EFFECTS OF ANTIDROMIC ACTIVITY IN GUSTATORY NERVE FIBERS ON TASTE RECEPTORS OF THE FROG TONGUE

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ABSTRACT

EFFECTS OF ANTIDROMIC ACTIVITY IN GUSTATORY NERVE FIBERS ON TASTE RECEPTORS OF THE FROG TONGUE

By

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It has been previously shown that interactions between individual gustatory units with overlapping peripheral receptive fields modify the stimulus-response functions of these taste fibers. Antidromic activity in peripheral collaterals of taste fibers can produce lateral interactions between sensory units innervating a common fungiform papilla on the frog tongue.

Antidromic electrical stimulation of the lingual branch of the IX nerve of the frog was conducted while recording intracellular potentials of taste disc cells in order to assess the possible role of antidromic sensory fiber activity in the modification of receptor cell bioelectric properties.

Antidromic activation of sensory fibers resulted in depolarization of cells of the papilla surface and hyperpolarization of the subsurface receptor cells. These potential changes exhibited latencies greater than 1 second which could not be ascribed to the conduction times of any of the fibers in the IX nerve. They also showed summation, adaptation and

post-stimulus rebound. This rebound was of a polarity opposite to the initial change produced by antidromic stimulation and of the same direction as the change accompanying adaptation.

Depending on stimulus conditions antidromic activity was able to produce depression or enhancement of chemosensory fiber discharge in response to taste stimuli. Different stimuli were found to have potentiating or depressing effects on the antidromically elicited potential changes of taste disc cells.

The results of these experiments favor the model of lateral interaction between sensory units of the tongue as functionally mediated by bioelectric effects on the receptor cells consequent to antidromic activity in taste fibers innervating them.

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This work is dedicated to my family, who have held unswerving faith in me throughout the years, to my grandfather who instilled in me the love of science, and to my grandmother who encouraged its pursuance.

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I. LITERATURE REVIEW

Anatomy of Frog Tongue Taste Receptors

Fungiform Papillae

The localization of the sense of taste in the receptors of the frog tongue fungiform papillae has been suggested by anatomists (Gaupp, 1904; Hammerman, 1969; Kolmer, 1910). These receptor cells have been described as located in the specialized end disc forming the dorsal surface of the papilla (Ecker, 1889; Gaupp, 1904; Hammerman, 1969). Physiological studies have corroborated this view (Pumphrey, 1935; Kusano and Sato, 1957; Zotterman, 1949).

The structures responsible for taste reception are located in the fungiform papillae of the tongue (Ecker, 1889; Gaupp, 1904; Hammerman, 1969) as well as in discrete buds dispersed throughout the epithelium of the buccal and pharyngeal cavities. Those of the tongue are solely in the taste disc of the fungiform papillae while those located in other areas are of different morphology, and histologically resemble the lateral line receptors of the lower vertebrates (Ecker, 1889; Pumphrey, 1935).

The fungiform papillae have been reported to number 500-600 on a frog tongue (Kusano and Sato, 1957) and 400-500 by Rapuzzi and Casella (1965). Robbins reports the density of

such structures to be on the average 4.9 per square millimeter of tongue surface (Robbins, 1967a). The interpapillary distance is between 100 and 400 microns (Kusano and Sato, 1957; Rapuzzi and Casella, 1965). Hammerman (1969) in a histological study of their development reports the size of a single papilla to be 142-160 microns in diameter and the height above the tongue surface 200-230 microns. The papillae studied by Kusano and Sato were 100-200 micra in diameter (Kusano and Sato, 1957) and those reported by Rapuzzi and Casella (1965) averaged 100 micra across the surface of the taste disc.

Innervation

Ariens Kappers et al. (1936) report the amphibian tongue to be innervated by cranial nerves V, VII, IX. The sensory components responsible for taste are primarily located in the IX nerve (Ecker, 1889; Gaupp, 1904; Kusano and Sato, 1957; Pumphrey, 1935; Rapuzzi and Casella, 1965; Strong, 1895), although Robbins (1967a) mentions a contribution to the base of the tongue by cranial nerve VII. No taste components have been reported in the branches of cranial nerve V innervating the frog tongue.

Autonomic fibers are described in the IX nerve of the frog (Chernetski, 1965; Herrick, 1925; Strong, 1895) but these have been considered vasomotor (Pumphrey, 1935). Chernetski (1965) has reported their origin in the anterior sympathetic ganglion. They have light, or no myelin sheaths, and their

conduction velocities range from 0.2-0.6 meters per second (Chernetski, 1965) as compared to 1-15 meters per second for sensory fibers (Rapuzzi and Casella, 1965).

Sensory fibers in the IX nerve each divide into 4-6 collaterals (Rapuzzi and Casella, 1965) before entering the fungiform papillae. Each papilla receives 5-10 (Gaupp, 1904), 7-14 (Rapuzzi and Casella, 1965), 6-7 (Robbins, 1967a) afferent fibers, collaterals traveling to and innervating neighboring fungiform papillae. Rapuzzi and Casella (1965) confirm anatomical assertions (Ecker, 1889; Gaupp, 1904) that each papilla contains only one nerve fiber serving the sense of touch. Although Pumphrey (1935) reported sensitivity to touch over the entire tongue surface, Rapuzzi and Casella (1965) and Taglietti et al. (1969) describe it as residing only in the fungiform papillae. The nerve fibers entering the papillae lose their myelin sheaths and undergo tortuous convolutions before ascending to the region of the cells making up the taste disc of the fungiform papilla (Gaupp, 1904; Hammerman, 1969).

Taste Disc

The three morphological cell types composing the majority of the taste disc of the fungiform papillae in the frog have been described and differently labeled by various authors. Although the cells of the topmost layer appear homogeneous they have been variously called goblet (Ecker, 1889; Hammerman,

1969), cylinder (Gaupp, 1904; Robbins, 1967a), associate (DeHahn and Graziadei, 1971), supporting (Uga, 1966; Uga and Hama, 1967), and mucous cells (Rapuzzi and Casella, 1965; Stensaas, 1971). They apparently contain mucous granules and do not serve a direct sensory function (Hammerman, 1969). The layer of cells below these is composed of two general morphologic types. One has been labeled the forked cell (Ecker, 1889; Gaupp, 1904; Hammerman, 1969) because it divides into at least two peripheral processes which extend upward toward the surface of the taste disc. Rapuzzi and Casella (1965) refer to this type as supporting cell while Stensaas names it sustentacular (Stensaas, 1971). The other major cell type has its cell body located below the surface and on a level with that of the forked cell. It has a process extending up to the surface of the taste disc and due to its similarity to the visual receptor cell of the frog retina has been called the rod cell (Hammerman, 1969, Stensaas, 1971). It has been considered the taste receptor of the frog tongue (Beale, 1869; Ecker, 1889; Kolmer, 1910), a view supported by modern microanatomists (Hammerman, 1969; DeHahn and Graziadei, 1971; Stensaas, 1971) and physiologists (Kusano and Sato, 1957; Rapuzzi and Casella, 1965). It has variously been called rod (Hammerman, 1969), cylindrical (Ecker, 1889), goblet (Gaupp, 1904), sensory cell (Rapuzzi and Casella, 1965) and bipolar rod cell (Stensaas, 1971).

Without discriminating between rod and forked cells the layer of cells containing these two types has been described as that of the gustatory cell (Kusano and Sato, 1957; Uga, 1966; Uga and Hama, 1967) or sensory cell (DeHahn and Graziadei, 1971). For the purposes of the present work the terminology of Hammerman (1969) seems most suitable, and will be used. The topmost layer of cells will be referred to as goblet or mucous cells, while the lower layer will be considered to contain rod and forked cells. Hammerman (1969) describes a total of seven cell types composing the taste disc, all but the previously mentioned three forming a ring around the periphery of the disc and not directly related to the functional aspects of the sense of taste in the frog.

Because of their implication in the peripheral process of taste reception by different authors the anatomy of the three cell types most populous in the taste disc of the frog will be reviewed. Of the average 700 epithelial cells forming the taste disc on the surface of the fungiform papilla, 200 are estimated to be goblet cells (Robbins, 1967a). They are described as 10-20 micra in diameter and 20-24 micra long (Ecker, 1889), 19-22 micra long and 10-12 μ in diameter (Hammerman, 1969) and Stensaas (1971), reports them to be 6-10 microns in diameter and extending from the surface of the taste disc where they compose most of the surface down to a depth of 25-30 microns, tapering as they descend. Between the basal processes of these goblet cells reside the bodies

of the rod and forked cells which send thin processes upward between the goblet cells to the surface of the taste disc.

The forked cells are somewhat smaller than most of the other cells of the taste disc. Ecker (1889) gives their dimensions as 6-8 μ in diameter with 1-2 μ apical processes. This is in agreement with the dimensions of Hammerman (1969) and Stensaas (1971) who report dimensions of 5-8 micra for the cell body and 1-2 micra for the diameter of the apical processes.

Rapuzzi and Casella (1965) describe a taste disc as containing 40-50 rod cells. Ecker (1889) reports a taste disc as having several hundred of these cells. From the tip of their processes (that project to the surface) to their base these cells are 32-38 micra long. At their widest part they are reported to be 5-7 micra (Hammerman, 1969; Kusano and Sato, 1957) and 7-9 micra (Stensaas, 1971). The rod-like processes taper from four micra at the base to one micron at the apex (Hammerman, 1969; Stensaas, 1971).

Nerve Branchings

It is well-established that single nerve fibers of the glossopharyngeal nerve branch both within a single fungiform papilla and before entering the papillary stalk (Ecker, 1889; Gaupp, 1904; Herrick, 1925). Rapuzzi and Casella (1965), estimated that a single sensory fiber branched 4-6 times before entering the fungiform papillae and that each branch

furthermore formed 5 terminals within a single papilla. If each branch innervated a different sensory cell, a single fiber was estimated to be in contact with about 30 gustatory receptors. Kusano and Sato (1957) add that each taste receptor is apparently innervated by more than one gustatory fiber. Rapuzzi and Casella (1965) tracing the neural interconnections between papillae on the frog tongue by electrically stimulating one papilla and recording the action potentials of collateral branches of nerve fibers innervating this and neighboring papillae found that from 6-29 papillae may be interconnected with any one papilla. They also state that most of these interconnected papillae have 2-4 nerve fibers in common with each other. Taglietti et al. (1969) reported that chemical stimulation of a single papilla with $CaCl_2$ would lead to collateral nerve fiber discharge in 2-3 commonly innervated fungiform papillae nearby. Thus, it is evident that single nerve fibers not only innervate multiple papillae and receptor cells, but that their receptive fields overlap those of other gustatory fibers both within a single and among several fungiform papillae on the surface of the frog tongue. Such an anatomical arrangement is amenable to functional interaction which may be occurring between gustatory units on the surface of the tongue.

Receptor-Nerve Junctions

Hammerman (1969), Rapuzzi and Casella (1965) and Stensaas (1971) report patches of contact between sensory fibers and the rod cells of the taste disc which include loose intertwinings, apposition, and increased density of cell membranes in the areas of such contacts. DeHahn and Graziadei (1971) and Uga (1966), Uga and Hama (1967) describe such contacts as synaptic, having tight junctions, 200 angstrom synaptic clefts, and thickened membranes in the area of synapsis (Uga, 1966; Uga and Hama, 1967). Such junctions are polarized in their microanatomy. The sense cells of the taste disc contain dense granules aggregated near the synapse. These have been reported by Rapuzzi and Casella (1965), Stensaas (1971) and Uga (1966), Uga and Hama (1967). These vesicles are considered to contain a transmitter substance and have been reported to be 500-900 angstroms (Uga and Hama, 1967) or 800-1000 angstroms (DeHahn and Graziadei, 1971) in diameter. DeHahn and Graziadei (1971) further report that these vesicles contain norepinephrine as identified by microhistochemical techniques. This supports the suggestions of adrenergic functions proposed for these vesicles by Uga and Hama (1967). Another aggregation is that of clear vesicles which are found primarily in the nerve terminals and serve to distinguish sense cell from nerve. These vesicles are 200-600 angstroms in diameter (Uga and Hama, 1967), found only in the nerve terminals (Stensaas, 1971) and have been found by histochemical methods to be cholinergic

(DeHahn and Graziadei, 1971). These structures suggest a bidirectional function of such synapses similar to those found in rat olfactory bulb (Andres, 1965).

Receptor-Receptor Junctions

DeHahn and Graziadei (1971) describe tight and gap junctions between peripheral processes of taste disc receptor cells. These have been described as appositional by Hammerman (1969) but the former authors suggest that they may serve to provide a pathway for interaction between peripheral processes of receptor cells.

Receptor-Goblet Cell Junctions

Described as unspecialized by DeHahn and Graziadei (1971), the presence of tight junctions (zonulae occludentes) between the goblet cells and peripheral rod-like processes of the presumed receptor cells of the taste disc has been suggested to subserve a functional alteration of receptor function by providing a low resistance pathway for ionic currents between receptor process and goblet cell (Stensaas, 1971).

Goblet Cell-Goblet Cell Junctions

The many tight junctions between adjacent mucous cell membranes of the taste disc are similar to those described above for receptor-goblet cell junctions. Though not morphologically similar to those believed to subserve chemical

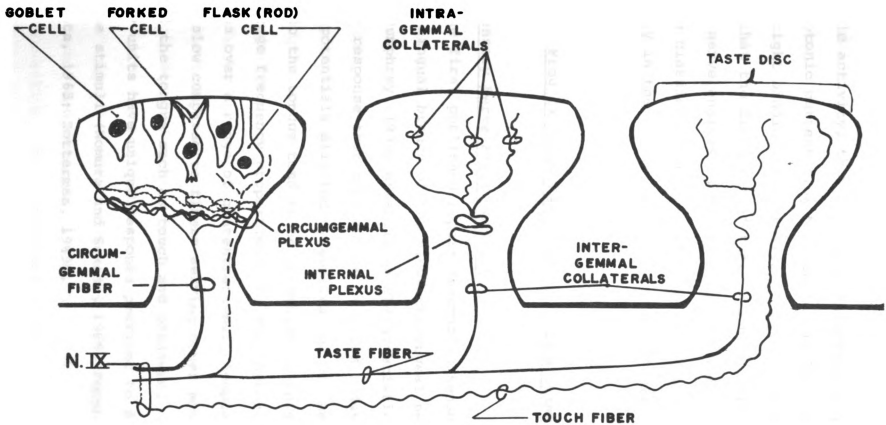


Figure 1. Schematic representation of peripheral taste structures in the frog tongue.

synaptic activity, they have been suggested as pathways for electrotonic current flow by Stensaas (1971). Furthermore, their tight binding of epithelial cells has been taken to prove that the diffusion of taste stimuli through the papilla to the nerve endings is highly unlikely, thereby strengthening the classical assertion of receptor properties as residing only in the specialized cells of the taste disc (Stensaas, 1971).

Frog IX Nerve Responses to Tongue Stimulation

Chemosensory Nerve Fiber Activity

The first published report describing sensory activity in the lingual branch of the glossopharyngeal nerve of the frog (Pumphrey, 1935) established characteristics of chemosensory response that continue to be valid today. Nerve action potentials elicited by application of chemical solutions to the tongue tend to be irregular in interval but with an average frequency proportional to the intensity of the stimulus over a range of concentrations. Adaptation is relatively slow compared to fibers serving other sensory modalities of the tongue such as touch and pressure. Individual sensory units have unique response profiles to different adequate stimuli (Nomura and Sakada, 1969; Pumphrey, 1935; Yamashita, 1963; Zotterman, 1949).

Adequate stimuli for chemosensory activity in IX include sodium chloride and other monovalent salts (Kusano, 1960; Kusano and Sato, 1957; Yamashita, 1963) as well as many divalent salts such as calcium chloride and magnesium chloride (Kusano, 1960; Kusano and Sato, 1957; Robbins, 1967b). Organic and inorganic acids may also stimulate the chemoreceptors (Kusano, 1960; Kusano and Sato, 1957; Robbins, 1967b; Zotterman, 1949). Bitter alkaloids such as strychnine (Zotterman, 1949) and quinine hydrochloride in solutions applied to the tongue can elicit chemosensory discharge (Kusano, 1960; Kusano and Sato, 1957; Zotterman, 1949). Studies by Kusano (1960), Kusano and Sato (1957), and Robbins (1967b) indicate that in contradiction to earlier reports by Pumphrey (1935) and Zotterman (1949) sucrose is itself an adequate chemosensory stimulus to the frog tongue. In addition, Kusano and Sato (1957) report that the sodium salt, saccarin, can stimulate the chemoreceptors, but believe its effect to be primarily on sensory units which normally respond to low salt concentrations. They also add that unlike the case in mammals, units which respond to sucrose are not receptive to saccarin. Yamashita (1963) reported a chemosensory response to sodium glutamate and confirmed by him in another report (Yamashita, 1964). That the frog taste receptors respond to application of water appears to be a well-founded generalization (Kusano, 1960; Kusano and Sato, 1957; Nomura and Sakada, 1969; Robbins, 1967b; Yamashita, 1963; Zotterman, 1949). The report by

Zotterman (1949) also describes a response to extracts of macerated flies prepared in water or frog saliva.

Tactile and Thermal

The touch fibers in the IX nerve are found to discharge rapidly in response to tactile stimulation of the fungiform papillae (Kusano and Sato, 1957; Nomura and Sakada, 1969; Rapuzzi and Casella, 1965; Taglietti et al., 1969). First described by Pumphrey (1935) they are rapidly adapting, a single touch rarely able to elicit more than two action potentials in the same fiber. Fibers responding to thermal stimulation of the frog tongue contribute to whole nerve activity as recorded with gross electrodes only above 25°C. (Yamashita, 1964). Thermal receptor activity is rare in the range from 10-20°C. (Chernetski, 1964).

Peripheral Interactions of Stimuli

Some solutions which may be adequate stimuli in themselves are found to depress the response of chemosensory fibers to other stimulus solutions. Zotterman (1949) reported the depression of the frog tongue response to water by previous application of saline solutions and quinine. This depression of the water response is reported for solutions of cobalt chloride, most monovalent salts, saccarin, quinine and acetic acid solutions (Kusano and Sato, 1957; Kusano, 1960). Yamashita (1963) adds magnesium chloride, barium chloride and

strontium chloride to the list of depressants. Ringer's solution used as a bathing solution for the tongue was found by every previous study to be generally a poor stimulus, and in many cases quieted the spontaneous chemoreceptor discharge from the frog tongue. Yamashita (1963) adds that in addition to depression of the water response, Ringer's decreased nerve discharge to sodium chloride and calcium chloride solutions on the tongue of the frog. Kusano (1960) and Kusano and Sato (1957) reported no depressant effect on the water response by solutions of magnesium chloride, calcium chloride and sucrose. Zotterman (1949) reported that the frog tongue response to water was even enhanced somewhat by previous exposure to calcium chloride. Yamashita (1963) describes an inverse relationship between sensory nerve response and calcium chloride concentration at stimulus solutions greater than 0.016 molar.

Enhancement of response has been shown for other stimulus combinations. The discharge of the IX nerve chemoreceptors is enhanced in its response to salt solutions when the tongue is previously stimulated with water (Kusano, 1960; Yamashita, (1963). A more peculiar effect is seen when the frog tongue is stimulated by acetic acid and rinsed with Ringer's. In this case, there appears to be an augmented sensory fiber discharge when the Ringer's is applied. This has been termed an "off response" by Yamashita (1964) and has been found to be related directly in magnitude with the temperature of the tongue as well as the concentration of the previous solution of acetic acid.

Temperature Effects

Chemoreceptor discharge in response to standard solutions on the tongue increases as a function of increased temperature over a range of 10-35°C. (Yamashita, 1964). In that study, it was also reported that the rate of adaptation increased with temperature. The slope of the response function for a series of various concentrations of one stimulus species increased with temperature.

Pharmacologic Actions on Chemosensory Discharge

Using either tap water or a 2 percent sodium chloride solution as a stimulus to the frog tongue, Landgren et al. (1954) reported the following effects of various cholinergic and anticholinergic drugs applied to the tongue surface on the IX nerve discharge. Acetylcholine produced increased excitability as did the cholinesterase inhibitors neostigmine, diisopropoxyphosphoryl fluoride (DFP) and tetraethyl hypophosphate (TEPP). Eserine was without definite effect. Cholinergic blocking agents such as curare, decamethonium and succinylcholine led to a decreased response to stimulation. Eserine was, however, found to have a potentiating response which was reversed by atropine applied to the tongue surface in another series of experiments reported by Rapuzzi and Ricagno (1970). The conclusion suggested by both papers is that chemosensory nerve fibers are activated by a synaptic transmitter liberated

presumably by the receptors of the taste disc and acting postsynaptically in a manner similar to acetylcholine.

A rather indirect suggestion of post synaptic action on chemosensory nerve terminals in the frog fungiform papillae was reported by Nomura and Sakada (1969). Abolishing the propagated action potentials with tetrodotoxin, they were able to record slow potentials presumably generated in the peripheral terminals of the fibers innervating a single fungiform papilla. Touch fibers responded to tactile stimulation with a consistent current proportional to the strength of the stimulus, whereas the potentials produced by chemical stimulation of the papilla were more variable as might be expected in a post synaptic element responding to quantal transmitter release.

Taste Disc Influence on Nerve Function

The findings of Robbins (1967b) that cutaneous nerves which were used to cross-innervate the frog tongue developed "gustatory" capabilities identical in every way to those of IX nerve fibers allowed to reinnervate the tongue, led him to recognize the importance of the influence of taste disc receptor cells on chemosensory function. When tongues were transplanted to the dorsal lymph sac and reinnervated by cutaneous nerves it was found that these fibers also developed "gustatory" characteristics, including a water response (Robbins, 1967b). Zotterman (1949) could find no sensitivity to water of frog cutaneous chemosensory fibers.

Single Gustatory Fiber Sensitivities

A final generalization which apparently is true for frog IX nerve chemosensory response to tongue stimulation is the concept of multiple sensitivities for individual units. Most fibers studied had a response to two or more different classes of stimuli on the tongue (Kusano, 1960; Kusano and Sato, 1957; Robbins, 1967b; Zotterman, 1949). Because responses to stimuli over broad concentration ranges were generally difficult to record due to the technical restrictions of these preparations comparisons of predominant sensitivities were not made.

Autonomic Effects on Peripheral Receptors

Sympathetic Enhancement

Tucker and Beidler (1956) reported that sympathetic nerve activity could be responsible for enhancement of olfactory sensitivity in the rat. Beidler (1961) suggested that such an effect could be a function of sympathetic modulation of blood flow in the sensory organ. Dodt and Walther (1957) in remarking on the enhancement of temperature receptor response in the tongue of the dog by electrical stimulation of the lingual nerve came to the conclusion that this effect was produced by blood flow changes in the tongue. Hellekant (1971) reported that the enhancement of taste responses in the rat was a function of increased blood flow in vessels supplying

the taste buds. He furthermore concluded that depression of taste responses was not a result of local oxygen supply to the taste buds, but rather directly a result of decreased flow of blood to the tongue.

Sympathetic activity has been found to enhance sensory nerve discharge from cutaneous receptors in the skin of the frog (Chernetski, 1964) as well as the amplitude of the generator potential in muscle spindle stretch receptors (Eldred et al., 1960). Sympathetic enhancement of afferent nerve discharge has been reported for the cochlear nerves (Spoendlin, 1966) and mammalian muscle spindle (Hunt, 1960). These sympathetic effects were not considered to be solely the result of changes in blood flow. The implication of adrenergic transmitter enhancement of sensory discharge has been proposed to explain some of these sympathetic effects (Chernetski, 1964).

Autonomic Effects on Taste

Esakov (1961) and Brush and Halpern (1970) have demonstrated reflex enhancement and depression of sensory discharges from the frog tongue elicited by distention of the stomach or the presence of peptone in the gastric contents. Esakov (1961) reported that the glossopharyngeal discharge produced by application of salt solutions or tap water to the tongue surface was enhanced by stomach distention and depressed by the presence of peptone in the gastric lumen. No effects on the

responses to quinine as a taste stimulus were found. Brush and Halpern (1970), however, do report a depression in taste sensitivity to quinine when the stomach was stretched. These depressant effects could be elicited by electrical stimulation of the peripheral stump of the glossopharyngeal nerve to the tongue. No differential effect of this sort of activity was reported, antidromic stimulation of the IX nerve leading to decreased afferent activity in both the stimulated and unstimulated (contralateral) nerve trunk (Esakov, 1961). Brush and Halpern (1971) also report the effects as being seen in both the left and right glossopharyngeal nerves when efferent impulses were initiated in only one of the two.

Stimulation of the cranial sympathetic trunk of the frog with high frequency electric shocks (50-100 per second) were reported to lead to enhancement of sensory discharge in taste fibers from the tongue which lasted 1-3 seconds longer than the electrical nerve stimulation (Chernetski, 1964). In these same experiments the fibers running in the IX nerve out to the tongue were identified as sympathetics with conduction velocities of 0.4 to 0.8 meters per second. Kimura (1961) reports a similar sympathetic enhancement of gustatory activity in nerve fibers from the tongue of the rat.

Esakov and Byzov (1971) reported that centrifugal stimulation of the IX nerve in the frog produced a hyperpolarization of the taste cells of the frog fungiform papillae. The latency of this hyperpolarization was in the range of 0.8-1.0

seconds and its amplitude directly related to the frequency of the electrical nerve stimulation over a range of 2-60 stimulus pulses per second. These authors suggested that the fibers responsible for both depression of afferent sensory discharge in taste nerves and the hyperpolarizations of the receptor cells were the same, and are the autonomic efferents responsible for the reflex control of gustatory responses. No estimation of the conduction velocities of these fibers was made, and if they are the autonomics described by Esakov (1961) and Brush and Halpern (1971) these conduction rates would be expected to fall in the range reported by Chernetski (1964, 1965) as 0.2-0.8 meters per second.

Intracellular Recordings From Taste Receptors

Receptor Resting Membrane Potential

The first report of microelectrode recording from taste cells (Kimura and Beidler, 1956) reported that in the rat these cells displayed a resting transmembrane potential with the interior of the cell negative with respect to the surrounding tissue. Though polarized, these cells display highly variable membrane potentials. In the rat and hamster they have been reported to be from -30 to -50 millivolts with respect to the exterior tissue (Kimura and Beidler, 1961; Tateda and Beidler, 1964) with an average value of -40.1 millivolts (Ozeki, 1970, 1971). Ozeki also reported that there was

no statistical difference between the membrane potentials of gustatory and non-gustatory cells of the rat taste bud, a finding reported also for the toad tongue by Eyzaguirre et al. (1972). Ozeki (1971) reported, however, that the transmembrane resistance of gustatory cells was lower (536 ohms per cm.^{2}) than that of other epithelial cells (1.9 kohms per cm.^{2}) at rest. For cells of the frog taste disc Sato (1969) reported that cells in the receptor area at a depth of 20-30 micra below the surface had membrane potentials in the range of -10 to -35 millivolts while those measured by Eyzaguirre et al. (1972) in the toad gave values of -6 to -40 millivolts at rest. Esakov and Byzov (1971) report receptor resting transmembrane potentials in the frog taste disc ranging between -20 and -40 millivolts.

Receptor Potentials

Application of chemical stimuli to the surface of the taste bud usually caused a depolarization of the receptor cells impaled by the microelectrode (Kimura and Beidler, 1956, 1961; Tateda and Beidler, 1964) in the rat and hamster, a finding also reported for the rat by Ozeki (1970, 1971), the frog (Sato, 1969) and toad (Eyzaguirre et al., 1972). However, occasionally chemical stimulation would result in an increase in polarity (hyperpolarization) measured by the recording electrode (Tateda and Beidler, 1964; Eyzaguirre et al., 1972). Tateda and Beidler (1964) report that in the

rat sucrose, quinine, HCl, H_2O and alkali salt solutions produced a depolarizing potential while NaCl solutions between 0.02 and 0.01 molar could produce potential changes in the opposite direction (hyperpolarization). Ozeki (1971), however, reports that the rat taste cells depolarized to the above classes of stimuli but found they could also be caused to hyperpolarize by quinine at resting membrane potentials greater than -50 millivolts.

Eyzaguirre et al. (1972) report that in the toad acid and alkali salts generally produced depolarizations which often rose above the zero potential, completely reversing the membrane potential, making the inside of the cell positive (up to 20 millivolts in some cases). Water consistently produced hyperpolarization of the cells of the fungiform papilla as did quinine in cells with membrane potentials greater than -60 millivolts. At membrane potentials between -60 and +40 millivolts quinine produced little change, while above 40 millivolts, the application of quinine tended to drive the interior of the cell even more positive. A somewhat similar effect for quinine on rat taste cells is reported by Ozeki (1970). In contrast to the report by Sato (1969) that salts, acids, sucrose, quinine and water all produced only depolarizations in the frog taste cells, Eyzaguirre et al. (1972) report that sucrose did not produce any change in membrane potential, regardless of the cell resting potential, and the potentials produced by acid stimuli tended to be biphasic, first slightly

hyperpolarizing and then depolarizing. Acetic acid tended to be slightly hyperpolarizing, and often was not effective at all. Esakov and Byzov (1971) and Rapuzzi and Ricagno (1969) consider acetic acid a very good stimulus for the receptors of the frog fungiform papillae.

For salts the amplitude of the depolarizing receptor potentials are generally directly proportional to the level of the resting potential (Eyzaguirre et al., 1972; Ozeki, 1971; Sato, 1969; Tateda and Beidler, 1964). This holds with less consistency for acids (Eyzaguirre et al., 1972; Ozeki, 1971). This direct relationship also holds generally true with occasional exceptions (Tateda and Beidler, 1964) for the amplitude of receptor response and concentration of stimulus.

The reports of Eyzaguirre et al. (1972) and Ozeki (1970, 1971) indicate that quinine may produce no effect in resting cell transmembrane potential over a broad range but may induce further depolarization in already depolarized cells or induce further hyperpolarization in already hyperpolarized cells. The response of the taste disc cells to water is not modified by changes in transmembrane potential over a wide range (-54 to +6 millivolts) and because of its hyperpolarizing action, can enhance the depolarizing receptor potentials produced by other stimuli, whereas quinine tends to have just the opposite effect (Eyzaguirre et al., 1972). From all the reports on intracellular responses to taste stimulation the consistent finding of multiple sensitivities for single cells of the

taste bud is evident. Thus while not all cells respond to all of the various classes of stimuli, each usually does produce a response to more than two different stimuli. The magnitudes of these responses differ among cells and within the same cell at different times. Each cell appears to have an individual, and constantly shifting sensitivity profile, although the qualitative relationships between response sensitivities to the various classes of stimuli appear to be consistent. Receptor responses to stimulation by concentration series of the adequate stimuli have not been reported. Although receptor cells appear to be multiply sensitive, it is important to point out that this sensitivity has been measured in most cases at only one point in the response function of any one cell. Therefore, determination of a predominant maximum sensitivity to any one class of stimuli for these receptors has not been made.

Extracellular Potentials

Kimura and Beidler (1956, 1961) and Tateda and Beidler (1964) describe potentials in the rat produced by chemical stimuli as occurring only within cells of the taste bud. Sato reported that in the frog only cells of the taste disc responded with receptor potentials during chemical stimulation of the tongue (Sato, 1969). These authors' results were cited by Ozeki (1971) in support of his finding gustatory cells only within the taste bud.

Esakov and Byzov (1971), Kimura and Beidler (1956, 1961), Rapuzzi and Ricagno (1969), and Tateda and Beidler (1964), report that currents of polarity opposite to that within the taste cells could be recorded with the tip of the electrode located extracellularly. These potentials were considered reflections of the receptor potentials of the taste cells.

Eyzaguirre et al. (1972), Ozeki (1971), and Sato (1969) reported that the potentials recorded in response to chemical stimulation were only of intracellular origin, the first paper ascribing them as a characteristic of all epithelial cells of the toad tongue, and the other two papers attributing these potentials only to receptor cells.

Esakov and Byzov (1971), Eyzaguirre et al. (1972), and Rapuzzi and Ricagno (1969), however, report currents measurable outside the specific borders of the taste bud in response to chemical stimulation. The first authors discount these potentials as reflections of the receptive process, the second paper attributes them to chemical stimulation, but only of intracellular origin, while the third paper describes them as directly a result of receptor activity within the taste disc of the fungiform papillae, electrotonically spread in the surrounding tissue.

Effects of H_2O and Quinine

Eyzaguirre et al. (1972) showed that, in the toad, water applied to the tongue produced an intracellular

hyperpolarization. Since the amplitude of the response to depolarizing stimuli is a function of the cell transmembrane potential at the time the stimulus is applied, it would be expected that previous washing of the tongue with water would increase the amplitude of the depolarizing potential produced by subsequent application of a standard salt solution. This was indeed shown to be the case.

Quinine also was found to exert a hyperpolarizing effect on the cells of the taste disc. In this case, however, the authors found a decreased response to applied depolarizing stimuli. Even when no change in resting potential was seen with application of quinine, the depression of response to depolarizing stimuli applied subsequently was quite evident.

The hyperpolarizing effects of water and quinine were independent of resting transmembrane potential to a large extent. The authors suggested that water would tend to leech out cations from the taste cells but leave the responsiveness to stimulation unimpeded. Quinine on the other hand appears to produce a stabilization or inactivation of some components of excitable membranes (Falk, 1961) and was used by Graham (1935) to unmask the prolonged subnormal period of nerve response which is apparently the result of an electrogenic pump mechanism in the axon membrane. Eyzaguirre et al. (1972), report that quinine produced an increase in membrane resistance of taste disc cells and suggest that different taste stimuli may have different mechanisms of action in taste

chemoreceptors since both quinine and depolarizing stimuli (which decrease cell resistance) are adequate to initiate afferent sensory discharge in taste fibers of the tongue.

Taste Receptor Transmembrane Conductance

The quinine-produced increase in transmembrane electrical resistance of taste cells found in the toad by Eyzaguirre et al. (1972), is also confirmed for the taste cells of the rat (Ozeki, 1970, 1971). The suggestion by Grundfest (1961) that receptor activity is not dependably reflected by the receptor potential and that receptor activity may not be accompanied by an electrical sign appears to be the case for the taste receptors of the rat. Ozeki (1971) has shown that the receptor transmembrane conductance to ionic flow was increased by depolarizing taste stimuli. The effect of quinine (which can hyperpolarize or depolarize) was to decrease the ionic conductance. After stimulation with NaCl, a depolarizing stimulus, the recovery of the normal resting potential was accompanied by a decrease in conductance. However, although the transmembrane potential recovered in 10-50 seconds after cessation of stimulation, the conductance took up to 180 seconds to recover its normal value. In addition, during adaptation of a cell to stimulation by NaCl, the conductance change of the cell membrane was not correlated with the adapting amplitude of the receptor potential. Because membrane potential changes due purely to ionic permeability changes

would be reflected in the conductance of the cell membrane, Ozeki (1971) suggests that the actions of different chemical stimuli on the receptor cell may involve different mechanisms of excitation, and that adaptation is not produced simply by a change in permeability to some specific ions but also by inactivation of the mechanism producing conductance changes.

Active Transport Electrogenesis

Hyperpolarization

It is known that cell membrane potentials are the sum of passive and metabolic components, the former attributable to membrane permeability and capacitance, the latter usually ascribable to active transport or ionic pumps (Marmor and Gorman, 1970). Many of the metabolically activated electrogenic systems produce hyperpolarization of the affected cell. Bourgoignie et al. (1969) describe a hyperpolarization of toad bladder cells activated by cyclic adenosine monophosphate (C-AMP) and other cyclic nucleotides that had a latency of 3-5 minutes and persisted for 15 minutes. The smooth muscle of guinea pig taenia coli exhibits a hyperpolarization in response to stimulation of the nerves innervating it (Burnstock et al., 1963). This hyperpolarization displayed latencies as high as 500 milliseconds and the rise time to half-amplitude of the response ranged from 0-8-1.2 seconds. On recovery the membrane potential often could be seen to overshoot the

baseline membrane potential resulting in a depolarization of the cell that could lead to excitation and the initiation of action potentials in the smooth muscle cell. Summation of hyperpolarizing responses was observed with stimulus frequencies of one per second and more. The antidromically elicited hyperpolarization of a molluscan nerve cell reported by Gorman et al. (1967) was not sensitive to changes in cell membrane polarization, and resulted in decreased excitability of this cell. It was suggested that this hyperpolarization was the result of activation of a metabolic pump mechanism since passive ionic currents would be changed by potential gradients across the cell membrane and the hyperpolarizations did not exhibit this characteristic. Libet and Tosaka (1969) described a postsynaptic inhibitory hyperpolarization of rabbit sympathetic ganglion cells that could be activated by a single electric shock to the presynaptic trunk and which often lasted as long as 3-25 seconds after the stimulus was ended. The time course of this hyperpolarization was similar to that reported by Graham (1935) for the subnormal period following activation of the frog sciatic nerve.

Tasaki and Spyropoulos (1957) showed that the passive electrical properties of frog nerve were relatively independent of temperature over a range from 14-25^{o}C. The response of the frog tongue to taste stimulation, however was reported to be temperature sensitive, peaking in the vicinity of 25^{o}C. (Yamashita, 1964). In their report on the effects of the

metabolic component on the membrane potential, Marmor and Gorman (1970) found that in the mollusc cell they were studying, warming produced a hyperpolarization which was reversed with the metabolic inhibitor ouabain. Considering the receptor-nerve complex, Grundfest (1961) theoretically considers the effects of receptor potential polarity on the signaling of stimulus characteristics to the nerve terminals and reports various mechanisms by which hyperpolarization may lead to excitation and inhibition of the afferent sensory terminals.

The sympathetic ganglion cell hyperpolarization could also be depressed by ouabain or lowered temperature. The hyperpolarization was independent of membrane potential over a wide range and was considered by the authors to be the result of activation of an electrogenic metabolic pump (Nishi and Koketsu, 1968). A similar mechanism apparently operates in the rat cerebellum Purkinje cell. Siggins et al. (1971) describe a hyperpolarization of these cells produced by C-AMP and norepinephrine which was not sensitive to levels of cell polarization. They suggested that activation of a metabolic pump by norepinephrine utilizing C-AMP was the factor responsible for the hyperpolarization observed upon application of norepinephrine or C-AMP. Torda (1972) has found evidence for a similar mechanism in the rabbit superior cervical ganglion cells. She considers the hyperpolarization produced both by electrical stimulation and application of C-AMP to result from

activation of the enzyme diphosphoinositide kinase leading to the initiation of ionic transport, coupling in time and space such transport and the consequent hyperpolarization.

The effect of glycine on cells of the cat medulla is a slow hyperpolarization (105 seconds) that suggests an ionic mechanism not simply due to passive qualities of the cells (Hosli and Haas, 1972). Siggins et al. (1971), however, attribute the hyperpolarization seen with glycine and gamma-aminobutyric acid (GABA) to an increased conductance to potassium whereas the hyperpolarization produced by C-AMP is attributed to a decreased conductance of the cell coupled with activation of the metabolic electrogenic pump.

The observations of Hellekant (1971) and Perri et al. (1969) that taste receptors soon lose their sensitivity when deprived of blood flow for 15-20 minutes seem to indicate that a metabolically dependent mechanism plays an important role in the function of tongue gustatory receptors.

Antidromic Mechanisms

Single Fiber Effects

Dodt and Walther (1957) reported that antidromic stimulation of the lingual nerve produced depression of cold receptors in the tongue of the dog. This depression had an immediate onset and lasted up to 500 milliseconds depending on the basal activity of the fiber and frequency of antidromic

stimulation. Macdonald (1971) found a similar time course for inhibition of taste fibers in the frog tongue due to antidromic activity. An initial facilitation (5-20 milliseconds post-antidromic stimulus) was followed by a period of depressed excitability that lasted 300-400 milliseconds. Lindblom (1958) described a decreased excitability in tactile fibers of the frog skin 10-50 milliseconds after a collateral branch of a fiber was stimulated either electrically or by touch. The conduction of impulses antidromically into the terminal branches of the tactile unit was regarded as the cause of this subnormal period of excitability. Lindblom and Tapper (1967) confirm that such an antidromic inhibition occurs in the tactile fibers of the cat and monkey foot-pad.

Miller (1971) proposed a mechanism for the inhibition of single afferent taste neurons of the rat that depends on decremental non-propagated electrotonic effects in the terminal branches of the same sensory fiber. This proposal differs from the above mechanisms in that it does not invoke antidromically propagated action potentials to produce excitability changes in nerve terminals.

The first four reports show that the depression occurred only in collaterals of the same fiber. There was no apparent interaction between different sensory units. The depression was dependent on the generation of a propagated action potential and subliminal stimulation of one collateral was not effective in changing the sensitivity of the other collaterals

either to natural or to electrical stimulation. In all cases the depression lasted for a relatively short time, falling in the range reported for post-activity subnormal periods of nerve fibers themselves (Gasser, 1935; Graham, 1935).

Antidromic Motor Effects

It is established that antidromic activity in sensory fibers can elicit vasomotor effects. Lewis has shown this to occur on activation of sensory fibers of the skin (Lewis, 1942). This is the concept of the "axon reflex". The vasodilitation (flare), local edema (wheal) and hyperalgesia of sensory fibers in the skin are apparently due to release of a kinin or acetylcholine-like substance from the terminals of the collateral branches of the sensory fibers in the vicinity of stimulation. Erici and Uvnas (1951) report that the vasodilitation produced in the cat tongue by stimulation of trigeminal sensory fibers is an antidromic effect. Although the release of a neurohumor from these terminals was not proven, considering the physiology of the vasculature, this would be a logical assumption.

Antidromic Activity Effects Across Fibers

Casella and Rapuzzi (1963) and Taglietti *et al.* (1969) reported that chemical stimulation of all the papillae of the tongue of the frog which were interconnected by collateral branches of sensory fibers could lead to depression of the

response of any one papilla of the group. Any two interconnected papillae have on the average 2-4 fibers in common (Rapuzzi and Casella, 1965). Such lateral effects, therefore, were proposed as an effective mechanism for reciprocal interactions between sensory units with overlapping receptive fields (Taglietti et al., 1969). This was strengthened by the demonstration of Filin and Esakov (1968) that electrically initiated activity traveling antidromically in a sensory fiber innervating a fungiform papilla of the frog could produce prolonged (5 minutes) depression of the responsiveness to chemical stimulation of an adjacent taste fiber sharing the same receptive field.

Wang and Bernard (1969) found that taste fibers of the cat tongue, each with a different predominant sensitivity to one of the four basic taste stimuli (salty, sweet, bitter, sour) displayed either depression or enhancement depending on the adapting and test stimuli. This reciprocal influence between individual gustatory nerve fibers with overlapping receptive fields led to the hypothesis of lateral inhibition by antidromic conduction of impulses in collaterals of taste fibers sharing common taste buds (Bernard, 1971, 1972). Rall and Shepherd (1968) present physiological evidence for such a lateral inhibitory effect in the rabbit olfactory bulb. That both orthodromic and antidromic stimulation of mitral cells of the bulb led to this hyperpolarizing inhibition led these authors to propose bidirectional synaptic conduction

between mitral and granule dendrites, the presence of which appear to be anatomically confirmed (Reese and Brightman, 1965; Reese, 1966), and bear similarities to those described for the frog taste disc by DeHahn and Graziadei (1971), Uga (1966), and Uga and Hama (1967).

II. STATEMENT OF PROBLEM

The gustatory sense is able to discriminate an almost infinite number of discrete tastes. The response functions to a sapid stimulus may be modified by the presence of other substances which may or may not be particularly effective taste stimuli in their own right. Physiologic conditions and disease processes can affect the sensory responses of the gustatory system.

A large majority of afferent taste fibers respond to more than one of the four primary categories (salty, sweet, bitter, sour). Intracellular studies of taste receptor cells indicate that they also are not strictly stimulus-specific. The lack of absolute receptor and nerve fiber specificity has led to the theory of spatio-temporal nerve discharge pattern, unique for each stimulus, to explain the encoding of taste stimuli by the peripheral gustatory system.

Evidence for a neo-Mullerian view has, however, been recently augmented by results suggesting that there is, rather than an absolute specificity, a predominant sensitivity of the peripheral taste unit to any one of several taste stimulus categories (Wang and Bernard, 1969). Thus, a unit which has a lower threshold to one stimulus category may be considered to have a physiological specificity for this stimulus class.

Wang and Bernard (1969) found that some of the response functions of individual taste units showed decrements with increasing stimulus strength. In addition, adaptation of the tongue with one class of stimuli could enhance or depress the response of a taste unit to another stimulus, depending on the length of the adaptation period and the specific stimuli used. The response of a single taste fiber to whole tongue stimulation was less than the sum of the responses to individual stimulation of the papillae that made up its receptive field. Filin and Esakov (1968) found that antidromic activity in the collaterals of a taste nerve fiber could exert a prolonged inhibitory effect on the response of a neighboring taste fiber to chemical stimulation.

Consideration of these across-fiber reciprocal effects led to the postulation by Bernard (1972, 1971) that antidromic activity in taste fibers with overlapping receptive fields leads to peripheral interactions between individual gustatory units of the tongue. Such interaction could occur between the terminals of nerve fibers in close proximity within the taste bud, or, by some trans-synaptic action antidromic impulses could affect the response characteristics of the receptor cells multiply innervated by different sensory fibers.

The purpose of the present investigation was to test the functional existence of the latter model. Experiments were planned to determine whether the observed lateral interactions

produced by antidromic activity in sensory units of the tongue could occur by producing an effect on the receptor cell itself and whether these changes in bioelectric properties of the receptor cell could be associated with both the inhibitory and facilitatory effects observed in the response functions of individual gustatory units.

III. STATEMENT OF OBJECTIVES

1. To determine the effect of efferent activity in the glossopharyngeal nerve on the bioelectric characteristics of cells in the taste disc of the frog tongue fungiform papilla.

2. To localize the area within the taste disc demonstrating such an effect.

3. To characterize the qualities of these bioelectric responses in taste disc cells.

4. To determine if such an effect has the ability to modify the afferent sensory discharge in the sensory nerve fibers of the tongue subserving chemical sensitivity in taste.

5. To test the effect of taste stimuli on the receptor surface cell response to efferent activity in the sensory fibers of the IX nerve.

6. To examine the influence of excitation of taste disc cells in a papilla on the bioelectric properties of receptor cells in a neighboring unstimulated papilla of the frog tongue.

7. To study the effect of changes in cell polarization on the mechanism of peripheral modulation of these receptor properties.

IV. MATERIALS AND METHODS

Experimental Animals

Common grass frogs (Rana pipiens sp.) of body length 3-4 inches were used for all experiments. A stock was maintained in a cold-room tank at 4°C from which the individuals were chosen for each experiment. The frogs were unfed. Prior to surgical preparation the frogs were double-pithed. This procedure in most cases did not appreciably depress blood flow in the fungiform papillae of the tongue. Blood flow was visually monitored throughout the course of the experiments through a binocular operating microscope (Carl Zeiss Inc.) and if stasis was observed in the papilla under the recording electrode either another papilla was chosen or the preparation discarded. The temperature of the preparation equilibrated to one or two degrees Centigrade below room temperature. Prior to, and during recording, the temperature of the tongue as recorded by a YSI model 425C tele-thermometer (Yellow Springs Instrument Co., Inc.) remained between 20-23°C.

Electrical Nerve Stimulation

In those experiments requiring electrical stimulation of the glossopharyngeal nerve to the tongue, the cut peripheral

trunk of the nerve was laid on a pair of platinum-iridium (80% Pt. 20% Ir.) wires .008" in diameter (Medwire Inc.). These stimulating electrodes were mounted in a glass tube affixed to a 3-plane manipulator (Narishige Inc.) and led by shielded wires to the output terminals of a SD9 electronic stimulator with built-in stimulus isolation from ground (Grass Instrument Co.). The nerve was arranged on the electrodes such that the anode of the bipolar electrodes was always nearest the cut end of the nerve. Periodically the nerve was lifted from the body fluids into the air. Stimulation was only applied at these times and the current flow to adjacent tissues was minimized. If the stimulating sessions were of long duration, the nerve was intermittently sprayed with physiological saline from an atomizer in order to prevent desiccation.

Electrical Stimulation of Fungiform Papillae

When a single papilla on the tongue was to be stimulated electrically, a monopolar stainless steel electrode with a tip electrolytically etched to a diameter of 5-10 microns was applied to the taste-disc of the papilla. This electrode was insulated to within 5 microns of the tip with a varnish of high dielectric properties (Insl-X E-33 Clear Insulating Dip, Insl-X Prod. Co.). These electrodes had a D.C. resistance of 250 thousand ohms. An indifferent ground was placed on the

leg of the frog to complete the electrical circuit. The electrode for stimulation was visually placed with its tip just touching the surface of a taste disc utilizing a 3-plane manipulator.

Chemical Stimulation

For application of taste stimuli to a small area (usually one or two papillae) single drops of about 10 microliters were delivered from the tip of a 30-gauge hypodermic needle. Whole-tongue stimulation was accomplished by filling the chamber in which the tongue was affixed with the stimulating solution until the surface of the tongue was immersed. When changing solutions the chamber was drained through a port in its bottom, a wash of Harris-Ringers saline was applied then drained, and the next stimulus was used to fill the chamber (Figure 2).

Chemical Solutions

Solutions used for chemical stimulation of the tongue were made up in double distilled water. When buffered, Tris (Sigma Chemical Co.) was used as the buffer. All solutions were tested for pH with a model 701 digital pH meter (Orion Research Co.) and combination electrode No. 4858-L15 (Arthur H. Thomas and Co.). Harris-Ringers saline was buffered with phosphate buffer to the pH of frog saliva.

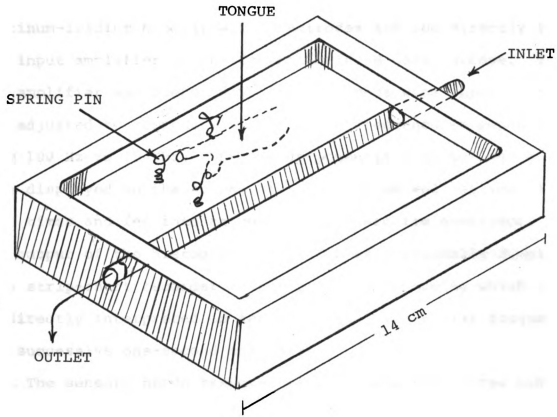


Figure 2. Chamber used for immobilization of frog tongue during microelectrode recording from cells of fungiform papillae and recording sensory discharge of lingual branch of the glossopharyngeal nerve. Chemical solutions were introduced at the inlet and drained using suction at the outlet of the chamber.

Glossopharyngeal Sensory Nerve Recording

Action potentials from afferent fibers were picked up on platinum-iridium bipolar wire electrodes and led directly to the input amplifier of the Tektronix 561-B oscilloscope. The 3A9 amplifier was operated in the A.C. mode with input filtering adjusted to reproduce signals with frequency components from 100 Hz to 10 KHz. The action potentials of the nerve were displayed on the screen of the 561-B as well as amplified 1000 times and fed into an event/time histogram generator. The output of the histogram generator was graphically displayed by a strip-chart recorder. This produced a tracing which could be directly interpreted in terms of action potential frequency for successive one-second intervals.

The sensory nerve response was analyzed over three time periods each 15 seconds in duration. During the first, or basal period, the tongue was bathed in Harris-Ringer saline. This saline was used as a rinse in all chemical stimulus experiments. The second interval consisted of the first 15 seconds immediately following application of one of the chemical stimuli. Ten seconds were allowed to elapse before activity was measured for the third interval. This sequence allowed comparison of two components of the response (the initial or rapidly adapting transient, and the slowly adapting or steady-state activity) with the basal activity recorded prior to stimulation.

Following a 3-period control series as described above, the tongue was again washed in saline and allowed to rest until nerve discharge reached a relatively steady baseline rate. In one minute the baseline had usually been reached and values for the first time period of a new series were collected. Following this, the glossopharyngeal nerve was antidromically stimulated with electrical pulses found in previous tests to elicit slow-wave potentials in taste disc cells. The stimulus train duration, Ds, was 15-25 seconds and at its termination the tongue was immediately stimulated with the same chemical solution applied in the control series. Activity values for the two response intervals were collected as before. The values of the two response intervals of a series were then expressed as a percentage of the activity averaged over the pre-stimulation basal period.

D.C. Microelectrode Recording

Micropipettes were pulled to tip diameters of 1-0.5 micra on a model 700 C Vertical Pipette Puller (David Kopf Instruments) from 1.0 mm O.D. X 0.5 mm I.D. precision diameter borosilicate glass tubing (type 7740, Corning Glassworks). They were filled with either 3 M-KCl or 1 M-potassium acetate solution using a technique modified after Tasaki *et al.* (1954). The impedance of these electrodes was in the range of 10-50 megohms as tested with a low-current (1.0 nanoampere) D.C. or

A.C. (1 KHz) signal. The electrolyte solution was filtered through a millipore filter (Gelman Inc.) which removed crystals and dust particles before the solution was used to fill the electrodes. This was found necessary for passing current intracellularly since a small particle could easily obstruct the tip of the electrode and lead to a very high resistance input.

Each electrode was tested before use both visually under a microscope and electrically in saline to assess its suitability for use. Electrodes with cracked or broken tips and those containing crystalline or amorphous inclusions were discarded. Tip diameters of acceptable electrodes were measured under the microscope and were one micron or less in diameter. Patency was tested by applying a one nanoamp D.C. current to the electrode immersed in saline. The ability of the electrode to pass this current through the electrical circuit between the indifferent and recording electrode for a time in excess of one second was taken as an indication of electrical continuity between the lumen of the microelectrode and indifferent electrode. Impedance was measured by imposing a microvolt 1 kilohertz square wave signal to the recording electrode. After internal compensation in the amplifier for attenuation of the signal due to the capacitative components of the recording circuit, the amount of attenuation was used to calculate by Ohm's law the impedance of the recording electrode due to the resistive component of the circuit. An electrode impedance

in the range of 10-50 megohms was considered acceptable, signifying a sufficiently small tip for cell penetration. In preparations requiring the passage of current through the electrode tip in order to manipulate cell membrane potentials the resistance of the microelectrode was periodically measured during the course of the experiment. When the electrode resistance increased over 100 megohms it was assumed that blockage of the tip had occurred and the electrode was discarded.

Electrodes found acceptable by the above criteria were mounted in a combination half-cell:electrode holder (W.P. Instruments) which also contained a silver-silver chloride half-cell. When used in conjunction with a similar electrode holder for the indifferent electrode, junction potentials between the two electrometer amplifier inputs could be decreased to several millivolts. The electrode holder was mounted on a HP-2A micrometer advance drive (Pfeiffer Co.) which was modified to operate through the activation of a small D.C. motor via a remote switch. The microelectrode could be advanced through calibrated distances as small as 1 micron with this drive. The electrode was put in contact with the surface of the tongue visually and then advanced with the remote-control drive while observing the electrical potential being displayed on the oscilloscope. When a sudden shift in the potential was observed it was taken to indicate an impalement of a cell (see Eyzaguirre et al., 1972) and the

experimental procedures were commenced. Note was usually made of the calibrated depth indicated on the manipulator advance mechanism, in order to have some idea of the relative position of the cell with respect to others recorded from in the same area. When the electrode was withdrawn subsequent to the test procedures, often a sudden shift in the potential toward the initial baseline would indicate the exit of the electrode from the cell.

The indifferent electrode was a capillary filled with three molar KCl. It was connected via a silver-silver chloride half-cell to the ground circuit of the M-4A electrometer probe (W. P. Instruments). The indifferent electrode was usually positioned in electrically neutral tissue at the apical mandibular suture of the frog. The output of the electrometer was the difference between the potential of the indifferent and the potential of the active (recording) electrode. The animal was grounded to the electrometer by a platinum-iridium ground pin inserted near the site of the indifferent electrode.

The signal of the electrometer was led to the 3A9 Tektronix D. C. amplifier, suitably amplified, and displayed both on the screen of the 561-B oscilloscope as well as graphically reproduced by a two-channel oscillographic strip-chart recorder (Texas Instruments Inc.). The second channel of the recorder was used to mark stimulus periods.

Manipulation of Cell Membrane Potential

The M-4A circuitry enabled passage of small inward or outward currents through the recording microelectrode (Figure 3) while simultaneously recording cell potential. These currents were in the range of 50-200 nanoamperes.

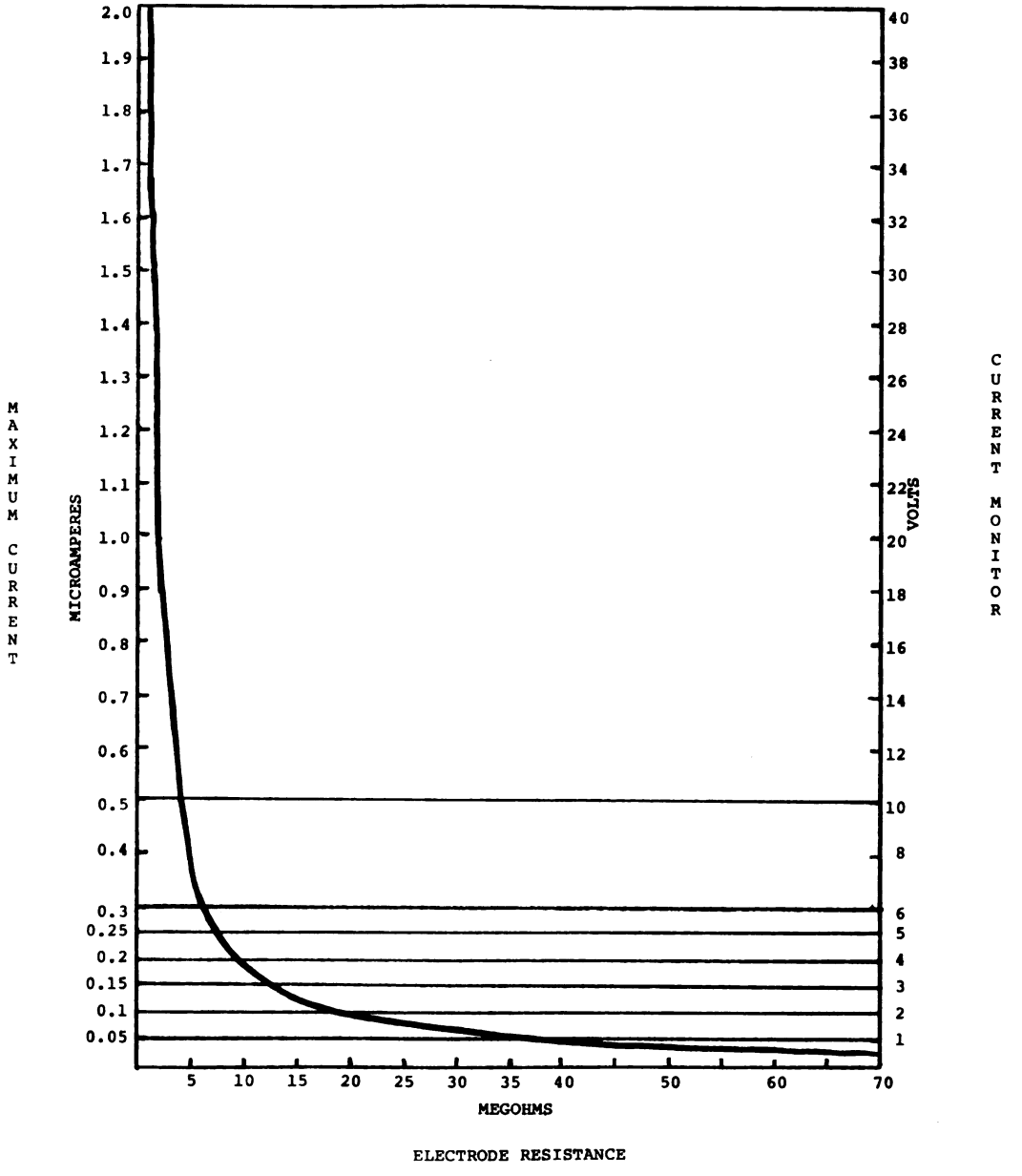


Figure 3. Current-voltage plot for microelectrodes of various resistances. Right ordinate: voltage applied to electrode. Abscissa: electrode resistance. Left ordinate: current passed through microelectrode.

V. EXPERIMENTAL PROCEDURES

Glossopharyngeal Nerve Stimulation and Taste Papilla Responses

In this as in all subsequent experimental preparations the frog was placed in a supine position after destruction of brain and spinal cord. The glossopharyngeal and hypoglossal nerves were exposed via a ventral approach through the lower jaw. Both nerves were bilaterally cut as near to their exit from the skull as could be accomplished (Figure 4). Because of the importance of adequate blood flow in the tongue for the response of the receptors (Hellekant, 1971) the circulation was carefully left intact as the tongue was reflected anteriorly from the mouth and pinned, dorsal surface upward, in the plexiglass chamber described earlier.

One of the glossopharyngeal nerves was placed on the stimulating electrodes and a double-barreled saline-filled capillary, similar to that described by Rapuzzi and Casella (1965), was placed over a single fungiform papilla of the extended tongue forming a small chamber 100 micra in diameter with three openings. One of the openings is connected by flexible tubing to a small syringe which is used to produce a negative pressure that draws a single papilla up into the chamber through a second opening. The third opening is fitted

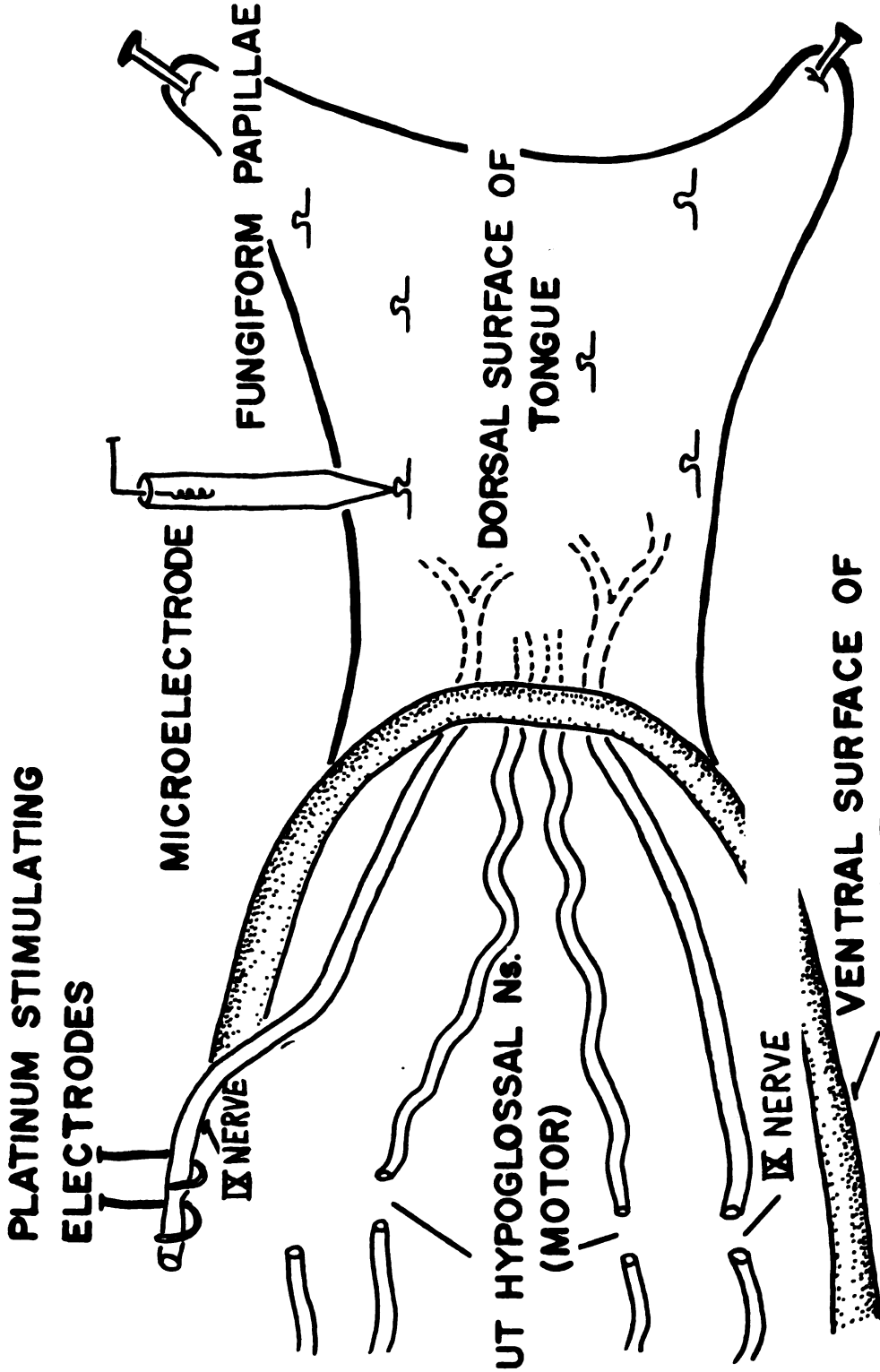


Figure 4. Diagrammatic representation of preparation used for microelectrode recording from cells of fungiform papilla taste disc and antidromic stimulation of lingual branch of the IX nerve.

with a metal electrode which serves to sample electric currents produced in the chamber (Figure 5). Substitution of chlorided silver for the platinum used by Rapuzzi and Casella facilitated recording of both fast and slow potential changes.

The glossopharyngeal nerve was stimulated electrically and action potentials were recorded from the nerve fibers supplying the papilla in the chamber electrode. This allowed determination of stimulus thresholds and most effective pulse durations and frequencies for adequate excitation of fibers innervating the taste papillae. Conduction velocities for these fibers were also calculated.

In addition, under some stimulus parameters slow potential changes could be measured from the whole papilla. These were recorded on the strip-chart oscillograph after the high frequency action potentials were electronically filtered out in the input stage of the amplifier. These slow waves were then related to the excitation of the various fiber groups recorded earlier.

Other experiments substituted microelectrodes for the chamber recording electrode. Changes in the electrical currents measured in single cells were likewise related to the stimulus parameters found adequate to elicit both the nerve fiber action potentials and the whole-papilla slow potentials.

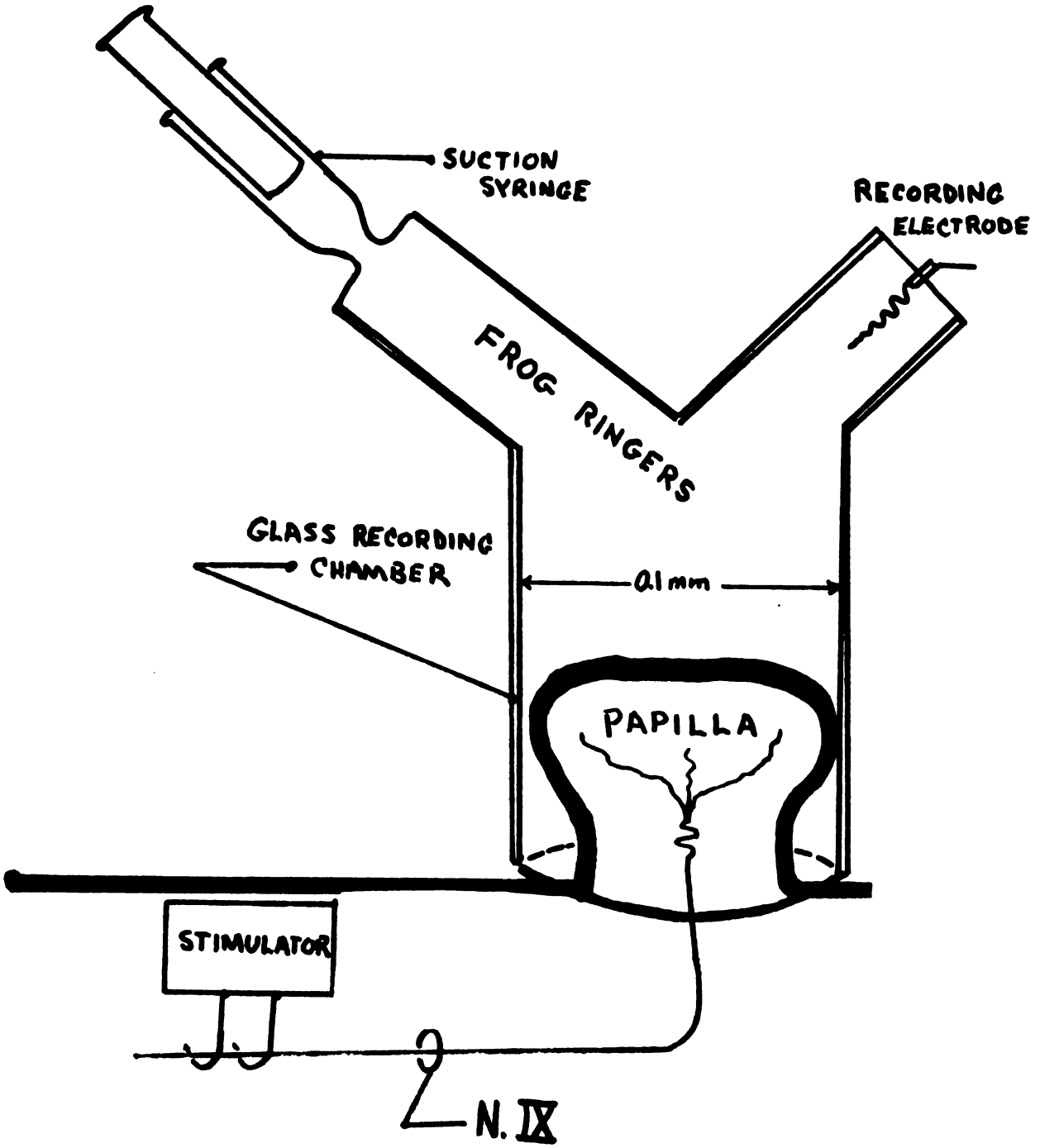


Figure 5. Representation of preparation used for recording action potentials invading fungiform papilla on electrical stimulation of the IX nerve. With high-frequency components of the signal filtered out this preparation was also used to record slow-wave activity elicited in papilla by antidromic stimulation of the glossopharyngeal nerve.

Characterization of Sources of Slow Potentials in Papillae

Impaling cells on the surface of the taste disc with microelectrodes was performed as described earlier. Once a cell had been penetrated, as evidenced by a sudden potential change and stable resting level, the stimulation of the ipsilateral glossopharyngeal nerve was commenced and the current flow in the impaled cell measured and displayed on the strip-chart recorder. During the course of the experiments the electrode was often advanced through several membrane layers, some forming a seal around the electrode tip adequate to prevent great leakage of intracellular fluid to the area outside the cell. The depth of the electrode as indicated on the micrometer advance mechanism would be noted for those cells which maintained their integrity sufficiently to respond to the stimulation of the IX nerve. Cells of the tongue surface outside the area of the taste disc and fungiform papilla were also examined and their responses compared to membrane potential changes recorded within the papilla.

Characterization of Membrane Potential Responses

Records were analyzed for resting membrane potentials (Em), amplitudes of membrane potential responses to nerve stimulation (Ep), polarity of these potentials (negative or positive), latency of onset of response (Lr) and in cases

where nerve stimulation was prolonged the potential at time of stimulus cessation was measured (Eacc). The exponential time constant (time for the response to reach 63% of its peak value) was measured for the initial change in potential and designated τ p.

The values for Ep were plotted against τ p for negative (hyperpolarizing) and positive (depolarizing) potential changes. Stimulus-response functions were plotted for Ep vs. stimulus frequency, and Ep vs. number of stimulus pulses delivered at a frequency of one per second.

Efferent Electrical Stimulation and Afferent Sensory Activity in IX Nerve

The bipolar electrodes on which the peripheral trunk of the severed glossopharyngeal nerve was laid could be connected in rapid succession to either the electronic stimulator or the input amplifier of the oscilloscope utilizing a rocker arm switch. For these experiments the nerve was first stimulated at frequencies and voltages known to initiate action potentials in fibers which innervated the taste papillae. Immediately after the period of stimulation (usually 15 seconds) recording of afferent action potential activity in this same nerve was begun. Concomitantly a chemical stimulus was applied to the surface of the tongue. The afferent activity was electronically analyzed by an event/time histogram generator. The output of this analyzer was recorded on

an oscillograph as number of action potentials for successive one second intervals. This enabled the comparison of sensory nerve response to chemical stimulation of the tongue with and without antecedent antidromic activation of these sensory fibers. Comparison was made of sensory response to chemical stimulation of the tongue and the effect of antidromic nerve stimulation on this response. In one experiment, nerve stimulation and chemical tongue stimulation were applied simultaneously and the subsequent nerve activity was compared to activity from chemical stimulation alone. This was performed to test the effect of collision of afferent sensory and efferent antidromic action potentials on the response characteristics of the tongue sensory units.

Interaction Between Taste Stimulation and Antidromic IX Nerve Stimulation on Taste Disc Cell Potentials

Single cells of the taste disc were impaled with microelectrodes. After a suitable baseline potential was established the papilla was stimulated by a single drop of either Harris-Ringers saline, distilled water, 0.5-1.0 M NaCl, 0.03 M HCl, or 0.03 M quinine hydrocholride (No. Q1125, Sigma Chemical Co.). The IX nerve was then electrically stimulated as previously described and changes in the cell potentials were recorded. Any effect of previous chemical stimulation of the papilla on the response of the impaled cell to nerve stimulation was noted.

Electrical Stimulation of Taste Disc and Recording of Effects on Response of Neighboring Papillary Cells to Antidromic IX Nerve Stimulation

These experiments utilized a preparation similar to that described above. Instead of chemical stimulation of the papilla under the recording microelectrode, however, electrical stimulation of adjacent fungiform papillae was performed. Using a fine-tipped monopolar stainless steel electrode, biphasic electric currents were applied to the taste disc between pairs of IX nerve antidromic stimulation periods. Monitoring of potential changes in cells of the papilla next to the one under the monopolar stimulating electrode was accomplished with micropipette electrodes as described before. Ep was plotted against τ p for pre- and post-stimulus periods.

Displacement of Membrane Potentials During Antidromic Stimulation of the Glossopharyngeal Nerve

Cells of the taste disc were impaled by the recording micropipette and currents passed through the electrode utilizing the constant current source of the M-4A electrometer. The membrane potential could be thus displaced in either a hyperpolarizing (inside made more negative) or depolarizing (inside more positive) direction. The currents available were a function of the electrode-cell resistance. The magnitude of displacement was therefore variable from cell to cell.

The effect of membrane potential on Ep of the response to antidromic stimulation of IX was plotted in terms of magnitude and sign of Ep at positive and negative values of the membrane potential Em.

VI. DEFINITIONS

Eo: Zero Potential. That baseline established when both the active recording electrode and indifferent electrode were electrically continuous in the bathing medium of the preparation.

Em: Membrane Penetration Potential. The potential difference between the indifferent electrode (referred to Eo) and the recording electrode after the recording electrode had penetrated a membrane (presumably a cell membrane) separating an area of electric potential from the extracellular compartment.

Ep: Peak Response Potential. The maximal change in Em produced in response to electrical or chemical stimulation of the sensory structures of the tongue.

Eacc: Accommodation Potential. The value of Em after a prolonged period of electrical stimulation to the IX nerve.

Lr: Response Latency. The time period from the onset of a stimulus to the time an incipient change in Em became evident.

tp: Time to Peak. The time period from incipient post-stimulus change in Em to the time the response was maximal (Ep).

tacc: Time of Accommodation. The time period from Ep to Eacc.

τp: Time Constant of Ep. The time for a change in Em to reach 63% of Ep.

τacc: Time Constant of Eacc. The time for Em to fall to a value of 37% (Ep - Eacc.)

Slope Eacc: Slope of Accommodation. The ratio of (Ep - Eacc) to the duration (Ds) of a prolonged stimulus. The change in Em after the peak response Ep has been obtained while stimulation is still being applied.

$\frac{Post}{Pre}$Ep: A ratio comparing the value of the response of taste disc cells to antidromic IX nerve electrical stimulation before (pre) and after (post) subsequent electrical stimulation of a neighboring papilla.

$\frac{Post}{Pre}\tau p$: A ratio of the value of the time constant of the rising phase of the response of taste disc cells to antidromic IX nerve electrical stimulation before (pre) and after (post) subsequent electrical stimulation of a neighboring papilla.

% Response: The value of afferent sensory nerve discharge elicited in response to a stimulus, expressed as a percentage of the frequency of action potentials recorded in a basal or non-stimulated condition.

Ds: Stimulus Pulse Train Duration. The time period over which continuous electrical pulses were delivered by stimulus electrodes.

Dp: Stimulus Pulse Duration. The duration of the individual pulses composing a stimulus pulse train.

f: Stimulus Pulse Frequency. The rate of delivery of single stimulus pulses within a train.

V: Stimulus Voltage. The amplitude of the individual pulses delivered by the stimulating electrodes.

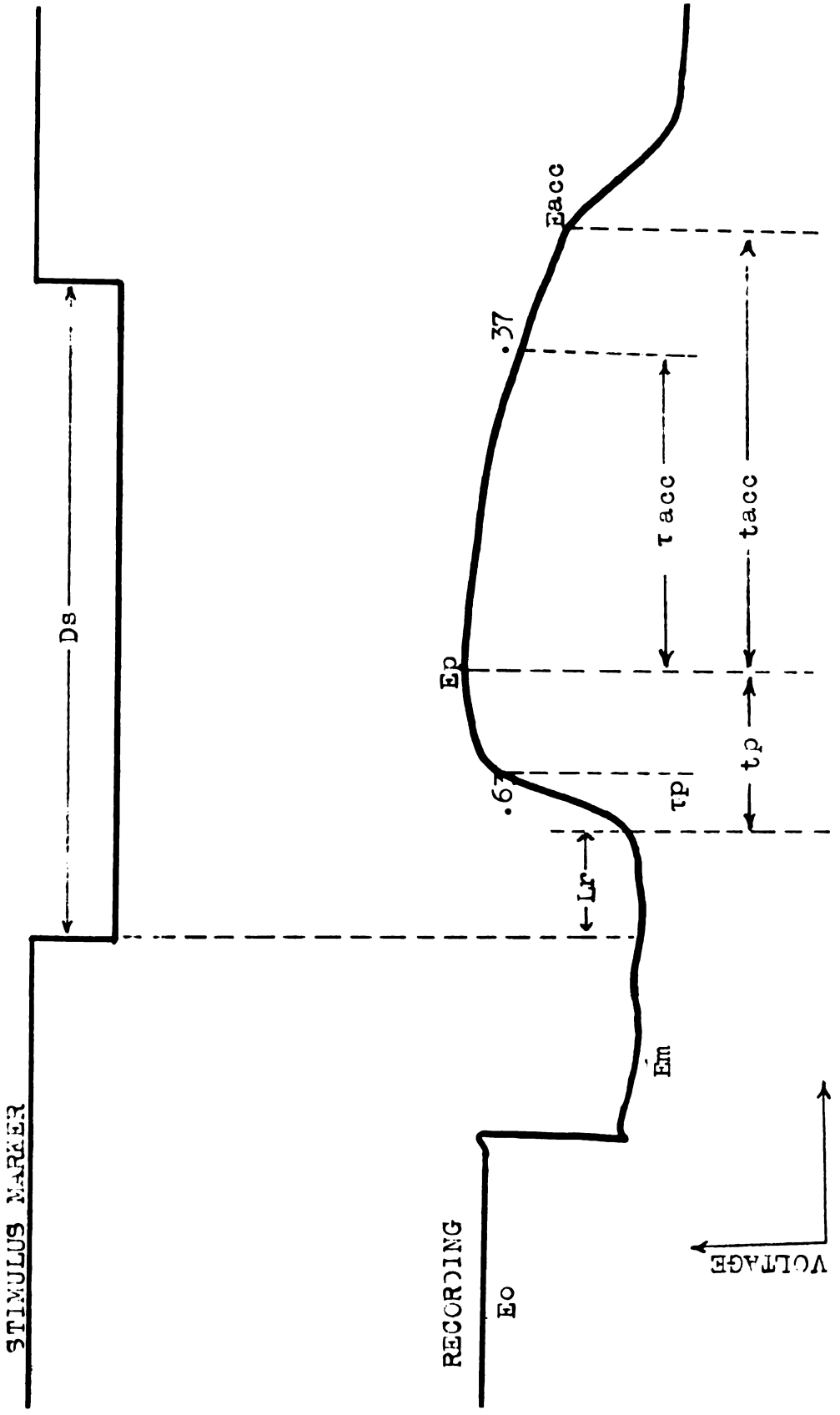


Figure 6. Graphic representation of parameters characterizing temporal and scalar properties of stimulus and response records used for analysis.

VII. RESULTS

Glossopharyngeal Nerve Stimulation and Slow Potentials in the Fungiform Papillae

If antidromic activity in sensory fibers of the IX nerve exerts an effect on the bioelectric properties of the taste disc cells innervated by these fibers, such an effect should be reflected in currents generated in the fungiform papilla. Currents of physiological significance in the function of the taste receptors would be expected to be localized in the sensory cells of the fungiform papilla and not likely found in cells of other tongue structures not innervated by taste nerve fibers.

The properties of the fibers responsible for effects on the sensory cells should correspond to those of sensory afferent nerve fibers if the mechanism is to be considered an antidromic one. Two easily determined qualities differentiating such fibers would be threshold voltage and conduction velocity.

The experimental arrangement of Figure 5 yielded records similar to those shown in Figure 7. The polarities of such currents were variable in amplitude and sign. Different papillae would produce differing waveforms even though stimulus parameters effective in eliciting such currents were held

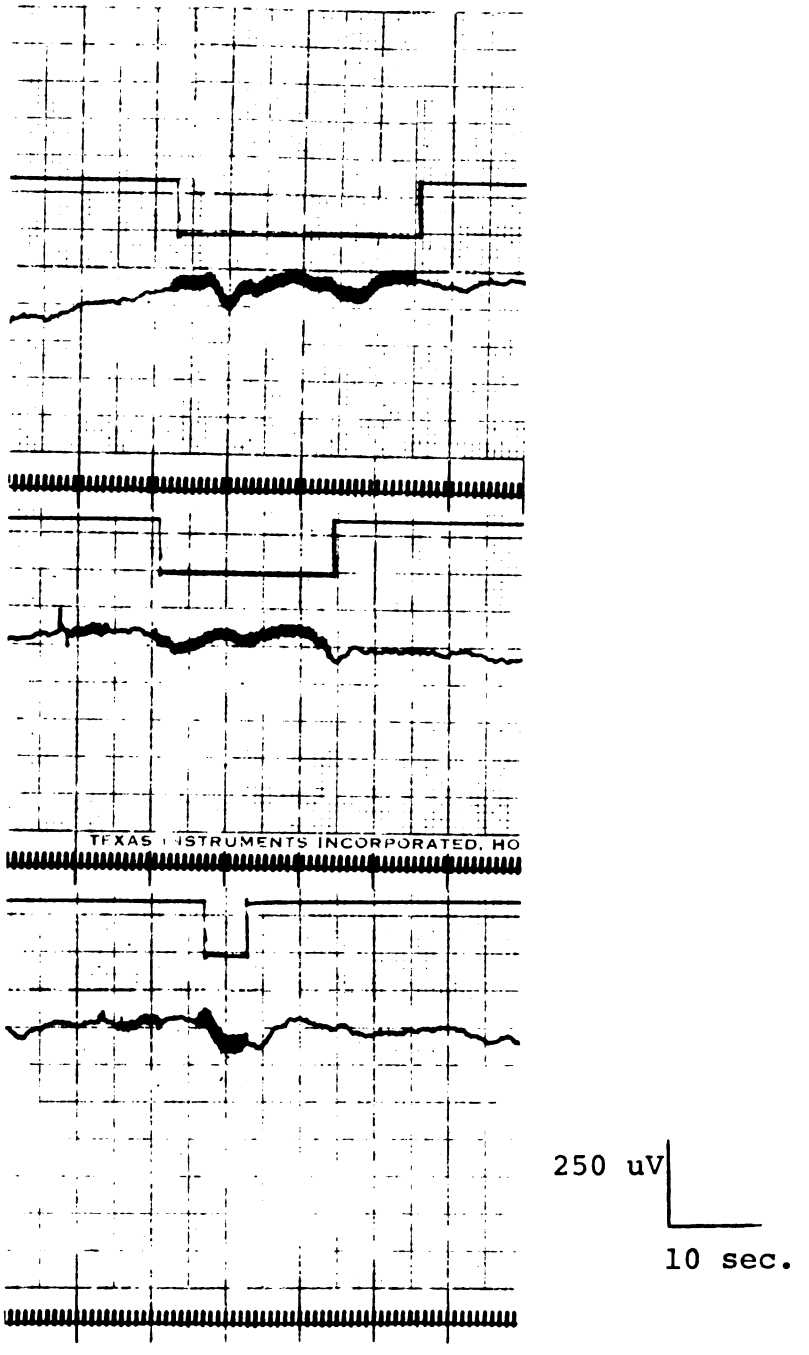


Figure 7. Three representative records of slow-wave bioelectric currents recorded from fungiform papillae using the gross chamber electrode illustrated in Figure 4.

Parameters of electrical stimulation of IX nerve: V = 1 volt; f = 2/second; Dp = 1 millisecond.

constant. The one factor which did relate to these currents was the stimulus parameters which most readily produced them. Application of relatively low-voltage (1-3 V.) square-wave (duration 0.1-1 msec.) pulses to the IX nerve at frequencies of 2-60 per second was usually adequate to elicit such slow wave responses. These parameters are similar to those reported by others (Esakov and Byzov, 1971; Rapuzzi and Ricagno, 1970). In experiments using electrolyte-filled micropipettes for intracellular recording, sharply defined currents of similar nature were seen on the surface of fungiform papillae and nearby. Large-tipped electrodes (as in some cases when the micropipette had been inadvertently broken off at the tip) registered currents of more variability than those seen extracellularly with small-tipped (and presumably more selective) recording electrodes.

Small-tipped electrodes were seen in all cases to display positive potentials (electrode tip more positive than indifferent) when placed on the tongue surface in the presence of stimulation of the glossopharyngeal nerve. Figure 8 shows the time and amplitude characteristics of such a current. The evidence of these slow potentials on the surface of a fungiform papilla was used as an indicator of adequate nerve stimulation in all of the experiments utilizing microelectrode recording. Papillae damaged in preparation, poisoned by toxic substances (alcohol, tissue fixative, strong acids or salts) or with no blood flow rarely displayed these slow potentials

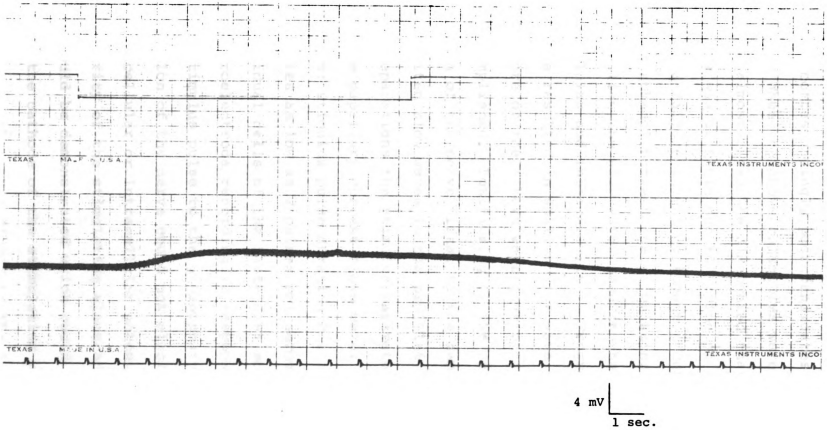


Figure 8. Positive potential recorded from surface of fungiform papilla taste disc by microelectrode. The top tracing shows the duration of electrical stimulation of the IX nerve. The middle tracing is the response potential to antidromic stimulation, and the bottom trace is a 1-second time marker.

Stimulus parameters: V = 1 volt; f = 20/second; Dp = 1 millisecond.

and were correspondingly poor candidates for intracellular studies. When the above calamities were not substantiated, it could almost always be discovered that the stimulation of the nerve was at fault due to drying of the nerve, constriction, or a failure in the equipment. Cutting the nerve or applying ethanol between the stimulating and recording sites would abolish the slow waves seen in the papillae (both extracellular and intracellular). Progressive crushing of the nerve resulted in smaller slow wave potentials, diminishing as more of the fibers were destroyed until complete block was accomplished. Positioning of the stimulus electrodes on a section of the nerve distal to the damage would reinstate the elicited slow waves in the papillae of the tongue. In 26 nerve preparations the stimulus parameters optimal for eliciting these papillary current flows were: stimulus strength 1-3 volts; stimulus pulse duration 0.1-1.0 milliseconds; frequencies as low as 2 pulses per second. Slow waves recorded in individual cells of the taste disc sometimes could be seen with microelectrode recording in response to delivery of a single stimulus pulse to the glossopharyngeal nerve. D. C. stimulation of the nerve could not elicit a measurable change in extracellular or intracellular potentials. By reversing the polarity of the stimulating electrodes on the nerve anodal block could be demonstrated which could be reversed by reinstating the cathode of the stimulating pair of electrodes in a position on the nerve distal to the anode. Stimulation of

the left glossopharyngeal nerve could not elicit a response on the right side of the tongue although there seemed to be some overlap of left and right nerves on papillae located near the midline. The stimulus parameters used were not found to alter blood flow in the capillary loop of the papilla. In all experiments from which data were used, the papillae were visually monitored with a 40-power binocular microscope. Individual blood cells could be seen coursing through the papillary capillary. When such flow was observed to stop or noticeably diminish in the papilla it was absolved from further inquisition and a new papilla with viable circulation substituted.

Action potentials of glossopharyngeal nerve fibers innervating the papilla could also be recorded with the gross chamber electrode. Figure 9 is a photograph of five superimposed sweeps, each initiated by one stimulus shock to the IX nerve. Action potentials with consistent latencies of 3 and 4 milliseconds are evident (arrows). The length of the nerve from cathodal stimulating electrode to papilla in this case was measured as 4.1 centimeters. Calculation of the conduction velocity in these fibers yielded values of 10 and 13.6 meters per second. Measurements in three frogs gave conduction velocities in the range of 6-14 meters per second with a mean of 10.7 and standard deviation of $\pm$ 3.0 (6 nerves). The thresholds and most effective stimulus parameters for

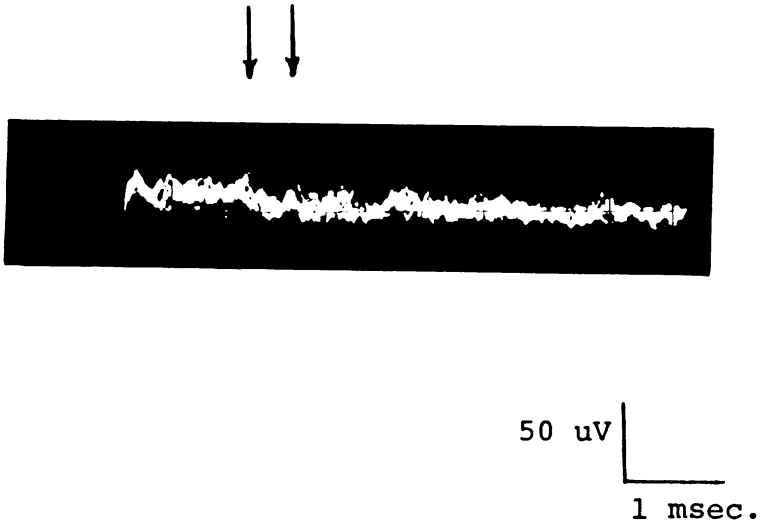


Figure 9. Oscilloscope tracing of 5 superimposed sweeps showing compound action potentials recorded in fungiform papilla at 3 and 4 milliseconds (arrows) after electrical antidromic stimulation of the glossopharyngeal nerve containing sensory fibers innervating that papilla.

initiating these compound action potentials were identical to those for eliciting the slow potentials recorded in the fungiform papillae.

Some intracellular responses to IX nerve stimulation were of a depolarizing nature (recording electrode becoming more positive) while other cells of the taste disc demonstrated hyperpolarizing responses (recording electrodes becoming more negative). Figure 10 a,b illustrates some typical responses. Both types were found to have thresholds. These were characterized by stimulus parameters described above for nerve and extracellular papillary responses.

Depolarization and Hyperpolarization in Cells of Fungiform Papillae

The taste disc of the frog fungiform papilla is composed of essentially two cell layers. The top layer, which is occupied primarily by goblet-shaped cells extends from the surface to about 15 microns, while the second layer, which contains the cell bodies of the rod and forked cells begins at this depth and is about 20 microns thick (Figure 11).

Measurements of the resting membrane potential of taste disc cells were obtained in 23 frogs. An estimate of depth within the papilla was made for some of these cells by noting the distance the electrode was inserted beyond the surface by the micrometer advance mechanism. The response potential (Ep)

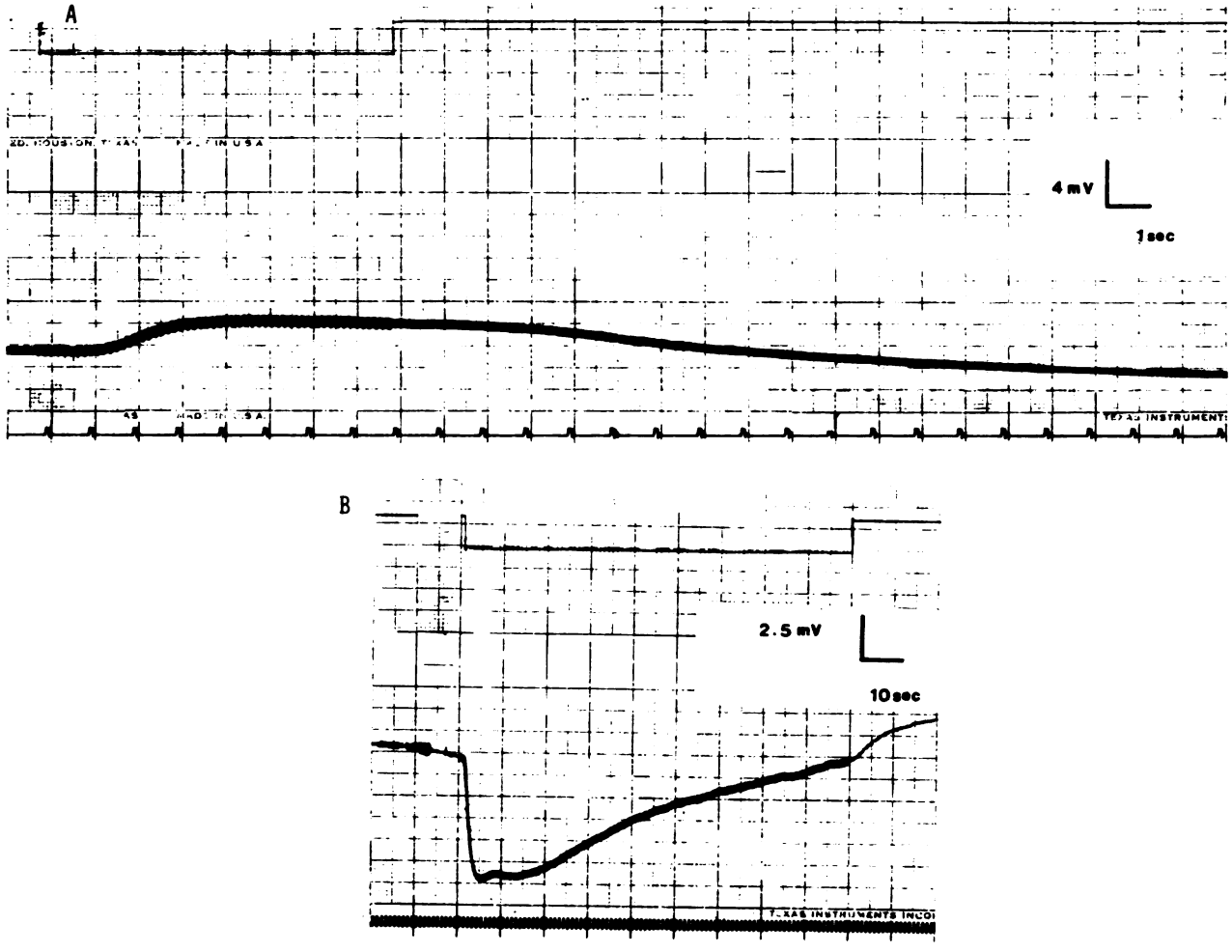


Figure 10a,b. Depolarizing (A) and hyperpolarizing (B) changes in membrane potentials of two different cells in the taste disc. These responses typified the two classes of taste cell responses found to be elicited by antidromic stimulation of the glossopharyngeal nerve.

Figure 11. Photomicrograph of a saggital section through a taste disc of a fungiform papilla used in microelectrode recording. The surface of the disc is on the top. The two layers of nuclei denoting the cellular stratification may be easily seen. Depolarizing cell responses to antidromic nerve stimulation were most commonly found in the most superficial layer (A). Hyperpolarizing cell responses to antidromic nerve stimulation were only seen at electrode depths corresponding to the deeper pseudostratified cell layer (B). Scale: 10 microns.

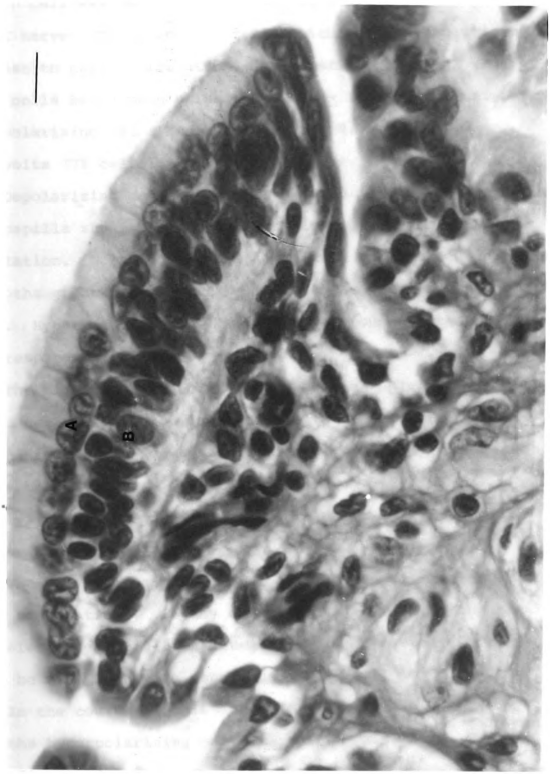


Figure 11

of each cell was recorded following electrical stimulation of the IX nerve. There were 98 cells which gave a depolarizing response to nerve stimulation. The resting potential (Em) of these cells had a mean value of -10.46 $\pm$ 8.4 millivolts. Hyperpolarizing cells had a mean Em of -8.86 $\pm$ 5.5 millivolts (79 cells).

Depolarizing cells were always found in the surface layer of a papilla showing a response to glossopharyngeal nerve stimulation. Occasionally depolarization was noticed in cells at depths greater than that believed to be occupied by goblet cells. Hyperpolarizing cells were never found at the surface of a responding papilla.

The mean amplitude of the depolarizing potential (Ep) in twelve cells of the top layer of the taste disc was +16 mV $\pm$ 2.7 and that of twelve hyperpolarizing cells penetrated at depths below 15 micra from the surface was -12.6 mV $\pm$ 3.5. These values (disregarding sign) were not significantly different ($p = .05$).

The relative magnitude of the response potentials varied among preparations. In any one papilla, however, using the same electrode for all measurements, relative comparisons could be made with some assurance.

In the course of this investigation it became evident that the hyperpolarizing cells were found only within the taste disc, and at some depth beneath the surface. Depolarizing cells were seen on the surface of the disc, at depths

within the disc, and in areas not considered to contain taste receptors. These extrapapillary depolarizations were seen in cells located near and around the fungiform papillae.

Figure 12 illustrates the data obtained from an experiment planned to make comparative measurements of these responses in and around a fungiform papilla. The response amplitudes (Ep) for twenty-one cells exhibiting depolarization on IX nerve stimulation are plotted with respect to their distance from the center of one fungiform papilla. The two vertical bars delineate the area of the taste disc. As can be seen, the depolarization exists in cells within and to either side of the fungiform papilla but the relative amplitude of this depolarization falls off rapidly as the electrode is moved from the border of the taste disc. From this data one would expect that either these cells are also innervated by fibers of the IX nerve, or that they serve as passive sources for a current sink located within the papilla.

Characteristics of Slow Potential Responses in Taste Disc

Both hyperpolarizing and depolarizing slow potentials were recorded in taste disc cells of the fungiform papillae. If these two waveforms are different reflections of a common mechanism they would be expected to display common characteristics in terms of latencies, stimulus-response functions, adaptation or fatigue, thresholds and risetimes.

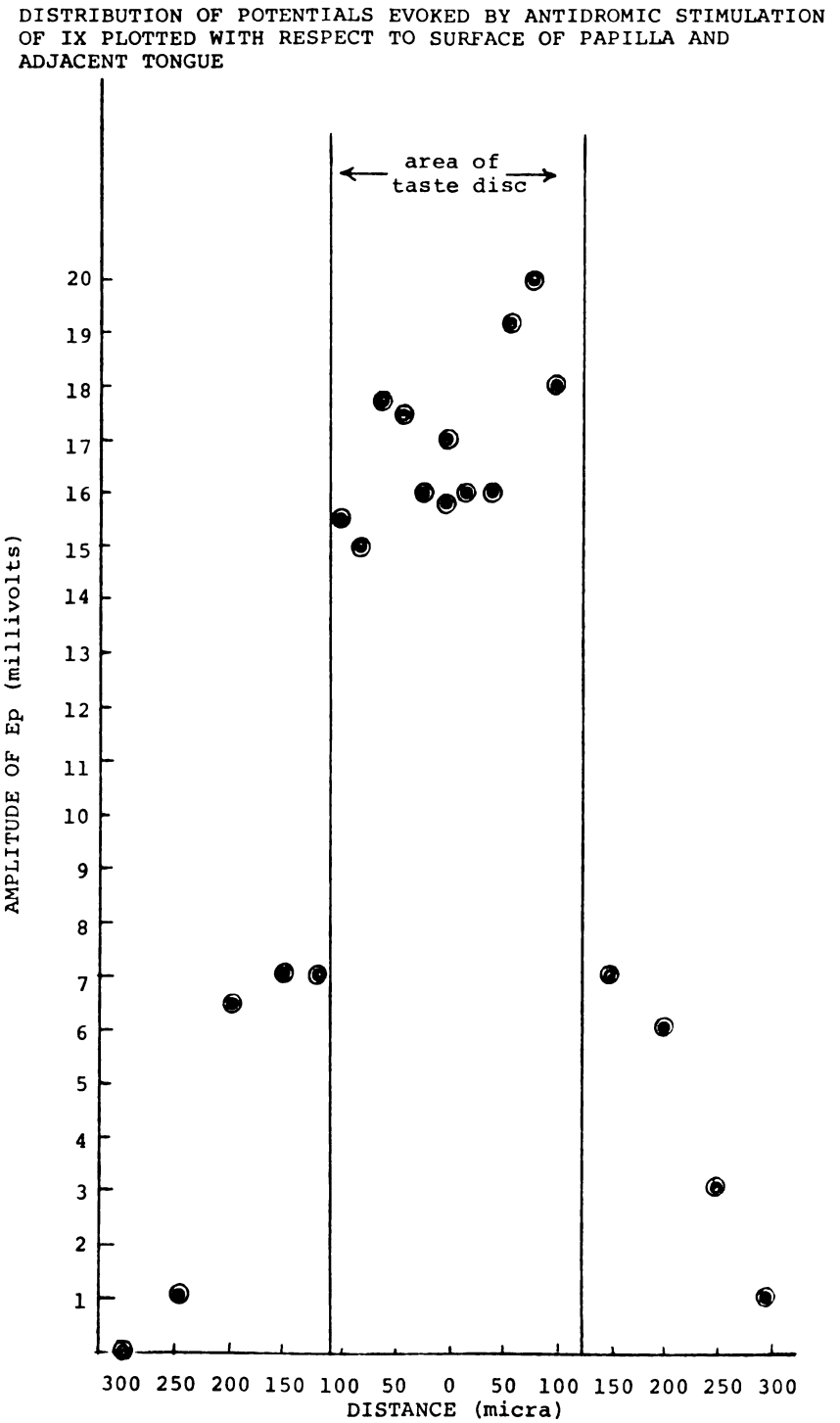


Figure 12. Response potentials (Ep) of cells penetrated on the surface of the taste disc and adjacent non-gustatory epithelium surrounding a fungiform papilla. The two vertical lines delineate the area of the taste disc.

Latencies of initial response as measured in 75 depolarizing cells and 67 hyperpolarizing cells had identical means, 1.3 seconds. This is well above the conduction time measured for nerve impulses to travel from the stimulating electrodes to the taste disc (3-4 msec.). These latencies compare with similar values published by Esakov and Byzov (1971). Thresholds to nerve stimulation were also identical.

At a frequency of one stimulus per second the peak amplitude of both depolarizing and hyperpolarizing responses increased with the increasing number of pulses delivered in a stimulus train up to about ten pulses. However, the response increment per stimulus pulse ($\partial|Ep|$/pulse) decreased as the number of pulses delivered to the glossopharyngeal nerve increased. In a train of pulses, therefore, each successive pulse was less effective than the preceding in producing a voltage change in the cells of the taste disc. Figure 13 illustrates this relationship. The summation effectiveness of repetitive pulses in nerve fibers innervating the papilla appear to be optimal at low rates. Frequencies of this order are similar to those elicited in gustatory sensory nerve fibers by weak, near threshold taste stimuli. Figure 14, a,b,c shows responses of a depolarizing cell to 1,3,4 stimulus pulses delivered to the IX nerve respectively.

Figure 15 illustrates the latency, peak response time, adaptation and habituation characteristics of hyperpolarizing cells. In most cases these were similar to those qualities

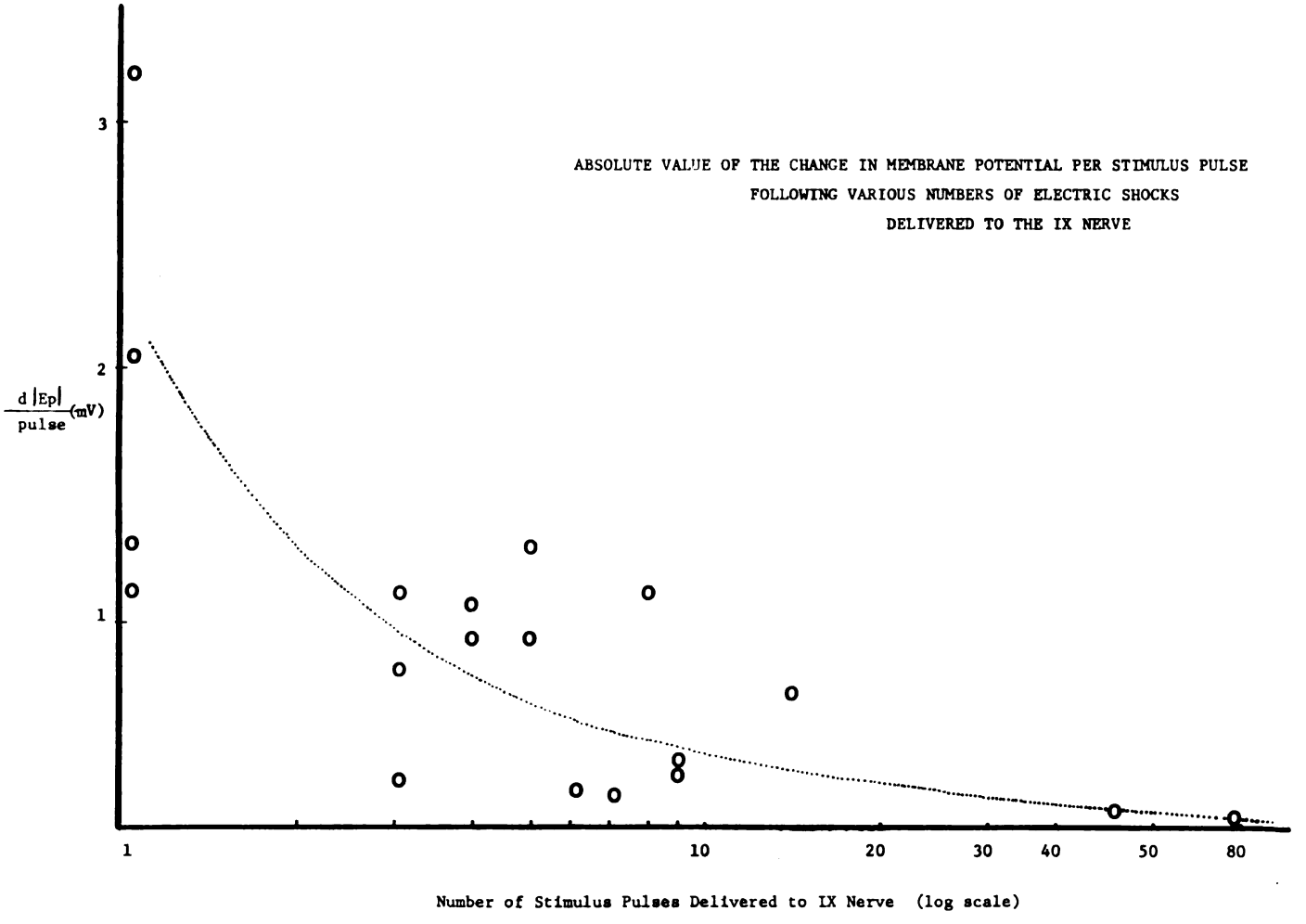


Figure 13. Taste disc cell response increment per antidromic stimulus pulse plotted as a function of the number of stimulus pulses delivered to the IX nerve over a range of 1 to 80 pulses. The value of 11 depolarizing responses and 8 hyperpolarizing responses are plotted together, disregarding sign.

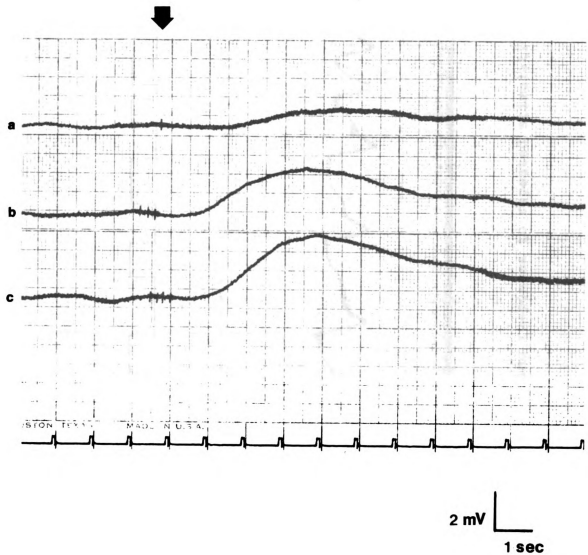


Figure 14a,b,c. Changes in the depolarizing response potential of a taste disc cell when the IX nerve is antidromically stimulated with one (a), three (b) and four (c) pulses. Time to peak (tp) for each response as measured from onset of first stimulus pulse is 4.5 seconds.

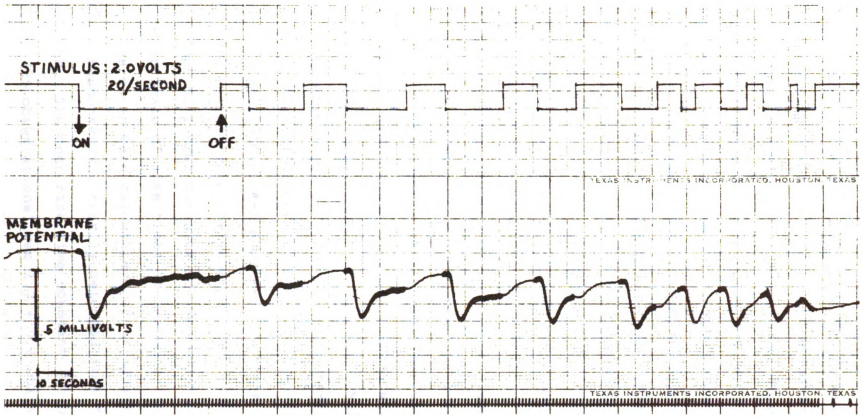


Figure 15. Typical hyperpolarizing responses to repeated stimulation of the IX nerve.

observed in depolarizing cells except for polarity. Regardless of stimulus strength the latencies of the initial response in both cell types were similar. Latencies of 75 depolarizing cells in 9 experiments gave an average of 1.3 $\pm$ 0.32 seconds, including the conduction time for IX nerve impulses of 3-4 msec. Latency of response for hyperpolarizing cell responses as measured in 67 cells averaged 1.3 $\pm$ 0.35 seconds including nerve conduction time. Because time intervals could only be measured in most cases with an accuracy of 0.3 seconds, the latency values can be considered identical, the differences falling well within the experimental error.

Stimulus train durations longer than 4-5 seconds did not enhance response potential amplitudes over the value reached in the first 1.5-4 seconds. Thus, response Ep reached a peak in 4-5 seconds, and despite continuing stimulation of the nerve fell off to some relatively constant level in the face of nerve stimulation. The Ep continued to rise in cases where stimulus train durations were less than the characteristic response times of 4-5 seconds. In these cases, Ep values were reached with the same interval required as when stimuli were of longer duration for any given cell. A more accurate estimate of rise times is the time constant, τp.

Values of τp were calculated for 43 antidromically evoked slow wave responses arbitrarily selected from five different experiments. The mean rise time constant for hyperpolarizing responses (25 observations) was 1.7 $\pm$ 0.6 while that for 19

depolarizing responses was 1.6 $\pm$ 0.4 seconds. The value of the Ep in these cells was not a determinant of the rise time of the response. Within any one cell the antidromically elicited hyperpolarization or depolarization reached its peak in approximately the same time from its initiation regardless of the amplitude of the response. The slope of the rising phase of the Ep was, therefore, a function of the magnitude of the Ep. This may be appreciated by referring to the responses displayed in Figure 14.

Figure 15 illustrates another characteristic of the taste cell response to IX nerve stimulation. Repetitive trains of high frequency stimulation delivered in short intervals led to a progressive decrease in response magnitude Ep. Typical of both hyperpolarizing as well as depolarizing responses the effect of such stimulation was to drive the resting or post stimulus membrane potential in the direction of the Ep. Given sufficient recovery time, the Em of these cells would often return to values near those recorded on penetration of the cell, but due to the technical difficulty of remaining in the cell with the recording electrode for such extended periods (5-10 minutes) few measurements of such long term processes were successful.

A consistent characteristic of both hyperpolarizing and depolarizing taste disc cells is the decrease in response potential level shortly after attainment of peak amplitude in the presence of continuing stimulation of the glossopharyngeal

nerve. This may be consonant with adaptation. With very long stimulus train durations such adaptation often was seen to return the intracellular potential not only to the baseline level but beyond. The rate of adaptation in hyperpolarizing cells was somewhat more rapid than in depolarizing cells but could not be shown to be statistically different. The slope $\partial E/\partial t$ for 20 measurements of hyperpolarizing responses in two experiments had a mean of 0.908 millivolts/second and 16 measurements of depolarizing responses in three frogs had a mean of 0.214 millivolts/second. The rate of adaptation varied among cells sampled. Rebound of the membrane potential occurred in both cell types at the end of a period of stimulation during which it had reached a steady state level. The latency of this rebound was approximately the same order of magnitude as the Ep but its polarity was opposite. That is, if the adapted cell was a depolarizing cell, the rebound phenomenon changed the membrane potential in a hyperpolarizing direction and in hyperpolarizing adapted cells, the rebound tended to produce depolarization of the cell beyond the adapted level of the Em. Figure 16 illustrates both the adaptation and rebound overshoot phenomena as recorded from a hyperpolarizing cell.

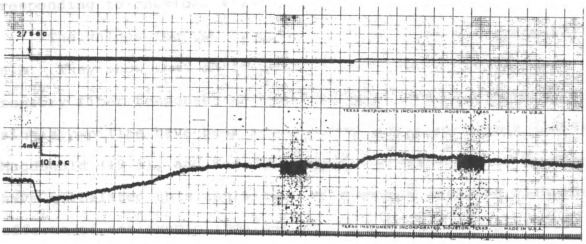


Figure 16. Overshoot during adaptation to a prolonged stimulation of the IX nerve and rebound after stimulus cessation frequently seen in responses of taste disc cells. The hyperpolarization produced in this cell by antidromic nerve stimulation results in an adaptation overshoot and post-stimulation rebound in a depolarizing direction.

Antidromic Stimulation and Responses of Afferent Nerve Fibers to Taste Stimuli

Antidromic stimulation of the frog glossopharyngeal nerve to the tongue produces hyperpolarization and depolarization in different cells of the taste discs. If these antidromic effects have significance in modification of receptor cell sensitivities to taste stimuli, such an effect should be reflected in the afferent discharge rates of sensory fibers responding to chemical stimulation of the tongue.

The values for two preparations are shown in Figure 17 a,b. They show that antidromic nerve activity can depress sensory fiber responses to taste stimuli. Similar results for single papillae have been reported by Filin and Esakov (1968), Macdonald (1971), and Taglietti et al. (1969). None, however, have correlated this inhibition of afferent response with the effects of antidromic impulses on cells of the receptor surface of the taste disc.

Figure 17 c shows the results of an experiment performed in one frog. In this series, hydrochloric acid was applied to the tongue as a chemical stimulus concurrently with electrical antidromic stimulation of the IX nerve. Because stimulation and recording could not be carried out simultaneously in this preparation, activity values in the first response interval are not shown. Chemical stimulation alone produced an increase in discharge rate of 191% (referred to prestimulus

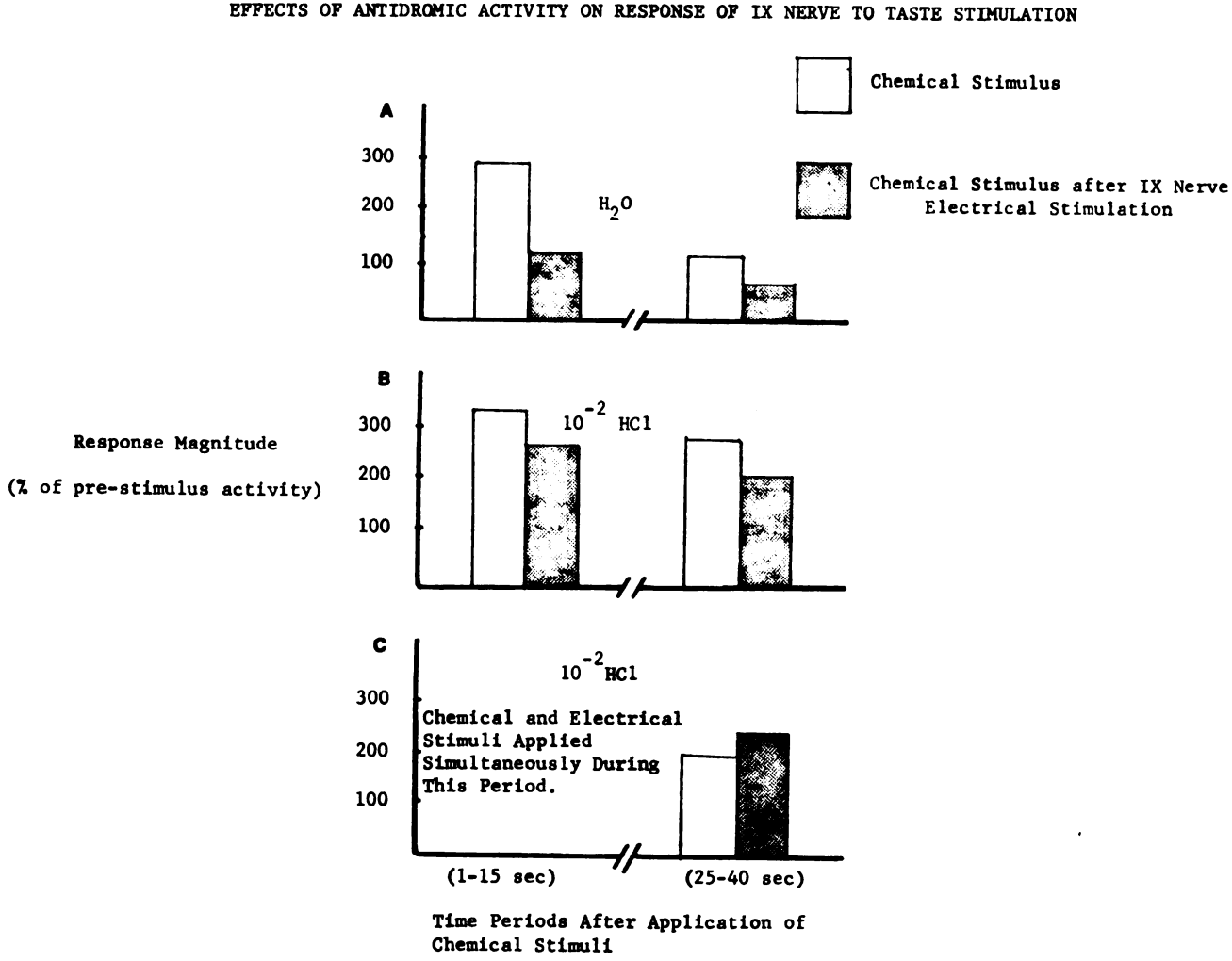


Figure 17. Frequency of gustatory nerve response to chemical stimulation of the tongue and the effects of antidromic electrical stimulation of these nerve fibers on their activity.

baseline rate). Chemical stimulation applied in the presence of antidromic IX nerve stimulation resulted in a post-stimulation discharge of 227%. Macdonald (1971) reported a period of enhanced excitability following antidromic stimulation of sensory fibers in the bullfrog tongue but this enhancement was only seen for a period of 5-20 milliseconds after stimulation. The enhancement seen in Figure 17c occurs at a time post-stimulation that compares with the rebound depolarization seen in the membrane potential of taste cells following prolonged antidromic stimulation (Figure 16).

Intracellular Response of Taste Disc Cells to Direct Chemical Stimuli and Its Effect on Antidromically Elicited Slow Potentials

If there is a mechanism for peripheral interaction among sensory units of the taste disc, and if such a mechanism involves changes in the bioelectric characteristics of the receptor cells, chemically elicited activity in the taste disc might be expected to modify the intracellular responses of receptor cells to antidromic stimulation of the glossopharyngeal nerve.

Cells of the taste disc of the fungiform papilla were impaled by microelectrode and records were obtained of their electrical response to chemical stimuli applied by microsyringe. The results of 72 observations in four frogs are shown in Figure 18. Records obtained at various depths within

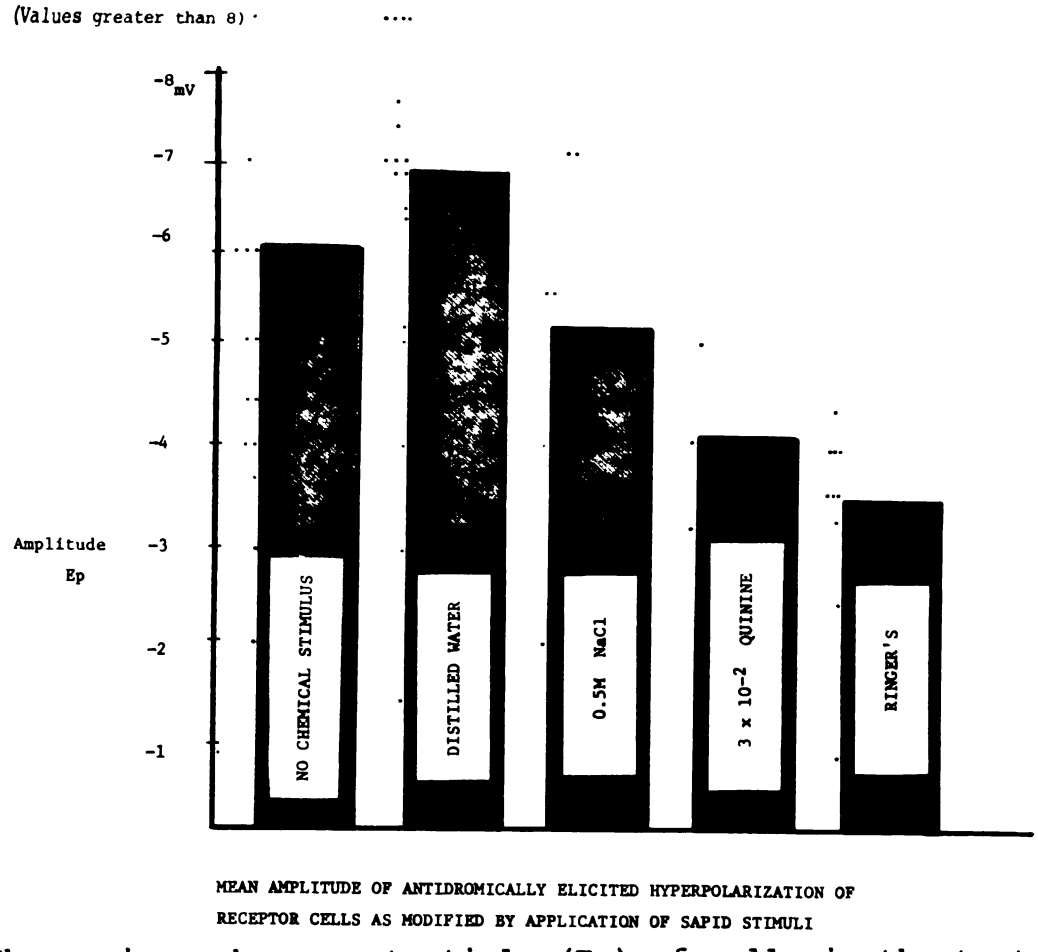


Figure 18. Change in membrane potentials (Em) of cells in the taste disc produced by application of chemical solutions to the surface. Responses of cells impaled by microelectrode at random depths in the taste disc were qualitatively similar. Data points used to calculate mean response are marked along left border of each column.

the papilla were not qualitatively different with respect to depth. Not all cells penetrated at depths below 15 micra responded to chemical stimuli, but no effort was made to identify these cells.

The IX nerve was antidromically stimulated in these preparations in the presence and absence of chemical stimulation of the papilla containing the cell impaled by the microelectrode. The amplitude of the slow wave response (Ep) of the cell was compared in conditions of taste stimulation and no taste stimulation. Figure 19 illustrates the average responses from 51 observations in 4 frogs. Salt solutions and quinine usually produced a depression of the antidromic response, while double distilled water was seen often to lead to an augmented antidromic response. If the antidromic effect was dependent on the cell membrane potential, one would not expect to see the opposite effects of quinine and water on the Ep since both these substances cause hyperpolarization of the receptor cells. The hyperpolarizing effect of water has been considered a result of leaching out intracellular cations from the receptors (Eyzaguirre et al., 1972).

Effect of Electrical Stimulation of Neighboring Papillae on the Response of Taste Disc Cells to Antidromic IX Nerve Activity

In the frog action potentials elicited in a sensory fiber innervating one papilla can travel antidromically up a collateral branch into a neighboring papilla (Rapuzzi and Casella,

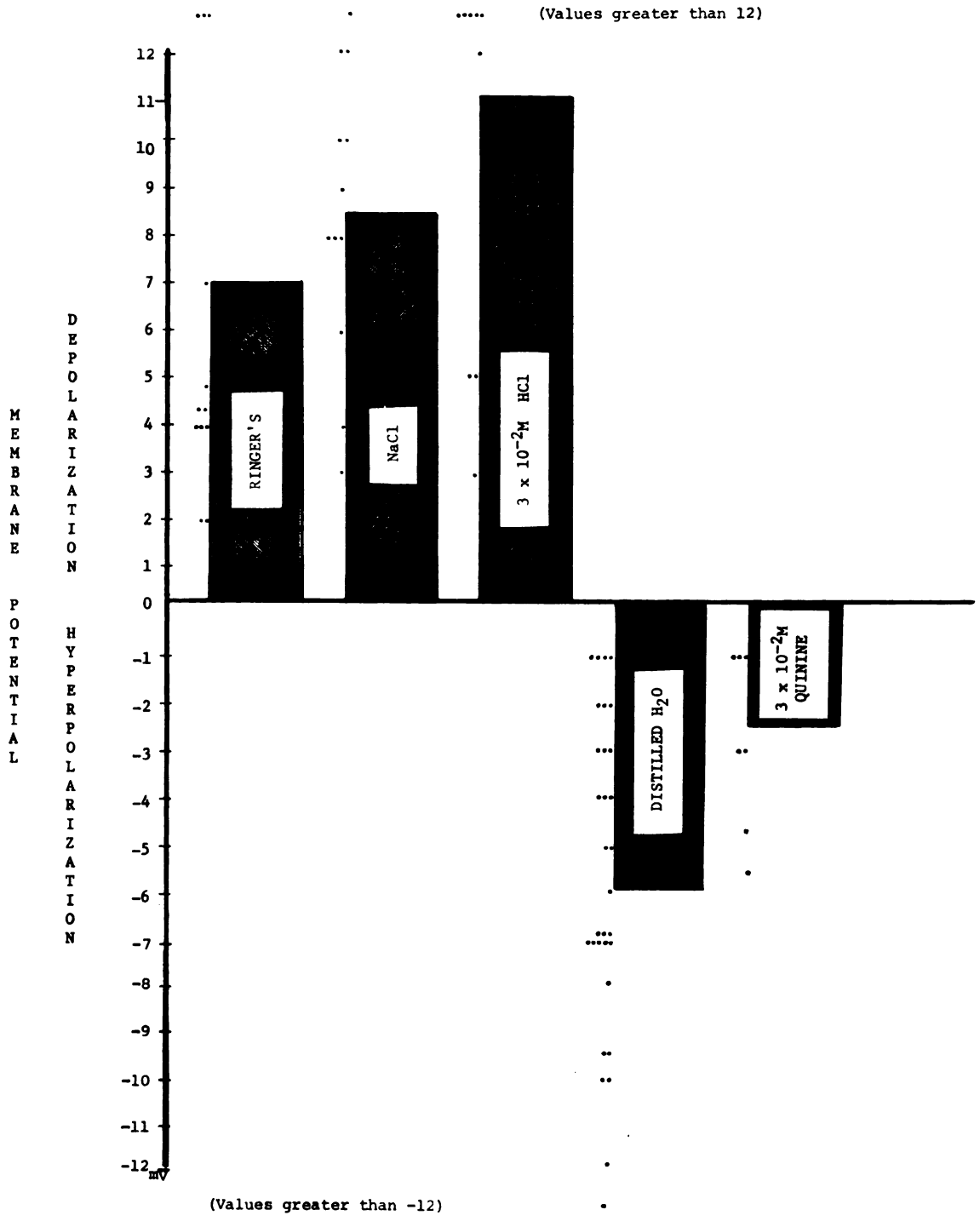


Figure 19. Effect of prior application of sapid solutions on the amplitude of antidromically elicited hyperpolarizations of taste disc cells. Individual data points for each stimulus class are are marked along left border of column depicting mean effect.

1965) and are capable of depressing afferent activity of other sensory fibers innervating the antidromically invaded papilla (Filin and Esakov, 1968). If such depression is mediated via an effect on the bioelectric properties of taste disc cells, it could be reflected in changes of the transmembrane potentials recorded from these cells.

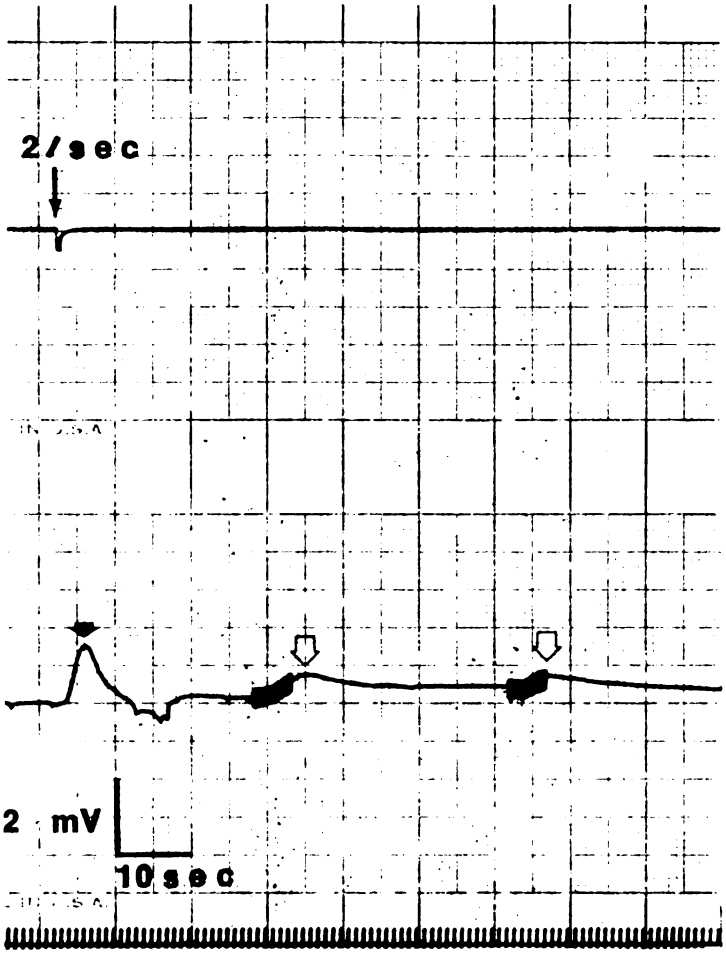
The tracings shown in Figure 20 illustrate small potentials which could be recorded from depolarizing (Figure 20a) and hyperpolarizing (Figure 20b) cells of a fungiform papilla when an appropriate neighboring taste disc was electrically stimulated via a fine-tipped stainless steel monopolar electrode. The direction of potential change in each of these two classes of cells is the same as that produced by antidromic electrical stimulation of the IX nerve. The large initial potential deflections of each tracing identify the cell response to antidromic IX nerve stimulation.

Neighboring papilla stimulation can increase or decrease the response of taste disc cells to antidromic IX nerve stimulation (Figure 21). The data from two of the preparations which were found to respond in this way is plotted in Figures 22 and 23. In these figures the Ep and τp are compared for the period immediately before and at varying times after stimulation of the neighboring papilla, and plotted as the ratio of the pre-stimulus:post-stimulus value at various times following stimulation. The ratios of the post to pre-stimulus Ep

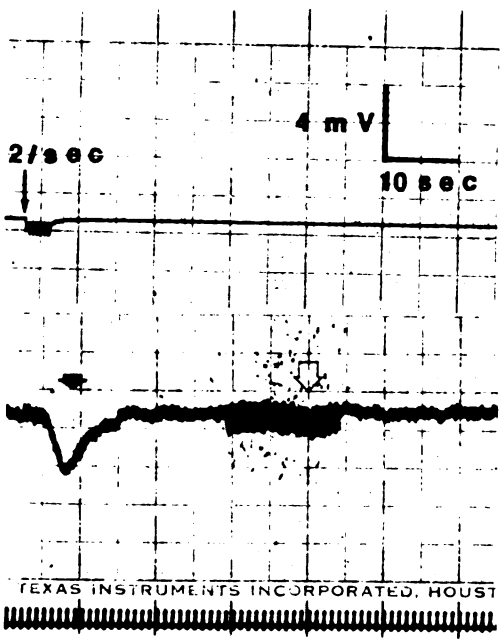
Figure 20a. Small depolarizations produced in a cell of the taste disc by electrical stimulation of a taste disc on a neighboring fungiform papilla. The initial depolarization under the solid arrow indicates the response of this cell to electrical antidromic stimulation of the IX nerve. The two smaller depolarizations under the white arrows are the result of direct electrical stimulation of the surface of a neighboring papilla.

20b. The hyperpolarization indicated by the solid arrow was produced by antidromic IX nerve stimulation and characterizes this cell as a hyperpolarizing cell, as referred to in the text. Direct stimulation of a neighboring papilla could produce the small hyperpolarization indicated under the white arrow.

a


2/sec
2 mV
10 sec

b


4 mV
2/sec
10 sec
TEXAS INSTRUMENTS INCORPORATED, HOUST

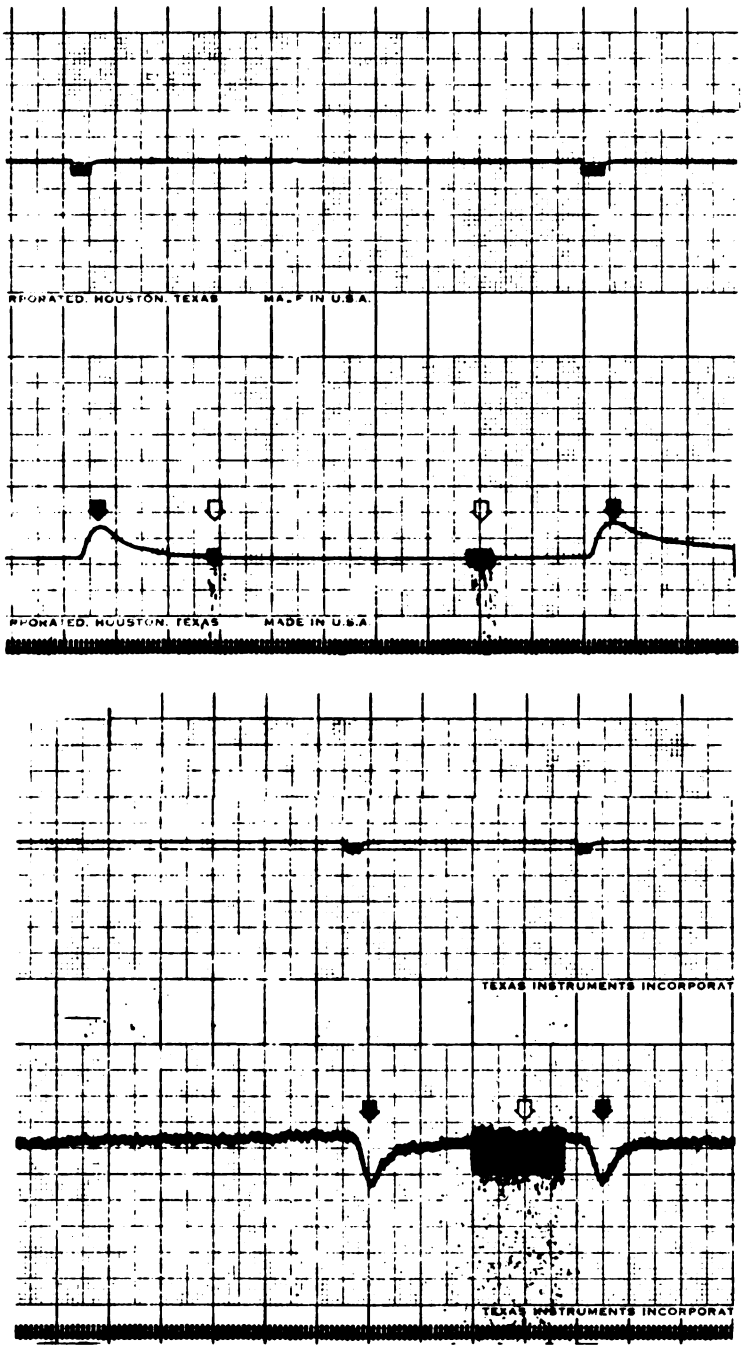


Figure 21. Effect of electrical stimulation of neighboring papilla on the antidromically elicited response of taste disc cells. Black arrows indicate the response of taste cell to antidromic stimulation of the IX nerve. White arrows indicate periods of adjacent papilla electrical stimulation.

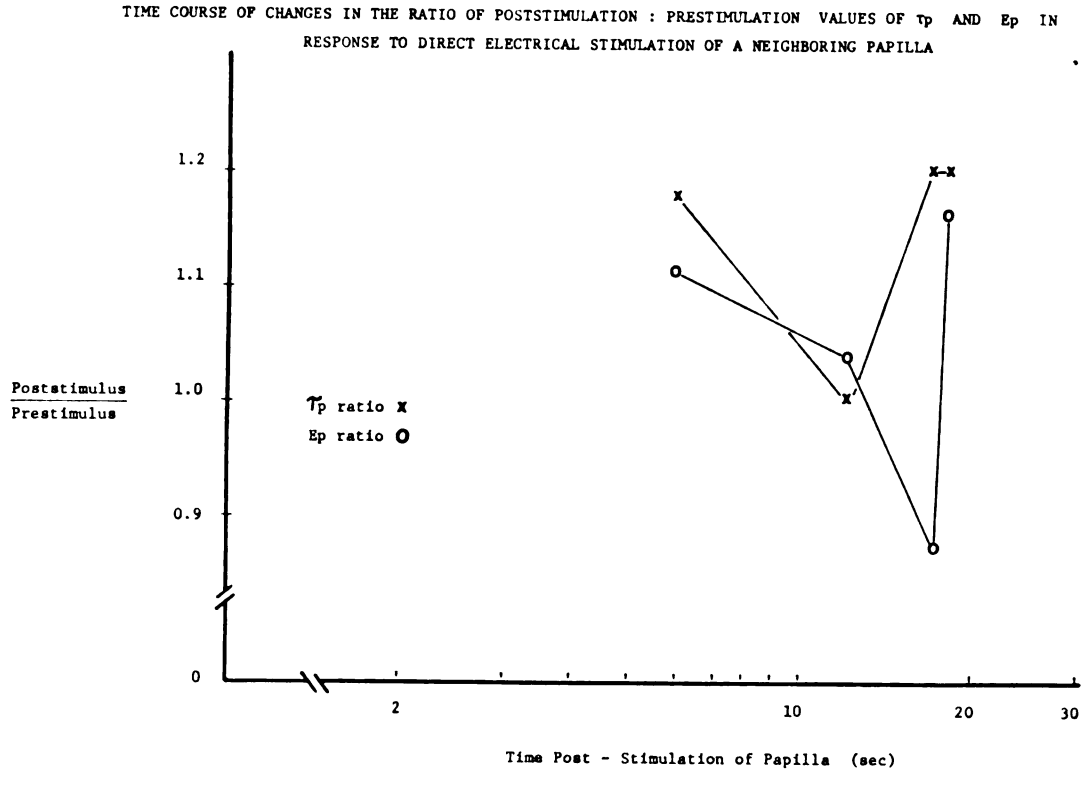


Figure 22. Time course of effects of neighboring papilla stimulation on the Ep and τp of the depolarizing response to antidromic stimulation of the IX nerve.

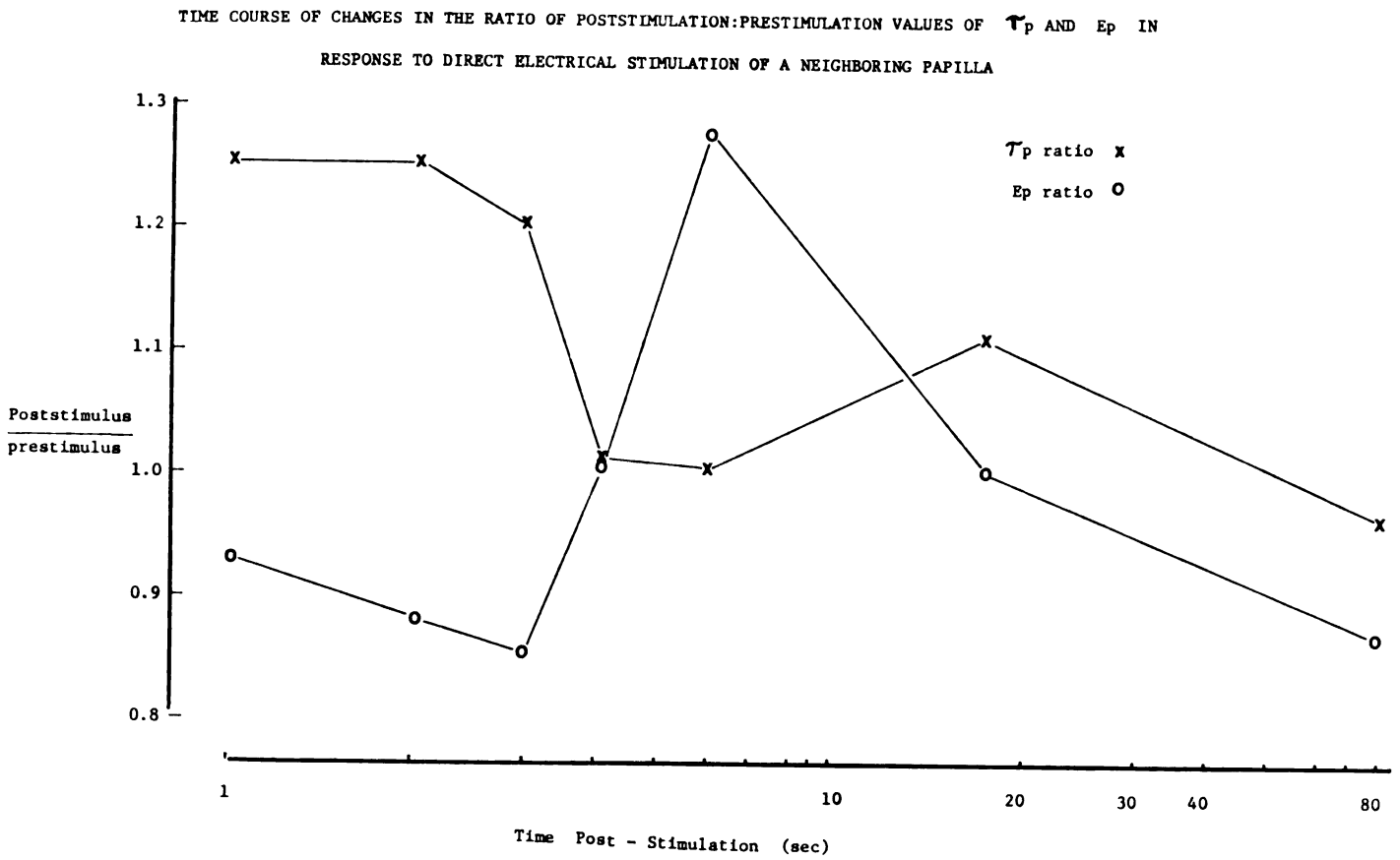


Figure 23. Time course of effects of neighboring papilla stimulation on the Ep and τp of the hyperpolarizing response to antidromic IX nerve electrical stimulation. The ratioes of Ep post:prestimulation of a neighboring papilla are plotted versus the time elapsed after neighboring papilla stimulation that Ep poststimulation was tested. The τp values of these responses are similarly plotted. The two curves show a negative correlation of -.533 from 1 through 18 seconds after electrical stimulation of a neighboring papilla.

and post to pre-stimulus τp are plotted at progressive times after neighboring papilla stimulation. This affords inspection of the time course of any changes in the bioelectric responses of the depolarizing and hyperpolarizing cells impaled by the intracellular recording electrode. In the depolarizing cells the changes in Ep and τp roughly follow each other but in the hyperpolarizing cells, such a relationship is not seen until 17 seconds after neighboring papilla stimulation. Although the time periods studied (especially for the depolarizing cells) are unfortunately limited by technical problems always present when dealing with such small cells, the recognition that there are time-dependent changes in cell properties after antidromic stimulation can be important in the analysis of sensory transducer events occurring in the peripheral gustatory elements. Until recently, gustatory responses had been studied under steady-state conditions without regard to the importance of the dynamics of receptor function in the peripheral analysis of chemical stimuli.

Effect of Taste Cell Membrane Potential on the Response to Antidromic Stimulation of the Glossopharyngeal Nerve

If the cellular mechanism responsible for the current flows reflected by the slow potential changes seen on antidromic stimulation of the IX nerve is dependent on conductance

changes to ionic charges separated across the receptor cell membrane, then alteration of the resting membrane potential, Em, in these cells would be expected to change the response potentials, Ep.

In three depolarizing cells (Figure 24) and two hyperpolarizing cells (Figure 25) of the taste disc, response potentials elicited by antidromic stimulation of the IX nerve were measured during manipulation of the cell transmembrane potential by passing current through the recording intracellular electrode. In depolarizing cells the amplitude of the response to antidromic stimulation remained relatively constant over a range of membrane potentials from -72 millivolts to +54 millivolts. Similarly, in hyperpolarizing cells that were clamped at membrane potentials in a range from -5 millivolts to +6 millivolts, the response potential remained constant and did not exhibit the slope that would be seen if the mechanism of this response was membrane-potential dependent.

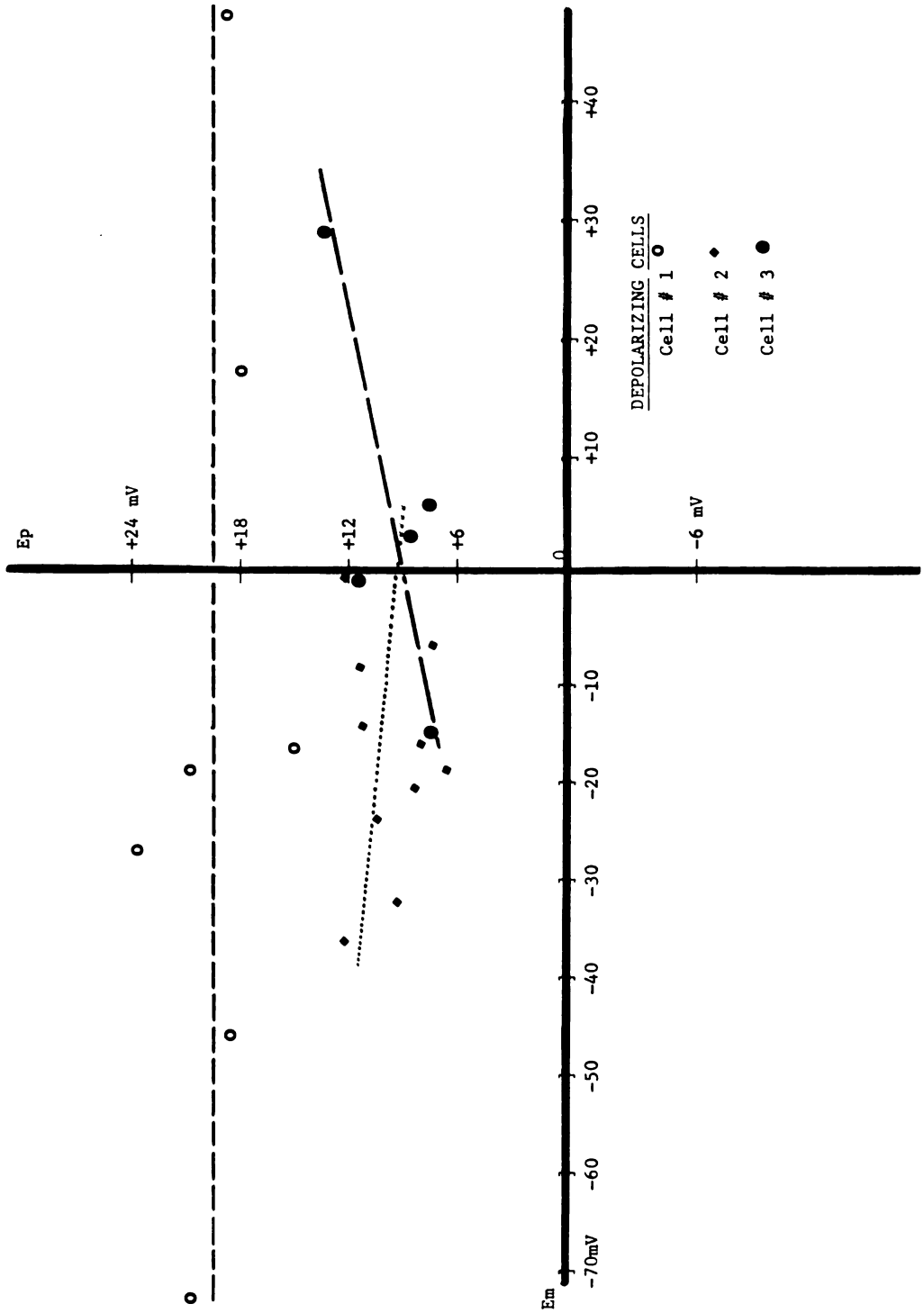


Figure 24. Effect of membrane potential (Em) on the response of three normally depolarizing cells to the antidromic stimulation of the IX nerve.

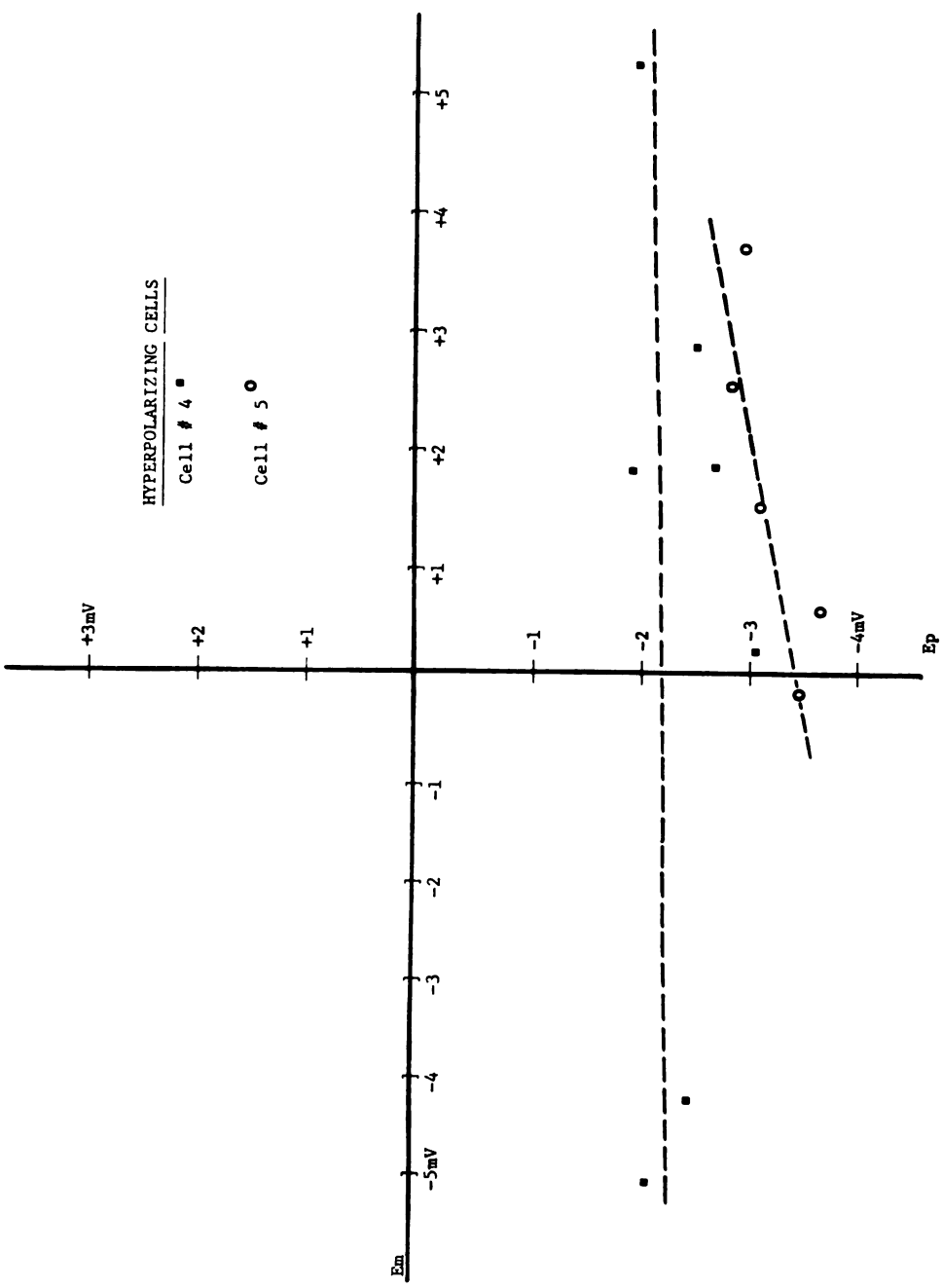


Figure 25. Effect of membrane potential (Em) on the hyperpolarizing response (Ep) of two cells. The membrane potential was varied by passing current through the recording microelectrode and the evoked potentials (Ep) were produced by antidromic stimulation of IX nerve.

VIII. DISCUSSION

Peripherally originating interactions among gustatory units have been reported (Filin and Esakov, 1968; Macdonald, 1971; Miller, 1971; Taglietti et al., 1969; Wang and Bernard, 1969). The functional element subserving communication between interacting sensory structures has in all cases been indicated to be the afferent sensory nerve fiber. Because one fiber innervates several sensory papillae via collateral branches (Ecker, 1889; Gaupp, 1904; Herrick, 1925: Rapuzzi and Casella, 1965) it was proposed that such peripheral interaction required the antidromic conduction of action potentials along these collaterals to other terminals of the sensory unit (Bernard, 1971a,b; Filin and Esakov, 1968; Macdonald, 1971; Rapuzzi and Casella, 1965; Taglietti et al., 1969) or local, non-propagated currents in the collaterals (Miller, 1971). Figure 26 shows how such antidromic action potential activity may invade neighboring papillae in a sensory unit (Bernard, 1971 a,b).

Antidromic Activity in IX

The lingual branch of the IX nerve in the frog has been found to contain sensory (afferent) and autonomic (efferent)

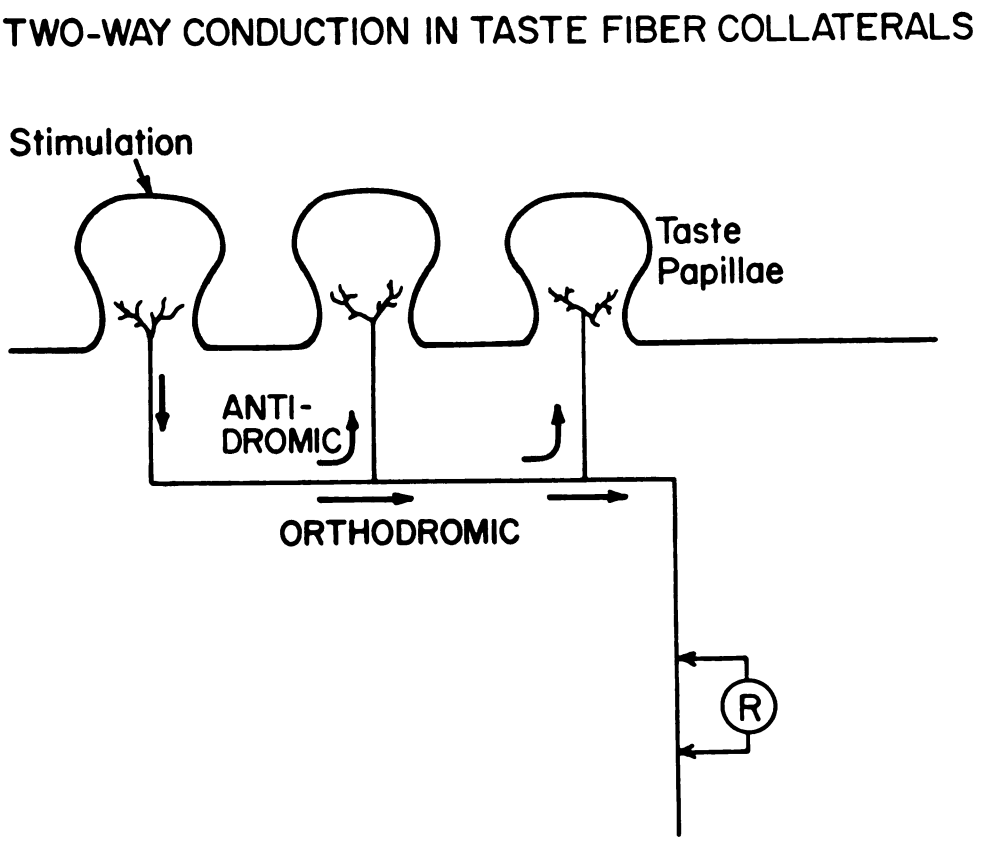


Figure 26. Representation of the pathways for orthodromic and antidromic conduction of action potentials in a single taste fiber innervating three papillae on the surface of the tongue. (Bernard, 1971)

nerve fibers (Ariens Kappers et al., 1936; Herrick, 1925; Strong, 1895). Sympathetic efferent activity has been found to enhance the afferent sensory activity of fibers from the tongue (Chernetski, 1964; Halpern, 1967; Kimura, 1961) and to moderate blood flow in tongue structures (Erici, Folkow and Uvnas, 1951; Erici and Uvnas, 1951). Other autonomic centrifugal fibers were found to enhance or inhibit afferent taste fiber discharges from both the ipsi and contralateral tongue receptors (Brush and Halpern, 1970; Esakov, 1970; Halpern, 1967). The conduction velocities in these efferent fibers is found to be in the range from 0.2-0.8 meters per second (Chernetski, 1965). Taste afferents in the frog have conduction velocities of 1-15 meters per second (Rapuzzi and Casella, 1965), and do not cross the midline to the contralateral side of the tongue to any great extent (Strong, 1895).

The slow potentials recorded from taste papillae presented in the results of the present work are most likely, therefore, to be the result of primarily antidromic activity in sensory fibers of IX, because the stimulus parameters used to stimulate the cut peripheral stump of IX initiated action potentials in fibers with conduction velocities of 6-14 meters per second, and did not affect blood flow in the tongue. In addition, the effects produced were ipsilateral and no papillary bioelectric activity could be elicited on the contralateral side of the tongue on stimulation of the IX nerve with parameters effective in producing responses in ipsilateral

papillae. The nerve activity was not able to produce changes in the sensory discharge of the contralateral IX nerve as described by Esakov (1961) when he stimulated efferents in the glossopharyngeal of the frog.

The relatively long latencies of the papillary cell responses to this antidromic stimulation of the IX nerve are too great to be accounted for even by the slow conduction velocities in the autonomic fibers. Assuming even the slowest of these fibers were responsible for the effect, with a conduction velocity of 0.2 meters per second and a maximal nerve length of 5 centimeters the latency due to conduction in the slowest fibers would only be 25 milliseconds. The argument, therefore, that the long latencies indicate activation of autonomic fibers is not a likely one.

It is known that touch fibers may innervate more than one papilla per fiber. Rapuzzi and Casella (1965) found that a tactile sensory fiber innervated an average of 2.7 papillae. Each papilla, however, had only one tactile fiber in it, and the fiber did not branch within the papilla. The diameter of these fibers is about the same as the gustatory fibers and their conduction velocities fall within the range observed for those fibers activated in the present experiments. However, the gustatory fibers each innervate from 5-6 papillae, and are estimated to branch again within each papilla about five times (Rapuzzi and Casella, 1965). Therefore, one gustatory fiber can be expected to have about 25-30 peripheral terminals as

compared to only 2-4 for a tactile fiber. It is reasonable to assume that the relatively large currents associated with the slow potentials in the whole papilla (Figure 7) could not be produced by the single tactile fiber innervating it, and that the responses observed in the papilla are more likely a result of activity in the chemosensory fibers, and that these fibers were conducting action potentials centrifugally, a direction antidromic to their afferent sensory conduction.

Inhibitory Effects of Antidromic Activity in IX

Antidromic activity in the glossopharyngeal nerve was found to decrease the afferent activity of chemosensory fibers from the frog tongue (Figure 17). Such inhibitory effects of activity in peripheral taste fibers have been described for the frog tongue by Filin and Esakov (1968), Macdonald (1971), Miller (1971), and Taglietti *et al*. (1969). There appears to be a time-dependency of such inhibition. Initial effects (2-20 milliseconds) were facilitatory and were followed by a period of depression (Macdonald, 1971). The depression had a prolonged time course of up to 5 minutes (Filin and Esakov, 1968).

Two sites for such depression effects are evident. First, nerve terminals of the sensory fiber collaterals may exhibit a refractoriness due to depolarization produced by antidromically

traveling action potentials. These effects would be expected to have their major influence over a period of not more than 400-500 milliseconds at the most since the recovery of these nerve fibers following activity does not exceed this time (Gasser et al., 1938). Such depression times are in agreement with the findings of Macdonald (1971). Similar effects of antidromic depression of nerve terminals for cold receptors in the tongue of the dog are reported by Dodt and Walther (1957). The inhibition of peripheral tactile units by antidromic invasion (Lindblom, 1958) follows a similar time course. The initial facilitation reported by Macdonald (1971) is in agreement with the supranormal periods of nerve fibers of this size (Gasser et al., 1938). The long time course of depression found by Filin and Esakov (1968), and this study, cannot be explained by the refractory periods of the nerve terminals and must therefore be dependent upon another functional mechanism.

The second site where depression may be manifest is at the peripheral receptor itself. Such a mechanism would require the postulation of a trans-synaptic effect which would outlast the relatively short depression period seen in the nerve terminals. In addition, since antidromic action potentials in afferent fibers are responsible for the depression, either an inactivation of the neuronal post-synaptic membrane or an inhibition (synaptic) of the receptor must be assumed.

No evidence for the former hypothesis is available and consideration of the latter would require either the presence of an inhibitory interneuron which would exert a depression on the receptor or a population of both afferent and efferent synaptic structures on the same sensory neuron.

For the frog taste papilla both afferent and efferent types of synapses between nerve fibers and taste receptor cells have been anatomically described (DeHan and Graziadei, 1971; Uga, 1967; Uga and Hama, 1967). These have been considered junctions between receptor and sensory neuron without mention of the known efferent fibers to the papilla. However, since it has been shown that antidromic activity in sensory fibers can produce a change in the bioelectric properties of the receptors (Figures 10, 13, 14, 15, 19, 20) and that orthodromic activity in chemosensory fibers is dependent on chemical transmission between receptor cells and taste fiber endings (Landgren et al., 1954; Rapuzzi and Ricagno, 1970), the functional possibility of reciprocal transmission at the receptor-neuron junction is an acceptable hypothesis. Anatomical and physiological evidence has been presented for the existence of such a dual system in the rat olfactory bulb (Andres, 1965; Rall and Shepherd, 1968; Reese, 1965; Reese and Brightman, 1954).

It is also known that the same transmitter may be responsible for excitatory as well as inhibitory effects on the same postsynaptic cell, the predominant effect being a function of

the relative activity of inhibitory and excitatory postsynaptic sites (Wachtel and Kandel, 1967). Such a system does not violate the principle of Dale (Eccles, 1957) which states that a neuron may liberate only one species of transmitter at all its synapses. Postsynaptic inhibition by the same cell which also elicited excitation of the postsynaptic neuron in the trigeminal nucleus of the cat was shown to be a function of the frequency of the presynaptic discharge (Dubner, 1967). Wachtel and Kandel (1967) showed that a single neuron could be excitatory at some of its synaptic terminals and inhibitory at others. They also demonstrated that a single presynaptic element can exert both excitatory and inhibitory postsynaptic effects at the same site on the postsynaptic membrane. Such mechanisms may be utilized in the peripheral interactions among sensory units of the tongue. Wang and Bernard (1969) found that afferent nerve discharge of chemosensory units in the chorda tympani nerve from the tongue of the cat showed decreasing activity with increasing stimulus concentration. These results can be explained by invoking mechanisms of peripheral modulation of activity among receptor-nerve elements of the tongue.

Antidromic IX Nerve Activity and Taste Receptor Cell Bioelectric Changes

Hyperpolarization of nerve or receptors has generally been shown to result in inhibition of activity in these

elements while depolarization is associated with a state of enhanced excitation (Boeckh et al., 1965; Jenerick and Gerard, 1953; Kuffler and Eyzaguirre, 1955; Lorente de No, 1947). Nomura and Sakada (1969) found that afferent activity and slow depolarization of tactile nerve fibers in the papillae of the frog were directly related. However, taste fibers did not produce such a reliable correlation between afferent impulse activity and terminal depolarization on stimulation of the papilla with water, sometimes showing action potential generation without a concomitant "generator potential." The slow potentials recorded by Nomura and Sakada were considered to be changes in sensory nerve fiber currents and not currents produced in the receptor cells of the taste disc.

Figures 10b, 15, 16, 20b, 21 illustrate that hyperpolarization was produced in cells of the taste disc by antidromic stimulation of sensory fibers of the glossopharyngeal nerve. Figure 17a,b shows the inhibition of sensory activity in nerve fibers produced by such antidromic stimulation. Salt solutions were found to produce depolarization of cells of the taste disc, as did HCl (Figure 18). This finding supports those reported by Eyzaguirre et al. (1972) and Sato (1969) for the toad and frog, respectively. Similar effects were reported in intracellular studies of the rat taste bud (Kimura and Beidler, 1961; Ozeki, 1970, 1971; Tateda and Beidler, 1964). Figure 19 shows that depolarizing stimuli such as these were able to lessen the hyperpolarizing effects of antidromic nerve

stimulation. Eyzaguirre et al. (1972) showed that pure water exerted a hyperpolarizing effect on cells of the toad taste papillae. Figure 18 shows the similar effects obtained for water in the frogs used for the present experiments. In Figure 19, furthermore, it is seen that previous application of water to the taste papilla of the frog potentiated the hyperpolarizing effects of antidromic nerve stimulation. On the other hand, Figure 17c shows that the simultaneous application of a depolarizing chemical stimulus (HCl) to the papilla with antidromic stimulation of the IX nerve led to a small potentiation of the chemosensory discharge. It would seem that the potentiating effects of antidromic activity on the response to HCl is not reconcilable with the concept that afferent activity in chemosensory fibers is a simple function of receptor cell membrane potential.

By studying the adaptation of a taste papilla cell to prolonged antidromic stimulation (Figure 16) it can be seen that the hyperpolarization decreased in spite of the continuing antidromic activity. The membrane potential of this cell is seen to overshoot the initial resting potential in a more depolarizing direction. If the membrane potential is an accurate reflection of the bioelectric state of the cell, it could be considered to be completely adapted in about 90 seconds where the slope of the change in potential is seen to be practically flat. If such adaptation was a function merely of changes in ionic conductances, cessation of the antidromic

activity would be expected to allow either a return to the original resting membrane potential, or the cell would remain at nearly the same potential as found when completely adapted. Figure 16 shows, however, that at the termination of antidromic activity the cell membrane potential bounces upward, further depolarizing it. This "rebound" phenomenon indicates that even after the membrane potential adapted to a steady state, the antidromic nerve activity was producing an effect on the cell, recovery from which involves changes in electrogenic components of the cell that produce a depolarizing change in the membrane potential. Figure 10b shows another example of this rebound phenomenon where the adaptation is sufficient to unmask this interesting phenomenon. Apparently when antidromic rates are high, as in Figure 15, the ability of the cell to adapt is unequal to the task and the rebound appears to be an approach to pre-stimulation potential. The observation that the waveform of the Ep varies with stimulus conditions may indicate at least a two-component mechanism of antidromic effect, one a fairly rapid change (5-20 seconds) which is reflected in the change in membrane potential, and a more prolonged effect (5-10 minutes) that is less labile. This is supported by the fact that the cell which has exhibited a depolarizing rebound will begin to return to its initial resting potential level. In the cases where this return has been followed, it was observed to approach asymptotically the same Em as it had prior to antidromic activation, the time

course of this return being several minutes. This effect on the taste cells by antidromic activity in the sensory fibers does not appear to have an equilibrium potential and changes in resting cell membrane polarization even to the complete reversal of the Em (Figure 25) had little effect on the Ep. The modulation of the antidromic Ep by taste stimuli (Figure 19) therefore is not likely to be mediated by changes in Em produced by these chemicals.

If, however, sensory fiber discharge is a function of receptor membrane potential change, then the effects produced by antidromic activity on Em may serve to modulate the discharge rates of afferent chemosensory fibers. The hyperpolarizing as well as depolarizing components of these antidromically activated potentials could be seen to participate in both depression and enhancement effects of stimuli at the peripheral level. An example of these opposite effects on the sensory response to HCl is seen in Figure 17b,c. Wang and Bernard (1969) have reported peripheral enhancement and depression to chemical stimulation in the tongue of the cat, and Miller (1971) and Beidler (1969) have shown this effect in the rat.

The effect of a stimulus on the receptor cell Em, the effects on antidromic Ep, Eacc, τp and τacc as well as the latent electrogenic component responsible for the "rebound phenomenon" may be considered additional factors in the coding

of stimulus quality which classically has been ascribed to the spatio-temporal effects of the stimulus.

Taste Receptor Potentials

The fungiform papilla taste disc contains two layers of cells which may be responsible for the slow potentials recorded upon antidromic stimulation of the sensory nerve fibers of the IX nerve. The surface layer of goblet shaped cells is not considered to have a sensory function but the "rod" cells of the deeper layer have been classically reported as the sensory receptors of the taste system in the frog (Beale, 1869; DeHan and Graziadei, 1971; Ecker, 1889; Hammerman, 1969; Kolmer, 1910; Stensaas, 1971). Synaptic junctions between cells of the taste disc and nerve fibers have been reported only for the "rod" cells (DeHan and Grasiadei, 1971; Stensaas, 1971; Uga and Hama, 1967; Uga, 1966).

Figures 8 and 10a,b show that antidromic stimulation initiated depolarization in surface cells and hyperpolarization in cells at a depth corresponding to that of the "rod" cells of the fungiform papilla. In addition, Figure 12 shows that the depolarizing responses were not confined to cells of the taste disc, but could be found in cells of the tongue epithelium not associated with taste sensibility. These epithelial cells, however, are joined by "zonae occludentes" or tight junctions among themselves and with the "rod" cell

processes they envelop within the taste disc itself (Dehan and Graziadei, 1971; Stensaas, 1971). The fact that the depolarization of these surface cells followed the potential changes of the "rod" cells with little difference in latency (Figures 22, 23) and similar stimulus-response characteristics may indicate a passive role in current flow within the papilla. Such a hypothesis is suggested for papilla elements in amphibia by Herrick (1925) and for the toad by Eyzaguirre et al. (1972). The theoretical considerations on the effects of passive elements by Llinas and Bloedel (1969), and Lorente de No (1947) are applicable to the depolarizing responses recorded from surface cells within and without the fungiform papillae of the frog tongue. If they serve as passive current sources for the receptor elements within the taste disc, the depolarizing cells would contribute to the peripheral modulation of the effects of taste stimuli on the receptors. Such functions have been considered for smooth muscle by Tomita (1966) and in the integration of activity in the olfactory bulb of rat, rabbit and cat (Rall and Shepherd, 1968). This is especially significant in view of the findings that epithelial cells respond to chemical stimuli by a change in electrical potential (Eyzaguirre et al., 1972). While time courses of changes in potential and τ_p in epithelial cells follow each other rather closely (Figure 22), it can be seen that cells which are in the receptor region of the taste disc (Figure 23) do not exhibit such a relationship in the initial phases of response to

antidromic invasion. The passive elements of a volume conductor would be expected to follow the potential changes of the electrotonically-coupled active elements. Potential changes in the depolarizing cells of the papilla show such a relationship to the hyperpolarizing cells. A metabolic effect of antidromic stimulation which affects the time constant, τp of current changes in the cells directly affected by such activity would not be expected to be passed to the passive element of the volume conductor. Figure 23 shows that the time constant changes in the hyperpolarizing cells did not follow the Ep changes of these cells. However, for the same time periods (6-17 seconds post antidromic activation), the Ep and τp changes in depolarizing cells are similar and follow the Ep changes of the hyperpolarizing cells. Such an effect would be expected in a system where cells are electrotonically coupled but not metabolically coupled. The taste disc of the frog fungiform papilla appears to satisfy the theoretical requisites for such a system.

Evidence for a Metabolic Component of Taste Cell Response

The long latencies of taste cell response to antidromic nerve stimulation indicate a delayed effect not consonant with conductance changes produced by synaptic transmission in electrochemical systems (Hubbard, Llina's and Quastel, 1969).

Single electrical shocks to the IX nerve did not elicit a potential change in the taste disc cells until 1-1.5 seconds post-stimulus (Figure 14). In some cases these effects persisted for several minutes beyond cessation of stimulation. The time course of the effects of repetitive stimulation shows that relatively little augmentation of response Ep occurred after the first four seconds of stimulation (Figures 8, 10, 15, 16). The Ep was more sensitive to the number of low frequency pulses (1-5 per second) than to the frequency of the stimulation *per se* (Figures 13, 14). Blood flow in the papilla was important for the preservation of the antidromic effects. Hellkant (1971) found that rat taste bud function was also dependent on adequate arterial blood flow.

Activation of a metabolically driven sodium pump has been described for synaptic activity in bullfrog sympathetic ganglia (Nishi and Koketsu, 1969), mammalian sympathetic ganglia (Libet and Tosaka, 1969) and mammalian smooth muscle (Burnstock *et al.*, 1963). The post-synaptic effect of nerve stimulation in these systems was hyperpolarizing, inhibitory, and of a latency on the order of seconds. Gorman *et al.* (1967) reported that the hyperpolarization produced in ganglion cells of a marine mollusc by antidromic nerve stimulation was the result of activation of such a pump, and that this pump was not sensitive to the membrane potential of the cell. Marmor and Gorman further showed that this metabolic component of the cell was temperature dependent, warming of the cell

producing an enhanced hyperpolarization. Similar metabolic pumps have been demonstrated in Purkinje cells of the rat cerebellum (Siggins and Oliver, 1971), in the medulla of the cat (Hosli and Haas, 1972) and in the superior cervical ganglion of the rabbit (Torda, 1972). Bourgoignie et al. (1969) showed that in cells of the toad bladder this hyperpolarizing pump was dependent on cyclic AMP and that the change in membrane potential did not follow the increase in transmembrane current produced by the metabolic sodium pump. The cyclic AMP activated pump is not dependent on the transmembrane potential and produces hyperpolarization which may persist for several minutes (Siggins et al., 1971, Torda, 1972). The activation of C-AMP is intrapostsynaptic (Torda, 1972) and therefore a result of activity initiated by release of some transmitter from the presynaptic terminals (Hosli and Haas, 1972).

Reviewing the characteristics of the hyperpolarizing response of taste disc cells to antidromic nerve stimulation, the similarity of this response with those described above for cells utilizing a metabolically activated electrogenic pump seems evident. The long latency and prolonged duration outlasting the nerve activity by several orders of magnitude; the slow following rate to nerve stimulation; the dependency on adequate circulation; the depolarizing rebound after adaptation to long stimulus trains; the independence of the Ep from the membrane potential, Em; the differential change in

τp and Ep over time; the opposite effects of some chemical stimuli on the Em and Ep of the taste receptors--all these are evidence for a mechanism utilizing an electrogenic metabolic pump for the peripheral modulation of sensory information in the gustatory system of the frog.

Significance of This Work

On theoretical grounds Grundfest (1965) suggests of receptors: "Occurrence of a response of indefinite amplitude and duration depending upon the stimulus, sometimes of depolarizing and other times of hyperpolarizing potentials, depending upon the cell and/or the stimulus represents a group of properties which also provide indirect evidence (*of receptor function as being more than that able to be accounted for by purely passive ionic components*)."* Considering the receptor potential: "It should not be forgotten however that the potential need have no function, and indeed that the activity of the receptor cell need have no electrical sign." (Grundfest, 1969.)

The interpretation of the data presented in this dissertation supports Grundfest's hypothesis as applied to the receptors of the frog taste disc. Furthermore, in addition to the factors affecting receptor response described by Grundfest, this work indicates that a metabolic component,

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*Italics mine.

activated by antidromic activity in chemoreceptor nerve fibers can exert an important effect on the receptor, and contribute to the peripheral processing of sensory information in the frog gustatory system.

If similar peripheral mechanisms are functional in other vertebrate taste systems, the findings presented in the present work may serve as a model for taste interactions in general. Cognizance of this effect may help to explain some of the differential effects of similar taste substances (NaCl and KCl) as well as similar tastes of different molecular species (sucrose and lead salts). This model is attractive in that it offers a mechanism for the peripheral enhancement or depression of taste fiber response as well as for the lateral effects seen in taste afferents with overlapping receptive fields. The change in apparent taste qualities over the course of adaptation to prolonged stimulation becomes more clear when one recognizes the prolonged effects of relatively small amounts of nerve activity on the receptor apparatus. This obviates the necessity of postulating nerve fiber interactions and behavior which have not been found in any other neural sensory system. In addition, application of the concepts of this model for peripheral interaction can help amend the discrepancy between theoretical transform functions and observed results for the initial phasic and prolonged tonic components of afferent chemosensory nerve responses. The relative independence of this peripheral

effect from the receptor transmembrane potential may help absolve sensory neurophysiologists studying taste from the apparent inconsistencies in coupling of receptor potential changes with sensory nerve fiber discharges. It is believed that this model can serve as a useful tool for understanding the sense of taste and some components of the disease processes in those afflictions such as cystic fibrosis, adrenal dysfunction, vitamin deficiencies and familial dysautonomia which are reflected in taste abnormalities.

IX. CONCLUSIONS

1. Electrical stimulation of the lingual branch of the IX nerve of the frog produced changes in the membrane potential of cells forming the taste disc of the fungiform papillae. These effects were due to the antidromic activation of sensory fibers and not associated with efferent activity in sympathetic fibers.

2. Currents in cells of the taste disc surface were depolarizing and appeared to be electrotonically coupled to the hyperpolarizing potentials recorded from the cells of the receptor layer.

3. Antidromically elicited potential responses exhibited latency, summation, adaptation and post-stimulation rebound. This rebound was of a polarity opposite to the initial change produced by antidromic stimulation and of the same direction as the change accompanying adaptation.

4. Depending on stimulus conditions, antidromic nerve activity was able to produce depression or enhancement of chemosensory fiber discharge in response to taste stimuli.

5. Different taste stimuli were found to have potentiating or depressing effects on the antidromically elicited potentials of taste disc cells.

6. Electrical stimulation of single taste papillae led to potential changes in taste disc cells of a neighboring papilla belonging to a common receptive field. These potentials were similar to those produced by antidromic stimulation of the whole lingual nerve but were of less amplitude, presumably due to the fewer number of nerve fibers antidromically activated.

7. The antidromically elicited potentials did not exhibit a dependency on the resting level of polarization of the taste disc cells.

8. The properties and behavior of these antidromically elicited responses were characteristic of those postulated for sensory receptors by Grundfest and demonstrated in other neural systems to be produced by a metabolically activated electrogenic pump.

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