory receptors to vapors has also been demonstrated in the tobacco hornworm (9).

The importance of delineating the latitude of specificity of chemoreceptors lies in the basic relation of specificity to mechanisms of sensory transduction and to behavior that depends upon unambiguous discrimination among many chemicals that may act as gustatory stimuli or pheromones.

The observation that the four types of labellar chemoreceptors are differentially responsive to the vapors of a variety of nonpolar compounds, such as limonene, citral, benzene, and benzaldehyde, prompted Cherkin to suggest in a personal communication that qualitative differences in responses to vapors of different nonpolar compounds might be reproduced by regulated vapor concentrations of a single nonpolar compound. One of the compounds suggested for testing was halothane ($CF_3CHBrCl$), an inhalational anesthetic. This, in common with similar compounds, tends to stimulate at low concentrations and inhibit at high.

Following Cherkin's suggestion we have examined the electrophysiological and behavioral responses of the blowfly *Phormia regina* to halothane and several other nonpolar compounds in the vapor and fluid states.

MATERIALS AND METHODS

Electrophysiological responses of the labellar chemosensory hairs were studied by a modification of the side-wall technique of recording originally perfected by Morita and Yamashita (10). The isolated head of a fly was impaled on a glass micropipette containing Calliphora Ringer's solution (11), which served as a salt bridge to a silver wire. This was the reference electrode. A similar pipette containing 0.05 M LiCl serving as a recording electrode made contact with the dendrites through a crack in the side of the hair, thus leaving the apical pore available to any stimulus. All recording was extracellular. Thirteen flies were tested. A total of 26 of the largest labellar hairs, including numbers 1 to 10, were examined.

Compounds to be tested were placed in a glass pipette (tip diameter about 50 μm) which was then moved slowly toward the tip of the hair. Neural activity was monitored continuously as the pipette approached to within 200 μm of the hair or was then either withdrawn or placed directly on the tip. After each stimulation, receptors were allowed to return to their basal rate of activity. Periodically they were stimulated with water, NaCl (0.2–2.0 M), and sucrose (0.5–1.0 M) to ascertain whether or not they were still responding normally to physiologically adequate stimuli.

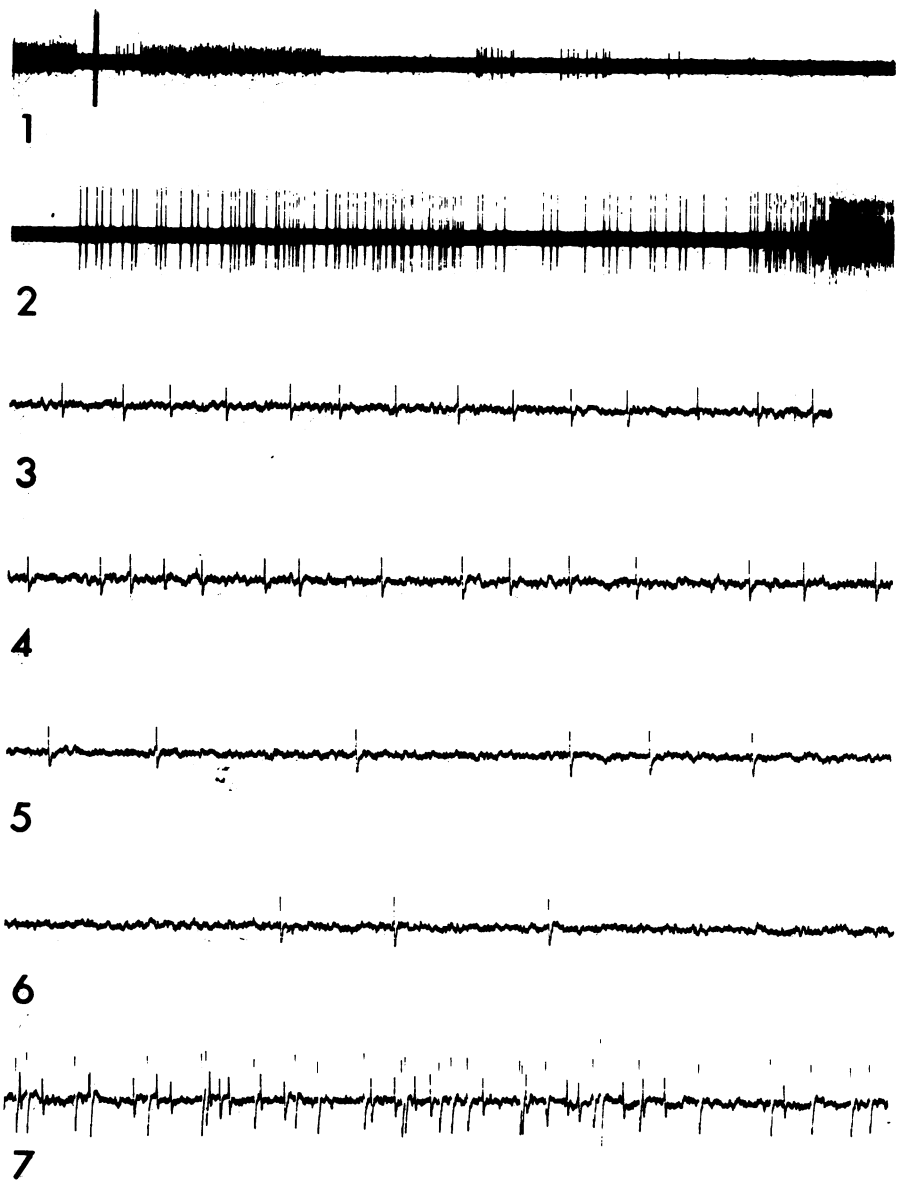
RESULTS

When halothane was applied as a vapor at a concentration of 1% (vol/vol), 200 μm from the tip of the hair, no electrophysiological responses occurred. When the concentration was increased to 5% (vol/vol), the water receptor responded for a period of 10–60 sec (Fig. 4). The average of 23 tests was 19 sec. The same result was obtained when halothane was applied as a liquid (Figs. 1 and 2).

The response in both situations was attributed to the water receptors for the following reasons: the amplitude and wave form of the spike were the same as those observed when water was used as a stimulus and different from the spikes elicited by salt and sugar; when a suspension of halothane and 0.5 M or 2.0 M sodium chloride was applied, two different spikes appeared initially, one of which exhibited the characteristics of the water spike and the other the features of the salt spike (Fig. 10); after 100 msec the larger (salt) spike ceased or was reduced to a rate of about one per sec; and when a mixture of halothane and 1.0 M sucrose was applied, the sugar spike alone appeared during the first 200 msec, after which the water spike appeared while the sugar spike gradually ceased (Figs. 8 and 9). Considering the fact that liquid halothane tastes sweet to man, it is interesting that it does not stimulate the sugar receptor of the fly. Unexpectedly there was no cross-adaptation between water and halothane, as the Figs. 11–15 illustrate.

In general, the response to halothane differs from that to water: there is a latency of about 0.5 sec as compared with 5 msec with water; there is no initially rapid phasic response; and there is a gradual acceleration reaching a maximum rate of 21 per sec in the first second as compared with a maximum of 65 obtained with water.

The response to halothane, whether applied as a 5% (vol/vol)



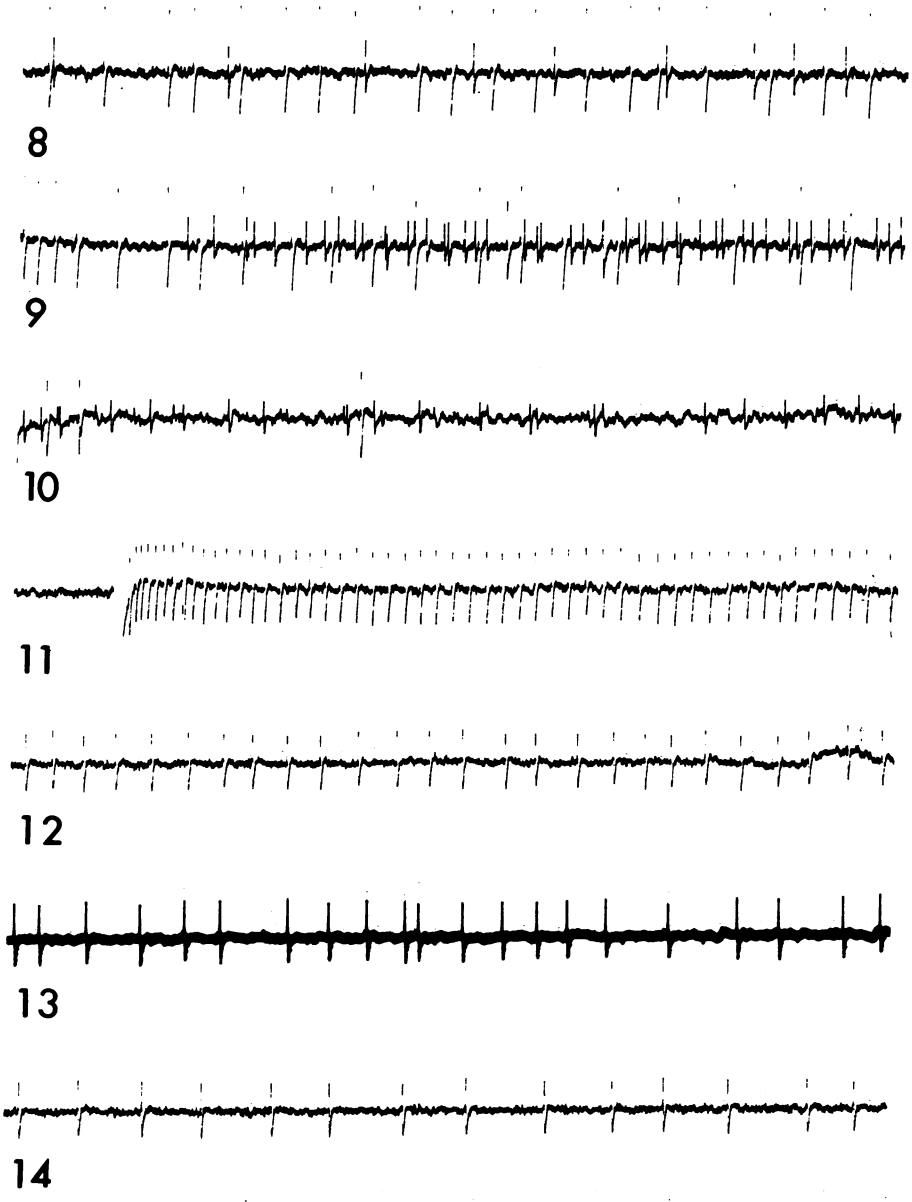
FIGS. 1–7. *Fig. 1.* Response of the water receptor to liquid halothane applied to a labellar hair for a period of 3.5 min. Only the first 32 sec are shown. The large vertical line indicates the beginning of stimulation. Preceding this is a brief stimulation by water. Duration of record in this figure is 35 sec. *Fig. 2.* The last 35 sec of stimulation continued from the preceding figure. Note the response of the salt cell (large spike). At the extreme right it is accompanied by a response from the water cell (small spike). *Fig. 3.* Response to water. Only the water cell is active. The duration of this and all subsequent records is 0.875 sec. The record begins at the onset of stimulation. *Fig. 4.* Response of the same water receptor to halothane vapor. *Fig. 5.* Response to chloroform vapor. *Fig. 6.* Response to carbon tetrachloride vapor. *Fig. 7.* Response of at least three receptors after the removal of carbon tetrachloride, which had been applied to the hair for 3 sec.

vapor (Fig. 4) or as a liquid, consists of three phases (Figs. 1 and 2). First, the water cell responds. This period of activity, lasting 19 sec on the average, is followed by a period of no activity lasting from 15 sec to 2 min and 55 sec (average 80 sec). At the conclusion of this silent period the water cell resumes activity at a low frequency punctuated by occasional activity from the salt cell. The salt cell then begins to respond in bursts. Often the salt and water cells give alternate bursts. Within 2–10 sec of this resumption of activity all four receptors in the hair respond at high and irregular rates. If the stimulus is removed during the silent period, all cells respond at "off."

The three phases of response also occur when chloroform is the stimulus. With carbon tetrachloride there is no silent period; at "off" three cells respond. Neither of these compounds is as effective as halothane in activating the water cell (Figs. 3–7). Other compounds that act in the vapor phase in one way or another are: octanol, butanol, isobutyraldehyde, citral, and chloral hydrate. With the first three there is no response until 2–10 sec have elapsed, after which all fibers respond, salt usually first. Chloral hydrate stimulates all fibers immediately. Chlorotone stimulates none.

In all cases in which three phases of response occur the first two are reversible; that is, the respective receptors respond normally within 20–30 sec to water, sugar, and salt. Phase three is reversible only if it is not permitted to continue for more than 2 sec. If it continues longer, the cells are still unresponsive 1 hr later.

None of the compounds tested are naturally encountered by the blowfly, yet observations of behavioral responses to them reveal a close correlation with the different phases of electrophysiological activity. A fly that has been deprived of water to the extent that it will extend its proboscis in response to water



FIGS. 8–14. *Fig. 8.* Response of a largest labellar hair to 1.0 M sucrose. The large spike is from the sugar cell; the small spike, from the water cell. *Fig. 9.* Response of the same hair to an emulsion of halothane and 1.0 M sucrose. Note the increased activity of the water cell. There is no change in the response of the sugar cell at this time. *Fig. 10.* Response of another hair to an emulsion of halothane and 2 M NaCl. The three large spikes are those of the salt receptor; the small spikes, the water receptor. *Fig. 11.* Initial response of another hair to water. *Fig. 12.* The same hair after continuous stimulation by water for 1 min. After 2 min of continuous stimulation the water cell ceased responding. *Fig. 13.* The response of the same cell to liquid halothane after it had completely disadapted (10 min). The record begins at the first spike. *Fig. 14.* The response of the same cell to liquid halothane after it had been stimulated continuously by water for 2 min. The record begins at the first spike.

applied to the tarsi or labellum will also extend its proboscis in response to halothane vapor or liquid halothane applied to labellar hairs. The extension appears to be normal in every respect, and the thirsty fly attempts to drink. If permitted to do so, however, it suffers irreversible damage to the labellum. The labellum curls and shrivels and eventually becomes brittle. Furthermore, receptors on the oral surface (the hairs are aboral) apparently react differently from halothane in that rejection occurs when they are stimulated by it. If the fly is prevented from drinking, and the application of halothane continues, vigorous extension continues for approximately 25 sec, after which the proboscis is retracted. It remains retracted for about 2 min. At the end of this time extension recurs; however, it is now erratic, is accompanied by regurgitation, and often is combined with rubbing of the labellum by the prothoracic legs.

If the same behavioral experiment is done with a fly that has been satiated with water, there is no initial extension of the proboscis; however, continued exposure to halothane for 2 min or more results in the erratic extension and regurgitation already described.

DISCUSSION

While it is clear that there is a preferred stimulus for each of three of the four chemoreceptors in the labellar hair, it is equally clear that none is narrowly specific. Spectra of preferred specificities enhance the potential for widening the che-

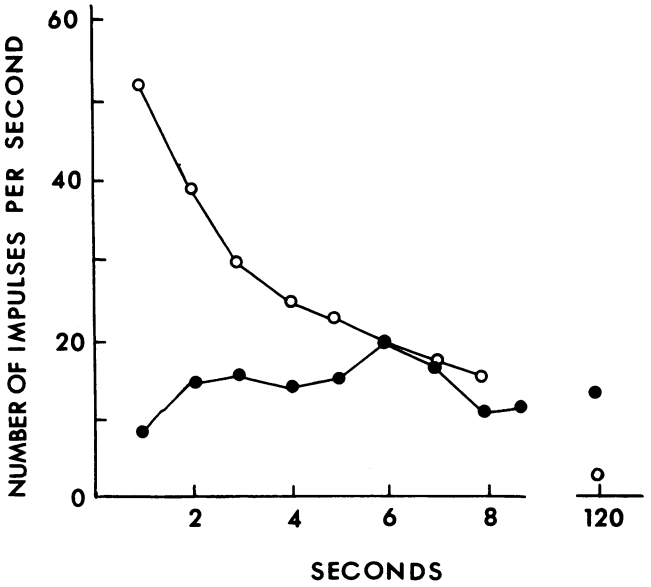


FIG. 15. Change in the frequency of spikes from the water receptor during prolonged stimulation with water and with liquid halothane. Open circles, water; closed circles, halothane.

mosensory horizons of the fly while at the same time permitting single receptors to trigger specific pattern of behavior. These potentialities find expression in two hypotheses regarding coding that are currently in vogue (12, 13). The labeled line hypothesis states that one neuron (in this case one receptor cell) transmits one message in response to whatever chemical affects it and, by implication, that this message can elicit a particular response or sensation. The across-fiber hypothesis holds that populations of receptors, each encoding a particular message, together produce a combined message, a pattern of input. These hypotheses are often expounded as though they are mutually exclusive, whereas this is not so in fact. Both methods of coding could occur under appropriate normal circumstances in any system. Actually, it is not at the receptor level that the issue is decided, but in the central nervous system where the incoming messages are decoded and where patterns would have meaning.

The responses of the fly to halothane support the idea that labeled lines and multiple fiber input each carry significant information insofar as behavior is concerned. It has long been known that water stimulating one receptor in a hair can initiate drinking behavior in a thirsty fly and that sugar stimulating a different receptor can initiate feeding in a nonthirsty hungry fly. The fact that halothane, a nonphysiological stimulus, can elicit a normal drinking reaction in a thirsty fly by causing the water receptor to generate spikes supports the hypothesis that labeled lines play a significant role in behavior.

The fact that higher concentrations of halothane cause all receptors to generate ragged bursting patterns of firing and that a different kind of behavior ensues supports the hypothesis that combined activity also has meaning insofar as the central nervous system is concerned. This observation confirms the conclusion of McCutchan (1), who demonstrated that combined irregular firing by many receptors resulted in characteristic aversive behavior.

The triple effect of halothane observed electrophysiologically may be explained by assuming that it stimulates at low concentrations, narcotizes at high, and at still higher concentrations damages receptor membranes possibly by dissociating lipid–protein complexes. Although these effects were produced by a single ambient concentration applied for different durations rather than by three discrete concentrations applied singly, the inference that the effects observed represented different concentrations at the receptor site with increased time is reasonable.

Excitation followed by narcosis is a phenomenon common to many anesthetics. Halothane has produced this effect on neurons of the mollusk *Aplysia* (14). Here excitation, as measured by the generation of action potentials, is preceded and accompanied by depolarization; narcosis is related to hyperpolarization. While we have not measured slow potentials in *Phormia*, the conclusion that the situation is similar to that in *Aplysia* is supported by observations that in flies the generation of action potentials in response to NaCl parallels the course of depolarization and the blocking of action potentials by $CaCl_2$ parallels hyperpolarization (15). When calcium is removed there is a postinhibitory rebound with the development of transient depolarization accompanied by action potentials from the salt cell. In the butterfly *Vanessa* inhibition of sugar and salt receptors also is accompanied by hyperpolarization (Tateda, quoted in ref. 15). Hyperpolarization followed by depolarization might also explain the rebound that often occurs in the salt receptor of *Phormia* after brief stimulation by water. Response of the salt receptor is not dependent upon the water receptor having produced action potentials because the rebound often occurs even when the water receptor has not spiked.

Alternatively it might be argued that water has diluted the extradendritic fluid and that its removal is followed by a surge of returning ions resulting in brief stimulation. Rebound by the salt cell has not been observed after prolonged (2 min) stimulation by water. It is possible in this instance that the extradendritic supply of ions has been too depleted to allow for a rapid recovery. A similar situation might be expected to ensue with stimulation by sugar, but no rebound has been observed in this case.

It is clear, however, that rebound can occur in those instances in which the stimulating compound is unlikely to reduce the concentration of ions. The salt receptor, and occasionally the sugar receptor, rebound after stimulation by halothane, xylene, citral, linalool, octanal, and a number of other nonpolar compounds. This phenomenon could be explained in terms of hyperpolarization or a blocking effect on ions during stimulation followed by a brief depolarization when the stimulus is removed. Certainly in *Aplysia* narcosis is related to hyperpolarization. On the other hand, Wolbarsht and Hanson (16) concluded that the blocking of impulses in the salt fiber of the blowfly by xylocaine, cocaine, procaine, chloral hydrate, and tetrodotoxin is not due to hyperpolarization. These anesthetics act as stimulants or depolarizing agents while still blocking the conduction of impulses in the dendrite.

The ability of nonpolar compounds, halothane, chloroform, and carbon tetrachloride, to stimulate the water receptor does not necessarily explain how water itself stimulates but it does provide a means by which some hypotheses relating to this problem may be tested. For example, Rees (17), in attempting to explain how the membrane of the water receptor can be depolarized when there are no ions in the stimulating fluid, postulated streaming potentials as a source of biological potential. According to this hypothesis, applied water lowers the osmotic pressure of the extradendritic fluid and ions flow along the osmotic gradient and through charged pores in the receptor membrane. This passage causes a difference in potential between the inside and the outside of the membrane, which could be a starting point for depolarization. It is unlikely that halothane, citral, and others alter the osmotic character of the ex-

tradendritic fluid. Even were it the case, the alteration would be in the opposite direction to that required by Rees' hypothesis. On the other hand, among the postulated changes in membranes that halothane, chloroform, and other neutral anesthetics effect are some that are not incompatible with Rees' hypothesis. According to Seeman (18) neutral anesthetics may cause, among other things, increase in membrane Ca^{++}, decreased facilitated diffusion of Na^+ and K^+ during the nerve impulse, increased diffusion of such neutral solutes as water, and increased passive leakage of K^+ or Na^+. An increased hydraulic flow of water could well initiate the streaming potentials required by Rees' hypothesis. On the other hand, since increased diffusion of Na^+ is also presumed to result from anesthetic occupation of the membrane, depolarization could be initiated directly by this mechanism.

A comparison of the characteristics of stimulation by water and by halothane reveals marked differences. First, the latency after application of halothane is much longer than that for water (compare Figs. 1 and 11). The delay could reflect the time required for halothane to partition between the ambient phase and the extradendritic fluid or the time to achieve a critical concentration at or in the dendritic membrane. Second, there is a reduction in the frequency of action potentials in response to water during the first 9 sec (up to 2 min) of stimulation (Fig. 15). This decline of response was not observed by Evans and Mellon (19) or by Rees (20) because they did not continue stimulation beyond 600 msec. During the initial period of 9 sec the frequency in response to halothane increases (in the first second) and then remains essentially constant, there being no evidence of adaptation. At its maximum effectiveness the rate of firing to halothane is only equal to the rate to water after it has been reduced to 38% of its initial value. This occurs at 6 sec. After continuous stimulation for 2 min the frequency of firing to water drops to 3.8% of its initial value while that to halothane (in those cases where it did continue to stimulate for as long as 2 min) is unchanged from the starting value. Additionally, after the receptor has been exposed to water continuously for 2 min and then stimulated with halothane, the frequency of firing is the same as if there had been no previous exposure to water.

Rees (20) proposed that adaptation of the water receptor of the fly *Protophormia terraenovae* might be the result of a localized accumulation of a solution of lower osmotic pressure than the bulk of the contents of the dendrite. Earlier, Morita (15) had presented evidence that the decline in the frequency of spikes was not due to adaptation of the spike generator nor of the receptor potential. Whatever the basis of adaptation in the first few milliseconds (usually referred to as the phasic portion of the response), adaptation from that period on probably results from a depletion of ions in the fluid surrounding the dendrite.

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