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An Electrophysiological Study of the Oral Plate Sensory Organs of the Honey Bee (Apis mellifera L.)

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AN ELECTROPHYSIOLOGICAL STUDY OF THE ORAL PLATE SENSORY ORGANS OF THE HONEY BEE (APIS MELLIFERA L.)

by

Rodney R. Seeley

A thesis submitted in partial fulfillment
of the requirements for the degree

of

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in

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Approved:

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Major Professor

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Committee Member

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Rodney R. Seeley

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ABSTRACT

An Electrophysiological Study of the Oral Plate Sensory Organs of the Honey Bee (Apis mellifera L.)

by

Rodney R. Seeley, Master of Science

Utah State University, 1971

Major Professor: Dr. Raymond T. Sanders
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The oral plate sense organs of the honey bee, Apis mellifera L., have been investigated employing an electrophysiological technique which allows simultaneous stimulation and recording.

The results of the study present evidence that the four bipolar sense cells innervating the sensory papillae on the oral surface of the hypopharynx are chemoreceptors. The sensory papillae respond to cations, glucose, water, and amino acids. Evidence for the absence of a mechanosensory cell is presented. The data concerning the sensory structures agree with the current theories of chemoreception.

(62 pages)

INTRODUCTION

Chemoreception has, in the past, been divided into three classes: (1) gustation or contact chemoreception, (2) olfaction, (3) a common chemical sense. Moncrieff (1967) explains that the common chemical sense was probably the first of the chemical senses to evolve. Gustation and olfaction are differentiations which occurred at a very early stage in evolutionary development. The common chemical sense is a general response to irritating compounds. Gustation is identified with a response to stimuli of relatively high concentrations in a liquid medium, and olfaction is identified with a response to relatively low concentrations of stimuli in a gaseous medium. Although the criteria for separating the different chemical senses leave much to be desired at the cellular level, the definitions are adequate for the present investigation.

A substantial amount of data has been accumulated relative to chemoreception in lower animal forms (Dethier, 1963) and in higher forms (Moncrieff, 1967). Insects have been used extensively because their chemoreceptor cells offer special advantages to the investigator. Some of these are: (1) single chemoreceptor organs are often easily accessible, (2) the innervation of the receptor organs is by a primary receptor cell, that is, the receptor cell receives the stimuli directly, generates the subsequent action potentials, and carries them to the central nervous system without involving any synapses, and (3) prominent development and sensitivity of the chemical senses in insects.

Investigations of chemoreceptors in insects, particularly the blowfly, Phormia sp., have produced great strides in contact chemoreceptor physiology.

The earliest investigations of chemoreception in insects were based mainly on the morphology of the sensory organs (Wolff, 1875 and McIndoo, 1916). Emphasis on behavioral studies increased as it became evident that classification of sensory organs on the basis of morphology alone was inadequate. Correlations between behavior and receptor physiology subsequently became very important in determining the function of receptor organs (Minnich, 1932, Frings and Frings, 1949, and von Frisch, 1950). Most of the information about insect chemoreception prior to 1955 was a result of these kinds of investigations. Behavioral responses to stimuli offer several disadvantages when one is attempting to investigate the response of a single receptor organ. Some of these disadvantages are: (1) it is difficult to tell whether the stimulant is acting on olfactory or gustatory receptors, (2) large areas of the insect's body are often exposed to the stimulant, (3) the response to a single stimulus is not always the same, (4) the behavioral response which is monitored is often a function of other parameters, e.g., touch, sight, etc., and (5) the accumulated data are often difficult to interpret.

According to Dethier (1963, p. vii), "the two most powerful modern tools placed at the disposal of the sensory physiologist are electronic apparatus for detecting electrical events in nerve tissue and the electron-microscope." Electronic apparatus has shed light on the chemoreceptors, photoreceptors, and mechanoreceptors, and "electrical recording from neural tissue has greatly expanded our rather conservative estimation of sense organs in general." From

qualitative and quantitative electrophysiological studies have come theories of sensory reception (Beidler, 1954; Davies and Taylor, 1969; Davis, 1961; Duncan, 1963; Goldman, 1965; Grundfest, 1965; and Vinnikov, 1965).

The honey bee belongs to the order Hymenoptera, the second largest order of insects. Its social organization is very well developed and its economic importance is significant. Conclusive evidence has been accumulated showing that olfactory, gustatory, and mechanosensory receptors are located on the antennae (Minnich, 1932; Kunz, 1933; Marshall, 1935; Frings, 1944; von Frisch, 1950; Fischer, 1957; Slifer and Sekhon, 1961; Simpson, 1963; Lacher, 1964; Boeckh, Kaissling and Schneider, 1965; Butler, 1967; Kaissling and Renner, 1968; and Ruttner and Kaissling, 1968). However, little information is available concerning receptor sites other than the antennae of the honey bee.

Stauffer (1969) studied mechanoreception and contact chemoreception on the labial palpus of the worker honey bee using electrophysiological techniques.

Since the sensory organs of the oral plate are in an ideal location to receive contact chemosensory stimuli, and since they have been recognized as sensory receptors, this study assumes the task of investigating, electrophysiologically, the response of the oral plate sensory papillae to several kinds of stimuli.

HISTORICAL REVIEW

Morphology

McIndoo (1916) reported, that unless the adult worker honey bees are able to discriminate on the basis of olfaction, they must sample some food with their mouth-parts in order to determine its acceptability. He concluded that bees possess a gustatory sense located somewhere in the mouth-parts. The two kinds of sense organs that he reported to be found on the mouth-parts are innervated papillae and innervated pores. McIndoo considered the innervated papillae as probable tactile receptors and chemoreception was not considered as a possible function because their cuticular covering is highly impermeable to aqueous solutions. McIndoo, therefore, considered that the innervated pores were responsible for the gustatory sense in the mouth-parts of the honey bee.

The mechanism by which stimulating solutions reach the nerve fibers in the sensillae of insects was partially resolved by Richards (1952). He postulated that the lipid content of the epicuticle and incomplete sclerotization in some types of insect sensilla imply that easy penetration of solutions into the sensilla may occur. The electron microscopic studies of Adams, Holbert, and Forgash (1965) on the innervated papillae of the mouth-parts and legs of the stable fly, Stomoxys calcitrans, show that nerve fibers appear to terminate, unmodified, beneath a pore in each papilla. The pore diameters for tarsal

contact chemoreceptor papillae range from 0.05 microns to 0.2 microns. They concluded that the site of action of chemical stimuli is probably the nerve endings in the papillae which are exposed to the exterior through the pores in the tips of the papillae. These results are in contrast with McIndoo's original assumptions. They also reported finding the attachment of a single nerve fiber to the epicuticle at the base of the tarsal tactile receptor.

Thurm (1964) studied the cervical hair plate receptors of the honey bee in an attempt to gain information about the cellular elements involved in the transducer process of mechanoreceptors and the kind of mechanical distortion which is effective in stimulating the structure. He found that each hair was innervated at its base by a single bipolar neuron and that the physical and chemical properties of the joint membrane of the hair suggested that it consists of resilin. He found that a ciliary structure separates a terminal segment of the distal nerve process from the remaining distal fiber and he concluded that compression of the nerve end is probably the normal stimulus for the mechanoreceptor. Vinnikov (1965) reports that a general characteristic of the sensory terminals of vertebrates and most invertebrates is a more or less modified cell provided with a ciliary structure.

Behavior

Kunz (1927) found that the acceptance threshold which bees have for sugar depends greatly upon their nutritional state and that $HgCl_2$, $NaCl_2$, KI, phosphoric, formic, and acetic acids, among other compounds, irritated bees.

Bees are, thus, capable of discriminating among compounds and their nutritional state is often involved in the discrimination.

By using proboscis extension as an indication of positive feeding response, Minnich (1932) found that stimulation of the first leg and antennae of the honey bee with sugar solutions produced positive feeding responses. Kunze (1933), using the same techniques, found that receptors for sugar are located on the eight distal joints of the antennae and he stated that antennal chemoreceptors are much more sensitive than the tarsal chemoreceptors. Marshall (1935) found that the antennal chemoreceptors normally respond to dilutions of the order of 1/12M and never fail to respond to 1/6M sugar solutions, whereas the contact chemoreceptors of the fore-tarsus require a 1 M solution of sugar to produce a positive feeding response.

Frings (1944) trained honey bees to associate the odor of coumarin with stimulation of the antennal and tarsal contact chemoreceptors with sugar solutions. Subsequently, bees were found to extend their proboscis upon exposure to only coumarin. The extension of the proboscis was used as evidence of stimulation by odor. Bees with one or both antennae were able to learn the response to coumarin, but bees with only three segments of the flagella were unable to learn the response. Frings and Frings (1949) concluded that olfactory receptors of the honey bee are probably located on the terminal eight segments of the antennal flagellum. Also, they reported that contact chemoreceptors are present on the mouth-parts of the honey bee. This was proved by experiments which consisted of stimulating separate portions of the mouth-parts with solutions

applied by fine glass-needles. Extension of the proboscis was recorded as a positive response.

Application of sugar solutions at the ends of three or four of the labial sensilla of the blowfly, Phormia regina, consistently produced a positive feeding response, i.e., extension of the proboscis (Grabowski and Dethier, 1954). Dethier (1955) reported that when a drop of sugar solution was coaxed up the shaft of the labellar and tarsal chemosensory hairs of the blowfly, in no case did the insect respond until the drop reached the tip of the hair. When the tip of the hair was amputated, the hair lost its sensitivity to sugar and water. He also found that different sugars did not elicit identical responses. Dethier found that sugars with α-linkages are the superior stimulants and he concluded that the relative position of the -OH and -H at the number four carbon are important also because of the different responses produced by α-D-galactose and α-D-glucose.

Theory

A theory of taste stimulation based on the assumption that the magnitude of response is directly related to the number of ions or molecules that react with the receptor membrane was proposed by Beidler (1954). The data which he obtained from electrophysiological studies on chemoreceptors of the rat's tongue indicates that physical rather than chemical forces are involved in the interaction between the chemical stimulant and the receptor site. This theory has become an important concept in dealing with phenomena of the receptor membrane in insects as well as in mammals.

Davies and Taylor (1959) state that the capability of a molecule to reach the receptor membrane is important in determining the magnitude of response to olfactory stimuli. The combination of a compound with a receptor site is thought to be capable of altering the cell membrane in some way, thus, causing a permeability increase and resulting in a depolarization of the receptor membrane. To be adsorbed at the receptor site, molecules must pass through an aqueous phase. The olfactory threshold, according to Davies and Taylor, can be calculated by knowing its absorption constant for molecules passing from air to the lipid-water interface and the value of the "puncturing" ability (1/P) of the odorant where P is the number of molecules which must be concentrated simultaneously on one of the receptor sites in order to cause a response.

Physiology

Arthropod sensory cells are often bipolar primary sensory cells. Electrophysiological investigations of these sensory structures are, therefore, simplified because the possibility of spatial summation and integration of impulse which may occur at the synapse is eliminated.

Hodgson, Lettvin, and Roeder (1955) developed a successful means of directly monitoring the electrical activity of an innervated labellar chemosensory sensillum of the blowfly by means of extracellular electrodes. In their investigations the reference electrode was placed in the crushed head of the fly. A micropipette containing the stimulating solution and recording electrode was placed over the sensillum. The potential changes were recorded by means of a push-pull cathode follower, preamplifier, and a cathode-ray oscilloscope.

Action potentials were recorded which corresponded to stimulation by salt solutions, and smaller spikes were seen when sugar was the stimulant. Mechanoreception was also identified in the sensillum.

Electrophysiological recordings from Limulus receptor organs illustrated that chemical responses can be abolished selectivity by treatment of the receptors with strong acid without harming thermal and tactile responses. The chemoreceptive surface is susceptible to injurious treatment, probably because those neurons which coarse to the surface of the cuticle or spine and are exposed to the environment are the chemoreceptors (Barber, 1956). These results are compatible with morphological (see page 4) as well as behavioral data (see page 7) collected by other investigators.

Hodgson and Roeder (1956) produced records from chemoreceptive and mechanoreceptive cells of Phormia which support the idea that the salt or L-spikes, sugar or S-spikes, and mechanical or M-spikes arise from separate cells and that each cell is very specific in terms of to which stimulus it will respond. Also, they reported that movement of the hair causes a frequency change in the L- and S-receptor cells. On the other hand, Wolbarsht and Dethier (1958) reported that no consistent change is recorded in the frequency of the salt and sugar spikes when the hair is stimulated mechanically. Wolbarsht (1958) reports further, that the activity of the S-fiber is not related to the electrical activity of the L-fiber. He suggests that the supposed electrical interaction depends only on the character of the stimulating solution and that addition of sugar to a salt solution alters the thermodynamic activity coefficient of the

salt, the diffusion coefficient of the salt, and may block receptor sites where salts act to stimulate the L-neuron. Hodgson and Browne (1960) have added to the conflicting information concerning the effects of the response of one fiber upon another. They recorded three types of responses from blowfly labellar hairs: (1) an increase in frequency of the afferent impulses from L- and S-receptor cells while the mechanoreceptor is firing, (2) the mechanoreceptor firing while the L- and S-receptors remain unaltered, (3) an increased rate of firing for all three receptor cells. They also suggested that these characteristics cannot be accounted for by concentration changes of chemical stimuli during the testing procedure and that calculations of the expected frequencies of chance summation of L- and S-fibers during prolonged recording from these receptors agree closely with observed summation frequencies. Although conflicting evidence exists, it appears that electrical interaction in the blowfly sensilla may be capable of altering the response of the sensory cells.

Hodgson (1957), in an attempt to further elucidate the characteristics of the labellar sensory receptors in Phormia, reported that competition between highly stimulating and weakly stimulating sugars occurs and that more than one kind of specific receptor for sugar occurs at the receptor site of the S-fiber. Evans (1961) also presents electrophysiological evidence (blowfly labellar sensory receptors) which supports the hypothesis that the receptor membrane bears more than one type of receptor site for sugars. Each site has structural requirements for effective combination with the stimulant, e.g., glucose and fructose appear to combine with different receptor sites.

Comparison of the response of the fleshfly contact chemoreceptors to various sugars shows the following results: sucrose > fructose > glucose when the concentrations are below 0.3 M and sucrose > glucose > fructose when concentrations are above 0.3 M (Morita and Shiraishi, 1968). It was calculated that the response to glucose is not proportional to the number of 1:1 complexes formed between the glucose molecule and the receptor site. For glucose and fructose the experimental values are in good agreement with the theoretical curve of the 2:1 complex model. One has to assume strong competition between sucrose and glucose for the same receptor site. Glucose can also occupy the fructose receptor site. Formation of multimolecular complexes between stimulant molecules and the receptor sites seems probable.

Electrophysiology of the contact chemoreceptors of the blowfly labellum indicates, at supramaximally stimulating concentrations of chloride salts, an effectiveness of the cations in the sequence: K = Na > NH_4 > LI = Cs. At submaximally stimulating concentrations the paired anion is observed to markedly affect the cation receptor response. When testing different anions of potassium salts, the cation receptor was stimulated with an effectiveness sequence of: I = NO_3 > Br > C1 > F (Steinhard, 1965). A pure cation receptor, then, does not exist. The anion is important in determining the responses of the cation receptor. On stimulation of the salt receptor of the blowfly, the effectiveness of the anions increase monotonically with the atomic number. The effectiveness of the cations is greatest for potassium and declines as the atomic number increases. The response to a mixture of salts appears to be an average of

their concentrations (Gillary, 1966c). The differences between an acceptable and an unacceptable salt, electrophysiologically, appears to be simply the frequency of impulses produced by the salt receptor (Dethier, 1968).

Analysis of the differences between Ca^{++} and other hyperpolarizing ions in relation to Na^{+} and K^{+} which are depolarizing ions suggests that the hydrated size of the ion may have some effect on the reaction of the ions at the receptor site (Rees and Nobuaki, 1968).

A protein fraction from Bovine taste buds has been correlated with the sugar receptor site of the contact chemosensory cells (Dastoli and Price, 1966). There is, thus, a possibility that the stimulant molecules are reacting with a protein molecule at the receptor site and that the reaction, under certain conditions, produces a depolarization of the neuron.

Gillary (1966b) reports that when the ambient temperature is altered, the response of the blowfly salt receptor to 1.0 M NaCl stimulation increases at a rate of about 10% per 1 C within the range of 23 to 28 C, but it remains unresolved as to the effect on the frequency of response when the receptor membrane alone is varied in temperature. At a constant ambient temperature the response to 1.0 M NaCl increases with increasing relative humidity at a rate of between 0.5% and 1% for each per cent increase in the relative humidity (Gillary, 1966c).

Wolbarsht (1965) reports that the salt receptor in the blowfly labial sensilla is unaffected by a large range of pH's. The large tolerance of the receptor site to variations in the pH and salt concentrations suggests that the

concentration of the stimulant presented to the receptor membrane may be considerably less than that of the bulk phase of the stimulating solution. Gillary (1966a) suggests that the adsorption theory of taste stimulation may be an over-simplification of a potentially complex situation in the sensillum. In the blowfly, Lucilia, the latency period between response and the application of the stimulus varied between 5 and 13 milliseconds after the application of 0.25 and 0.06 M NaCl (Browne and Hodgson, 1962). The length of the hair had little effect on latency, therefore much of the latent period must be occupied by movement of the stimulant to the receptor site and by the excitatory process.

Wolbarsht and Dethier (1958) report that the L- and S-fibers of the blowfly may be stimulated after the tip of the hair is amputated, but that the response is not as great as it is in the normal receptor. The lowered frequency may explain the lack of behavioral response when the hairs are cut (see page 7), but a more general distribution of receptor sites may occur over the entire dendrite than was previously suspected.

The number of action potentials increases exponentially with an increase of anelectrotonus in Vanessa tarsal receptors (Morita and Takeda, 1959). The generator potential arises in the chemoreceptor and is negative at the recording point with reference to the hair base. It is concluded from the polarity that the generator potential is the depolarization of the chemoreceptor surface membrane which is located at the hair tip. This depolarization probably spreads electrotonically to the proximal part of the chemosensory neuron where impulses are iniated. Impulses are produced by the cathodal d.c. current at the initiated

site of chemosensory impulses; the site is located somewhere near the base of the hair. Upon ordinary chemical stimulation, the generator potential at the chemoreceptor surface may play the role of the source of this cathodal current (Morita, 1959). A train of impulses is never recorded without a sustained negativity in the generator region of the hair. The negativity increases in magnitude with an increase in the strength of the stimulus. Thus, it can be assumed that the negativity is the generator potential. Hyperpolarization was produced by application of $CaCl_2$, acetic acid, quinine, and other compounds all of which inhibit the initiation of impulses (Morita and Yamashita, 1959).

Evidence supporting the assumption that the action potentials are generated at the proximal part of the neuron and that the receptor site is at the tip of the neuron is given by Morita and Takeda (1959). In mechanosensory neurons that are sensitive to motion but not steady deformation (phasic receptors) the graded potential returns to the baseline between each burst of impulses. The slow potential of the mechanosensory hairs which respond to steady deformations (tonic receptors) is maintained and returns to the baseline only after the cessation of the deformation. Variations in the spike amplitude which occur, and have caused some confusion in interpreting the results because of the usual all-or-none characteristics of the action potential, are due to the shift of the baseline caused by variations in the slow potential (Wolbarsht and Gray, 1959). According to Davis (1961) adaptation of sensory receptors may occur as a result of either the decline of the receptor potential, its less effective spread, or as a result of a rise in the threshold of the initial segment.

In studies of a lobster axon, the transducer behavior of the axon in response to a mechanical input is an electrical output, i.e., a depolarization of the membrane. The response of the axon to mechanical stimulation is an increase in membrane conductance accompanied by a depolarization (Julian and Goldman, 1962). The transducer action of an olfactory receptor is affected by the three dimensional structure of the stimulant molecule in addition to its size (Amoore, 1963). It is possible that both mechanical and chemical stimuli may initiate an essentially similar process. It is hypothesized that the appropriate stimulant may initiate an enzyme reaction whose initial velocity is dependent on the intensity of the stimulus (Duncan, 1963). There is little support for the occurrence of an enzymatic reaction at the receptor site, but conclusive evidence is lacking.

Transduction of chemical stimuli to an electrical response involves, in the case of sugar reception, the sugar molecule combining reversibly with a receptor substance to form a complex at the initial excitatory site. The complex depolarizes the cell membrane and the resulting generator potential disappears rapidly upon removal of the stimulant (Hodgson, 1964). No evidence in support of the existence of an excitatory compound released at the receptor membrane upon stimulation of the sense cell has been obtained from investigations of primary receptor cells.

Goldman (1965) concluded that the detailed anatomical structure of the nerve ending and its surrounding tissue contributes to the determination of the extent to which the system is a phasic or tonic mechanoreceptor. He found

from studies on the lobster giant axons that the mechanosensory element is responsive to a distortion of the membrane which increases the membrane conductance. Removal of external sodium reduces the depolarization produced by a mechanical stimulus to a small fraction of its original value.

Studies on the cervical hair plate sensilla of the honey bee have shown that only a mechanical force which is directed transverse to the nerve terminal is effective in stimulation. The force in the direction which is normally caused by bending of the hair leads to a compression and to a shift of the nerve terminal. The compression component alone is probably the stimulating effect (Thurm, 1965b).

The frequency of impulses rises with an increased generator potential in a receptor cell, but not necessarily linearly. The impulses may continue while the generator potential persists or they may stop, depending upon the kinetics of the electrogenic mechanisms of the electrically excitable membrane (Grundfest, 1965).

When a receptor cell is present and is innervated by a sensory neuron, the sign of the receptor cell potential, or whether it generates a potential or not is unimportant. The primary function of the receptor cell is to excite the sensory neurons, and electrogenesis in many cases is merely a sign of secretory activity of the receptor cell. The primary sensory receptor is much more dependent upon the development of a receptor potential than a sensory cell which is innervated by a neuron.

Thurm (1965a) concluded that the lowered absolute dynamic sensitivity following a response is the remainder of a decrease in absolute dynamic

sensitivity which develops during a response. He found that after about 10 minutes of O_2 deficiency the receptor potential response is nearly abolished. Generation of the receptor potential depends on the metabolic energy, and metabolic energy is necessary only for the maintenance of the ionic concentrations in the axon.

There is a tight constriction near the base of the labellar sensilla of the blowfly which acts to prevent extracellular electrical leakage between the interior of the hair and the body fluid. Hence, the resistance pathway from the tip of the hair to the site of impulse is seen with an initial positive phase. However, when the impulse passes the constricting space and enters the lumen of the hair, the opposite situation prevails, and the impulse appears negative. Under the exposure to strong salts when the hair is deteriorating, the impulse is initially negative, or has a reduced positive phase. This indicates that the site of impulse initiation has shifted distal to the constriction, presumably due to extensive depolarization. Anesthetics which act as stimulants or depolarizing agents for the salt fiber while blocking impulse conduction cause a positive phase to be recorded passively through the dendrite which is not decreased in amplitude. Thus, the resistance along the dendrite membrane of the chemosensory neuron is not increased by the action of the anesthetic. Evidence indicates that impulse conduction along the dendrite is not necessary for the normal activity of the chemosensory neuron. Invasion of the dendrite by action potentials does occur, but does not imply that the chemosensory receptors themselves can conduct impulses. They may exist as nonconducting patches (Wolbrasht and Dethier, 1958).

It is suggested that the main depolarizing current at the generator site in dipteran chemoreceptors may follow a pathway in which a Nernst potential set up across the membrane of the distal tip of the receptor dendrite drives a current through the dendrite cytoplasm, the dendrite membrane at the action potential initiation site, the wall of the proximal extension of the scolopoid body, the tricogen cell vacuole, and the contents of the large lumen back to the membrane at the tip of the dendrite. It is not known yet whether the application of the stimulating solution provides the final link in this current pathway or if this link is pre-existent and such an application merely establishes the receptor potential (Rees, 1968).

METHODS AND MATERIALS

Experimental animals

All experiments were performed on worker honey bees, Apis mellifera L. The bees were provided by the Wild Bee Pollination Investigation Laboratory, ARS, USDA, Logan, Utah. They were maintained in a glass paneled observation hive in the laboratory at room temperature. A solution of 50% sucrose in water was available to the bees at all times in addition to their stored reserves of honey and pollen. A two inch pipe from the hive to the outside of the building allowed the bees to leave the hive. Experiments were carried out between October 1969 and May 1970.

Dissection

A worker honey bee was taken from the observation hive, its head was removed, and embedded in a wax reservoir on a glass microscope slide. The surface of the clypeus was oriented at a forty-five degree angle to the surface of the wax with the frontal part of the head upward. The integument was then cut around the periphery of the clypeus and labrum. Subsequently, the clypeus and labrum were pulled away with a pair of fine-tipped forceps. The epipharynx and part of the preoral cavity surrounding the surface of the oral plate were also pulled away. The oral plate and its sensory papillae were then easily accessible.

Extreme care in embedding the head in the wax was essential since excessive heat injures the nerves innervating the oral plate sensory papillae,

producing abnormal results. A pool of wax was melted and partially cooled before the most dorsal part of the head was submerged in it.

The head of the bee is heavily invested with nerve and muscle tissue. To avoid the high electrical activity resulting from the vast number of muscles and nerves which would be superimposed upon the desired recording, the indifferent electrode was placed through a hole cut in the mandible after damaging its interior with a hot needle. The mandible, in spite of having some electrical "noise," was found to be the most suitable area for the location of the indifferent electrode.

Preparation of the recording electrode

The electrode used to record from the oral plate was similar to the platinized silver-silver chloride electrode described by Cole and Kishimoto (1962). A six centimeter length of 20-gauge silver wire was cleaned by using fine sand paper, washing with 95% ethanol and by heating it with a flame. A bunsen burner was used to melt a small spherical knob on one end of the wire which would just fit into a glass tube 1.8 mm I.D. The silver wire (anode) was then placed in the center of a helical cathode in order to insure symmetrical charge density. They were then immersed in 0.5 M KCl and 1.62 coulombs were applied between the anode and cathode at a rate of .226 ma. The KCl was replaced with Kohlrausch's solution (Nastuk, 1963), the polarity was reversed, and .81 coulombs were applied at a rate of .226 ma. The Kohlrausch's solution was subsequently replaced with 0.5 M KCl and the polarity was again reversed, and at a rate of .226 ma, .06 coulombs were applied. The resulting electrode

combines the stable potential and low direct-current resistance properties of a silver-silver chloride electrode with the low high-frequency independence characteristics of a platinized platinum electrode.

Deterioration occurs in the platinized silver-silver chloride electrode after about one week. A decreased stability of the base-line on the CRT, a lowered amplification of the signal with respect to the electrical noise and a color change in the electrode from black to a whitish-gray were indications of electrode deterioration. The chemical reaction

$$\underset{\text{(black)}}{AgCl} + e^- \longrightarrow \underset{\text{(whitish-grey)}}{Ag^+ + Cl^-}$$

illustrates the reason for deterioration of the electrode and the change in its color.

After the electrode had been electrolytically plated, a sleeve of plastic insulation approximately one centimeter in length was placed over the electrode about one-half centimeter from the tip of the plated spherical knob. The insulation was used as a plug and also to support the electrode when placed in the glass micropipette. Dental Sticky Wax (Kerr Manufacturing Company) was used to make a seal between the silver wire and the insulation and between the insulation and the glass micropipette.

The glass micropipette was made with the aid of a mechanical electrode puller from 1.8 mm I.D. glass tubing. The tip of the pipette was broken off under a compound microscope with a calibrated eye piece so that the inside diameter of the tip was less than 10 microns. A port was made in the side of the pipette about

.5 centimeters from the tip by applying air pressure to the inside of the glass tubing while heating the side. As the glass was melted the air pressure forced a hole through the side of the pipette. Two small plastic tubes could then be inserted through the port and sealed in place with Sticky Wax. One of these tubes (the influx tube) was connected to a syringe containing the testing fluid. The other tube (the efflux tube) is for flushing the solution out. This arrangement allowed the perfusion of testing fluid through the influx tube and out the efflux tube resulting in the ability to expel solution through the pipette tip and the easy removal of air bubbles through the efflux tube.

The base of the micropipette with its secured electrode was placed in a six centimeter long glass tube (3 mm I.D.) containing mercury so that the unplated end of the electrode was in electrical contact with the mercury. At the opposite end of the mercury filled glass tube was a BNC connector with a silver wire attached to it. The silver wire connected to the BNC connector was, therefore, in electrical contact with the recording electrode and the BNC connector (Figure 1).

Wire gauze was used as an electrical shield and covered all except the tip of the recording electrode. Shielded cable was used as a connection between the electrode and the preamplifer.

It was important to be able to initiate the easy expulsion of testing solutions from the tip of the pipette. Evaporation at the end of the electrode rapidly concentrated the testing solutions. The only way to insure that the concentration of the solution at the tip of the pipette was almost the same as the concentration

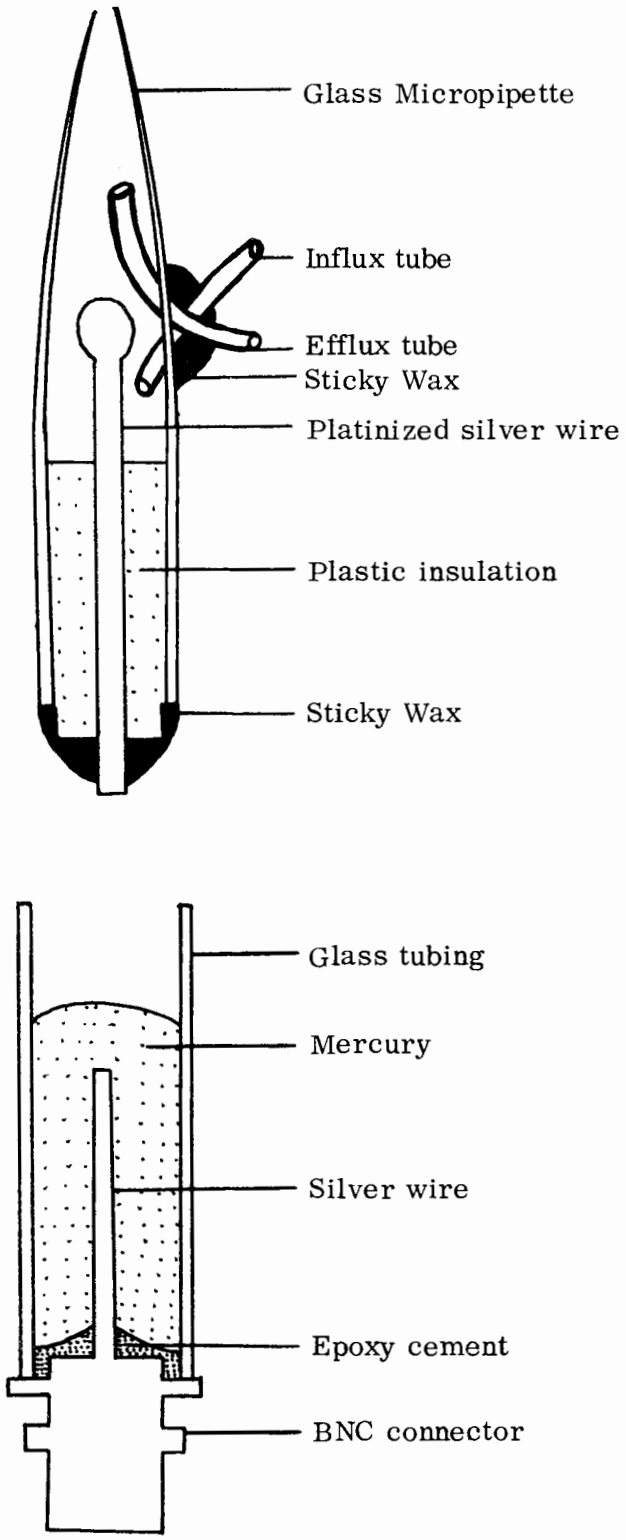

Figure 1. Section of electrode components used in the present investigation.

of the solution inside of the pipette was to wash the tip out immediately before touching the sensory papilla with the stimulating solution. If the sensory papillae were long enough to be inserted into the end of the pipette, evaporation would have been no problem. Since the sensory papillae on the oral plate are only about 7 microns in length, it is necessary for the stimulating solution to be a drop at the end of the micropipette. Evaporation and subsequent concentration of the stimulating solution is a possible source of error when one is trying to test a contact chemoreceptor with varying concentrations of one stimulant. Care had to be taken to avoid the phenomenon as much as possible.

Preparation of the indifferent electrode

The indifferent electrode was identical to the recording electrode with the exception of two features. First, the diameter of the micropipette tip was as much as .25 mm I.D. rather than 10 microns as in the recording electrode. Secondly, there was only one plastic tube in the port in side of the micropipette. This was possible because the tip diameter of the pipette was large enough to allow perfusion of enough solution through it to expel air bubbles.

Recording arrangement

The recording and reference electrodes were mounted on Brinkmann Micromanipulators. The different electrode was positioned so that it could be moved to any location on the oral plate. The indifferent electrode was positioned so that it could be placed in the hole produced within the mandible.

The head of the honey bee, which was embedded in wax on a glass microscope slide, was placed on a mechanical stage of a compound microscope which

was mounted on a plastic block at an appropriate level allowing easy accessibility to the micromanipulator. A compound light microscope which had its stage removed was used to observe the location of the electrodes. A beam of artificial light after being filtered by passing through a bottle containing distilled water was used to light the specimen. This precaution reduced the rate of evaporation of the hemolymph of the bee's head and of the stimulating solution at the tip of the glass recording electrode.

Stimulation and recording of the sensory papillae on the oral plate occurred simultaneously when the tip of the different electrode was flushed and then placed over a sensory peg. The potentials were amplified (Tektronix type 122 low-level dc preamplifier with low and high filters set at 0.8 and 1000 HZ, respectively), displayed on a CRT oscilloscope (Tektronix type 502), and photographed with a manual camera (Hewlett-Packard Model 197A). The permanent records were examined visually. The ambient temperature was 24-26^{o}C.

RESULTS AND DISCUSSION

Both the epipharynx and the oral plate of the honey bee have innervated sensory pegs, or more correctly, sensory papillae, on their "oral" surface. Food material passing to the inside of the bee (as nectar or pollen) or passing to the outside (as the royal jelly) must pass between the sensory papillae of the oral plate and those of the epipharynx.

The innervation of the oral plate is derived from the Nervus Labrualis which extends anteriorly and ventrally, parallel to the m. tentorio-oriscutalis. Before crossing over the hypopharyngeal suspensorium, it gives rise to a sensory branch called the Nervulus Tengumenti Labrualis I which is directed backward and connects with the sensory papillae of the oral plate (Youssef, 1966).

The area surrounding the sclerotinized oral plate is ensheathed in layers of circular and longitudinal muscle fibers which are strongly contractile (Snodgrass, 1956). The contractile nature of the muscle is easily seen when the oral plate is exposed in a living honey bee. Spasms in the muscle are of sufficient magnitude to actually bend the oral plate as well as make it vibrate. The instability of the oral plate produced by muscle movements is a source for much of the electrical "noise" seen in the recordings from the oral plate sensory papillae.

The oral plate is arched upward. It has two domes on either side separated by a groove between them. The sensory papillae which are grouped upon and restricted to these domes vary slightly in number from one honey bee to

another, an average being 90 pegs on an oral plate (McIndoo, 1916). The pegs are separated by approximately 55 microns and are of consistent density throughout each group. There is no difference in the external structure as far as can be determined with the light microscope or with the scanning electron microscope (Youssef, unpublished data). The papillae are approximately 7 microns in length and less than 1 micron in diameter at the tip. There have been no known studies to determine the mechanical or chemical properties of the cuticle of the sensory papillae, but the articulation at the base of the pegs appears to be rigid. The pegs, therefore, appear to be poorly adapted for side to side movement.

Recent unpublished results using electron microscopic examinations of thin cross-sections of the sensory pegs of the hypopharynx provide conclusive evidence that they are innervated by four dendrites and that all four of the dendrites pass toward the tip of the sensory papillae and they are bipolar, thus providing conclusive evidence that each papilla is innervated by four separate primary nerve cells (Youssef, unpublished data).

Mechanoreception

Each time a different stimulating solution was used in the recording electrode, an attempt was made to discover a mechanical response in the pegs. The results were consistently negative, but negative electrophysiological results should not be considered as conclusive evidence. There is always the possibility of an insensitive recording arrangement.

In previous studies of mechanoreceptors (Pumphrey, 1936; Wolbarsht and Dethier, 1958; Thurm, 1964) it has been found that the location of the dendritic ends are in a position to receive mechanical stimuli. For example, a mechanosensory sensilla in the blowfly exhibits a flexible articulation with the rest of the cuticle at the base of the peg where the dendrite of the mechanosensory cell terminates (Dethier, 1955). The sensilla acts as a lever and deflection of the lever from the resting position causes a flexion at the articulation. The dendrite termination on the flexible cuticle "senses" the movement and initiates a response in the sensory cell. The structure of the sensory papillae on the oral plate appear to be poorly designed for mechanoreception. From electron microscopic studies, the absence of a dendrite terminating at the base of the oral plate sensory receptors has been confirmed (Youssef, unpublished data). Hence, the morphological data and the electrophysiological data support one another, making it safe to report the absence of mechanoreception in the sensory papillae of the oral plate of the worker honey bee.

Chemoreception

When the stimulating solution in the recording electrode comes into contact with the sensory papillae, the reference electrode being in electrical contact with the lumen of the mandible, a large make-break artifact is produced on the cathode-ray tube. No make-break artifact is observed when the recording electrode comes into electrical contact with another portion of the oral plate. This is an indication that there is an area of low resistance at some point on the papillae and it is probably where the dendrites are exposed to the environment (Stauffer,

1969). From electron microscopic studies, it has been found that the dendrites in the oral plate sensory papillae may be exposed indirectly to the environment (Youssef, unpublished data). Again, the morphological data is compatible with the electrophysiological data. Evidence for the existence of a pore at the tip of an innervated contact chemoreceptor of the stable fly, Stomoxys calcitrans (Adams et al., 1965) makes the presence of a pore at the tip of an innervated sensillum or papilla a strong indication that the structure is a chemoreceptor (Dethier, 1963).

The oral plate of the honey bee is continuously bathed by a thick layer of liquid which is normally present in the preoral cavity. This liquid film covering the sensory papillae would present a formidable barrier to volatile chemicals. It is likely, therefore, that they respond to chemicals dissolved in the solutions which pass over the oral plate. Therefore, experiments designed to determine the function of the oral plate sensory papillae involved only aqueous solutions and no olfactory responses were investigated.

Response to chemical stimuli: Cations

McIndoo (1916) indicated that the sensory papillae on the oral plate of the honey bee are in a good position to receive gustatory stimuli, but he concluded, that because of the thick-walled cuticular structures the stimulus could not reach the neurons inside the papillae. The results of the present investigation show that the innervated sensory papillae do respond to aqueous solutions of sodium chloride. The recorded response is a train of action potentials in the positive direction. The amplitude of the spikes is relatively constant during a

single recording. Consistent results are obtainable and there is no observable difference in the response among the many papillae on the oral plate (Figure 5, b).

The amplitude of the spikes does vary from one recording to another. There are several factors responsible for the variations. The ionic concentration of the stimulating solution in the recording electrode was varied. The resistance of the solution in the recording electrode varies inversely with the ionic concentration and therefore effects the amplitude of the recorded impulses. Another cause for the variability is the difficulty in controlling the size of the electrode tip precisely. As the tip of the electrode becomes smaller, the resistance is increased greatly. Therefore, small variations in the size of the micropipette tip can cause differences in the recorded results. The platinized silver-silver chloride electrodes deteriorated over a period of about one week (see page 21). As deterioration proceeds the resistance and impedance characteristics of the electrodes change, giving varying results in the amplitude of the recorded impulses. Another important source of amplitude variation in separate records is the position of the electrode with respect to the sensory peg. Limited lighting of the specimen made it necessary to use low magnification (50-200X) of the specimen. A drop of stimulating solution suspended from the tip of the recording electrode was used to make electrical contact with the sensory papillae, but the distance of the tip of the micropipette from the tip of the papillae varied somewhat. As the distance of the micropipette increases, the resistance increases. This would also effect the amplitude of the recorded response. Measurement of the absolute amplitude of the action potentials was not

the purpose of the investigation. The identification of a response to varying stimulants satisfies the goal of the present investigation and therefore the exact resistance of the electrodes was not measured.

During the investigation an attempt was made to vary the concentration of sodium chloride used to stimulate the sensory papillae. One must be cautious when discussing concentrations using this kind of recording technique (see page 22). The concentration of the stimulating solution was a problem which could not be easily avoided, and for that reason one should treat reported concentrations as being relative to one another instead of being absolute values.

A series of sodium chloride concentrations ranging from .005 to 1.0 molal were used to stimulate the sensory papillae. The data were plotted using ionic activities instead of the molal concentrations. For 0.01 molal NaCl and lower concentrations the activity was considered to be equal to the molal concentrations. No typical NaCl response was observed when the stimulant was .005 M NaCl. Results obtained are similar to previous studies on other contact chemosensory organs of insects (Evans and Mellon, 1962). The responses were plotted as a parabolic curve with the magnitude of the response on the ordinate and the concentration of the stimulant on the abscissa (Figure 2). The results were displayed as approximating a linear graph of response versus the log on the concentration over a large concentration range (Figure 3). The response was taken as the rate of propagation of impulses at 10 seconds after application of the stimulus.

Beidler (1954) presented a theory of taste stimulation for rat contact chemoreceptors in which he assumes that the reaction of the stimulant with the

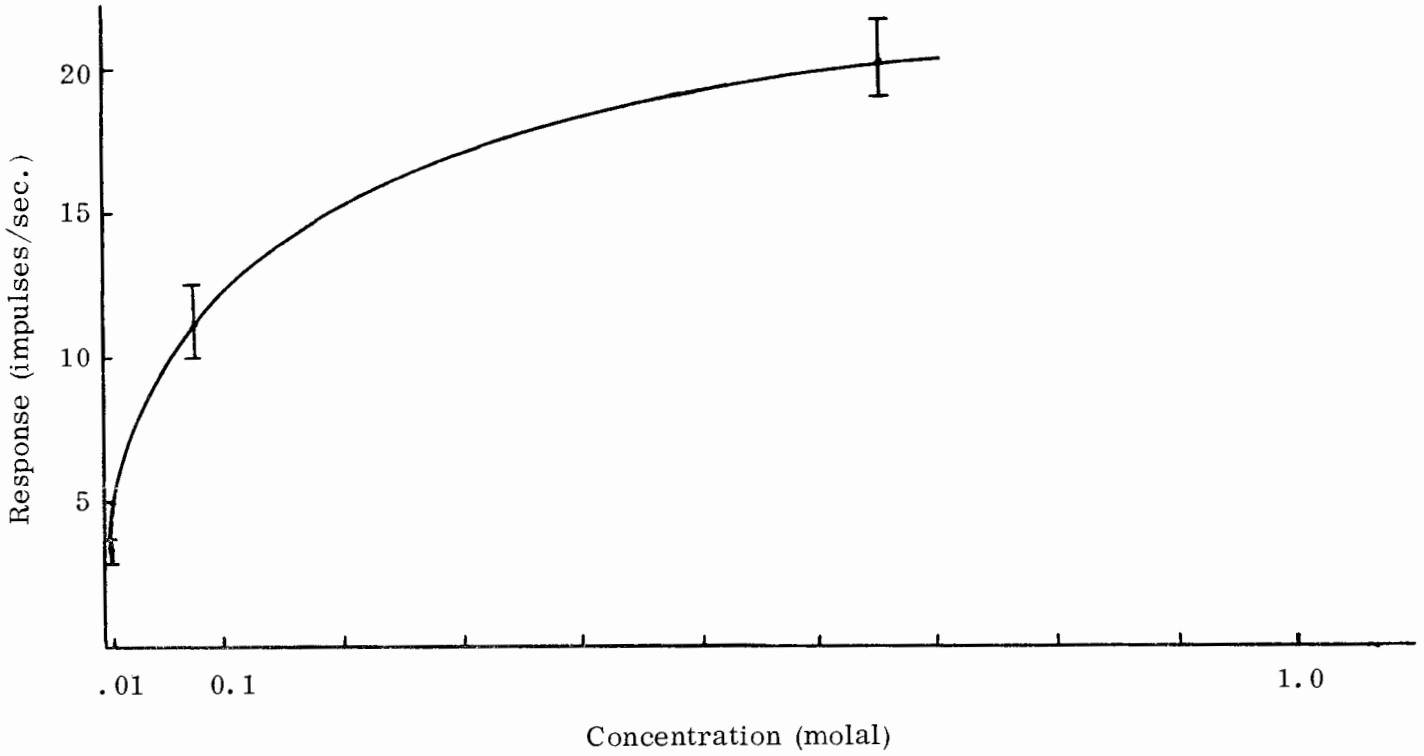

Figure 2. Stimulus-response curve. The activity coefficients of .01, 0.1, and 1.0 molal NaCl are taken as 1.0, .778, and .657 respectively.

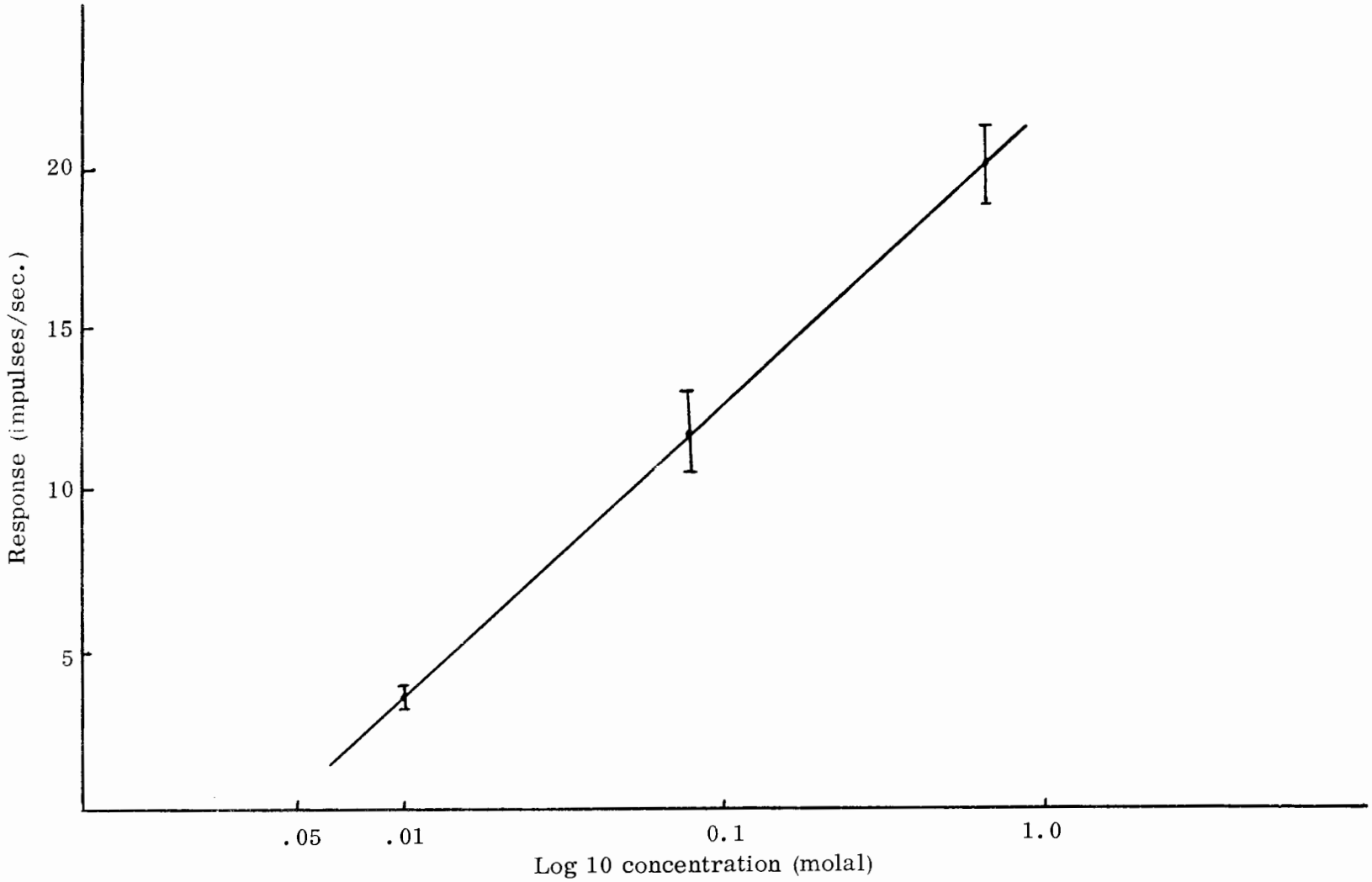

Figure 3. Log 10 concentration--response curve. Activity coefficients for .01, 0.1, 1.0 molal NaCl are taken as 1.0, .778, .657 respectively.

receptor site is based on the law of mass action, and that the magnitude of a response is directly related to the number of ions or molecules that react with the receptor surface. He represents the reaction of the stimulant with the receptor site as

1) $C + (S - N) \dashrightarrow N$

where C represents the stimulant concentration, S represents the total number of receptor sites on the receptor membrane, and N represents the number of receptor sites occupied by the stimulus. This reaction is similar to the expression representing the reaction between an enzyme and substrate.

$$E + S \longrightarrow ES,$$

One can solve for the equilibrium constant, K.

2) $$K = \frac{N}{C(S - N)} .$$

The values of N and S are impossible to determine. Beidler assumes that the maximum response, Rm, is proportional to the total number of receptor sites, S, and that a submaximal response, R, is proportional to the occupied receptor sites, N, thus deriving the two expressions

3) $R = aN$

4) $Rm = aS.$

where a is the constant of proportionality. Substituting these expression into equation 2 one can derive the expression.

$$6) \qquad \frac{C}{R} = \frac{C}{Rm} + \frac{1}{KRm}$$

If none of the inherent assumptions have been violated, a plot of C/R versus C should yield a straight line.

Data recorded from rat chemoreceptors stimulated by salts (Beidler, 1954) and from insect chromreceptors (Evans and Mellon, 1962; Stauffer, 1969) support this theory as does the present investigation (Figure 4). Calculated values for the equilibrium constant K are small which is consistent with the theory that the stimulus is adsorbed weakly to the surface of the receptor.

To determine if the salt receptor on the oral plate is similar to the cation receptor of Hodgson (1964), potassium chloride, sodium sulfate and choline chloride were used as stimulating solutions. No response was recorded when the stimulant was choline chloride. The sensory papillae did, however, respond to sodium sulfate and pottasium chloride. Replacement of the chloride ion with other anions does not eliminate the response, replacing the cation, sodium, with another non-stimulating cation, choline, completely abolished the response (Figure 12, c). This indicates that the sensory membrane is responsibe to small monovalent cations and relatively unresponsive to anions.

This investigation is compatible with the current trend to designate the classical term of salt receptor as a cation receptor (Hodgson, 1964). However the response to sodium sulfate was not equal to that of sodium chloride. The term cation receptor is therefore misleading. Rees (1968) reports that anions do effect the response of the sense cell to a cation and he suggests the replacement

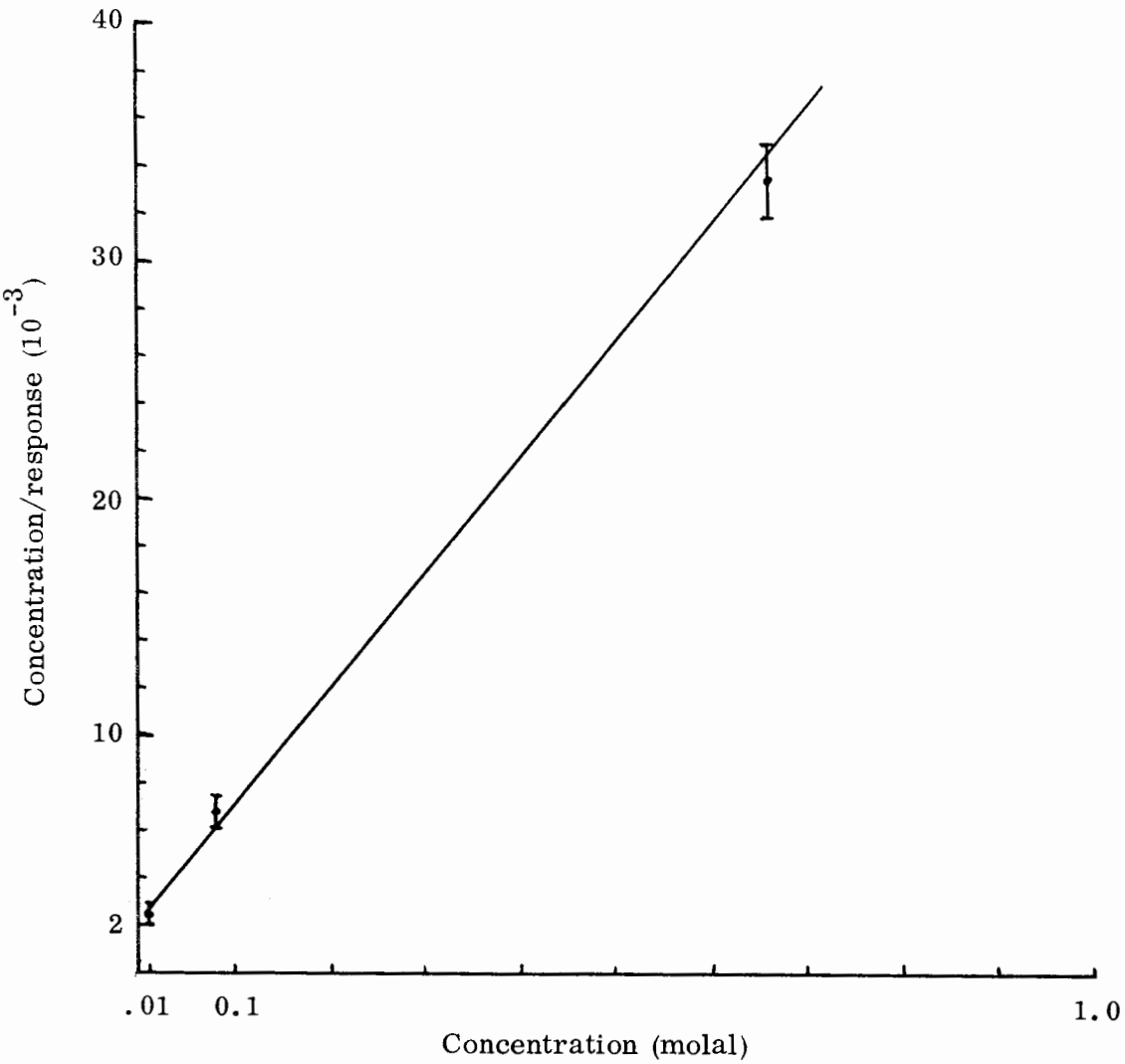

Figure 4. Concentration-response curve for NaCl. Activity coefficients for .01, 0.1 and 1.0 molal NaCl are taken as 1.0, .778, and .657 respectively.

of "salt," "sugar," "water," and "anion" receptors with type 1, type 2, type 3, and type 4 receptors respectively.

An attempt was made to determine if the sensory papillae are innvervated with a receptor which responds to variations in the pH of a solution. A series of solutions ranging from a pH of 11.4 to a pH of 2.4 and containing 1 molal NaCl were used as stimuli. One molal NaCl was used because the frequency of response made recording more convenient. The pH of the solutions were adjusted with hydrochloric acid and sodium hydroxide. No buffered solutions were used in order to avoid unknown effects which the buffering molecules may have on the chemoreceptor neurons. The response of the sensory papillae appeared to be normal to salt stimulation until extreme pH's were reached (Figure 10, 12 a, b). At a pH of 2.4 action potentials of several different amplitudes were recorded (Figure 10 a, b). The results indicate that a solution of pH 2.4 produces an injury response, and that all of the sense cells are propagating impulses. After a period of time, 1 to 2 minutes, no more impulses could be recorded from the sensory peg. At the basic end of the pH spectrum, normal responses were obtained until the testing solution became very basic. At a pH of 11.4, several spikes of large amplitude and inconsistent frequencies were observed, and after several seconds no more responses could be obtained (Figure 12 a, b). This, too, is considered to be an injury response. The oral plate sensory pegs appear to produce no response to variations in pH over a wide range of pH's until an injury response is evoked which ultimately leads to irreversible damage of the chemosensory neurons. The data are consistent with Beidler's theory for stimulation of taste receptors. The fact that large variations in pH have little influence

on the response to NaCl suggests that no enzymatic reaction is present in the response of the taste receptor.

Records of the response to salt solutions were recorded at various time intervals during the stimulation. The results show that the sensory receptor does adapt slowly to continued stimulation. However, one should remember that the evaporation occurring at the tip of the recording electrode causes the stimulant to become more concentrated as time passes (Figure 5 a, b, c). The rate at which the sensory papillae adapt to stimulation is, therefore, misleading. One should accept that adaptation does occur, and consider the time coarse of adaptation as being inaccurate for a single stimulant concentration.

Response to chemical stimuli: Water

In dilute solutions of sodium chloride two action potentials were recognized with very different amplitudes (Figure 7 a). The characteristic response to sodium chloride is represented by the larger spikes. The other impulse with a much smaller amplitude is a response to "water." A "water" receptor has been reported by Evans and Mellon (1962) on the labellum of Phormia and by Stauffer (1969) on the labial palpus of Apis mellifera. The response of the water receptor on the oral plate is similar. As the concentration of the stimulating solution increases, the response to water disappears. When the sodium chloride concentration was increased to 0.1 molal no impulses from the "water" receptor could be identified.

It is not clear what controls the response of the "water" receptor, but the most effective stimulant known seems to be water. Compounds such as sodium

chloride, choline chloride, sucrose, glycerol, and mannose inhibit the response of the "water" receptor at different concentrations suggesting that it is not a simple osmatic receptor (Evans and Mellon, 1962). It seems unlikely that water molecules stimulate the receptor site directly because it is always present in relatively high concentrations. More research is required in order to determine the characteristics of the "water" receptor.

Response to chemical stimuli: Sugar

Glucose is non-ionic and, therefore, a solution of glucose in distilled water exhibits a high resistance to electrical current. In order to record the response of the sense organ to glucose, a solution of 0.1 molal NaCl and 0.1 molal glucose was used as the stimulating solution. The oral plate sensory papillae of the worker honey bee responds to stimulation with the glucose and sodium chloride solution. The spike amplitude of the "sugar" receptor (Figure 7, b) with the "sugar" spike being slightly smaller. That there are two cells responsible for the propagated impulses is indicated by the frequency of the impulses. It is apparent that one spike often occurs during the refractory period of another spike (Figure 7, b c). A general characteristic of nerve cells is that they propagate impulses at a relatively constant rate over short periods of time and that the durations between the spikes is also relatively constant. The occurrence of one spike superimposed upon another in an oscilloscope trace is, therefore, an indication that the two spikes are iniated in different cells.

Evaporation at the tip of the recording electrode made it impossible to determine accurately the range of glucose concentrations to which the receptor responds.

Little is known about the reaction of sugars with the receptor membrane. Evidence suggests that the three dimensional structure of a sugar molecule is important in the reaction between the receptor site and the stimulant (Dethier, 1955; Morita and Shiraishi, 1968). This approach may produce supporting evidence for the stereochemical theory of olfactory reception proposed by Amoore (1963) and, at the same time, make it applicable to taste reception.

Response to chemical stimuli: Amino acids

No known electrophysiological evidence for a response to proteins or amino acids has been reported for Apis mellifera. It is difficult to obtain a solution of protein free of impurities. This makes it difficult for one to identify a protein as being responsible for a recorded response. However, amino acids can be obtained in a relatively pure state. It is possible, that if a receptor cell responds to amino acids, it will also respond to proteins.

Three solutions of amino acids (Sigma Chemical Company) were used as stimuli to test the oral plate sensory papillae. A solution of 0.1 molal sodium chloride and 2% L-asparagine at a pH of 6.8 gave a response with two definite spike amplitudes indicating a response to the salt and to the amino acid (Figure 11). Positive results were also obtained with .01 molal sodium chloride and L-valine, pH 6.4 (Figure 8) and with 0.1 molal sodium chloride and L-glutamine, pH 7.0 (Figure 9). The range of amino acid concentrations to which the sensory papillae responds is unknown. The number of amino acids which will initiate a response is unknown and the characteristics of the amino acids which are responsible for the positive response are also unknown. In the present investigation

three amino acids of quite different structures all produced positive results making it clear that an amino acid sensory receptor is present on the oral plate. It is likely that the amino acids cause a response in the fourth cell which innervates the oral plate sensory papillae. However, evidence exists which suggests that certain amino acids can stimulate the sugar and salt receptors of the blowfly and fleshfly (Shiraishi and Kuwabara, 1970).

Response to chemical stimuli: Queen substance

The sensory papillae were also stimulated with 9-oxodec-2-enoic acid in order to determine if they are important in recognition of the queen substance. A solution of 2% queen substance at a pH of 6.4 was used as a stimulating solution. Negative results were obtained consistently. A large make-break artifact occurred when electrical contact was made with the sensory peg, but no impulses were recorded. These results do not prove that a chemoreceptor for the queen substance does not exist on the mouth-parts of a honey bee, because there are several innervated setae which have never been investigated electrophysiologically on the mouth-parts. The data, however, indicate that the oral plate sensory papillae are not responsible for the recognition of queen substance.

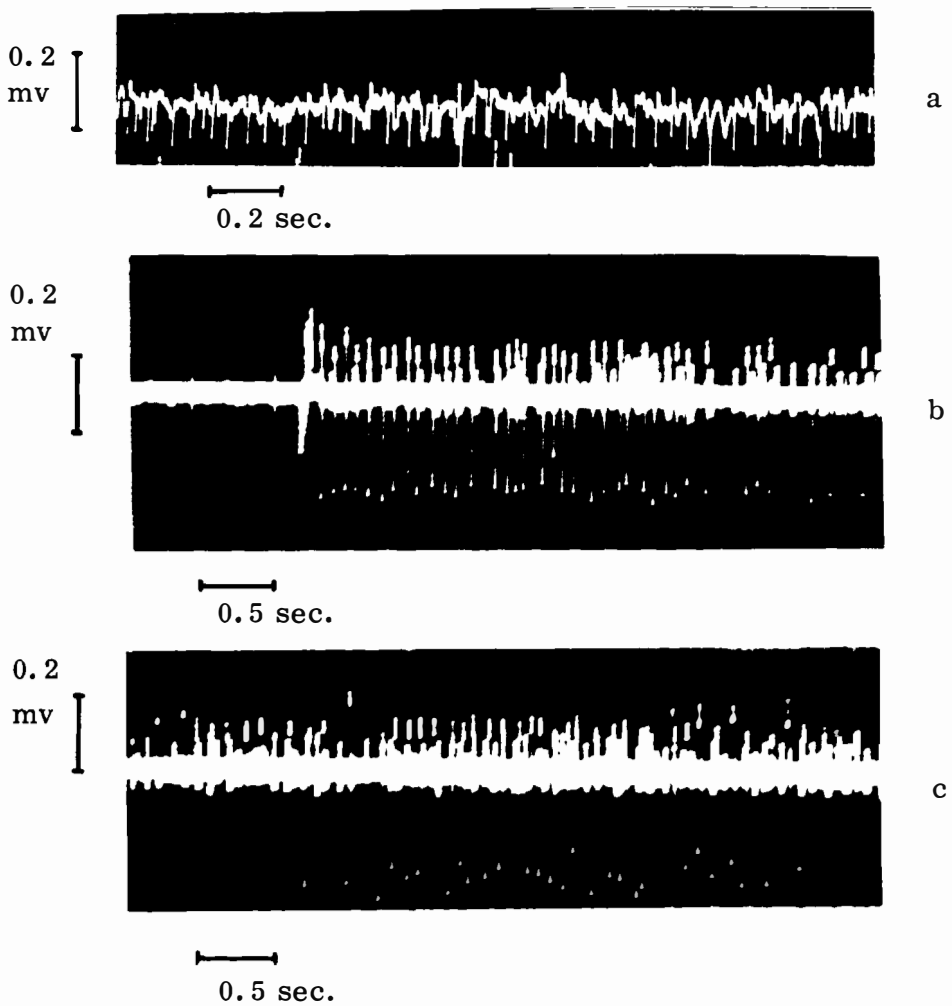

Figure 5. a. Response to 1.0 molal NaCl, 10 seconds after stimulation.
b. Response to 0.1 molal NaCl.
c. Response to 0.1 molal NaCl, 45 seconds after stimulation.

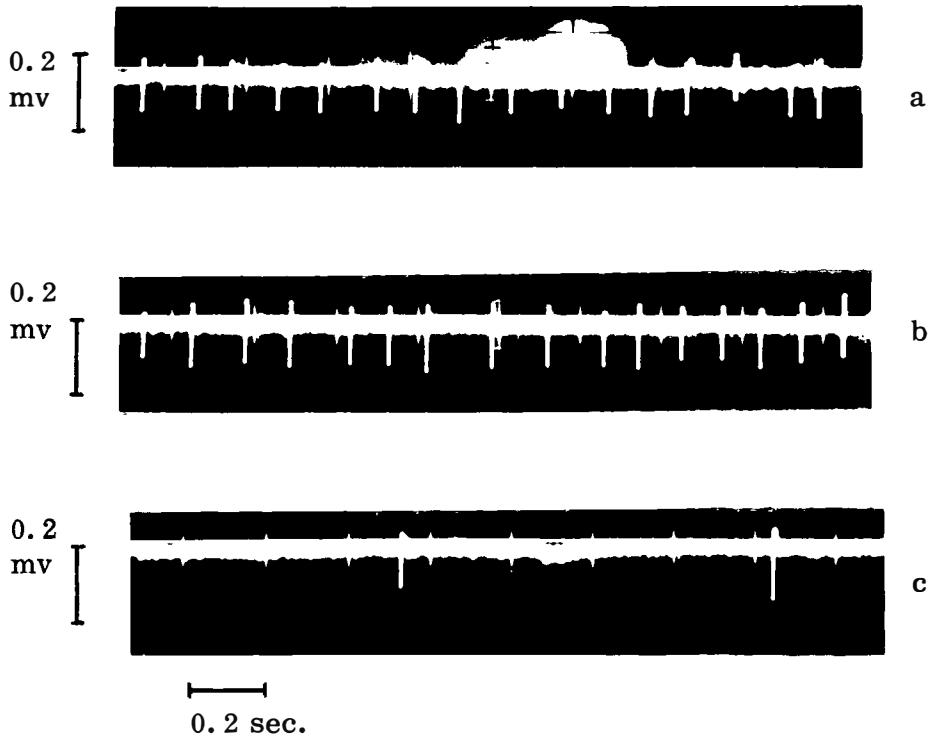

Figure 6. a. Response to 0.1 molal NaCl, 10 seconds after stimulation.
b. Response to 0.1 molal NaCl, 40 seconds after stimulation.
c. Response to 0.1 molal NaCl, 70 seconds after stimulation.

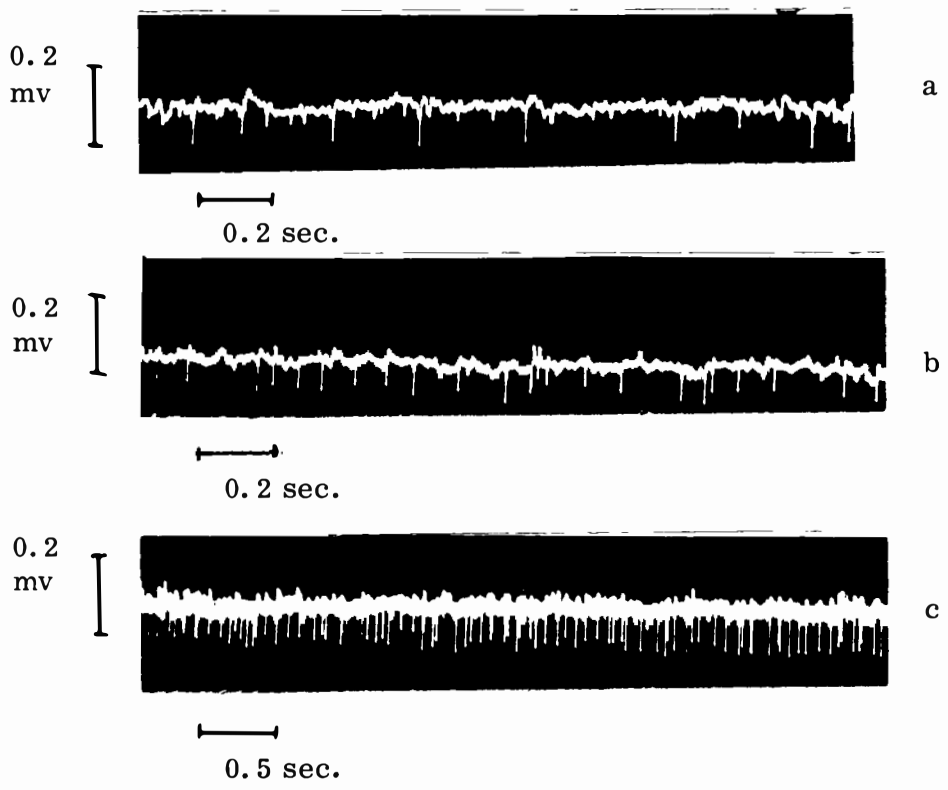

Figure 7. a. Response to 0.01 molal NaCl. Small spikes are responses to water.
b. Response to 0.1 molal NaCl and 0.1 molal glucose.
c. Response to 0.1 molal NaCl and 0.1 molal glucose.
Long spikes indicate response to salt.
Short spikes indicate response to glucose.

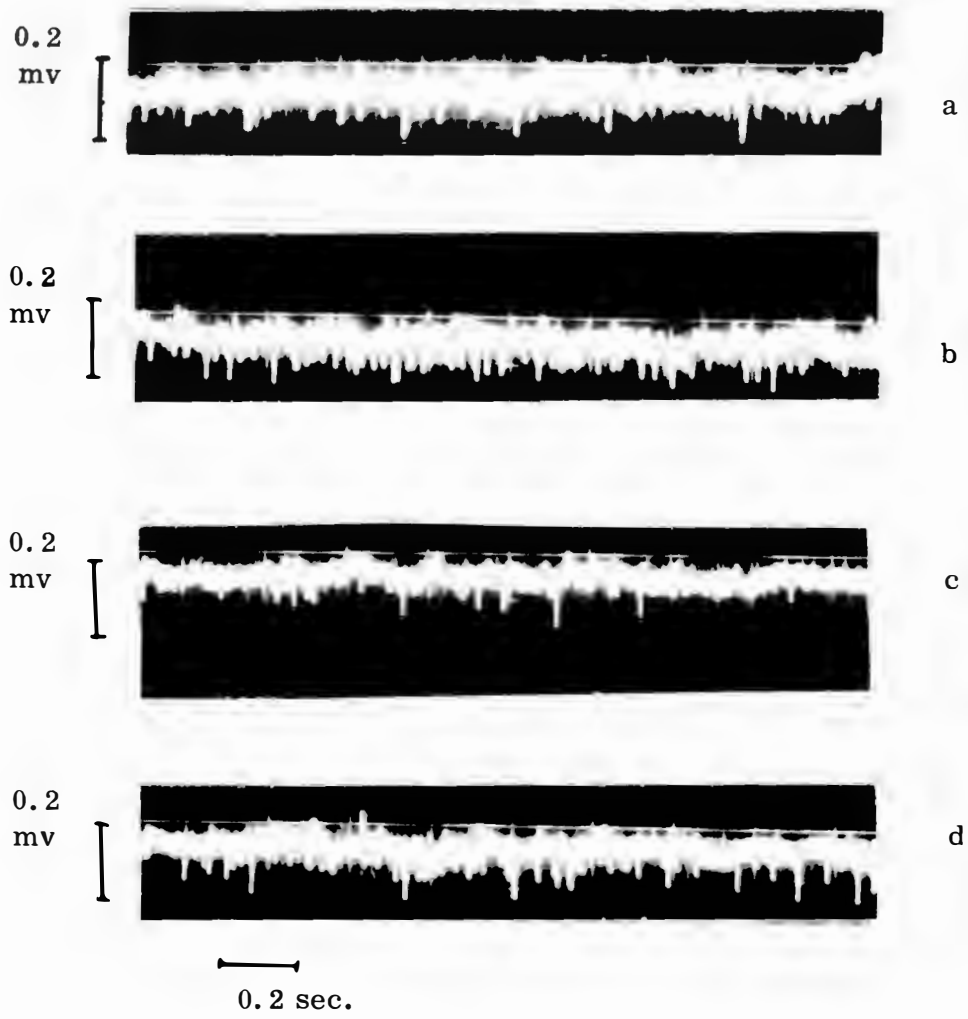

Figure 8. a. Response to 0.1 molal NaCl and 1% L-valine 30 seconds after stimulation pH 6.4.
b. Response to 0.1 molal NaCl and 1% L-valine 45 seconds after stimulation pH 6.4.
c. Response to 0.1 molal NaCl and 1% L-valine 60 seconds after stimulation pH 6.4.
d. Response to 0.1 molal NaCl and 1% L-valine pH 6.4.

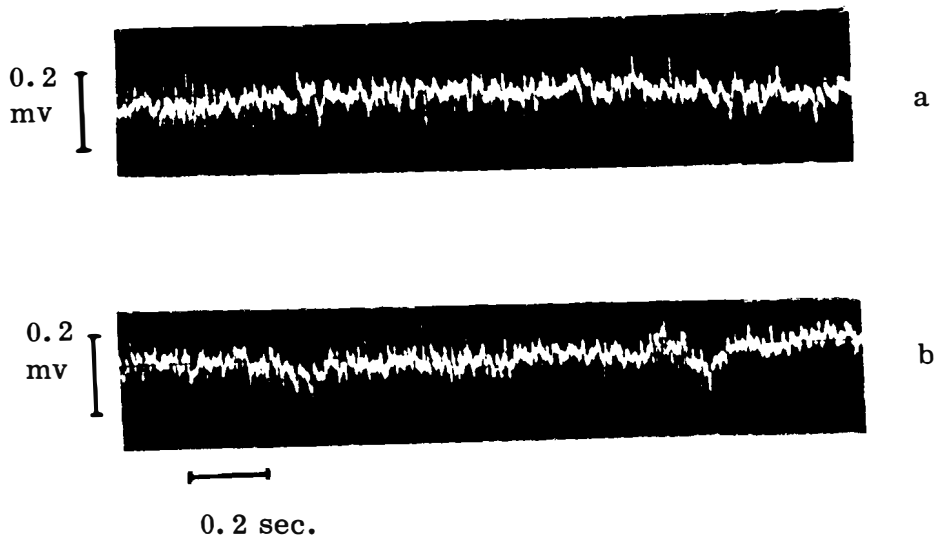

Figure 9. a. Response to 0.1 molal NaCl and 2% L-glutamine at pH 7.
b. Response to 0.1 molal NaCl and 2% L-glutamine at pH 7.

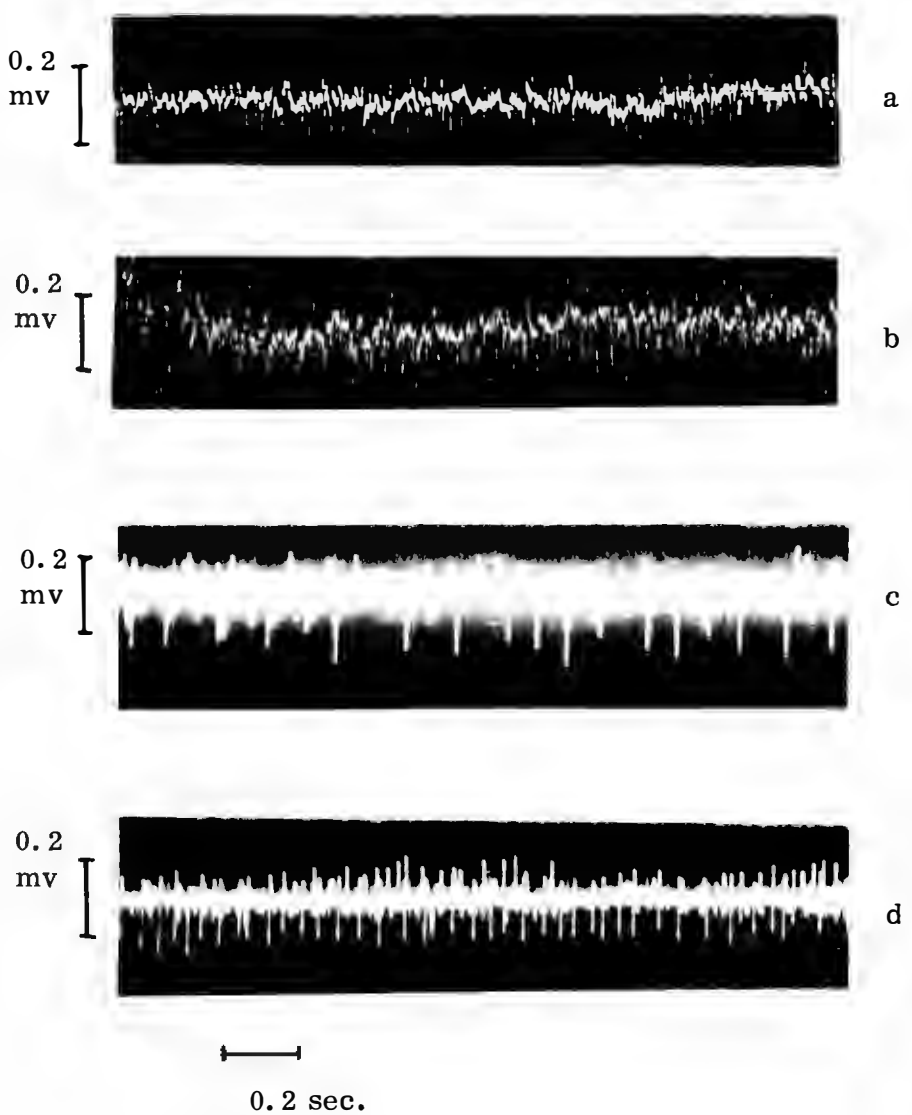

Figure 10. a. Response to 1.0 molal NaCl at pH 2.4.
b. Response to 1.0 molal NaCl at pH 2.4.
c. Response to 1.0 molal NaCl at pH 4.8.
d. Response to 1.0 molal NaCl at pH 7.4.

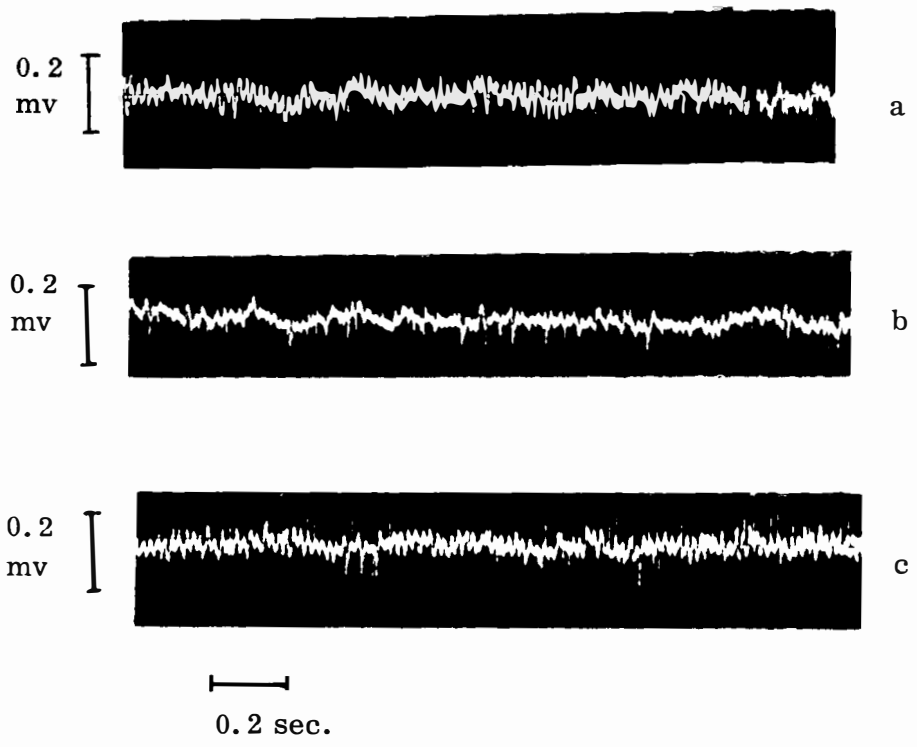

Figure 11. a. Response to 0.1 molal NaCl and 2% asparagine at pH 6.8.
b. Response to 0.1 molal NaCl and 2% asparagine at pH 6.8.
c. Response to 0.1 molal NaCl and 2% asparagine at pH 6.8.

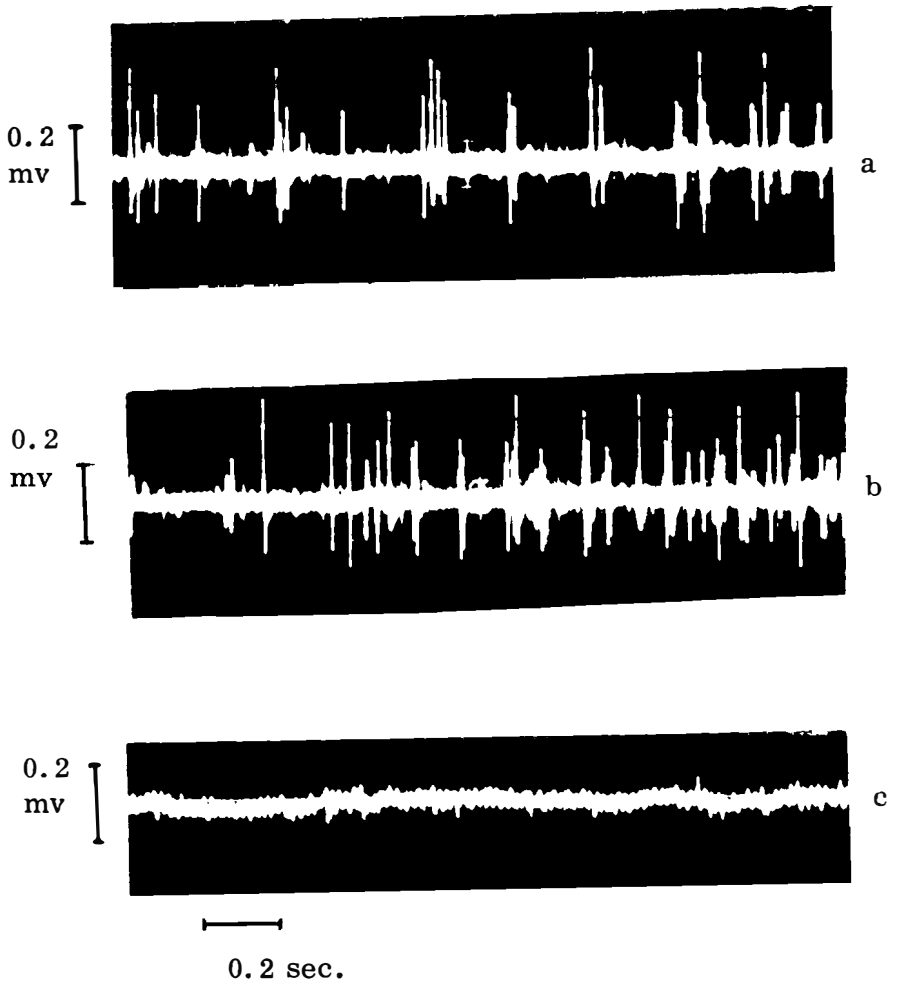

Figure 12. a. Response to NaOH pH 11.4.
b. Response to NaOH pH 11.4.
c. Negative response to 0.1 molal choline chloride.

SUMMARY

1. The heads of worker honey bees were removed and mounted on a glass slide, the oral plate was exposed, and the tip of one of the mandibles was removed and its contents injured with a hot needle.

2. A recording technique similar to the one used by Hodgson, Lettvin, and Roeder (1955) was employed to investigate the response of the oral plate sensory organs to several chemical stimulants.

3. A micropipette containing the recording electrode and the stimulant was placed over the individual sensory organs of the oral plate. The reference electrode was placed in the lumen of the mandible.

4. Each sensory papillae on the hypopharyngeal oral plate is innervated by four bipolar sense cells. The papillae are capable of responding to cations, amino acids, sugars, and "water." One cell responds to salt and another to "water." A third cell responds to sugar and possibly amino acids. Further study is required to determine the exact function of the fourth cell. No mechanical response was identified.

5. The data of the present investigation correlates well with the current theories of contact chemoreception.

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