ABSTRACT

THE ELECTROPHYSIOLOGICAL RESPONSES OF
GUSTATORY NEURONS OF
THE MUD PUPPY (NECTURUS MACULOSUS)

By

David William Samanen

The taste responses of the single fibers of the glossopharyngeal nerve of the mud puppy, Necturus maculosus, to an extended concentration series of NaCl, HCl, quinine hydrochloride and sucrose and to thermal stimulation were examined. The isolation of each neuron (unit) was evaluated by computer analysis with a program, FREDSAM, that analyzed the action potential amplitudes of the experimental records. An enumerator program, SAM-COUNT, using the neuron's amplitude window, counted impulses over the tests, and pre- and post-stimulus distilled water rinses. Latency was evaluated by monitoring the time of arrival of the stimulus solution to the neuron's receptive field. Stimulus-response (SR) and latency functions were calculated for each test series. The form of the gustatory response was also observed, i.e., whether the neuron responded with increased activity to stimulation, with decreased activity, or with increased activity during the water rinse.

The forms of the gustatory responses, their SR functions, and latency functions were found to vary among different stimuli and concentration as well as among nerve fibers. However, these parameters were observed in specific combinations, the most unique being a constant

SR and latency function for a single chemical series. Combinations of the response parameters were found which defined nine types of taste response. Most neurons responded to more than one of the chemical stimuli and therefore possessed multiple gustatory sensitivity. Their responses to at least two of the stimuli were of different type showing that the neurons also possessed a degree of chemospecificity.

THE ELECTROPHYSIOLOGICAL RESPONSES OF GUSTATORY NEURONS OF THE MUD PUPPY (*NECTURUS MACULOSUS*)

By

David William Samanen

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I. INTRODUCTION

Electrophysiological recordings from single gustatory neurons from many species have revealed a variety of neurophysiological taste responses. These have differed in form: 1) many neurons showing increased activity to stimulation, 2) others responding with decreased activity to stimulation, and 3) many neurons having increased activity with the presentation of the post-stimulus rinse. The response magnitude has been found to vary with the concentration of the stimulus in manners giving rise to widely different stimulus-response or SR functions. The responses of several neurons regularly increase with increasing stimulus concentration. Other fibers respond with regularly decreasing responses as stimulus concentration is increased. Some neurons have SR curves with both positive and negative slopes, that are U-shaped or bell-shaped. Yet the majority of experiments in taste sensory physiology have been performed using single, unequal concentrations of different stimuli to define the mechanisms of the first order peripheral afferent fibers of the taste system. A systematic investigation using an extended concentration range of chemical stimuli has not been reported for any animal.

The latency of the gustatory response of the single taste neuron has recently been studied as a parameter of the taste system (T. Sato, 1976). An inverse relationship between stimulus concentration and

response latency has been found but with implications that various forms of a latency-concentration function may exist. Accordingly, the experiments of this study were undertaken in order to define the taste response of single neurons according to three parameters: 1) the form of the response, 2) its SR function, and 3) its latency function.

The mud puppy has been chosen for taste research owing to the unusually large size of the cells of its taste organs, the taste buds. Intracellular recordings from the cells of the taste buds of Necturus have been completed in an attempt to discover their responses to chemical stimulation (West, 1976). However, only the general sensitivity of the gustatory neurons were known as revealed from whole nerve studies. The detailed responses of the individual neurons of the mud puppy's taste system are presented in this study.

II. LITERATURE REVIEW

Introduction

This review will consider initially the anatomy of the taste system of the mud puppy, Necturus maculosus. The innervation of its tongue by cranial nerves VII, IX, and X will be described. Then will follow a discussion of the neurophysiology of the taste system of Necturus as has been revealed by whole nerve electrophysiological recording. Finally, the taste responses of single gustatory neurons of several species will be reviewed. This will emphasize: 1) the forms of the single neuron's taste response (increased or decreased activity on stimulation or increased activity on post-stimulus rinsing), 2) the latency of the single fiber's taste response, and 3) the sensitivity of taste neurons to several chemical stimuli. For the latter, those experiments which use single concentrations of stimuli will be contrasted with those which use a range of concentrations, defining stimulus-response or SR functions.

Anatomy

General

The mud puppy, Necturus maculosus, is a completely aquatic, caudate amphibian. It prefers dark, cool habitats living on the bottom

of the fresh water lakes and rivers of Eastern North America consuming spawn, small fish, aquatic insects, and insect larvae. The adult mud puppy, possessing bushy, external gills, resembles the larval salamander or frog. Necturus at birth is 2.3 cm long, matures after eight years and may reach an adult length of 49 cm. Animals have lived as long as 23 years in captivity.

The physiologist's keen interest in Necturus is directed to the unusually large size of the cells of many of its organs. Knowledge of cellular function and response of the vertebrate kidney and retina have been advanced by studies of this animal.

Taste Organs of Necturus

Under the dissecting microscope, the mud puppy tongue glistens with a mucous coat and is smooth, lacking any obvious papillae (the well-differentiated structures which support the taste organs of most vertebrates). Only under indirect illumination or reflected glare can the lingual surface be resolved to show many low relief elevations or mounds (30 μ height, 250 μ diameter). These lingual eminences, identified by Farbman and Yonkers (1971), each hold a single taste bud. Figure 1 is a scanning electronmicrograph of two of these eminences (Samanen and Bernard, 1975). At the center of each mound can be seen the irregular surface of a taste bud, i.e., the taste organ of Necturus. The light micrograph of a sectioned eminence (Figure 2) shows that the taste bud extends completely to the lingual surface in contrast to the buds of most mammals which lie below the epithelium, contacting the surface environment only by way of a narrow taste pore of 5 μ diameter.

Figure 1. Gustatory eminences of the mud puppy. Necturus lacks well-differentiated papillae. Its taste buds lie within the low relief elevations or mounds seen in this scanning electromicrograph. The irregular surface of a single taste bud appears at the top of each lingual eminence.

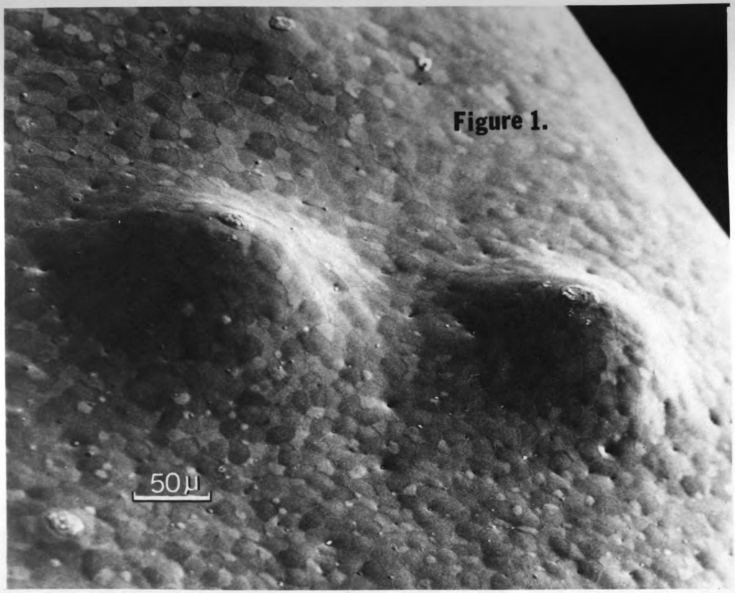


Figure 1.

Figure 2. Light micrograph of a gustatory eminence. Within each eminence lies a single, large taste bud, the taste organ of Nesturus, whose elongated cells extend to the lingual surface. A dermal papilla, containing nerve fibers and capillary (not shown),supports the bud. The section was made from epon embedded tissue, 5 μ thick, stained with toluidine blue.

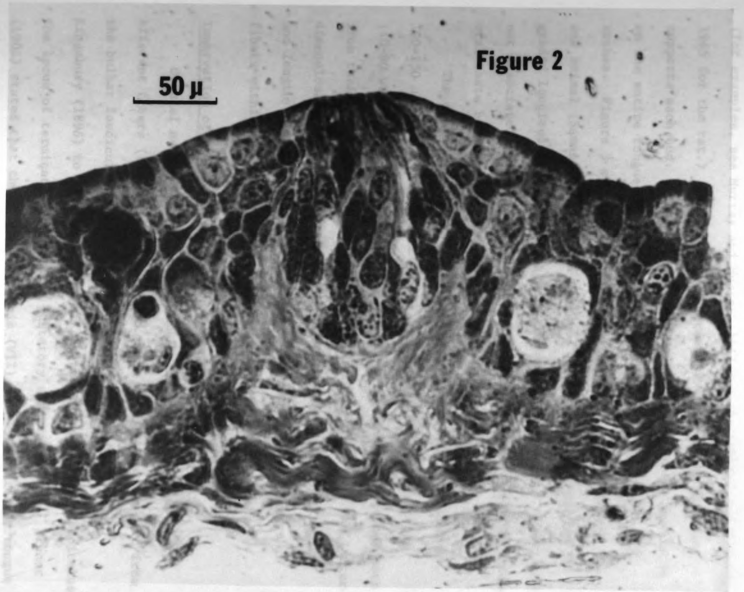


Figure 2

(For examples, see Murray and Murray, 1967 for the rabbit, and Farbman, 1965 for the rat.) In Necturus, a dermal papilla with a capillary supports each bud. In the oral cavity, gustatory eminences are found on the entire tongue, on much of the mucosa of the pharynx and gill arches. Figure 3 shows the distribution of 658 lingual eminences for one animal (Samanen, Kryda, and Bernard, 1975). The distribution is graded longitudinally, becoming more concentrated distally. Taste buds not associated with lingual eminences have not been reported. No extra-oral taste buds have been reported for the mud puppy.

The dimensions of the mud puppy's taste buds (90-120 μ wide by 100-150 μ height) are twice as large as those of other vertebrates (40-80 wide by 50-80 μ height). Their unusually large size arises both from their greater number of cells (80-100 versus 30-80) and the larger dimensions of those cells, reported to be twice as great in diameter and length (Farbman and Yonkers, 1971). The termination of nerve fibers within the taste buds is also described by these authors.

Innervation of the Mud Puppy's Taste System

Cranial nerves VII, IX, and X supply the mud puppy's gustatory afferent fibers (Figure 4). The facial nerve (VII) arises in part from the bulbar fasciculus communis, considered by Strong (1895) and Kingsbury (1895) to be an homologue of the mammalian solitary fasciculus, the locus of termination of primary gustatory afferent fibers. Drüner (1901) stated that the ramus alveolaris (VII) courses toward the tongue but largely terminates between the tongue and mandible. This contrasts with the major lingual innervation by the mammalian chorda tympani (VII).

Figure 3. The lingual distribution of gustatory eminences. 658 lingual eminences, identified for one mud puppy, are seen to cover the dorsal lingual surface. A slight longitudinal gradation exists, more gustatory eminences being concentrated at the distal tip (top of figure).

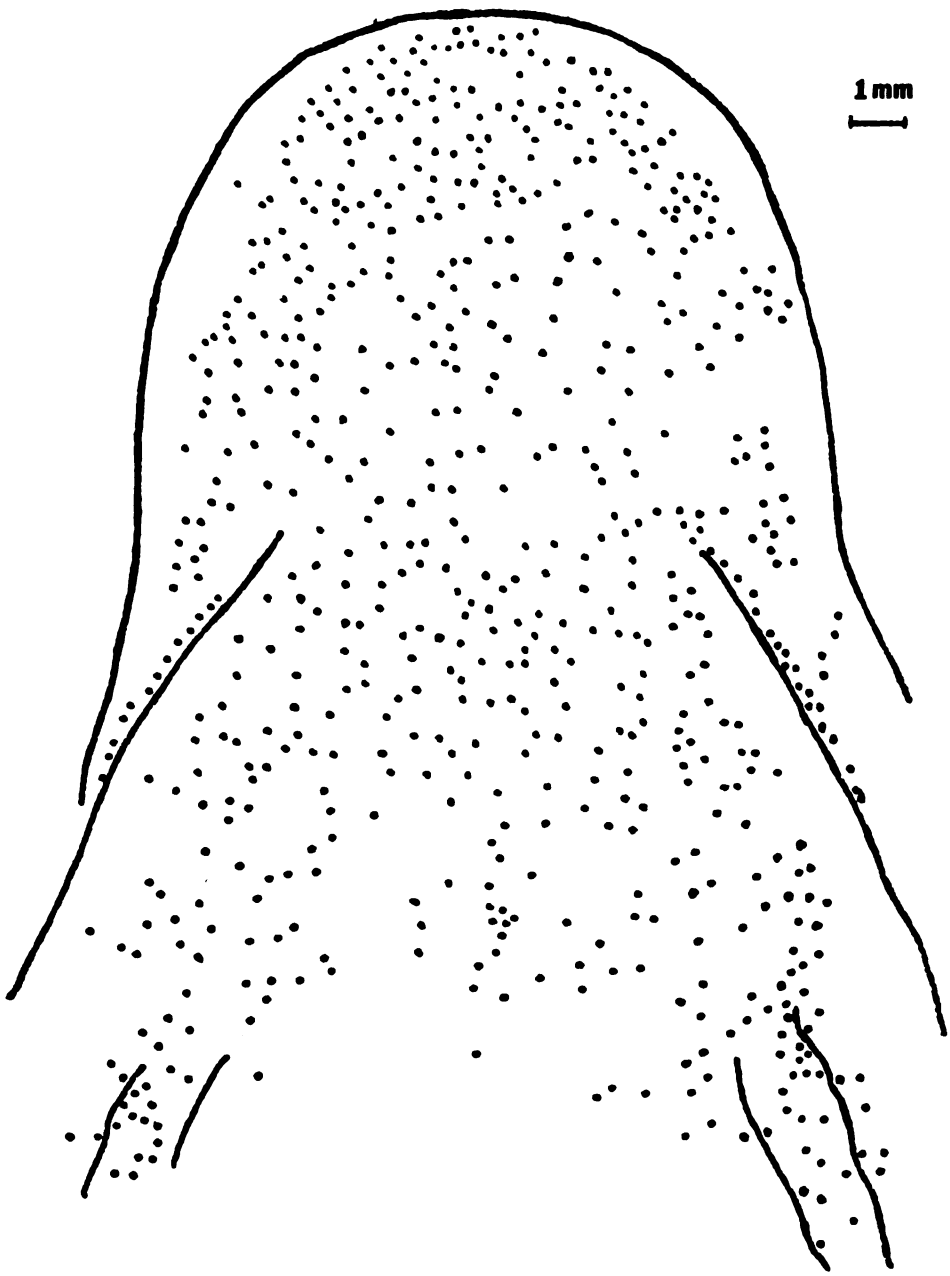


Figure 3.

Figure 4. Lingual innervation and gustatory sensitivity of the glossopharyngeal nerve. Illustrated on the left are the branches of the cranial nerves which contribute to the taste system of Necturus. The arrows show the approximate course and termination of branches of the facial nerve (the ramus alveolaris, VII, R. Alv.), the glossopharyngeal nerve (the pre- and post-trematic branches, IX, R. Pre. and IX, R. Post.), and the vagus nerve (the second and third branchial arch nerves, X_2 and X_3). To the right is shown the regional gustatory sensitivity of the glossopharyngeal nerve as determined by electrophysiological whole nerve recording. The relative responses to punctate stimulation with 0.5 M NaCl are shown (100 = maximum response). Decline of sensitivity at the tip, where taste buds are most concentrated (see text and Figure 3) suggests a receptive field shared by the facial and glossopharyngeal nerves.

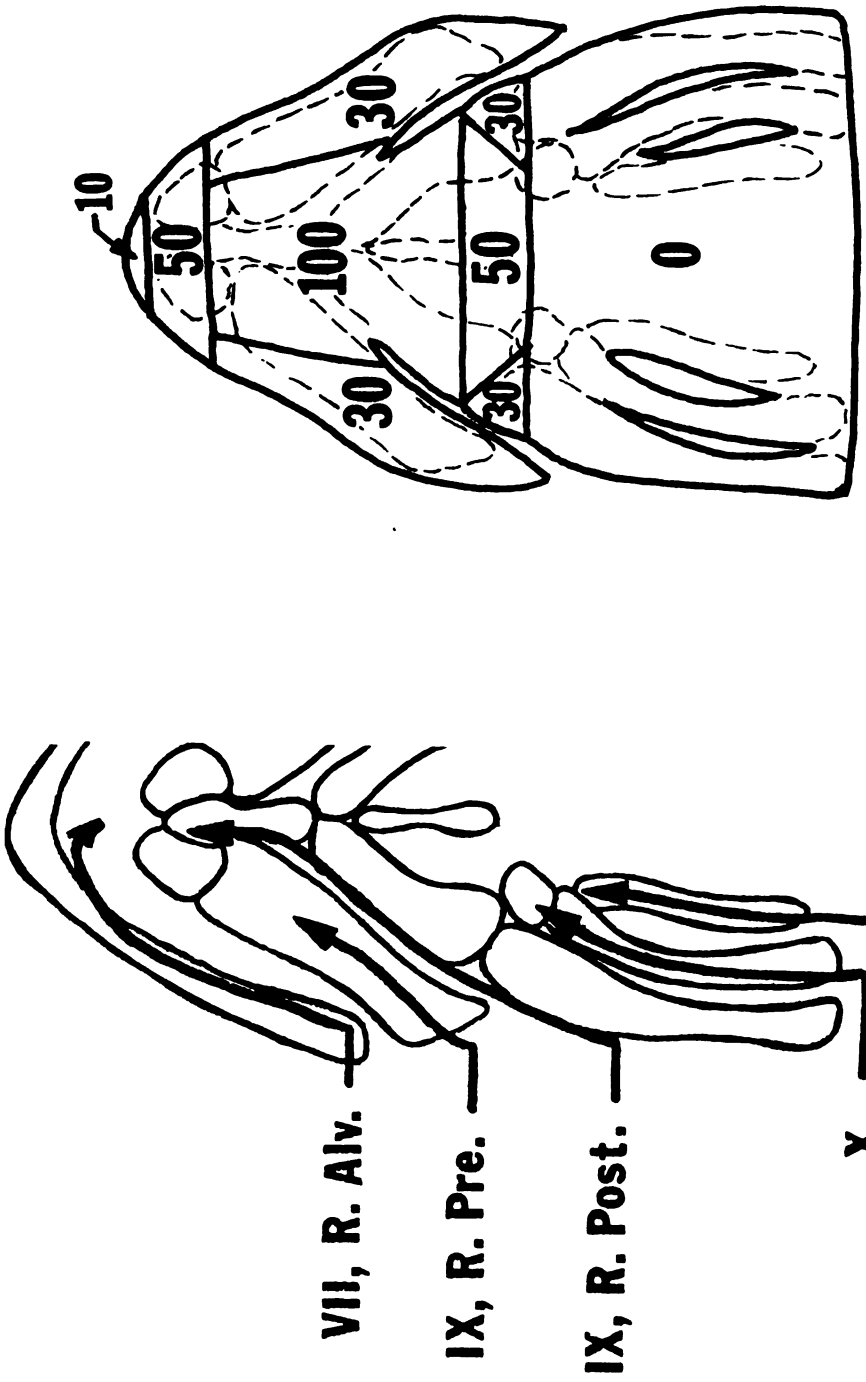


Figure 4.

Gesteland and Dixon have recorded from the facial nerve in Necturus (Dr. Robert Gesteland, personal communication). This gives electrophysiological confirmation to a possible role of VII in the mud puppy's taste system.

The glossopharyngeal nerve (IX) has been consistently described as part of the taste system of caudate amphibians (Drüner, 1901 and Francis, 1934). The rami pre- and post-trematicus (IX) terminate in epithelium above the lingual cartilages over most of the distal tongue, though the lateral and longitudinal extent of their regions of distribution are not known. Using punctate chemical stimulation 5-6 µl, 0.5 M NaCl, contrasted with distilled water) and whole nerve electrophysiological recording, Samanen, Kryda and Bernard (1975) found the area defined in Figure 4 to be the gustatory receptive field of IX. The decreasing sensitivity at the most distal area (5-10 mm caudally from the tip) is consistent with Gesteland and Dixon's recording from VII.

The vagus nerve (X), also called the second and third branchial arch nerves of Necturus (Drüner, 1901), terminates above the pharyngeal branchial cartilages of the proximal oral cavity (Drüner, 1901). While electrophysiological confirmation of its role in the mud puppy's taste system is lacking, it may provide gustatory innervation to the pharyngeal mucosa and gill arches.

Whole Nerve Electrophysiology

Pattern of the Whole Nerve Taste Response

The whole nerve response has been recorded in many animals. The activity from the many neurons is summated by an electronic integrator.

The summated taste response typically contains an initial phasic increase of activity upon stimulation, followed after 1-3 seconds by a lower tonic level of activity during continued stimulation. However, the typical phasic-tonic pattern varies among species, among stimuli, and even stimulus concentration. For example, NaCl and HCl test solutions produce the typical pattern from the chorda tympani of the cat, while quinine and sucrose do not (Pfaffmann, 1955). Tateda (1961) found the phasic response to occur proportionally with all suprathreshold concentrations of NaCl (from the catfish facial nerve) while the tonic response had a higher threshold (above 0.15 M). The mud puppy's glossopharyngeal nerve shows only a phasic response for all stimuli at all concentrations tested (Samanen, 1973). This is seen in Figure 5 for KCl up to 0.1 M.

Determination of Response Magnitude

Experimenters have chosen one of the phases of the taste response to estimate the size of the entire response. For example, Beidler (1953) chose the tonic level of activity as a magnitude estimator while Tateda (1961) used only the phasic portion.

Halpern and Tapper (1971) and Halpern and Marowitz (1973) have shown in the rat that taste quality information is available within the first second of stimulation. When conditioned to avoid 0.3 M NaCl, rats could detect, evaluate, and stop licking the conditioned stimulus within 138 to 600 msec. Faull and Halpern (1972) have shown a strong correlation between the initial rise of the phasic response and the concentration of the stimulus and note that this portion is most linear

Figure 5. The phasic whole nerve taste response of Necturus. Each panel in the upper row contains three records of the summated glossopharyngeal nerve responses to the repeated application of a single concentration of KCl solution. The superimposed traces show that the whole nerve taste response contains a consistent initial increase in phasic activity. The phasic response can be considered to be the response to only gustatory stimulation (and not to mechanical or thermal stimuli) because it is proportional to concentration and does not appear to distilled water application after water adaptation (lower panel). The slight curve of the initial rise of the phasic response to 0.1 M KCl is an artifact of the curvilinear data recorder.

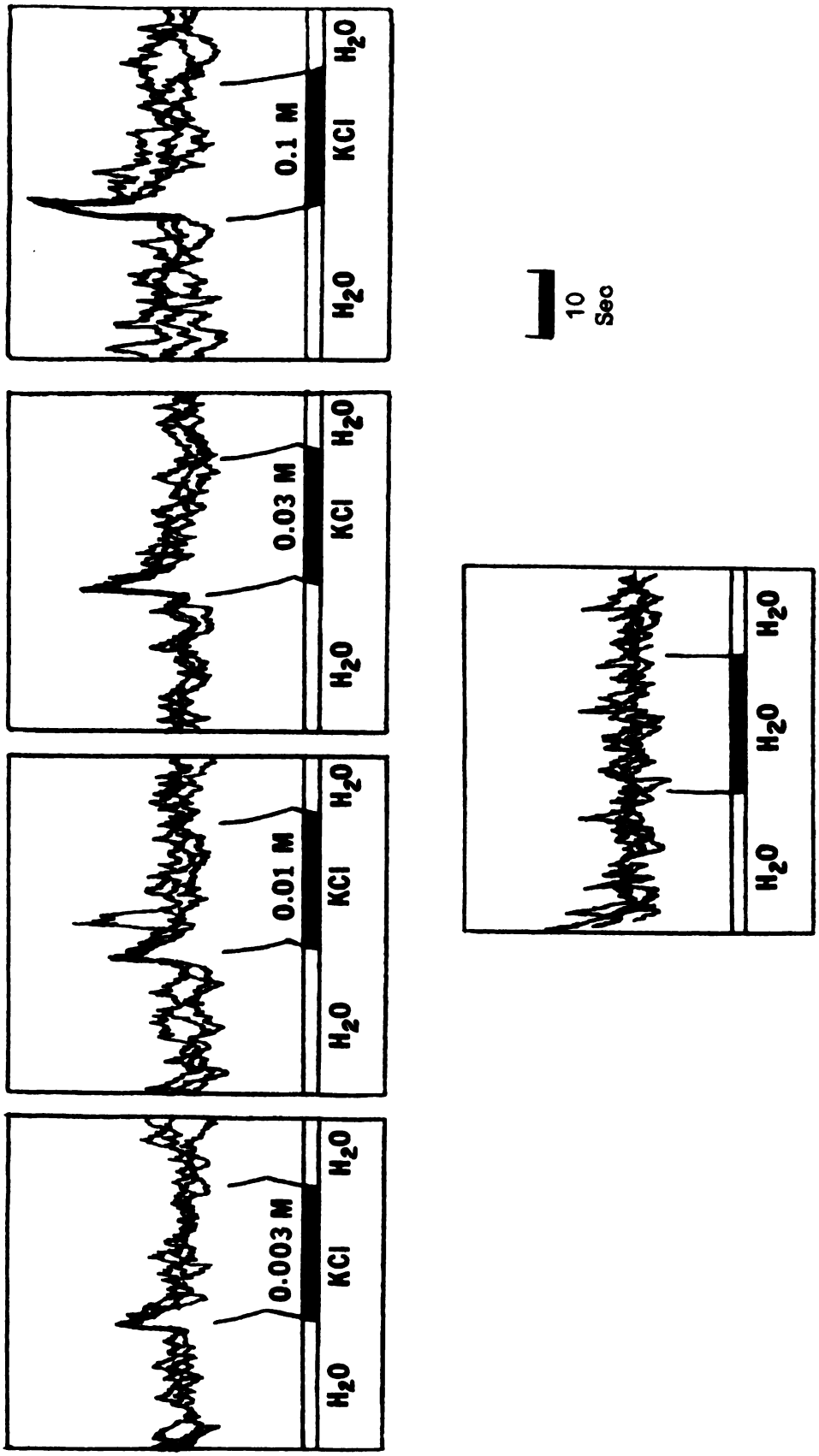

0.1 M
KCl
H_2O
H_2O
0.03 M
KCl
H_2O
H_2O
0.01 M
KCl
H_2O
H_2O
0.003 M
KCl
H_2O
H_2O
10
Sec
H_2O
H_2O
H_2O

Figure 5.

over time. Smith (1975) expanded these findings, also for the rat, using both electrical and chemical stimulation. The magnitude of the phasic response was proportional to the rate of rise of stimulating current, or the delivery rate of 0.1 M NaCl. Current of different amplitude delivered with the same rate of rise had equal phasic responses and proportionally unequal tonic responses. Thus for magnitude estimation, use of either phasic or tonic component alone may result in different conclusions. The tonic activity is proportional to steady concentration of the stimulus; whereas, the phasic is proportional to the rate of change of stimulus concentration and contains taste quality information.

Gustatory Stimulus-Response Functions

Gustatory intensity functions, called the stimulus-response or SR functions, have provided useful information about an animal's taste system. These include the relative effectiveness and thresholds of stimuli. Figure 6 is a composite graph showing the intensity functions for eight stimuli in the mud puppy's glossopharyngeal taste system. For all chemicals, the response is a regularly increasing function of concentration. HCl at all concentrations is the most effective stimulus and has the lowest threshold. (The threshold is the concentration at which the response exceeds the water-adapted, pre-stimulus level of activity. The threshold for HCl, not fully attained in the function of Figure 6, is below 0.0003 N or -3.5 Log N.) Sucrose has the highest threshold and smallest responses. All other stimuli are of intermediate

Figure 6. Gustatory whole nerve SR functions of Necturus. These are shown for the chemicals tested (QHCl = quinine hydrochloride, Sucr. = sucrose). Values of the whole nerve responses to each concentration of each stimulus are expressed as a percent value of the response to 0.1 M NaCl. This eliminates contributions of responses to mechanical and thermal stimuli and facilitates comparison with other species. The baseline activity was determined from the activity occurring on water presentation to the water adapted tongue. A limited range (interquartile) of this water activity is shown (gray bar). After Samanen, 1973.

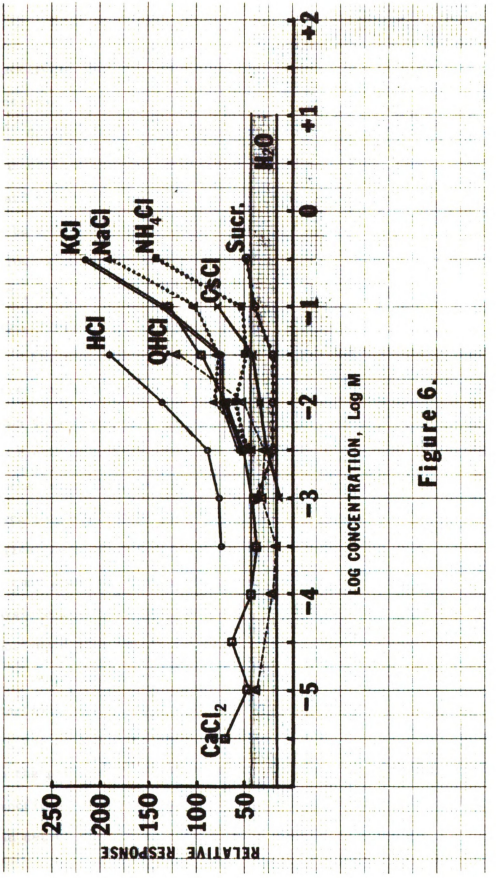

RELATIVE RESPONSE
250
200
150
100
50
KCl
NaCl
NH_4Cl
HCl
CsCl
Sucr.
H_2O
$CaCl_2$
-5
-4
-3
-2
-1
0
+1
+2
LOG CONCENTRATION, Log M

Figure 6.

effectiveness and no consistent correlations can be drawn from their SR functions.

One use of SR curves is to choose equally effective concentrations of test stimuli. For example, interpolation from Figure 6 at 150 relative response units shows that taste responses of equal magnitude can be expected from the glossopharyngeal nerve of Necturus for the following concentrations of stimulus; 0.2 M NaCl, 0.15 M KCl, 0.3 M NH_4Cl, and 0.02 N HCl (-0.7, -0.9, -0.4, and -1.85 Log concentration respectively).

The magnitude of the summated gustatory response has been well correlated with perceptual intensity. For example, recording from the human chorda tympani, the summated responses have shown: 1) that the electrophysiological and perceptual threshold for NaCl are the same, 2) that several sugars have the same relative electrophysiological and psychophysical effectiveness for any one person, 3) that electrophysiological adaptation closely parallels psychophysical adaptation, and 4) that gymnemic acid applied to the tongue abolishes the electrophysiological responses to sugars as well as the perception of sweet substances (Borg et al., 1969).

Whole nerve studies are limited in determining the more subtle functions of the taste system. For example, little information regarding the neurophysiological determination of taste quality is available in the pattern of the whole nerve response, or in its SR or latency functions. For example, near threshold concentration, human observers can detect the presence of a taste stimulus but cannot identify it.

It is only at higher concentrations that the quality of the stimulus can be recognized. There is nothing in the whole nerve response that explains this distinction. Understanding the neurophysiological mechanisms underlying these differences may require knowledge of the function of the individual elements of the taste system, the neurons and receptor cells and their interactions.

Single Neuron Electrophysiology

The gustatory responses of single neurons have been recorded at many levels in the nervous system: from the peripheral nerve fibers, in the geniculate ganglion of the facial nerve, at the nuclei for second order neurons (the nucleus tractus solitarius, the sensory trigeminal nucleus), from third order cells at the ventral thalamic nuclei, and at the cerebral cortex. Of the many preparations studied, the rat's chorda tympani is now classical by virtue of repetition. Individual gustatory neurons have been investigated in the rat, cat, hamster, guinea pig, rabbit, sheep, dog, frog, and rhesus, macaque, and squirrel monkeys, but not in humans. This review will emphasize the neurophysiology of the first order neurons.

Character of the Taste Response

Increasing Activity Upon Stimulation. Gustatory neurons generally respond with an increase in activity when stimulated. Yet their response patterns vary widely. The most typical pattern, first discovered by Pfaffmann (1941) in cat chorda tympani fibers, resembles the whole nerve response and consists of a phasic increase followed by a tonic

discharge throughout stimulation. He noted that the firing pattern of impulses was greatly irregular. This contrasted with both the regular tonic discharge of several sensory systems (e.g., the cat carotid baroreceptors) and the regularly decreasing frequencies of more rapidly adapting systems like the visual ganglion cells (Bronk, 1935). Fishman (1957) noted five distinct patterns when recording from rat chorda tympani fibers: 1) the typical phasic-tonic pattern, 2) an immediate rise to tonic discharge, 3) a slow rise to a peak response followed by gradual but complete decline, 4) a 0.2 second burst followed by quiescence and then a gradual build-up to maximum discharge, and 5) rhythmic bursting with regular intervals between impulse trains. All types occurred under constant stimulation. The response pattern has been found to vary with different stimuli, to vary with stimulus concentration, or to be invariant for a specific fiber. For example, a cat chorda tympani fiber responded with the typical phasic-tonic pattern to 0.1 M NH_4Cl but with only phasic discharge (complete, rapid adaptation) to 0.1 M NaCl (Beidler et al., 1955). Ogawa et al. (1974), found only phasic responses for one rat chorda tympani neuron with near threshold NaCl stimulation. With greater stimulus concentration, the tonic discharge appeared, becoming more regular with increasing concentration. A different chorda fiber responded to 0.5 M sucrose, 0.1 M NaCl and 0.02 M quinine hydrochloride with regular bursting activity. Thus, even though the whole nerve response displays a particular pattern, the underlying activity of the individual units may be widely different.

Decreasing Activity Upon Stimulation. Taste neurons are rarely completely inactive. They often fire at slow rates whether adapted to a rinse solution (usually distilled water) or in the complete absence of lingual stimulation. Mean rates of 0.5 to 0.8 Hz are common (rat and hamster chorda tympani neurons, Ogawa, Sato and Yamashita, 1968; Miller, 1971; Frank, 1973). Sato et al. (1975) noted a greater frequency for the macaque monkey chorda fibers, 1.96 Hz. The activity varied among fibers, with a range of 0-9 Hz, and 7 of 67 units above 4 Hz (all determined from 5 second averages). Boudreau et al. (1971) classified first order neurons of the cat's geniculate ganglion partly on their levels of spontaneous activity. Several units had very high rates of regular discharge (18-77 Hz), some in near synchrony with each other. These were not associated with lingual stimulation, several units responding to static displacement of the pharyngeal tissue, palate tissue, and eyeball, and several showing no response to any stimulus. Lingual units (responding to electrical, mechanical, or chemical stimulation of the tongue) varied widely in spontaneous activity, from 0-10 Hz (20 second averages). Their pattern of response consisted of various forms of bursting or a completely irregular discharge. Funakoski et al. (1972) observed rapid spontaneous activity of 15 Hz in the lingual sensory cells of the cerebral cortex of unanesthetized dogs and rats.

Spontaneous activity can be depressed or completely suppressed with specific gustatory stimuli, and therefore this represents a second type of response. Decreased spontaneous activity has been observed in responses to $CaCl_2$, quinine, NaCl, HCl, and sodium saccharin (Pfaffmann,

1941, Boudreau et al., 1971; and Sato et al., 1975). In the macaque monkey, a particular chorda tympani fiber showed normal responses (increasing activity) to sodium saccharin concentrations greater than 0.003 M, while the spontaneous activity was proportionally depressed by 0.001 M and 0.0003 M in the lower portion of the fiber's SR curve (Sato et al., 1975). Dog and rat cerebral cortical cells showed differential sensitivity to chemical stimulation by various changes in activity from spontaneous levels (0.1 M NaCl, 0.01 quinine, 0.5 M sucrose, or 0.05 N tartaric acid). Some cells were suppressed by all four, while others were selectively suppressed, excited (to rates above 40 Hz), or showed no response to one or more of the stimuli (Funakoshi et al., 1972).

The origin of the spontaneous activity is unknown. Pfaffmann (1941) observed that topical procaine hydrochloride abolished the spontaneous activity before the loss of taste responses. Sato et al. (1975) and Ogawa et al. (1968) observed a weak correlation coefficient ($r = 0.4$) between spontaneous activity and the response to cold stimulation in thermally sensitive gustatory neurons (rat, hamster, and macaque monkeys). They suggest that the spontaneous discharge represents ongoing thermal stimulation for these homeotherms, whose lingual surface temperature was only 25° C. Cohen et al. (1955) suggested that spontaneous activity may result from specific neurons responding to the rinse solution even after extensive periods of adaptation. Specifically, they found fibers in the cat chorda tympani that fired continuously under Ringer's rinse and that were also sensitive to NaCl.

Rinse Responses. A third category of response occurs not on application of the stimulus, but upon its removal with a rinse solution. For example: 1) after testing with sodium saccharin (hamster chorda tympani) in a single fiber that was sensitive to sucrose, 2) after NaCl in one fiber responding to the halides (hamster chorda tympani), and 3) in 21 of 26 fibers of the cat's chorda tympani after 0.3 M NaCl (Ogawa et al., 1969), after 0.03 N HCl (Yinon and Erickson, 1970), and after 1.8 M sucrose (Bartoshuk et al., 1971). Funakoshi et al. (1972) refer to a cerebral cortical neuron responding with brief "on" responses to the test stimulus and with "off" responses to the water rinse. Bartoshuk et al. (1971) feel that the underlying mechanism of the rinse response involves the specific stimulatory action of water. Ogawa et al. (1969) suggest that the sodium saccharin rinse responses arise from the re-stimulation of gustatory receptors which had been inactivated.

All three types of responses can be considered legitimate gustatory responses since they occur to specific chemical stimuli or in that they vary in magnitude according to the concentration of the stimulus. The mechanical and thermal stimulation that occurs with the test solutions and rinses are assumed to be equal for all stimuli.

Determining Response Magnitude

Given the variety of forms of the taste responses, investigators have differed in establishing criteria for defining and measuring a response. No standard exists. Some determine a criterion level of test activity such as a 50% increase (Frank and Pfaffmann, 1969) or an increase greater than one standard deviation (Sato et al., 1969) above

spontaneous activity. All investigators have used average spontaneous activity levels preceding all tests for comparison with the response to each single test. Most experimenters measure the spontaneous activity and test responses over equal intervals of time. This is an essential requirement for identifying lingering activity from previous stimulation. Responses of depressed activity are often only mentioned qualitatively. Miller (1971) separately calculated response depression and enhancement ratios.

With the variable form and frequency of the single unit taste response, a similar variety of magnitude calculations have been employed. Usually the average frequency of impulses over the test period is calculated (as impulses per second or per five seconds, etc.). Both Miller (1971) and Ganchrow and Erickson (1970) avoid the initial phasic response. They consider the later periods to measure the more stable or steady state response.

Any reviewer of taste literature should be aware of the differences in response magnitude estimation. The observations using more stringent criteria with respect to increases in activity above spontaneous levels may not correlate with other observations. Suppressed activity, a more subtle response may be simply ignored. The exclusion of portions of the response by considering only earlier or later events may over-emphasize the responses to specific chemical stimuli whose greatest activity is concentrated in a particular period. Elimination of the initial response period seems especially hazardous given the rat's demonstrated gustatory discrimination powers which encompass as

little as the first 200 msec. Finally, any averaging method obscures patterned discharge information which may be present in the taste response.

Patterns and Phases of the Taste Response

The various patterns and phases of discharge in the taste response have been briefly described above. Mistretta (1972) found rat chorda tympani fibers that showed periodic bursting with ten different stimuli. Other fibers she described as "differential" in that they responded to certain stimuli with bursting and to others with various patterns of random decay. Ogawa et al. (1974) similarly discovered that the gustatory response pattern could be stimulus dependent or fiber dependent (rat chorda tympani). Several of the response patterns were unidentifiable. They also found increased pattern stability with increasing stimulus intensity but no shift in pattern with concentration changes. Correlations between patterns and stimulus were low and they could not determine the role of pattern information with respect to taste quality.

Ogawa et al. (1974) contrasted the initial five seconds of ten-second gustatory tests with the last five seconds and found that the initial response magnitude was greater (macaque chorda tympani). The stimulus-response correlations drawn from either period alone could differ. However, with one exception, the differences were slight and not significant. They found that the different phases of the response had different relationships to stimulus concentration. The first five second period had a semi-Log relationship to concentration, while the second five seconds were linearly related to concentration on a Log-Log

scale. These findings did not apply to sucrose and quinine whose responses slowly increase to maximum frequencies. They suggest that the different response phases may arise by different mechanisms and represent different information. They conclude that phase and pattern information may relate to quality changes over time.

Latency of Gustatory Responses

To measure the latency of a taste response requires that the time of contact of the test solution with the receptive field of the neuron be accurately identified. Additionally, the time required for impulse conduction from receptor organ to the recording electrode should be determined. The physiological latency is then the interval between stimulus arrival and the occurrence of the first action potential, correcting for the impulse conduction time. Faull and Halpern (1972) measured solution arrival by monitoring reflectance changes of the illuminated tongue with a phototransistor. Ogawa et al. (1974) noted stimulus arrival by the reduction of a 10 Hz signal on the oscilloscope when electrically conducting test solutions reached the tongue. The signal source was capacitance coupled to the stimulus chamber surrounding the tongue and the signal, on stimulus arrival, was momentarily shunted through the test solution and animal to ground. T. Sato (1976) used a stimulus artifact potential that was produced when the stimulus solution flowed about a glass tube which contacted a gustatory papilla and contained a set of recording electrodes.

Faull and Halpern (1972) found a 30 msec latency for the rat's whole nerve (chorda tympani) response to NaCl and saw little change with

concentration (0.1 M = 27 msec, 0.5 M = 33 msec, 1.0 M = 27 msec). However, both Ogawa et al. (1974, for the rat) and T. Sato (1976, for the frog) showed that for single units, the response latency varied with concentration (see Figure 7). For both investigators the single fiber latency was inversely related to concentration, with the responses to weaker stimuli developing over several seconds (6 sec for 0.03 M sucrose, rat chorda tympani fiber, and 2.3 sec for 0.1 M NaCl, frog glossopharyngeal fiber). For any single concentration the latency differed among fibers (19-437 msec, mean = 158 msec for 0.5 M NaCl among 96 frog glossopharyngeal fibers). The latency-concentration functions may also differ among fibers, for while the regularly decreasing function of Figure 7 was based on several units, 7 of 13 gave no response to 0.1 M NaCl. Different latency functions may also underly the constant latency found for the whole chorda tympani by Faull and Halpern (1972, rat) while Ogawa et al. (1974) found an inverse relationship for single rat chorda fibers.

Multiple Gustatory Sensitivity of Taste Neurons

Pfaffmann's experiments on the cat chorda tympani and glossopharyngeal nerves (1941) were initiated in search of neurons exhibiting response specificity toward one of four stimuli: NaCl, HCl, quinine hydrochloride, and sucrose, which in man evoke the four primary taste qualities of salty, sour, bitter, and sweet. Pfaffmann discovered a property of taste neurons that would hold across all vertebrates--many fibers are not mono-gustatory, sensitive to only one of the four primary stimuli, but rather respond to several. He classified the fibers as

Figure 7. Latency function of single gustatory fibers to NaCl. The mean latency for 13 frog glossopharyngeal fibers is seen to be inversely related to stimulus concentration. Maximal latency of the gustatory response was 2.3 sec for 0.1 M NaCl, minimal latency was 16 msec for 1.0 M NaCl. (After T. Sato, 1976.)

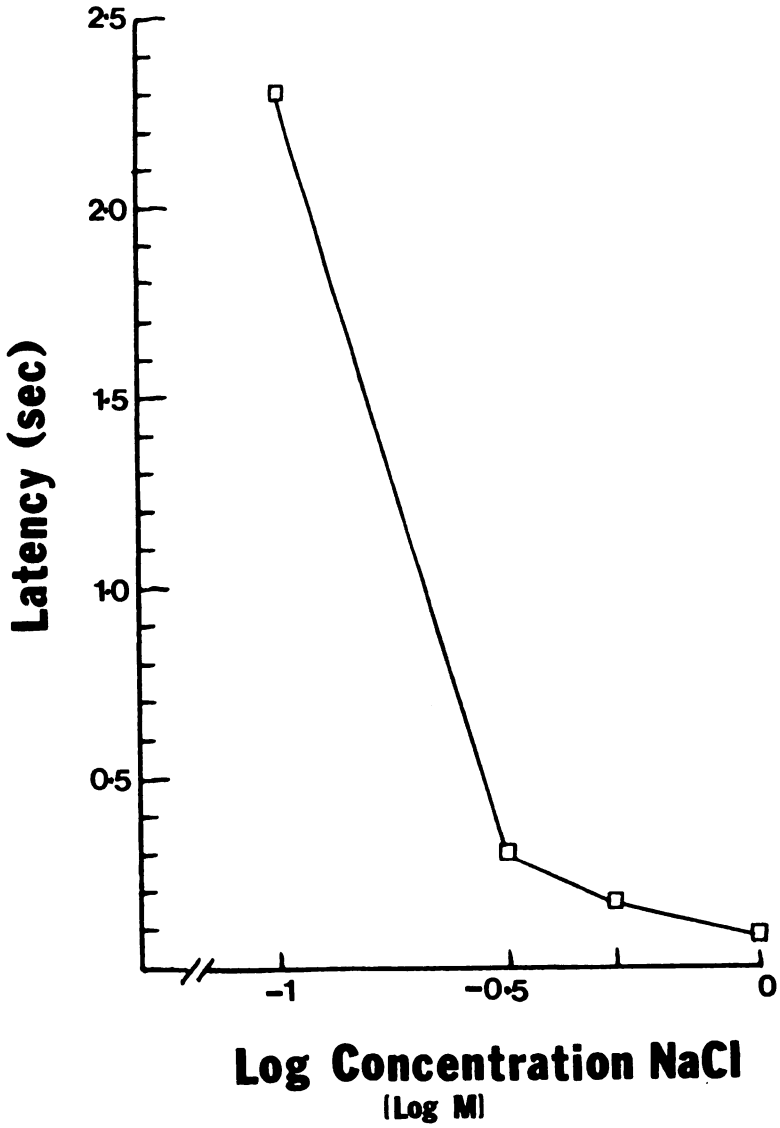


Figure 7.

"acid" sensitive (monogustatory), "acid-salt", and "acid-quinine" sensitive (multiply sensitive). The three fiber types revealed a "spectrum" of chemical sensitivity to the other chemicals tested. "Acid" fibers responded to HCL, acetic acid, and KCl, "acid-quinine" fibers to HCl, acetic acid, and quinine, "acid-salt" fibers to HCl, acetic acid, NaCl, sodium acetate, KCl and $CaCl_2$. The fibers could also be distinguished by the chemicals to which they were insensitive (e.g., NaCl and sucrose for the "acid-quinine" fiber).

Selection of Stimulus Concentration. Chemical sensory investigators do not know the physical parameters underlying perceptual quality. For this reason, intensity parameters are imprecisely defined. For example, cat chorda neurons have different thresholds for acetic acid and HCl (HCl: 0.007-0.07 N, pH 1.15-2.15; acetic acid: 0.03-0.01 N, pH 1.52-2.00; Pfaffmann, 1941).

In the past, unequal concentrations of the various taste stimuli were used. Table I lists the concentrations of the four primary stimuli employed by 11 authors studying rat and hamster taste systems. Next to each concentration is its relative effectiveness for a population of gustatory neurons as interpolated from the SR curves for summated chorda tympani responses (from Pfaffmann, 1955 and Frank, 1972). Effectiveness is rated on a relative scale with the whole nerve response to 0.1 N HCl equal to 100. The right column gives the range of effectiveness of the four stimuli determined in this manner. Ogawa et al. (1968) and Yinon and Erickson (1970) chose the most comparably effective stimuli (covering a limited range of 19 units of relative effectiveness). However,

Table I. Relative effectiveness[1] of four primary stimuli used in taste experiments on chorda tympani neurons.

Authors	NaCl Conc.	NaCl Effn.[1]	HCl Conc.	HCl Effn.	QHCl[2] Conc.	QHCl Effn.	Sucr. Conc.	Sucr. Effn.	Net Range of Effn., N.H.Q.S.[2]
				RAT					
Pfaffmann, 1955	0.1 M	95	0.03 N	75	0.01 M	35	1.0 M	25	95-25 = 70
Fishman, 1957	0.5	145	0.01	45	0.02	40	1.0	25	145-25 =120
Erickson, 1963[3]	0.1	95	0.03	75	0.01	35	1.0	25	95-25 = 70
Ogawa et al., 1968[4]	0.1	95	0.01	45	0.02	50	0.5	15	95-15 = 80
Frank & Pfaffmann, 1969	0.3	130	0.01	45	0.001	15	0.3	5	130- 5 =125
Wang, 1973	0.03	60	0.003	20	0.0003	7	---	---	60- 7 = 53
Frank, 1975	0.1	95	0.01	45	0.02	50	0.5	15	95-15 = 80
				HAMSTER					
Fishman, 1957	0.5	101	0.01	83	0.02	73	1.0	65	101-65 = 36
Ogawa et al., 1968	0.1	84	0.01	83	0.02	73	0.5	65	84-65 = 19
Frank, 1972[5]	0.3	94	0.003	58	0.001	36	0.1	43	94-36 = 58

[1] Based on the whole nerve effectiveness with 0.1 N HCl as 100% and interpolated from the SR functions from Pfaffman (rat chorda tympani, 1955) and Frank (hamster chorda tympani, 1972).

[2] N = NaCl, H = HCl, Q = QHCl = quinine hydrochloride, and S = sucrose.

[3] Also Erickson (1967) and Erickson et al. (1965) for the rat.

[4] Also Ogawa et al., 1968, for the hamster, below. Also Ogawa et al., 1969, for the rat and hamster, and Yinon and Erickson, 1970, for the hamster.

[5] Also Frank, 1973, for the hamster.

Ogawa et al. (1968) tested the same concentrations of stimuli on the rat as well as the hamster. For the latter, the same stimuli extended over 80 units of relative effectiveness. Frank (1973) stated that the stimulus concentrations were chosen to represent the midrange of the SR function for each stimulus. However, the SR maxima of different chemicals are not identical, since some show saturation below the maximum response level of others (e.g., sucrose versus HCl). Hence, midrange concentrations could be quite unequal in their electrophysiological effectiveness on peripheral neurons. Moreover, as mentioned previously, human perceptual gustatory intensity is correlated with the level of summated whole nerve activity. The relative magnitude of the summated nerve responses of the human subjects to six equimolar sugars directly matched their perceptual intensity scaling (Borg et al., 1968).

The major difficulty with the previously described methods is that single neurons often respond with SR functions quite unlike those of the whole nerve. Accordingly, in order to study responses in single units with the least error, it is necessary to test them with a range of concentrations that encompasses the entire range of the whole nerve response. If this is not done, the next best protocol would be to choose individual concentrations that are equally effective in the whole nerve population. The results in all other cases must be considered cautiously.

Functions and Relations with Single Concentration Tests. When a single concentration of each stimulus is used, gustatory neurons exhibit a wide variety of response specificity. Some fibers respond to

a few chemicals. These have been labelled narrowly tuned fibers (e.g., see Pfaffman, 1955 and Frank, 1975). Their proportion of the total population is small for most peripheral nerves. Of 101 frog glossopharyngeal fibers, 11 were monogustatory (11/105, Kusano, 1960) as were 1/15, 9/48, and 5/25 of rat chorda tympani fibers (Wang, 1972, Ogawa et al., 1968, and Frank and Pfaffmann, 1969, respectively) and 5/28 and 23/79 of hamster chorda fibers (Ogawa et al., 1968, and Frank, 1969, respectively) and 8/27 of rat glossopharyngeal fibers (Frank and Pfaffmann, 1969).

The majority of fibers show multiple sensitivity with a random distribution among the primary stimuli (among rat and hamster chorda fibers, Frank and Pfaffmann, 1969 and Frank, 1973, and the rat glossopharyngeal fibers, Frank and Pfaffman, 1969). The observed number of fibers responding to 1, 2, 3, or all of the primary stimuli does not differ from that predicted by random polynomial distribution of the sensitivities at the frequencies found in the sample population.

The property of multiple specificity is illustrated in Figure 8. The responses to three gustatory neurons are shown for the rat (Pfaffmann, 1955), hamster (Fishman, 1957) and frog (Kusano, 1960). The general responsiveness of the neurons varied greatly, one hamster neuron responding with 50 impulses/sec to NaCl, another with less than 10 impulses/sec to HCl and sucrose. The multiple sensitivities also varied between fibers and animals, three units (neurons) among the nine being clearly monogustatory (Figure 8, A and C Rat, and A Hamster). Notice that the testing of additional, non-primary stimuli (KCl for the

Figure 8. Multiple gustatory sensitivity of single neurons. The responses of three single neurons are profiled (A, B, and C) rat and hamster chorda tympani and frog glossopharyngeal fibers, after Pfaffmann, 1955, Fishman, 1957, and Kusano, 1960). Most fibers were multiply sensitive (only two rat, A and C and one hamster neuron A, are clearly monogustatory). Testing with non-primary stimuli revealed sensitivities greater than those to the primary stimuli (KCl causing the greatest response for one rat neuron, $MgCl_2$ for a frog neuron). Maximum responses varied greatly among the units.

KEY:
N = NaCl
H = HCl
Q = quinine hydrochloride
S = sucrose
HAc = acetic acid (frog)
K = KCl
Ca = $CaCl_2$
Mg = $MgCl_2$

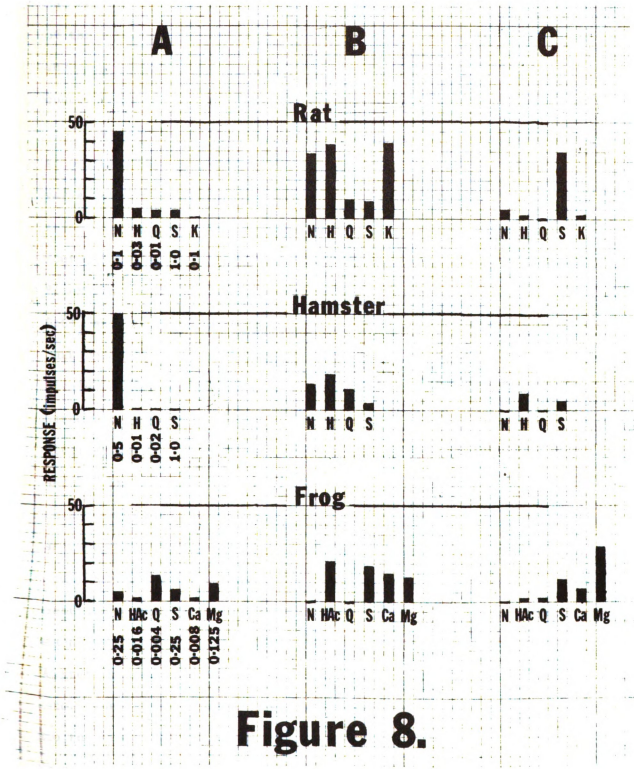

A
B
C
Rat
Hamster
Frog
RESPONSE (impulses/sec)
50
0
N H Q S K
0·1 0·03 0·01 1·0 0·1
N H Q S K
N H Q S K
N H Q S
0·5 0·01 0·02 1·0
N H Q S
N H Q S
N HAc Q S Ca Mg
0·25 0·016 0·004 0·25 0·008 0·125
N HAc Q S Ca Mg
N HAc Q S Ca Mg

Figure 8.

rat and $CaCl_2$ and $MgCl_2$ for the frog) reveals additional sensitivities of the neurons, in some cases with responses that were greater than to any primary stimulus. Testing was conducted with unequal stimulus concentrations and concentrations that are of unequal effectiveness.

Investigators have repeatedly attempted to classify neurons according to their observed response specificities. These schemes have varied even when applied to the same species, depending on the authors' choice of stimuli. For example, Pfaffmann (1941), testing mainly the four primary stimuli, found "acid", "acid-quinine", and "acid-salt" fibers in the cat's chorda tympani and mentioned their sensitivities to $CaCl_2$ and KCl only in passing. Kusano (1960) tested frog glossopharyngeal taste neurons with ten different monovalent cations, four divalent cations, HCl and acetic acids, and sucrose and sodium saccharin. He found four fiber types: "D-units", most sensitive to divalent salts; "M-units", most sensitive to monovalent salts, and "Q" and "A" units, with specificity to quinine hydrochloride and acetic acid respectively. He added that "D-units" were also sensitive to sucrose, quinine, acetic acid, and some monovalent salts.

Frank (1973) has developed a classification based on single concentration tests of each of the four primary stimuli. The responses to each stimulus are rated as a percent of the largest response. The latter is used to classify the fibers as "sucrose-best", "salt-best", etc. The responses of Figure 8 are transformed in Figure 9 according to the Frank classification. Transformation of the data in Figure 8 was accomplished as follows: 1) Only the responses to the four primary

Figure 9. Best taste profiles of nine single gustatory neurons. The response profiles for the single neurons of Figure 8 are transformed according to the procedure of Frank (1973) to determine their relative sensitivity to the four primary stimuli. Only the four primary stimuli are considered, the response to each being expressed as a percent of the maximum response to sucrose (S), NaCl (N), HCl (acetic acid for the frog, H), and quinine hydrochloride (Q).

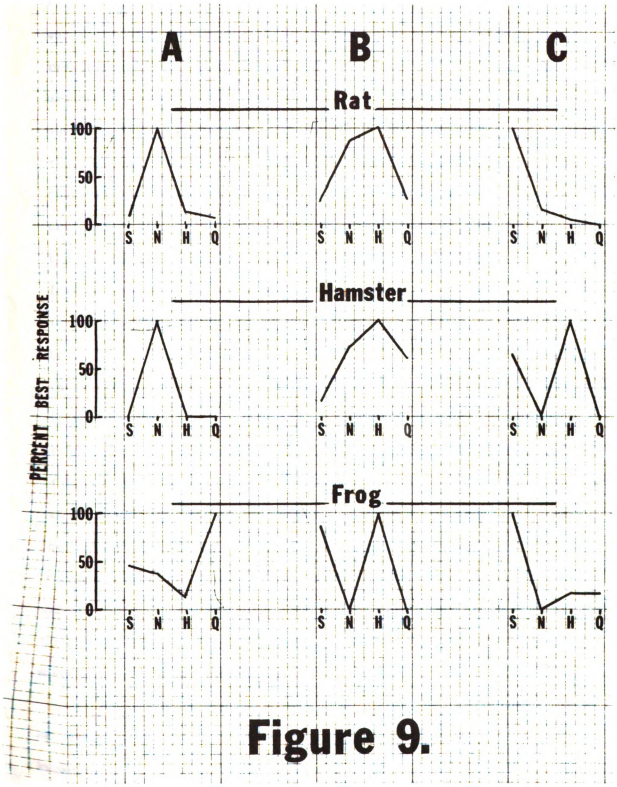


Figure 9.

stimuli were considered. 2) The maximum response to one of the four was identified for each unit (e.g., NaCl for rat neuron A). 3) The responses to the other three stimuli were expressed as a percent of the maximum response. 4). The responses were displayed in the order--sucrose, NaCl, HCl (acetic acid for the frog), and quinine. The neurons are thus displayed in Figure 9 and can be considered with respect to their sensitivities to the primary stimuli. However, the response profiles are now altered. As can be seen from the three hamster fibers, the response magnitudes are lost in transformation, the third unit (C Hamster) shows no difference in responsiveness from the first two (A and B). Among the three rat fibers, the middle fiber (B Rat), an H best fiber after transformation, is actually most sensitive to KCl (of those chemicals tested by Pfaffmann). The frog responses are similarly altered, especially the second (B Frog) which is classed a sucrose fiber after transformation but is twice as sensitive to $MgCl_2$. Frank (1973) calls attention to the limitations of this method and points out that the stimuli may not be homologous among all animals with respect to quality, dissimilarity, or purity of perceptual sensation. For the hamster chorda tympani Frank found four fiber types using this method of analysis. The fibers differed only slightly within each class, only the sucrose- and salt-best fibers differing with respect to their second best primary stimulus.

The power of Frank's analysis lies in the clear demonstration of a fiber's sensitivity toward the four primary stimuli. Thus for the hamster chorda tympani, Frank found that the sucrose-best fibers

responded least to the other stimuli. Quinine sensitivity was least clearly discriminated, few quinine-best fibers being found and those responding almost as well to one of the other stimuli. The lack of specificity to quinine was contrasted in a later study of rat glossopharyngeal and chorda tympani neurons (Frank, 1975), in which quinine-best chorda neurons were broadly sensitive (to HCL and NaCl), but glossopharyngeal quinine-best fibers were very specific. This is consistent with the whole nerve response of many mammals in which the tongues show regional sensitivity to the four primary taste stimuli. Bitter stimuli are most effective at the posterior lingual area which is innervated by the glossopharyngeal nerve; whereas, sweet stimuli excel at the tip of the tongue which is innervated by VII. Regional specificity was also demonstrated in the tests for independence of the distribution of the specific and combined sensitivities (Frank, 1972, 1973). While the degree of specificity (i.e., the number of effective primary stimuli) was again random, certain combinations of sensitivities were lacking or overemphasized. More units were sensitive to quinine along with NaCl or HCl and far fewer fibers were sensitive to sucrose along with other stimuli than predicted by a random polynomial distribution. Sucrose-best fibers were more specific, while quinine-best units were more broadly tuned than predicted by random distribution.

Single concentration tests are frequently used to draw correlations between different stimuli using graphical and statistical methods. Moderate correlation coefficients have been found between pairs of primary stimuli ($r = +0.30$ to $+0.58$). Sucrose is an exception for

chorda neurons with "r" values that were negative or less than +0.20 (Erickson et al., 1965; Frank, 1973). Correlation coefficients have also been used to compare the effects of non-primary stimuli. Strong positive values were found between NaCl and LiCl for the rat chorda fibers (r = +0.92, Erickson et al., 1965) and between HCl and citric acid for hamster chorda fibers (r = +0.82, Frank, 1973).

The use of correlation statistics with single concentration tests is of limited value. Investigators may be tempted to extend correlations between fiber sensitivities to physical properties of the stimuli that may underly perceptual taste quality. Yet the correlation statistics do not discriminate between properties that are ascribable to the fiber systems themselves (such as a specific insensitivity to a class of stimuli) and the properties of the stimulus molecules that are associated with a particular taste quality. For example, as cited above, rat and hamster chorda neurons lacked specificity to quinine, and the rat chorda neurons showed strong correlations between responses to quinine and organic acids (acetic and citric, r = +0.72, +0.66 respectively). Erroneous generalizations would be that acetic and citric acids have bitter perceptual properties to the rat or that the physical parameters responsible for "bitterness" are common to these three stimuli. Rat glossopharyngeal nerve fibers, in contrast, showed specificity to quinine. For the glossopharyngeal fibers, the bitter substances, urea and caffeine, were strongly correlated with quinine (r = +0.59 and +0.60) while the acetic and citric acid correlation coefficients fell drastically (r = +0.07 and +0.04; Frank, 1973, 1975).

Single Unit SR Functions. Several investigators have determined a single neuron's gustatory response to test stimuli through a range of concentrations. While no report shows a systematic investigation of several stimuli using this method of presentation, enough individual examples have accumulated to indicate several properties of single neuron SR functions.

SR functions of single neurons often differ from the SR curve of the summated whole nerve response. With the same stimulus, the threshold, slopes and maxima of SR functions vary among fibers (Pfaffmann, 1955; Cohen et al., 1955; Fishman, 1957; Ogawa et al., 1968; Wang and Bernard, 1969; and Ganchrow and Erickson, 1970). Examples are shown in Figure 10. In Figure 10A are the SR functions of four different rat chorda tympani fibers to NaCl (Pfaffmann, 1955). For all neurons, responses increase regularly with greater stimulus concentration, which is the most frequently observed relationship and the form of whole nerve SR functions. Figure 10B and 10C shows two frog glossopharngeal fibers for which the NaCl SR functions are quite different, one with negative slope and one which is U-shaped with a mid-range minimum (Kusano, 1960). A bell-shaped function for NaCl is shown in Figure 10D, cat chorda tympani neuron (Bernard, 1972).

For the same neuron, SR functions can differ with different stimuli, even among the four primary taste stimuli. In Figure 11 (A-C) are shown SR functions for individual neurons of the rat (Ogawa et al., 1969), hamster (Frank, 1973), and cat (Bernard, 1972). Nearly all the curves are positive and monotonic, showing different threshold and

Figure 10. SR functions for single neurons. These are shown for NaCl responses in seven different neurons of the rat, frog and cat (after Pfaffmann, 1955, Kusano, 1960, and Bernard, 1972, respectively). As can be seen, they possess widely different forms. In A the responses from four rat chorda neurons form regularly increasing functions of concentration, differing in threshold and maximum response. In B and C are two frog glossopharyngeal fibers with U-shaped and negatively sloping curves. In D is a bell-shaped function for a cat chorda neuron's responses to NaCl. The ordinates are not scaled equally but reflect each author's method of magnitude estimation. The abscissae have been standardized to Log concentrations to facilitate comparisons. D also includes the level of spontaneous activity (mean $\pm$ 1 standard deviation, shown by arrows).

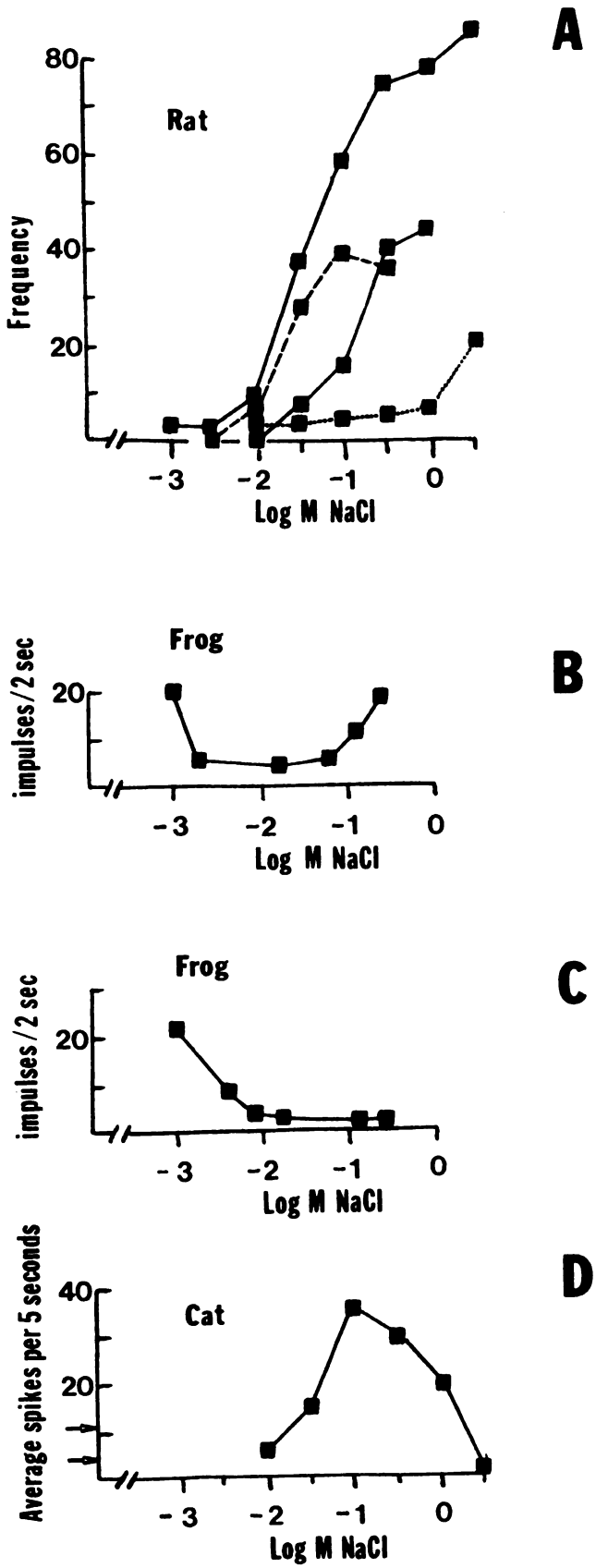
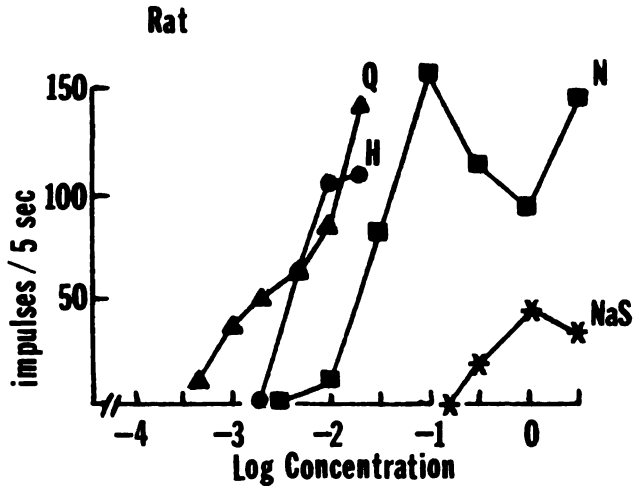

A
Rat
Frequency
80
60
40
20
-3
-2
-1
0
Log M NaCl
B
Frog
impulses/2 sec
20
-3
-2
-1
0
Log M NaCl
C
Frog
impulses/2 sec
20
-3
-2
-1
0
Log M NaCl
D
Cat
Average spikes per 5 seconds
40
20
-3
-2
-1
0
Log M NaCl

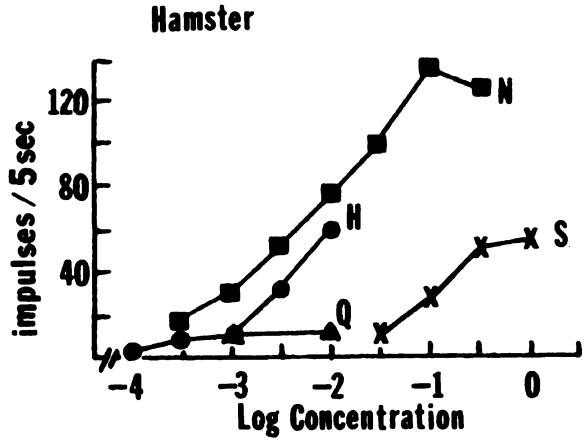
Figure 10.

Figure 11. The single neuron's SR functions to different stimuli. These are shown for a chorda neuron of rat (Ogawa *et al.*, 1969), hamster (Frank, 1973), and cat (Bernard, 1972) for the four primary stimuli as well as sodium saccharin (for the rat, NaS in A). While most functions have only positive slope, two are complex forms (N for the rat, H for the cat) and one is of zero slope (Q for the hamster). With such variety of forms and slopes, classification of the neurons according to the most effective stimulus is arbitrary since the stimuli are ranked differently by threshold or maximum response for each neuron. All responses were averaged over five seconds. C shows the spontaneous activity level (mean ± one standard deviation, by arrows from the ordinate).

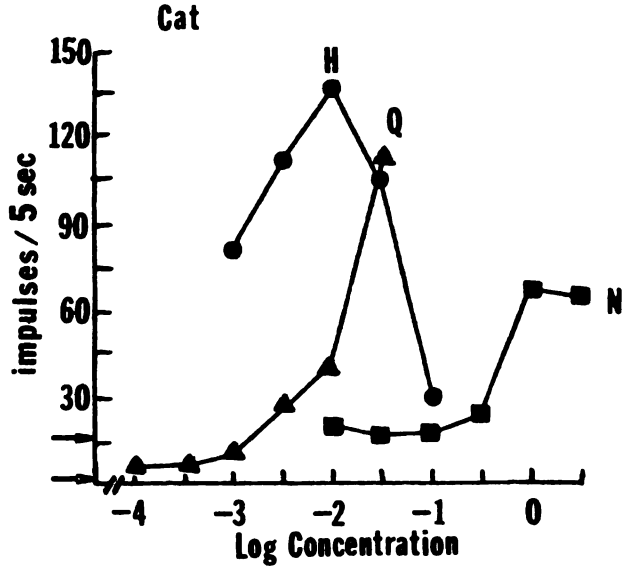
Key:
N = NaCl
H = HCl
Q = quinine hydrochloride
S = sucrose
NaS = sodium saccharin



A



B



C

Figure 11.

maxima. Noticeable exceptions are the NaCl SR curve for the rat, the HCl curve for the cat, and the quinine hydrochloride curve for the hamster. In the latter case, the curve for the quinine SR function had zero slope (Frank, 1973 for the hamster, Figure 11B). The responses to quinine were of a consistent, albeit low, level. The author considered the curve to represent the absence of any response, without stating the spontaneous activity level for this neuron.

Even with the variety of functions observed, Ganchrow and Erickson (1970) showed that the combined SR curve of a sample of 20-37 rat chorda neurons (averaged for each concentration) closely matched the reported summated whole nerve function (of Pfaffmann, 1955) both with respect to threshold and slopes for the four primary stimuli and KCl. While the individual SR functions were not shown, the authors reported them to have different thresholds and rates of increase.

The classification of a fiber from the full range of its SR function can be most complicated. For example, in Figure 11A, the rat chorda unit could be a quinine fiber (lowest threshold) or an NaCl fiber (with maximum response) and in Figure 11C, the cat fiber could be HCl-best or a quinine fiber, without knowing the HCl threshold. Each of the fibers responded in varying degrees to three of the primary stimuli and in the absence of further information such as the response latencies, suppression responses, or rinse activity, can only be arbitrarily classified. Recalling the body of literature relating to correlations and comparisons from single concentration studies, Frank's cautions are appropriate (Frank, 1973). She states that if many fibers

show drastic response reductions with increasing concentration, severe limitations must be placed on interpretation of data taken at any one moderate concentration and that if stimuli are variably strong or weak, different conclusions with respect to fiber specificity will be made.

Two experiments using extended concentration tests have shown that bell-shaped and negatively-sloped SR functions are not derived from arbitrarily strong or weak responses but are consistent functions. Moreover, these can be powerfully related to other SR functions and different response parameters to reveal more information about the taste system and its interaction with several stimuli.

Cohen et al. (1955) recorded from cat chorda neurons and observed prominent differences between two fiber types. The first showed spontaneous activity under adapting Ringer's rinse. It was also salt sensitive and responded with a regularly increasing SR function to NaCl with a threshold of 0.1 M (Figure 12A, "Salt Fiber"). The second neuron responded to distilled water (after Ringer's adaptation) and remained spontaneously active during water adaptation. To NaCl it responded with a regularly decreasing SR function with its responses declining to zero at 0.03 M NaCl (Figure 12A, "Water Fiber"). The two neurons possessed disparate sensitivities; distilled water did not affect the "salt" fiber and the "water" fiber did not respond to NaCl concentrations that were excitatory to the other. Seen together (Figure 12A) the SR functions of the two neurons are related, the responsiveness passing from one to the other as salt concentration is increased. The authors observed that discrimination over an entire range of salinity

Figure 12. Interrelated SR functions. Negatively sloped and bell-shaped SR functions have been correlated with other SR functions and different response parameters to reveal more information about the taste system.

A. Two fibers with opposed NaCl SR functions are seen to have maxima at opposite ends of the extended concentration range. Together the fibers' activity offers a broad sensitivity to salinity.

B. A single neuron's responses to sodium saccharin form a bell-shaped SR curve (NaS "on" curve). Its rinse responses to sodium saccharin have an SR function whose threshold is at the concentration for the maximum response to sodium saccharin (at 0.03 M, NaS "off" curve). From 0.03 to 0.3 M the responses to the application of sodium saccharin decline while its rinse responses proportionally increase. The authors suggest that this reciprocal relationship shows that "on" and "off" responses are related by a mechanism which can excite the neuron or inhibit it by the action of sodium saccharin molecules.

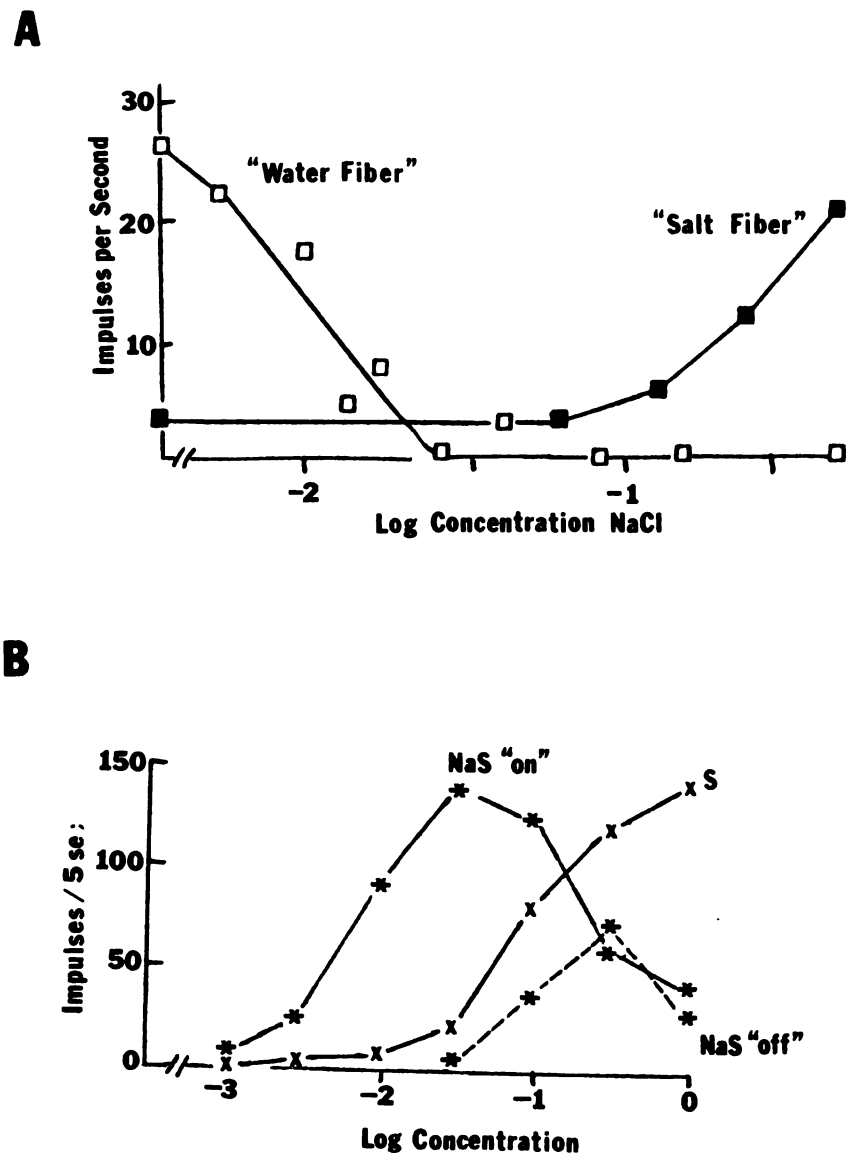

A
30
20
10
Impulses per Second
"Water Fiber"
"Salt Fiber"
-2
-1
Log Concentration NaCl
B
150
100
50
0
Impulses / 5 sec
NaS "on"
S
NaS "off"
-3
-2
-1
0
Log Concentration

Figure 12.

is thus afforded by two distinct fibers with response maxima at opposite ends of the range of concentrations.

The second example, from Ogawa et al. (1969) correlates different responses of a rat chorda tympani neuron with sodium saccharin. The neuron was classified as a sweet sensitive fiber by its criterion responses to sucrose over an extended range of concentrations forming a positive SR curve (Figure 12B, curve S). However, its SR curve to sodium saccharin was bell-shaped, with a threshold below that of sucrose (Figure 12B, NaS "on" curve). The maximum response to sodium saccharin was at 0.3 M and was equivalent in magnitude to the sucrose maximum at 1.0 M. The neuron also gave a rinse response after sodium saccharin, but only to concentrations that were greater than 0.03 M. The SR function for the rinse response was bell-shaped (Figure 12B, NaS "off" curve). The positive slope was nearly reciprocal to the declining portion of the sodium saccharin "on" function. Thus the negatively sloping portion of the SR function for NaS "on" was correlated with the rinse response SR function by: 1) the reciprocal slopes of their SR curves, and 2) the coincidental maximum of the sodium saccharin "on" responses with the threshold for the "off" responses. The authors suggest that these phenomena represent inhibition (the declining sodium saccharin responses from 0.03 to 1.0 M) and the removal of inhibition (the rinse response over the same concentration range of sodium saccharin). They propose a mechanism involving the dual action of sodium saccharin molecules at two distinct receptor sites, one which excites and one which inhibits the gustatory neuron.

Mechanical and Thermal Sensitivity of Taste Neurons

Many gustatory neurons respond to stimulation by two other modalities, mechanical and thermal. In mammals, mechanosensitivity appears to be confined to afferent fibers in the lingual branch of the trigeminal nerve. Chorda tympani fibers do not readily respond to mechanical stimulation. In the glossopharyngeal fibers of the sheep (Iggo and Leek, 1967), movement of a circumvallate papilla can enhance the neuron's chemical response though this may simply be a result of enhanced diffusion of the stimulus. The chemical sensitivity of trigeminal nerve fibers has not been clearly established. Degeneration studies (Beidler, 1969 and Miller, 1974) showed that the trigeminal nerve largely innervated the stalk and walls of the fungiform papillae of the rat. While no trigeminal neuron was clearly shown to innervate the taste buds (in the rat) the possibility cannot be completely excluded.

In lower vertebrates, e.g., the frog, glossopharyngeal gustatory neurons also respond to mechanical stimulation. Taglietti, Casella, and Ferrari (1969) have demonstrated that the receptive field of a neuron to mechanical stimulation, localized to several fungiform papillae, differed from the chemical receptive field, though the latter was determined only with $CaCl_2$.

Thermal sensitivity is commonly found in gustatory neurons. (For examples in the rat, hamster, sheep, and macaque monkey, see Fishman, 1957; Iggo and Leek, 1967; Sato et al., 1969; and Sato et al., 1975 respectively). Taste fibers that are sensitive to lingual warming are the exception for most species, while sensitivity to cooling is often

found. Sato et al. (1969) found that 48/50 gustatory chorda fibers in the rat were cold sensitive. Ogawa et al. (1968) observed a U-shaped thermal response function for a rat gustatory chorda neuron with a minimum at 35° C, ten degrees above lingual surface temperature. The authors concluded that the 25° tongue temperature (excitatory to such thermally sensitive gustatory units) may be the origin of spontaneous activity. Sato et al. (1969) found large correlation coefficients between spontaneous activity and cooling ($r = +0.503, +0.781$, rat and hamster) with significant differences (t test, $p < 0.01$) between the spontaneous activity of cold sensitive taste fibers and non-thermally sensitive chorda neurons.

The macaque monkey is an exception to the relatively large number of warm-sensitive chorda gustatory fibers (30%, versus 40% cold-sensitive). The warm and cold fibers responded to distilled water at temperatures above or below lingual temperature and together offer an extended range of thermal sensitivity (Sato et al., 1975).

III. STATEMENT OF THE PROBLEM

The neurophysiological taste response of the single gustatory neuron of the mud puppy, Necturus maculosus, has not been investigated. The taste response of single neurons of many vertebrates has been found to vary widely.

1. Three forms of the taste response of single neurons have been found: a) increased activity on stimulation, b) decreased activity with stimulus presentation, and c) responses to the water rinse after stimulation. Yet only the first form has been consistently recognized and quantified in analyzing the responses of the first order gustatory neurons. One objective of this study was to consider all forms in defining the taste responses of the single neurons of Necturus.

2. The magnitude of the taste response varies with stimulus concentration, defining the stimulus-response or SR function of a neuron for the taste stimulus. A single gustatory neuron has different SR functions for different stimuli. Among many neurons, dissimilar SR functions have been found for the same taste stimulus. Yet most taste sensory experiments employ single concentrations of stimuli. In part this arises from the limited ability to hold and test a single neuron. A small number of stimuli have been tested at one concentration to allow greater replication. A systematic investigation of SR functions

among many neurons has not been reported and is the second objective of this study.

3. Recent experiments have found that the latency of the taste response of single neurons is inversely related to the concentration of the stimulus (T. Sato, 1976). However, this relationship was based upon the average latency of the responses of several neurons. The responses of individual neurons were seen to have a wide range of latencies at a single concentration. The latency functions of the individual neurons were not investigated and is the third objective of this study.

Accordingly, the experiments of this study were undertaken to define the taste responses of single gustatory neurons of Necturus according to three different parameters: 1) the form of their taste response, 2) their SR functions, and 3) their latency-concentration functions. The four primary taste stimuli were employed: NaCl, HCl, quinine hydrochloride, and sucrose. These were not used to classify the neurons according to their sensitivities toward these stimuli, but were used to obtain a broad survey of taste responses from dissimilar taste stimuli. With single tests, the systematic investigation of these parameters sacrificed replication to gain breadth of testing by using the four stimuli over an extended range of concentration, 3.5 Log.

IV. METHODS

Smaller adult mud puppies, Necturus maculosus, 20-25 cm long were selected. These had been held in 20 gal (76 ℓ) plastic aquaria at 10-15° C in aerated, charcoal-filtered water in an artificial light cycle of 16 hours per day. Animals were held no longer than three weeks and were not fed during confinement. The antibiotic, tetracycline was dissolved in the aquarium water to eliminate possible infection by Saprolegnia fungus (see Samanen, 1973).

The animals were anesthetized with 5 gm % (w/v) urethan (aqueous ethyl carbamate solution) in two stages. The first was by whole body immersion for a maximum of five minutes to facilitate handling. The animals were then removed and rinsed with distilled water. Their tongues were extensively rinsed to reduce any depression of the taste system. For the second stage, their gills were placed in cups containing the urethan solution for an additional 10-15 min. Experiments were conducted at 22-24° C ambient temperature.

The dissection included first the exposure, transection, and desheathing of the peripheral branches of the glossopharyngeal nerve along the ventral aspect of the ceratohyal cartilage of the hyoid arch. Each branch was further divided into smaller (80 μ diam) bundles.

Standard differential monopolar recording techniques were employed using two identical, 300 μ diam, silver electrodes. The active electrode

conveyed the electrical potentials from the isolated nerve bundle. The indifferent electrode contacted nearby tissue rendering an optimal signal-to-noise ratio. Both were immersed in paraffin oil. The animal was grounded from its tail to the preamplifier's ground circuit.

The Grass P-15, AC, preamplifier (Quincy, Massachusetts) differentiated the potentials and then amplified (X100) and filtered the activity through a frequency bandwidth of 300 Hz-3KHz. A Tektronix, Type RM502-A, dual beam oscilloscope (Beaverton, Oregon) displayed the electrical activity which was then recorded by a Magnecord, Model 104B, stereo, AM tape recorder (Midwestern Instruments, Tulsa, Oklahoma)(see Figure 13).

Computer Determination of the Isolation of Neurons, FREDSAM

In order to determine that the recorded activity was from a single nerve cell, a computer program, called FREDSAM, was used. The only extracellular evidence for single neuron recording was the uniformity of action potential amplitude. Yet variance in spike amplitude is present in all records, arising from the cumulative effects of the recorded electrical noise. Accordingly, a computer program written by Mr. Marc Schneider for the LINC 8 computer (Digital equipment Corporation, Maynard, Massachusetts) was used to quickly and accurately determine the distribution of action potential amplitudes. The computer program named FREDSAM ("frequency distribution of spike amplitude") analyzed all diphasic potentials in the taped records, both the action potentials and the much smaller, irregular baseline or background electrical noise. The FREDSAM analysis was displayed as a

Figure 13. Electronic equipment for gustatory single neural unit recording. Differential AC amplification of the activity from two silver electrodes (Active and Indifferent) is accomplished by the pre-amplifier and oscilloscope. A permanent record is taped of the oscilloscope's output (Ch 1), of the stimulus timer's cycle, and of the experimenter's comments (Mic).

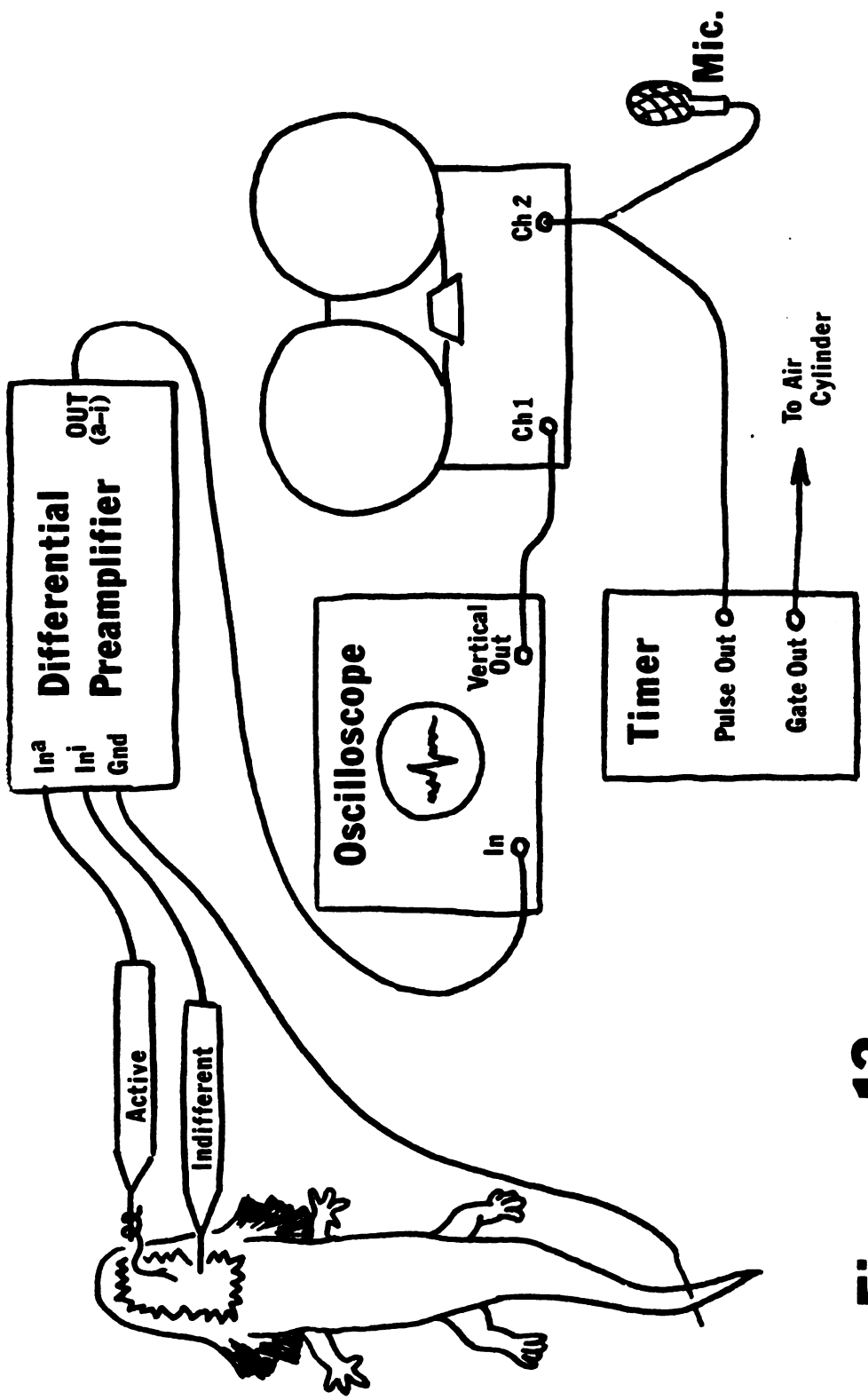

Differential
Preamplifier
In^a
In^i
Gnd
OUT (a-i)
Active
Indifferent
Oscilloscope
Vertical Out
In
Ch 1
Ch 2
Mic.
Timer
Pulse Out
Gate Out
To Air Cylinder

Figure 13.

population histogram (Figure 14). The bins of the histogram are displayed as single vertical lines along the abscissa. The width of the bins are based on a relative scale of amplitude which was calibrated for each experiment (Figure 16). Once the data was entered into the LINC 8, several options were available to vary the display, including: 1) scaling the ordinate to show from 25 to 6400 events, 2) displaying a limited number of bins, e.g., a left or right portion of the abscissa, or only the bins which encompass a single population, and 3) varying the bin width from 1 to 999 relative amplitude units per line. The latter option was always used to ensure the persistence of maxima and minima by changing the bin width over several relative amplitude units. (For other options, see Appendix I.)

The largest peak, displayed near the origin, was the small (usually 10 μV) background electrical noise. Uniform action potentials, indicating a well-isolated neuron, formed a distinct peak to the right of the baseline population. Multiple unit records had histograms with several peaks. When electrically distinct, they were separate from each other (Figure 15). The peaks of poorly isolated neurons were much smaller and less distinct. The histograms of the neurons of low responsiveness similarly had no clear peaks but only the random display of a few points. Therefore, records from poorly isolated neurons or those with low activity resulted in FREDSAM histograms with only a distinct baseline peak and its positively skewed tail and were rejected from further analysis (see Appendix I).

Figure 14. Computer analysis (FREDSAM) of a well-isolated neural unit.

Above. The action potentials from a well-isolated lingual neuron from three cold tests (4° C distilled water, only 1 of the 10 second tests is shown). Their diphasic amplitudes are uniform at 35 μV).

Below. The display of the FREDSAM analysis of the three tests giving a histogram of the distribution of diphasic amplitudes. Each vertical line represents the action potentials within a single interval of amplitude. The ordinate shows the number of events, the abscissa shows intervals of increasing amplitude. Amplitude intervals are defined on a relative scale (here each equals 0.6 μV). A calibrated scale is shown below the relative scale. Two distinct populations are seen: 1) the population of action potentials (right) and 2) the population of small baseline potentials (left).

The legend of the FREDSAM display contains:
a) a two-line statement identifying the display,
b) a row of numerals indicating--
   1) the maximum ordinate value (100 events),
   2) the total number of events displayed (625),
   3) the width of each interval (here equals 1 relative amplitude unit), and
   4) the lowest interval number displayed (1, at the origin).

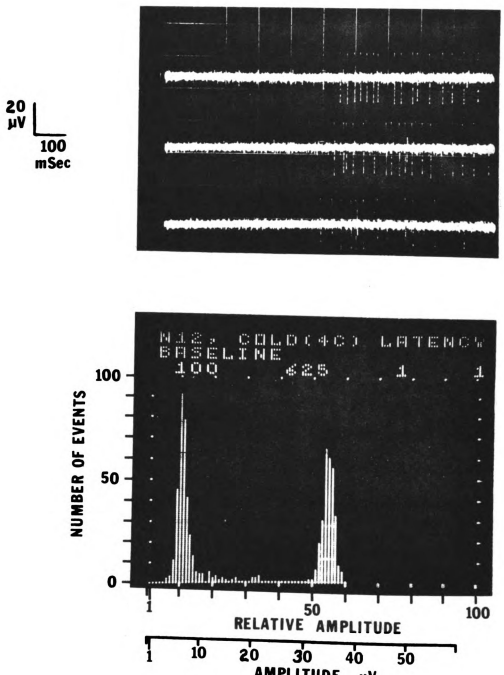

20
μV
100
mSec
N12, COLD(4C) LATENCY
BASELINE
100 . .25 . .1 . .1
NUMBER OF EVENTS
100
50
0
1
50
100
RELATIVE AMPLITUDE
1
10
20
30
40
50
AMPLITUDE, μV

Figure 14.

Figure 15. FREDSAM analysis of multiple isolated units.

Upper trace--a lingual branch responded with action potentials from several neurons (a, b, c, and d) when cold water (4° C) was applied to the tongue.

Display 1--The FREDSAM analysis of longer periods of the same thermal responses shows a multi-modal distribution with three distinct amplitude populations (b, c, d) and an asymmetrical baseline of small amplitude events (BL_1 and a).

Display 2--is equivalent to the right portion of Display 1 (right of the black arrow) and is amplified vertically 8X but has the same horizontal scale.

Display 3--lingual chemical stimulation (0.01 N HCl, 0.1 M NaCl, 0.03 M quinine hydrochloride, and 0.3 M sucrose) caused no responses from the same neurons, revealing a symmetrical baseline (BL_2) and indicated that the maximum, a, near baseline 1 (display 1) was due to responses from a thermal sensitive unit. The corresponding action potentials, a-d, are identified in the upper trace.

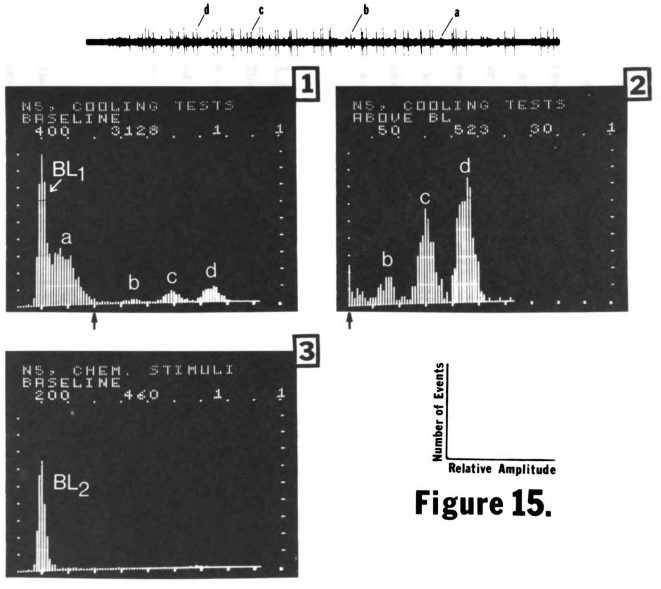

d
c
b
a
1
NS. COOLING TESTS
BASELINE
400 . 3128 . . 1. . 1
BL1
a
b c d
2
NS. COOLING TESTS
ABOVE BL
50 . 523 . 30. . 1
d
c
b
3
NS. CHEM. STIMULI
BASELINE
200 . 460 . . 1. . 1
BL2
Number of Events
Relative Amplitude

Figure 15.

During an experiment, FREDSAM was used to determine the gustatory neuron's isolation based upon the responses of a few chemical or tactile tests. Further dissection was performed if the neuron(s) were not isolated. Alternatively FREDSAM was used after an experiment to accept data from well-isolated neurons or to reject data from poorly isolated fibers and to determine the range of spike amplitudes defining a single unit for enumerating its response. To compare photographic and computer records, FREDSAM was calibrated for each experiment by recording a sine wave of known amplitude (from a Wavetek, Model 110, waveform generator). The sine wave is equivalent to a sequence of many diphasic events and appeared as a single, large population with little variance (Figure 16).

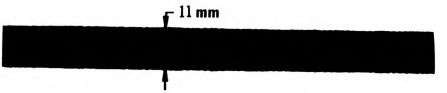
Stimuli and Protocol

The chemical stimuli were solutions of reagent grade NaCl, HCl, quinine hydrochloride, and sucrose dissolved in distilled water. I tested these over the following ranges of increasing ½ Log concentration.

NaCl	0.0001 - 0.3 M
HCl	0.00001 - 0.03 N
Sucrose	0.0003 - 0.3 M
Quinine hydrochloride	0.00003 - 0.1 M

All stimuli were applied for 20 seconds and were followed by at least a 40 second rinse of the aqueous solvent. The order of presentation of the four series of test solutions was varied among the experiments. However, to minimize the effects of adaptation, the solutions within each series were presented in the order of increasing concentration.

Figure 16. Calibration of the computer and photographic records. The photographic and computer data are calibrated for each experiment. The calibration signal, a sine wave of 1200 Hz and known amplitude, e.g., 20 μV (not shown) is both photographed and analyzed by the FREDSAM computer program. The 11 mm wide black bar (above) is a photograph of the sine wave when compressed on the time scale of 25 mm/sec. In the FREDSAM display, the sine wave occupies the 63rd interval of relative amplitude units. The variance of the sine wave's amplitude arises from electrical noise during recording and playback. Action potentials of any amplitude range defined by FREDSAM and enumerated with SAM-COUNT can be identified in their photograph according to their amplitude in μV, e.g., using the scale here of 20 μV = 63 computer amplitude units = 11 mm photograph.


11 mm

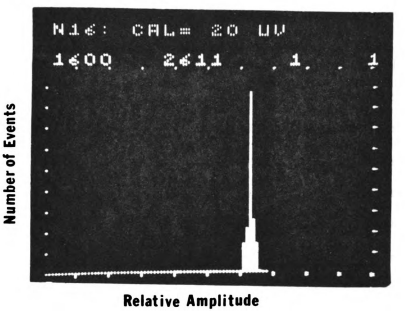

Ni⁶⁴ CAL= 20 UV
1600 2411 1 1
Number of Events
Relative Amplitude

Figure 16.

The thermal stimuli were heated and cooled distilled water (50-60° C and 4° C, respectively) which contrasted the room temperature rinse (22-24° C). Tactile sensitivity was determined by lightly touching the receptive field of the neuron with a glass probe (1 mm diam).

Stimulus Delivery

Stimulus and rinse solutions flowed separately from two elevated flasks through an air-driven valve toward separate outlets, one at the animal's tongue, the other into a waste bucket (Figure 17). The experimenter filled the stimulus flask by releasing pressurized nitrogen into one of 31 polyethylene storage bottles, driving the solution through its delivery tube and into the reservoir. Distilled water was used to wash the stimulus flask between different chemical series. A timer controlled the release of compressed air, repositioning the valve and alternating the fluids between the bucket and tongue. The timer could be adjusted to fix the rinse and stimulus period independently within a range from 0.2 to 300 sec. The timer emitted electric pulses which were used to identify the onset of the rinse and stimulus periods, and was recorded on the second channel of the tape recorder along with the experimenter's comments (Figure 13). The solutions were directed caudally over a lingual area including the receptive field of the neural unit. The flow rate was 1.4 ml/sec for both test and rinse solutions.

Data Analysis

Photographic. A Grass, Model C4N, oscilloscope recording camera (Quincy, Massachusetts) was used to photograph a continuous 30 second

Figure 17. The stimulus delivery system. Panels A, B, and C illustrate equipment which: (A) allow the selection of test solution (1, 2, or 3), and (B) supply distilled water rinse. Both liquids flow from their reservoirs into C where a valve driven by compressed air on signal from a timer, alternately directs one of the two liquids into the waste bucket or out of the test spout. Rate of flow was controlled by height of the stimulus and rinse reservoirs and by their stopcocks.

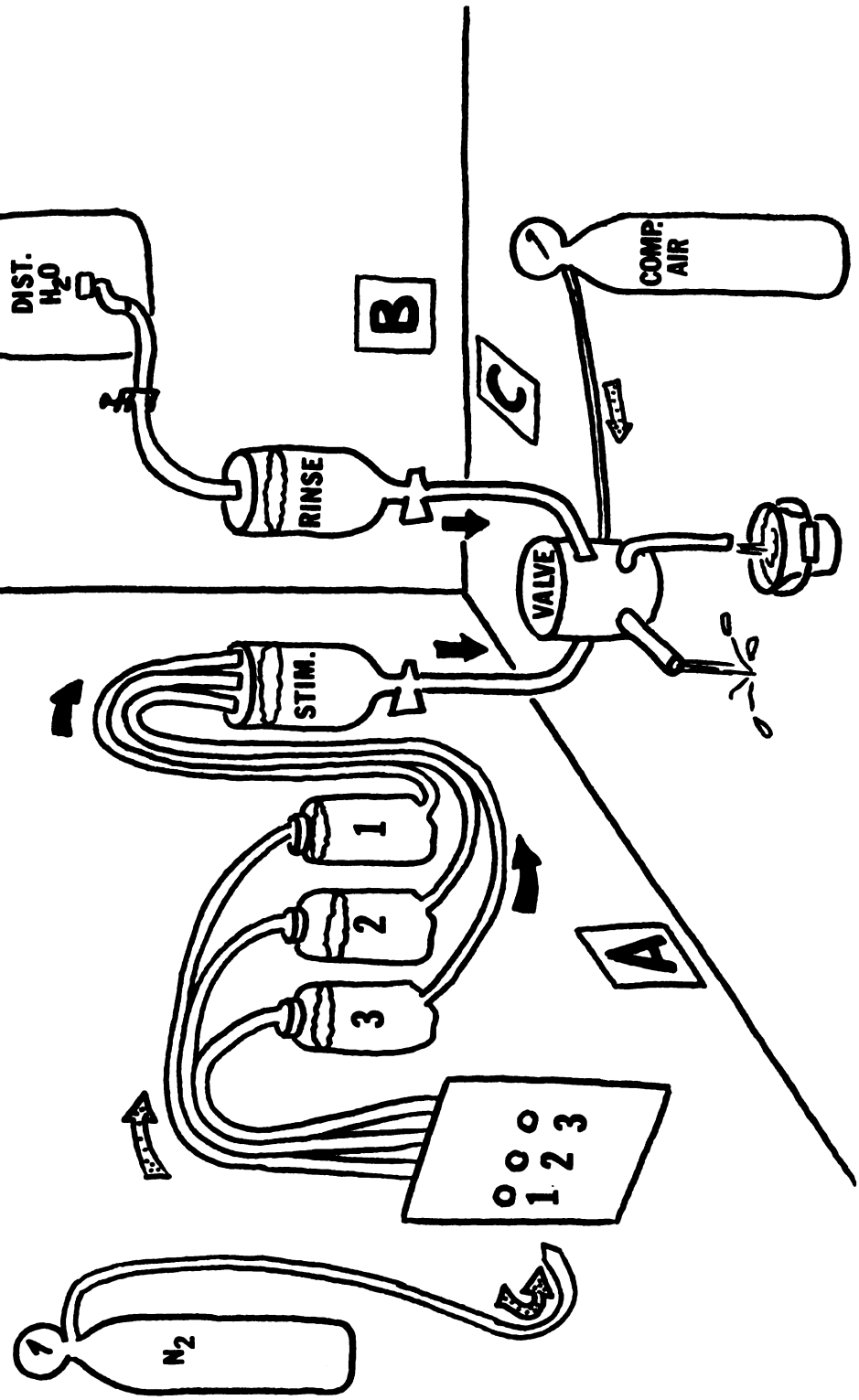


Figure 17.

record of each test (20 second test plus 5 second pre- and post-stimulus rinse) from the magnetic tape. The action potential photographs were compared to the computer data analysis. The sine wave calibration signal was photographed for correlation between computer and photographic records (Figure 16).

Post-Experimental Computer Data Analysis, SAM-COUNT. The object of analysis was to determine the total number of action potentials from a single neuron that occurred during a test and to compare this to its pre-stimulus activity during an adapting water rinse. Post-stimulus impulse counts were also enumerated to observe whether activity was maintained into the rinse period or whether rinse responses occurred. Impulse interval analysis was beyond the computer program's ability.

A second computer program, written by Mr. Schneider, utilized the amplitude parameters of FREDSAM to define the responses attributable to an individual neuron. The operator analyzed the neuron's activity after the experiment by selecting an amplitude window that defined the particular neuron according to FREDSAM. The enumerator program, SAM-COUNT (an acronym for "spike amplitude-count"), used the timer pulses to allow the operator to enumerate the activity over partial or whole periods as: a) the number of action potentials in a complete period (between timer pulses), or b) the number that occur immediately before or after a period. The latter partial analyses could be for any predetermined length in seconds up to a complete period. SAM-COUNT rejected those potentials that were not included in the amplitude window defining the neuron. The SAM-COUNT listing presented the number

of potentials from the neuron within the pre-designated test or rinse intervals in their order of occurrence. The taped record of each experiment was played once for every unit under analysis. All counts were compared to the photographic record to 1) eliminate discernible artifacts of the same size as the unit under analysis, 2) to reassign potentials which occurred between the initiation of stimulus delivery and the arrival of the test solution at the tongue, and 3) to reassign those action potentials which occurred between the initiation and arrival of water rinse. (See below for the method of measuring the solution delivery time, and Appendix II, SAM-COUNT, Evaluation and Background.)

Graphical Analysis of Enumerated Response Activity

From the SAM-COUNT listing, the 20 second test activity was graphed with the stimulus concentration to determine the SR functions of the neurons. The 5 second pre- and post-stimulus activity was used to indicate the spontaneous activity level of the preparation and the rinse response magnitude, respectively. Longer evaluation of both pre- and post-stimulus periods would have been desirable but was not possible since the magnetic tape record was often only clearly obtained for 5 seconds before and after the tests. For the latter case, the rinse response magnitudes that were calculated from 5 second post-stimulus activity were not seriously altered from 20 second estimates for their response pattern was very phasic, adapting completely within that time. In the former case, the 5 second averages of pre-stimulus or spontaneous

activity levels for most tests with most neurons were 1 or zero impulses/ 5 sec. With this value, most responses of increased activity exceeded criterion levels. The mean spontaneous activity + SE are displayed with each graph of the SR functions along the ordinate by an arrow (for example, Figure 22). For the responses of decreased activity, this criterion value was too small for statistical evaluation. Accordingly, for units with low level spontaneous activity, the photographic records of the tests were used for evaluation of decreased activity responses as in Figure 20. On occasion, longer determinations of pre-stimulus activity were required for a second reason. In this case, the pre-stimulus activity levels were larger and/or the test series succeeded a particularly effective stimulus and the test counts were suspected to reflect only the lingering activity from a preceding large response. In this case, 20 second pre-stimulus counts were obtained on separate runs with SAM-COUNT when the tape record allowed. Each interval of pre-stimulus activity that was used in analysis is noted with the graphs in the Results section.

Response Latency

Measuring Delivery Time. The timer pulses identified only the onset of events which led to the delivery of solutions to the tongue. Several hundred milliseconds were required for the compressed air to reposition the air valve, for the next solution to flow through the delivery tube aimed at the tongue and for the solution to contact the lingual surface. Although the delivery time varied across experiments, it remained constant during each, since the delivery system and animal's

head were securely clamped in position. The circuit in Figure 18B was employed to measure the exact delivery time. A Grass SD9 stimulator (Quincy, Massachusetts) supplied 50 mV, DC current through two leads (one placed in the stimulus reservoir, the other placed directly on the receptive field of the neural unit being examined). The circuit remained open as long as distilled water flowed through the system to the tongue. When an electrolyte replaced distilled water, the circuit was completed and the oscilloscope displayed a change in potential (Figure 18C). The timer pulse that marked the onset of flow of conducting solution (0.1 M NaCl) also triggered the oscilloscope sweep. The time required for the trace to begin its deflection thus represented the stimulus delivery time. The mean of 2-3 determinations was measured from a photograph of the oscilloscope display. Delivery time determinations were made after the experimental tests to avoid any effects of current on the gustatory response.

Conduction Velocity. The calculated latency values were not corrected for conduction velocity and no conduction velocity determination was made during the experiments. The time of conduction was estimated to be negligible considering the diameter of the fibers found in the glossopharyngeal nerve trunk. The myelinated fiber diameters were found to range from 1-15 μ, with a median of 3μ (Samanen, Kryda, and Bernard, 1975). This roughly corresponds to conduction velocities of 6-90 m/sec, with a median value of 18 m/sec. A maximum estimate for the distance between the tongue and recording electrode is 2.5 cm which would have required less than 5 msec for the smallest fibers.

Figure 18. Measurement of solution delivery time. In A, wire leads L_1 and L_2, placed in the stimulus reservoir and on the tongue, act as contact points of a switch in circuit B. The circuit, which includes the oscilloscope, V, and a 50 mV power source, is completed by redirecting the 0.1 M NaCl solution onto the tongue. The oscilloscope trace, C, triggered with the initiation of saline delivery, shows a change in potential (white arrow) at 280 msec identifying the arrival of the solution.

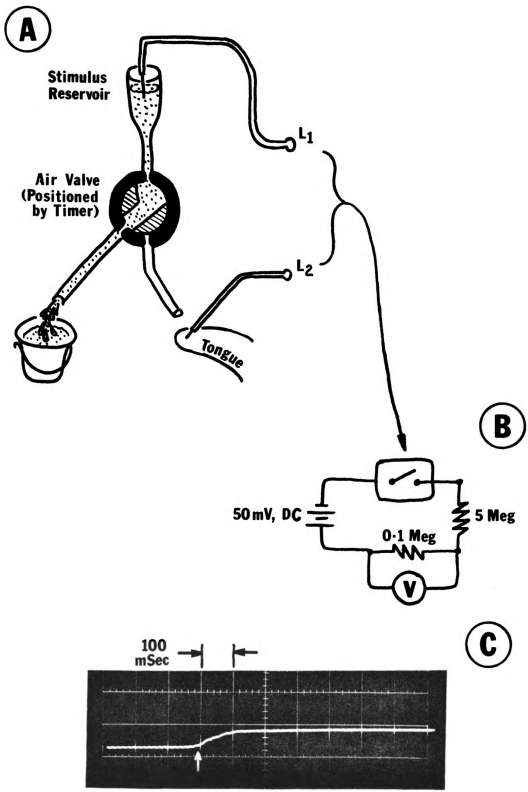

A
Stimulus Reservoir
Air Valve (Positioned by Timer)
L1
L2
Tongue
B
50 mV, DC
5 Meg
0·1 Meg
V
C
100 mSec

Figure 18.

Moreover, this small error was constant for any given unit and could only be a factor in inter-neuronal comparisons, analysis which was not attempted from these experiments.

Determining Physiological Latency. Response latency was determined from the photographic record by subtracting delivery time from the interval of time between the timer's onset pulse and the first action potential shown on the photograph. This remaining period of time was taken as the true physiological latency. Action potentials that occurred after the timer pulse but within the delivery time were subtracted from the computer counts of the response. Similarly, action potentials occurring between the rinse timer pulse and rinse delivery were reassigned to the test period from the computer's count of the post-stimulus rinse period.

V. RESULTS

The responses of 34 electrically isolated lingual neurons are described below. Of these, 14 were identified during the experiment by the action potentials that were seen on the oscilloscope occurring in response to chemical and tactile stimulation. These possessed receptive fields (20-40 mm^2) within different portions of the gustatory receptive field of the whole glossopharyngeal nerve (see Figure 4). Post-experimental analysis of their activity with FREDSAM revealed that 11 records were actually of multiple neural units (a range of 2-4 individual neurons in each record, with a mean of 2). The activity of the companion neurons of smaller diphasic amplitude had not been clearly observed during the experiment from the oscilloscope display. Nonetheless, their electrical isolation and chemoresponsiveness showed them to be acceptable and their responses were also evaluated with individual analysis with the SAM-COUNT program. Seven other experimental records were deemed acceptable during the experiment and even after testing by the evaluation of their photographic records. Yet the FREDSAM review of their tape recorded activity showed them to be poorly isolated and they were rejected from further analysis. The mean amplitude of the extracellular recorded action potentials of the isolated neurons ranged from 14-139.5 μV, with 27 of the 34 neurons having a more restricted range of 14-76 μV.

Spontaneous Activity

The afferent neurons could be grouped according to three different levels of spontaneous activity. The first and most common group was made up of 29 gustatory units which showed low rates or no spontaneous activity (0 impulses/5 sec for 657 of 844 tests). Only one neuron had a zero spontaneous activity level before each test. The inter-trial spontaneous levels for the other fibers varied with stimulus and/or concentration and ranged from 0-13 impulses/5 sec (0-2.6 Hz). The mean spontaneous activity over all tests of the 29 units in this group was 0.0125 impulses/5 sec.

The second and third groups of neurons displayed high levels of spontaneous discharge (1.25-30.00 impulses/5 sec). The second group consisted of fibers from a more posterior branch of the glossopharyngeal nerve. The activity of this branch was of a continuous irregular pattern (see Figure 19A). In one experiment, three neurons fired at rates of 24.3, 10.9 and 4.8 impulses/5 sec. The origin of this discharge is unknown and could not be altered by chemical, thermal, or mechanical stimulation of the tongue. Other lingual stimulation or stimulation of other oral or extra-oral regions was not attempted. Nonetheless, these may be considered a class of lingual afferent fibers since the recording was always from the severed distal portion of the branch. Of the 34 units recorded in the experiments, three fibers were of this class. Many more might have been studied but not being responsive to gustatory stimulation, they were abandoned when encountered in later experiments.

Figure 19. Activity and responses of glossopharyngeal afferent neurons.

A. Continuous spontaneous discharge at high rates from a posterior lingual branch. Three units are identified, V (whose activity is shown across the record), 2, and 3. These fired at rates of 24.3, 10.9 and 4.8 impulses/5 sec and were not affected by lingual stimulation.

B. The response of three gustatory neurons, a, b, and c, to 0.1 M quinine hydrochlorine (QHCl). The arrow identifies the onset of the stimulus cycle, presenting quinine after distilled water rinse. The response pattern is phasic, adapting to pre-stimulus levels in 10 sec (not shown).

C. The response of several gustatory neurons to 0.3 M NaCl (two larger units, I and II are identified by their numbers). The arrow coincides with the onset of the stimulus cycle. After a two second latency, the response builds gradually to maximum.

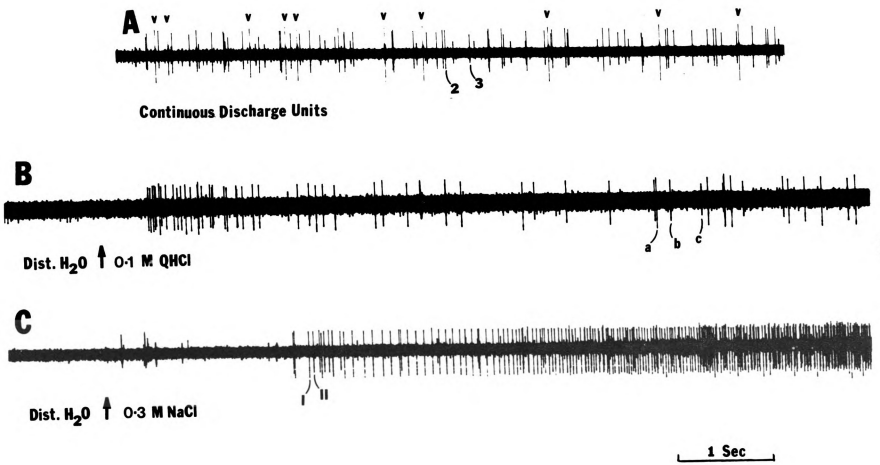

A
Continuous Discharge Units
2
3
B
Dist. H_2O
0·1 M QHCl
a
b
c
C
Dist. H_2O
0·3 M NaCl
I
II
1 Sec

Figure 19.

The third group of fibers fired at various pre-stimulus rates, which ranged from 0-14.75 impulses/5 sec. This group consisted of two companion fibers found in one experiment whose spontaneous activity declined throughout testing. The larger fiber fired initially at 12.75/5 sec, declined to a steady range of 3.5-8 impulses/5 seconds between tests, and then dropped to zero spontaneous discharge and became unresponsive after 18 tests. The smaller neuron showed a similar decline, but started at a lower rate (3.5 impulses/5 sec) and remained responsive through all tests despite its declining spontaneous discharge. Despite their unique occurrence, their responses are reviewed and discussed below, since they epitomize certain forms of gustatory response occurring in more typical taste fibers at drastically lower frequencies.

Forms of Gustatory Response

The three previously reported forms of gustatory response were observed: 1) increased activity upon stimulation, 2) decreasing activity upon stimulation, and 3) increasing activity with water rinse after stimulation. The most common response was increased activity upon stimulation which occurred with different impulse patterns. Even without impulse interval analysis, this could be judged from the photographic records. Regular bursting was not obviously seen. Peak response frequencies usually occurred with response onset but in some cases they increased gradually over many seconds (see Figure 19C). The largest response was 344 impulses/20 sec. A more typical response was 10-30 action potentials during the 20 second test period.

For the common gustatory unit with low level of spontaneous activity, the second form of response (depressed activity) was clearly observed in only one of 29 neurons. This resulted in part, because of the usually low level of spontaneous discharge and the limited ability to discern accurate criterion levels of spontaneous discharge from the taped records. (The five second determinations of pre-stimulus activity disallowed setting more exact response criterion levels.)

Depressed activity responses were abundant in two gustatory units with high level spontaneous activity (Figure 20A). The larger unit showed only depressed activity responses. The neuron with smaller amplitude action potentials showed both forms of response (increased and decreased activity). Its activity increased on presentation of stronger concentrations of HCl and NaCl. Its activity was depressed with sucrose and low concentrations of HCl.

Rinse responses were present in both of these neurons and were most obvious for both units after sucrose tests, both also showing activity depression to sucrose application. On water rinsing after sucrose, both fibers showed rebound activity which increased above pre-stimulus levels (Figure 20A, lower record). Rinse responses were also present for four of other units: for two neurons, only after sucrose; for one neuron, after HCl and NaCl; and for its companion, after HCl, NaCl and quinine hydrochloride. Rinse responses occurred after positive responses to the stimulus for the latter two neurons, and after periods of no activity for the former two. Figure 20, A and B, compares the rinse responses after depressed activity for a neuron with high level

Figure 20. Depressed activity and rinse responses.

A. (Upper) Two neurons (1 and 2) with relatively high levels of spontaneous discharge under distilled water rinse, responded to sucrose stimulation with complete suppression of activity.

(Lower) At the end of sucrose stimulation (record from A is continued) the neurons responsed to the water rinse with discharge above pre-stimulus levels.

B. (Upper) The response of a unit with low level spontaneous discharge (one action potential during pre-stimulus rinse) similarly responded with no activity during sucrose stimulation.

(Lower) The continued record shows that the water rinse caused a response far above the pre-stimulus activity level.

Thus the pattern of responses in A and B is exactly parallel, with B showing far lower pre-stimulus and rinse response rates.

Thus the pattern of responses in A and B is exactly parallel, with B showing far lower pre-stimulus and rinse response rates.

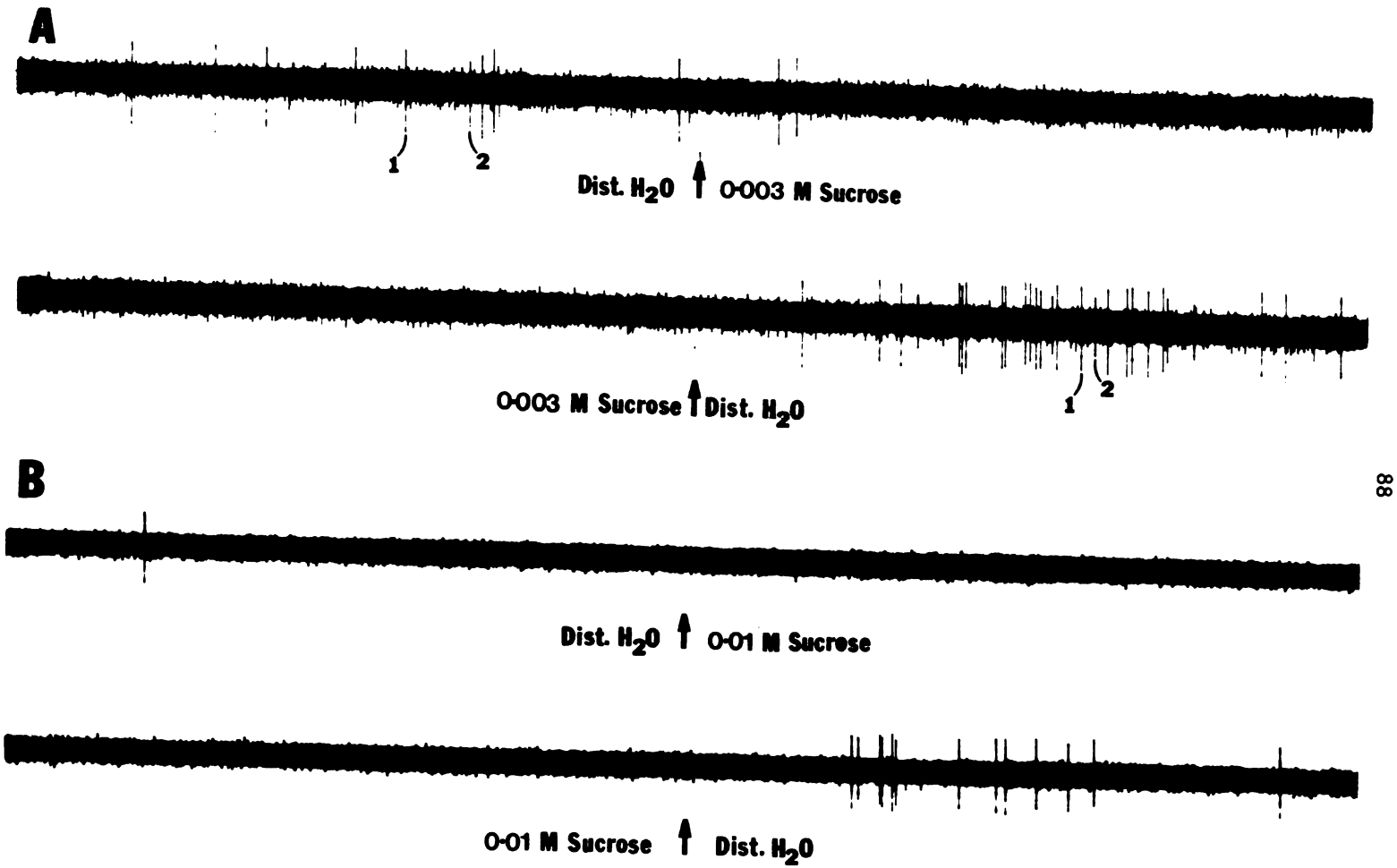


Figure 20.

spontaneous discharge with a common neuron with low level spontaneous discharge that gave a rinse response after no activity during the application of the stimulus.

Gustatory Stimulus-Response (SR) Functions

Neurons with Low Level Spontaneous Activity. More than one type of SR function was observed for the neurons. Examples of each form appear in Appendix IV and in Figures 22-24. The data were obtained using non-replicated tests in order to accomplish the extended series of tests within the critical life-span of a preparation, usually one hour, before desiccation or other factors altered the neurons spike amplitude or responsiveness. Accordingly, neither the slope nor the regularity of the SR functions could be determined statistically. With lack of rigorous statistical determination, many SR functions were judged to be indeterminate on the basis of their complex and irregular graphs, some of which were seen to fit an overall positive or negative trend (e.g., see Appendix IV, Figure IV-1, quinine hydrochloride test series for neuron 21.1). Others were very complex and were also judged to be of indeterminate form (Appendix IV, Figure 39). Of 29 units, 28 had at least one definable SR function (not including the two units of high level spontaneous activity). For the 29 common gustatory neurons with low level spontaneous discharge, 155 SR functions were examined, of which 67 were of identifiable form, 59 were indeterminate, and 29 were of no response (see Table II, with "Gustatory Latency Functions" in this section).

The gustatory neurons with definable functions most often (39 of 67 definable functions or 39/67) had positively sloped SR curves. Of these, 19/39 had the form of the whole nerve's SR function, having curves with positive slopes. Their responses showed a direct relationship to concentration, with larger responses being obtained with greater stimulus concentration. In Figure 21 is the photographic record of this type of response. Figure 22A is the SR function of this form (and Appendix IV, Figure 40). Saturation at the stronger concentration was occasionally seen (e.g., Appendix IV, Figure 40, unit 23.3).

Four neurons gave smaller responses with progressively more concentrated stimuli, i.e., their SR functions had negative slope. An example is shown in Figure 23A (open triangles) (and Appendix IV, Figure 41). For one fiber, the decreasing responses to the quinine test series occurred after a previously large response to 0.3 M NaCl (64 impulses/20 second test). To ensure that the declining responses for quinine were not solely due to the decaying activity of this previous test, 20 seconds of pre-stimulus activity was subtracted from each of the responses. The remaining activity still formed a negative function but of somewhat less regular slope (Figure 23A, filled triangles).

Positive and negative SR functions were also combined over a concentration series (for 10 of 39 neurons with positive SR functions), giving rise to a U-shaped function. The greatest responses were from the greatest concentrations. Examples are shown in Figure 24 (A and C) and Appendix IV, Figure 42. Three examples of the reverse combination

Figure 21. Gustatory responses with a positive SR and negative latency function. Three successive stimulations with increasing concentrations of HCl caused both greater response magnitude and shorter latency for the large unit identified by the inverted "Y's". The form of the SR and latency functions for the background units is not as regular.

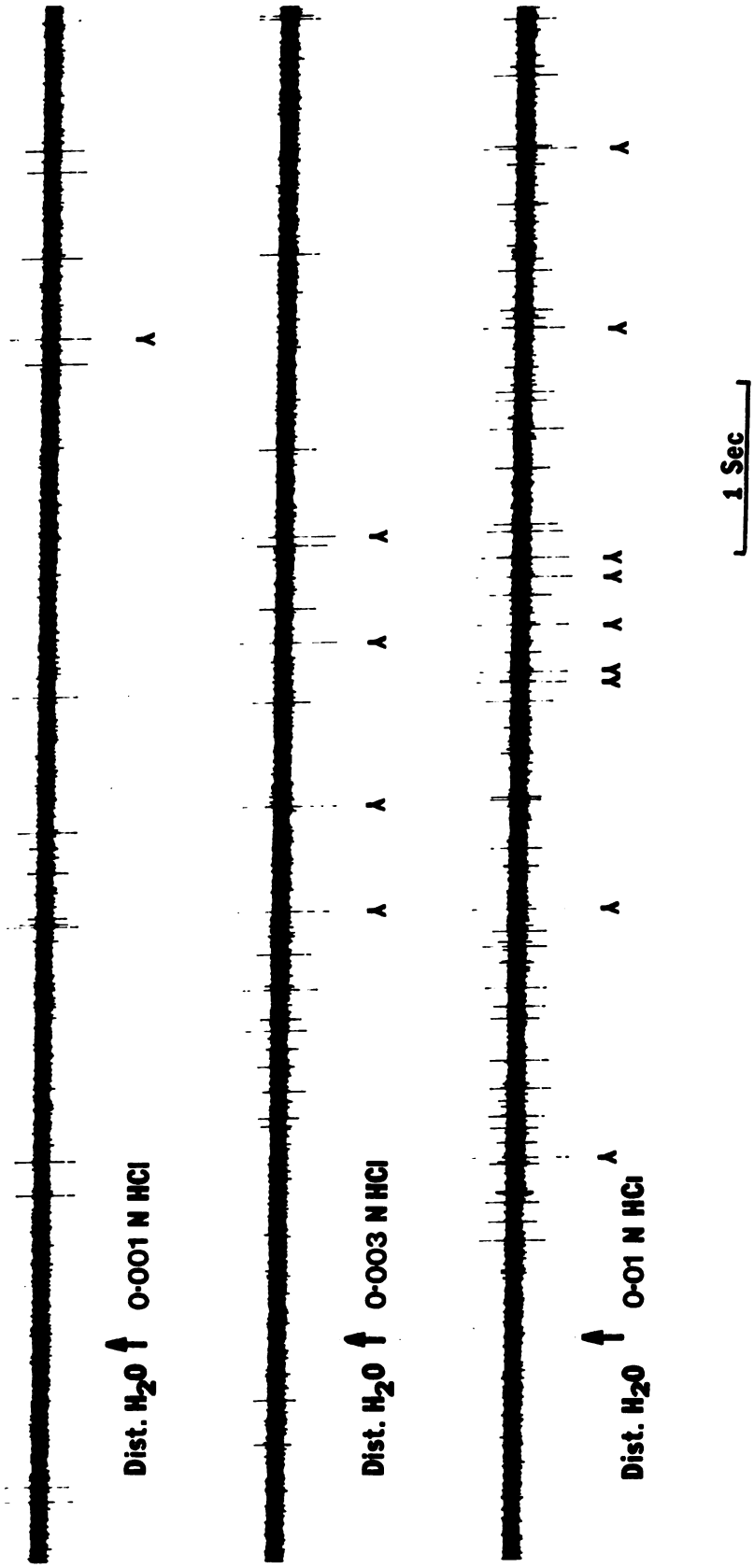


Figure 21.

Figure 22. Regular forms of SR and latency functions. A, C, E, and G, are graphs of gustatory SR functions with positive slope (A) and zero sloped SR functions (C, E, and G). The regular latency functions defined for the same neurons were of negative slope (B), zero slope (D), negative slope (F) and positive slope (H).

For this and Figures 23, 24, and 39-44 the following symbols are used:

Key: -●-●- = responses to HCl.

-▲-▲- = responses to quinine hydrochloride.

-X-X- = responses to sucrose.

-■-■- = responses to NaCl.

▲ = the whole nerve's response threshold for the same stimulus, placed along the abscissa.

----- = latency values for no response (equivalent to a 20 sec latency).

⟶ = the criterion level of pre-stimulus activity (mean inpulses/5 sec + SE), placed along the ordinate.

Responses are given as 20 second averages unless otherwise indicated in the legend. Latency is scaled in seconds except for graphs with thickened ordinate axes or those that are set apart in an inset of the graphs. These will be noted in the legend (e.g., D, the latency function graph for HCl stimulation of unit 21.1 has ordinate expanded to display only one second.

All concentrations are Log functions.

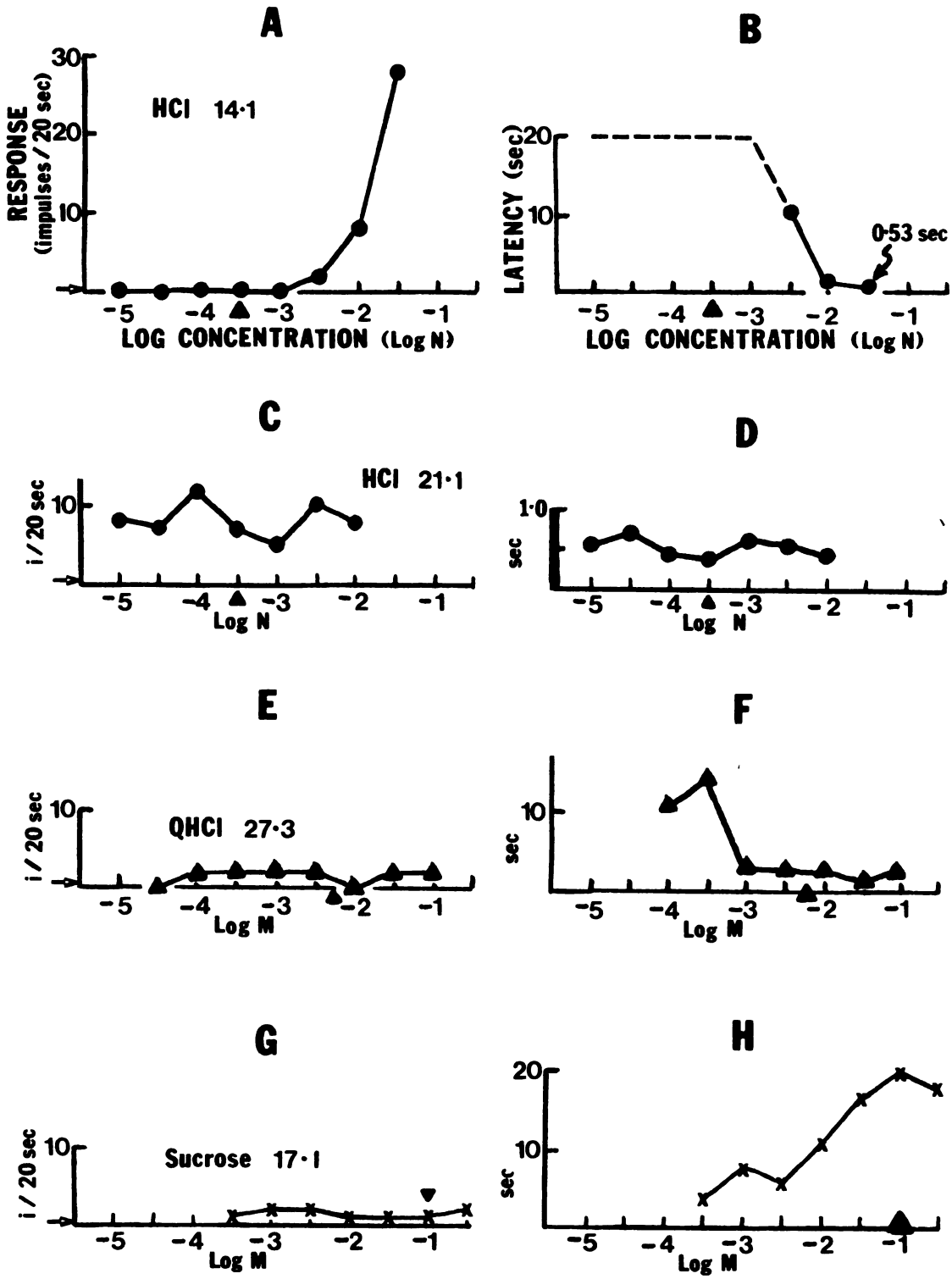

A
RESPONSE (impulses / 20 sec)
30
20
10
HCl 14·1
-5
-4
-3
-2
-1
LOG CONCENTRATION (Log N)
B
LATENCY (sec)
20
10
0·53 sec
-5
-4
-3
-2
-1
LOG CONCENTRATION (Log N)
C
i / 20 sec
10
HCl 21·1
-5
-4
-3
-2
-1
Log N
D
sec
1·0
-5
-4
-3
-2
-1
Log N
E
i / 20 sec
10
QHCl 27·3
-5
-4
-3
-2
-1
Log M
F
sec
10
-5
-4
-3
-2
-1
Log M
G
i / 20 sec
10
Sucrose 17·1
-5
-4
-3
-2
-1
Log M
H
sec
20
10
-5
-4
-3
-2
-1
Log M

FIGURE 22.

Figure 23. The negative SR and positive latency function of a unit.

A. The negative SR function of a neuron's responses to QHCl stimulation is corrected for declining activity from the preceding stimulus series by enumerating spontaneous activity over 20 seconds (filled triangles) as well as for the normal 5 second interval (open triangles). The negative slope persisted with either analysis.

B. The latency function for the same unit is of positive slope.

Symbols are the same as in Figure 22. (The dashed line represents the latency for no response, 20 sec.)

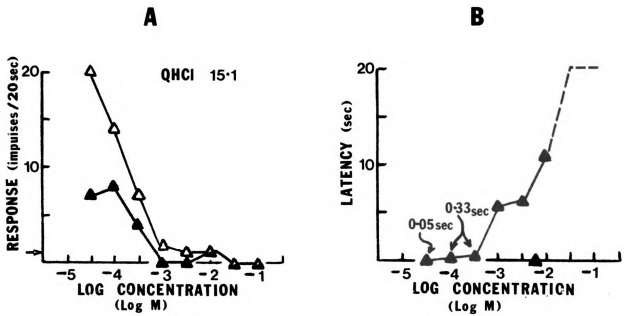

A
B
QHCl 15·1
RESPONSE (impulses/20sec)
LATENCY (sec)
20
10
0·33sec
0·05sec
−5
−4
−3
−2
−1
LOG CONCENTRATION
(Log M)

FIGURE 23.

Figure 24. U-shaped SR functions and bell-shaped latency functions. These are shown for two different units in response to HCl and NaCl stimulation. Notice that the SR minima and latency function maxima fall within 0.5 Log unit of the whole nerve threshold. Symbols are the same as in Figure 22.

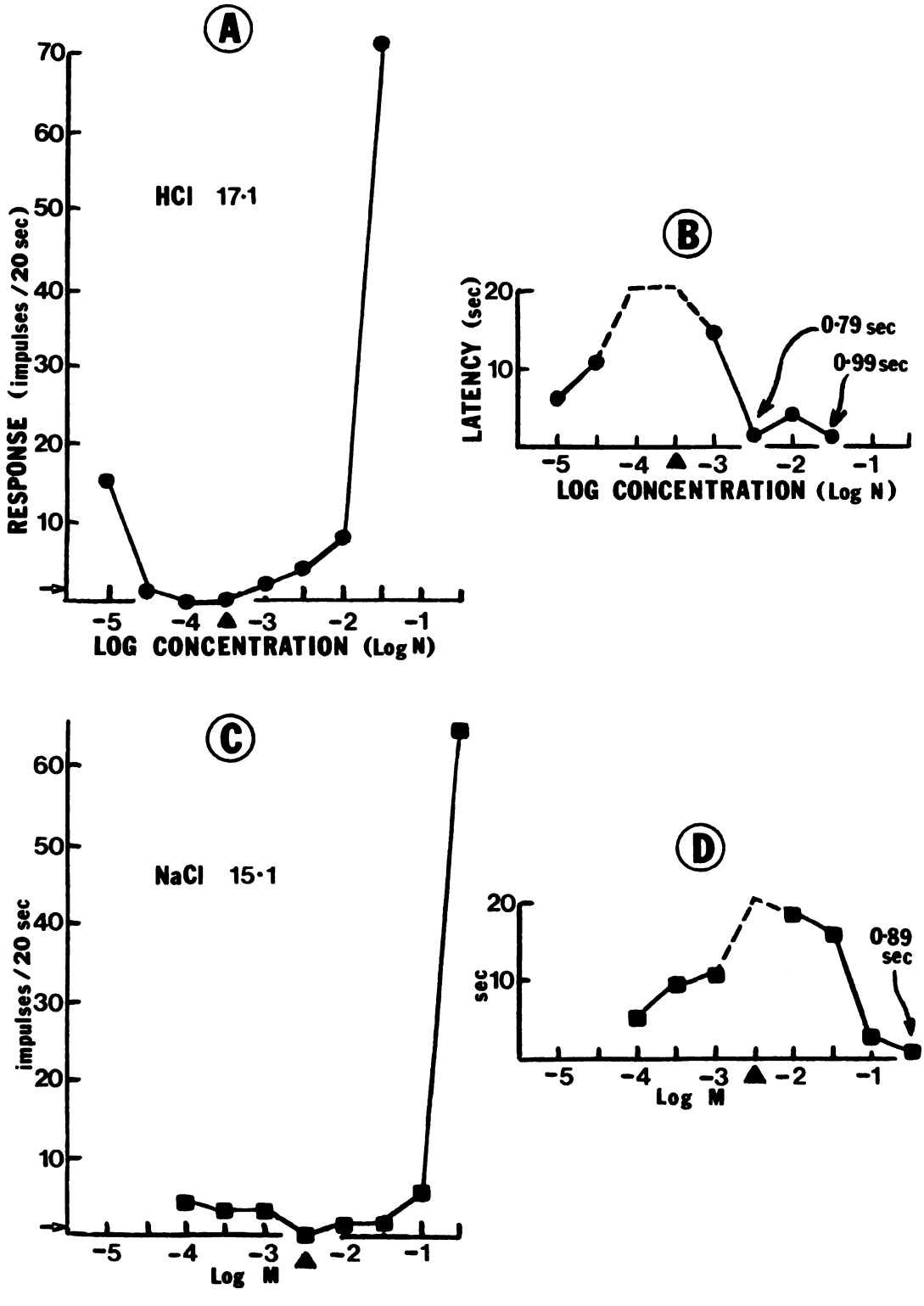

A
HCl 17·1
RESPONSE (impulses / 20 sec)
70
60
50
40
30
20
10
-5
-4
-3
-2
-1
LOG CONCENTRATION (Log N)
B
LATENCY (sec)
20
10
0·79 sec
0·99 sec
-5
-4
-3
-2
-1
LOG CONCENTRATION (Log N)
C
NaCl 15·1
impulses / 20 sec
60
50
40
30
20
10
-5
-4
-3
-2
-1
Log M
D
sec
20
10
0·89 sec
-5
-4
-3
-2
-1
Log M

FIGURE 24.

(positive and negative SR functions) forming a bell-shaped function were found. For two units this occurred with sucrose stimulation, for a third with HCl stimulation (Appendix IV, Figure 42, unit 24.2, sucrose tests).

Stimulus-response functions of zero slope were not unusual (12 of 67 functions with defined slopes). These are seen in Figure 22 (C, E, and G) and Appendix IV, Figure 41. The evidence that these are true gustatory response functions is as follows: 1) their response magnitude is large (2X to 8X above criterion levels) in several cases; 2) these responses were chemo-specific, since other stimuli produced different response functions, and 3) several tests of other chemicals gave responses that were much smaller or zero. This indicates that these were not small responses from mechanical or thermal stimulation which had been minimized by experimental controls.

Neurons with High Level Spontaneous Activity. The stimulus-response functions for the two neurons (units 29.1 and 29.2) with high level spontaneous activity were largely indeterminate. For one test series, the form was clearly recognizable, the responses of unit 29.1 to sucrose forming a U-shaped function (Figure 25) with the minimum representing the greatest depression of activity. In part, this occurred because the pre-stimulus activity level rose and fell through the series. However, the activity during depression was not constant, but decreased with each concentration over the first half of the series and increased over the last half. This is depicted in Figure 25A by the line graphs representing the pre-stimulus activity,

Figure 25. Gustatory responses of units with high level spontaneous activity.

A. The responses of unit 29.1 to sucrose are with depressed activity over the entire series. A line graph is drawn for each concentration. The left limb of the line graph at each concentration is the pre-stimulus activity level, the center is the test activity, and the right limb is the rinse response. (All activity levels are averaged over 20 sec.) The rinse response is seen to be greater than pre-stimulus activity except for the rinse response following 0.3 M sucrose.

B. The SR function for unit 29.1 ("net response" in the graph) is U-shaped. It arises in part because of changing pre-stimulus activity levels ("V" curve) but also with the U-shaped test activity levels (middle curve of inverted v's).

C. The SR functions to NaCl for unit 29.2 (also with high level spontaneous activity) is complex. It includes both depressed activity (at the weakest concentrations) and increased activity responses at greater concentrations. Squares are the net NaCl stimulus-response function (test activity minus pre-stimulus activity).

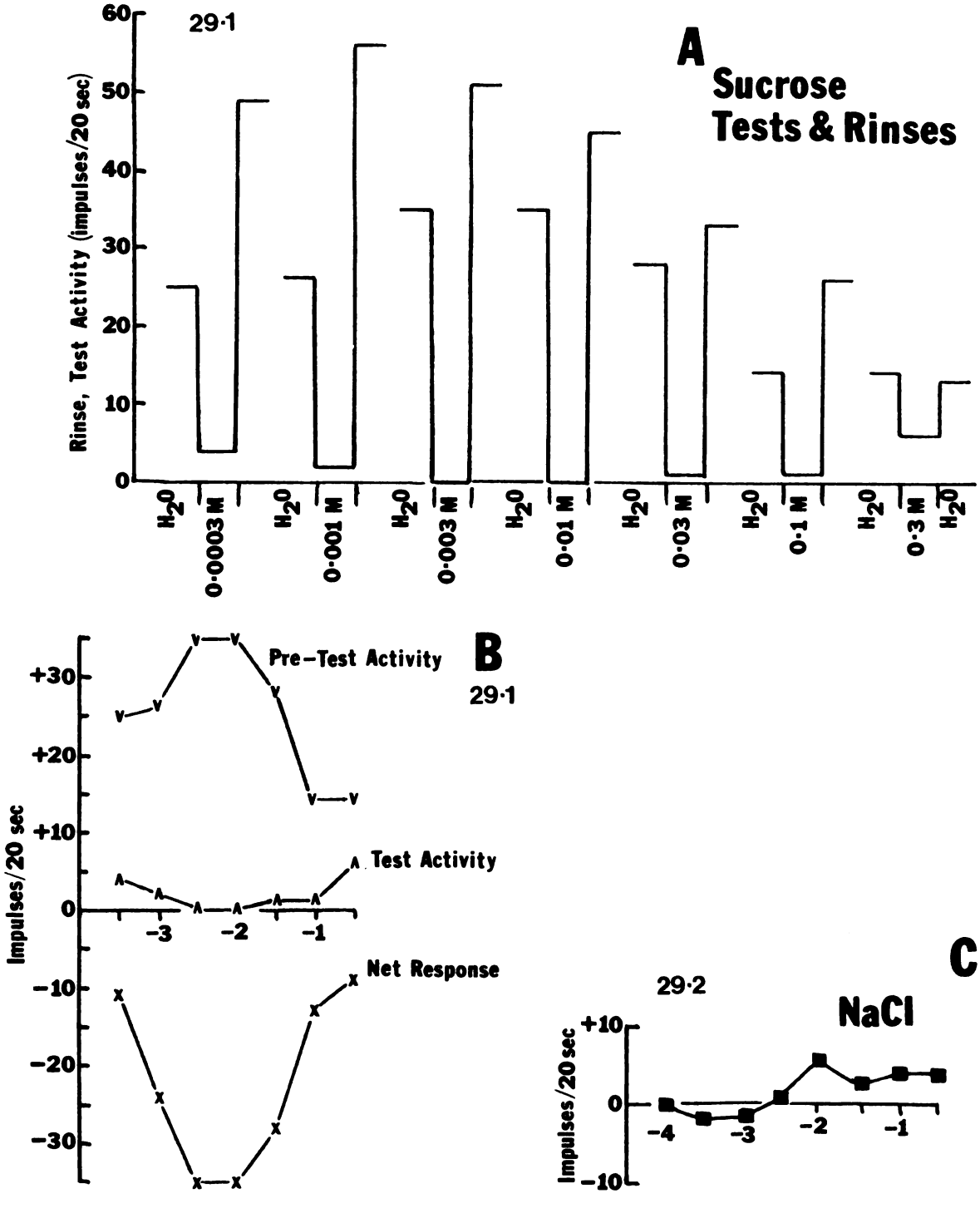

29·1
A
Sucrose
Tests & Rinses
Rinse, Test Activity (impulses/20 sec)
60
50
40
30
20
10
0
H2O
0·0003 M
H2O
0·001 M
H2O
0·003 M
H2O
0·01 M
H2O
0·03 M
H2O
0·1 M
H2O
0·3 M
H2O
B
29·1
Pre-Test Activity
Test Activity
Net Response
Impulses/20 sec
+30
+20
+10
0
-10
-20
-30
-3
-2
-1
C
29·2
NaCl
Impulses/20 sec
+10
0
-10
-4
-3
-2
-1

Figure 25.

response, and post-stimulus activity for each test. In Figure 25B, the SR curve ("net response") the pre-stimulus activity curve, and the curve showing test activity during stimulation for unit 29.1 are plotted to show their relationship (responses of SR curves = activity during stimulation minus pre-stimulus activity). The SR curves of unit 29.2 were more complex and less regular. The sucrose tests all caused activity depression. The HCl tests formed an irregular function with weak depression for the two lowest concentrations, with activity increasing for the greater HCl concentrations. The NaCl SR function (Figure 25C) was similar to that of HCl, with no response or very weak depression at the lower concentrations and increases only for the greater concentrations, tending to a zero slope over the last 1.5 Log range.

Rinse Response SR Functions. As noted above, six neurons exhibited rinse responses after one or more of the four classes of stimuli. The rinse responses were averaged over only five seconds. This does not seriously alter their comparative magnitude, for their response pattern was very phasic, adapting completely within that time. The property was chemo-specific for two fibers and broadly distributed in others (e.g., one unit gave rise responses to NaCl, HCl, and quinine hydrochloride, but not to sucrose). Two types of rinse responses were observed. The first type occurred after activity depression by the test stimulus (see Figure 26). Units 29.1 and 29.2 had high level spontaneous activity and responded with clear activity depression to the sucrose stimulus. Unit 21.1 also shows activity depression but is less obvious since the pre-stimulus activity was low. For all three

Figure 26. Rinse response SR functions. These are shown for four units, two with high level, pre-stimulus activity (29.1 and 29.2) and two with the more common low level, pre-stimulus activity (19.1" and 21.1). In some cases, rinse responses followed activity depression by the test stimulus (the solid line, test response, is below the pre-stimulus activity dotted lines, for 21.1, 29.1 and 29.2). The second type of rinse response occurred following positive responses (increased activity) to the test stimulus. (The solid line for 19.1" to NaCl is above the dotted line.) In all cases the rinse response (dashed lines) were of greater magnitude than the pre-stimulus activity or test responses.

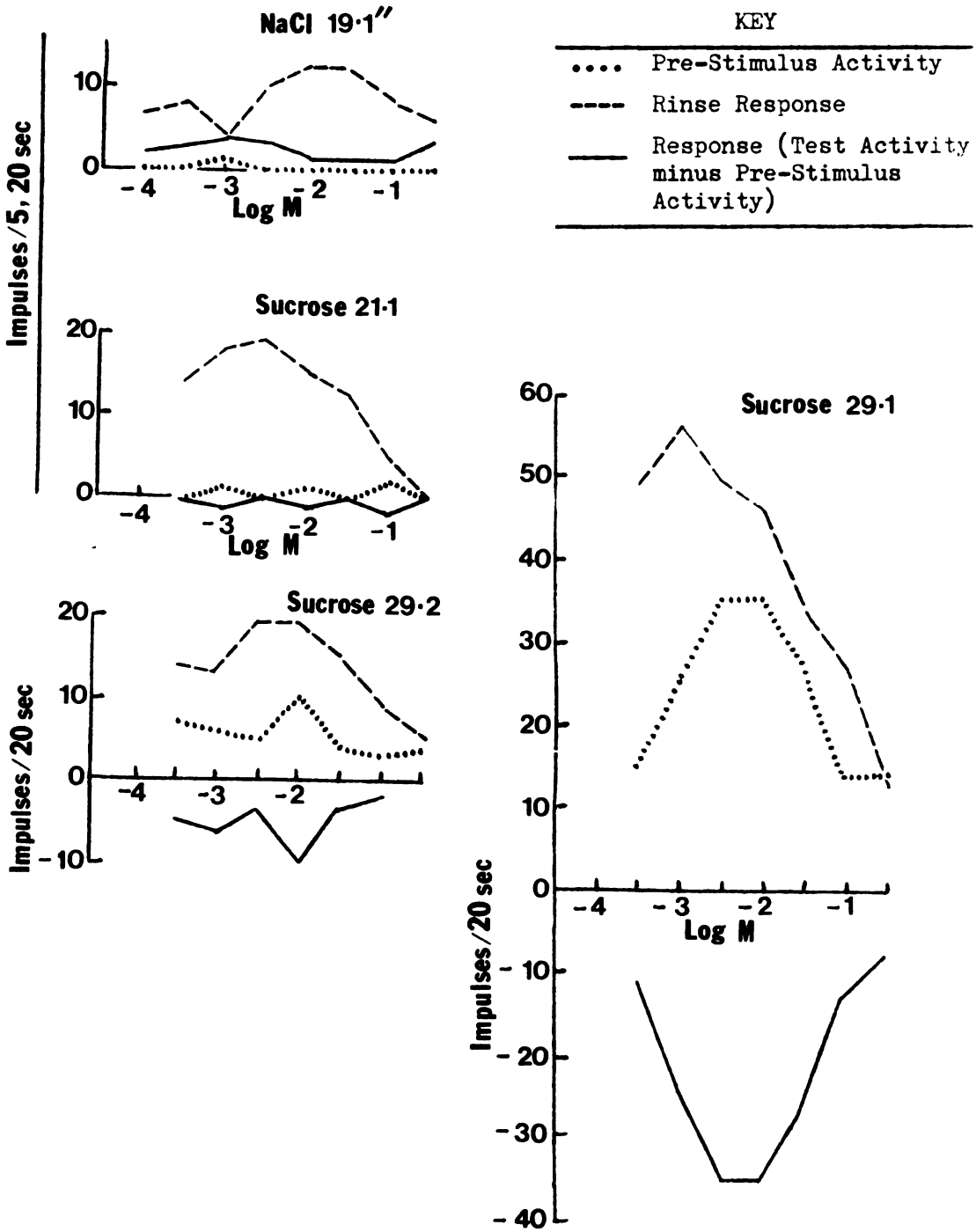

KEY
Pre-Stimulus Activity
Rinse Response
Response (Test Activity minus Pre-Stimulus Activity)
NaCl 19·1″
Sucrose 21·1
Sucrose 29·2
Sucrose 29·1
Impulses/5, 20 sec
Impulses/20 sec
Log M

Figure 26.

units, the rinse response not only increased activity to pre-stimulus levels but resulted in a rebound far above initial rates. The rinse response to unit 19.1" represents the second type of response, in which the neuron gave a positive response to the stimulus and was followed by rinse responses greater than test and pre-stimulus activity levels. The SR functions for the rinse responses to sucrose were bell-shaped (Figure 26, units 21.1, 29.1, and 29.2).

Gustatory Latency Functions

The physiological latency of the taste responses occupied a wide range, from 4 msec to nearly the entire test period, 19.75 sec. Figure 27 shows the relationship between the magnitude of the gustatory response and its latency. Larger responses tended to occur with shorter latencies. However, this relationship is variable (6 responses with a magnitude greater than 10 impulses/20 sec had latencies longer than 5 sec) and at best describes only a limited correlation. The smallest responses had latencies that ranged over the full test period (10 msec to 19.75 sec). The latency functions were of varied form, which helped to clarify the wide variation between the latencies of small and large responses.

As with the SR functions, several latencies functions had complex slopes (69 of 155 latency functions, Table II). These varied from those with a generally positive or negative trend to forms that were too complex to define by slope.

The most frequently encountered form was the negative latency function (30 of 57 latency functions with definable slopes), in which

Figure 27. Response magnitude versus latency. The latency (sec) and response magnitude (impulses/20 sec) for all tests are presented in the graph. Data follow no single consistent relationship but are limited by the inverse relation between latency and response magnitude. Thus while the larger responses tend to have shorter latencies, the smaller responses (1-2 action potentials) occur over the full range of latencies.

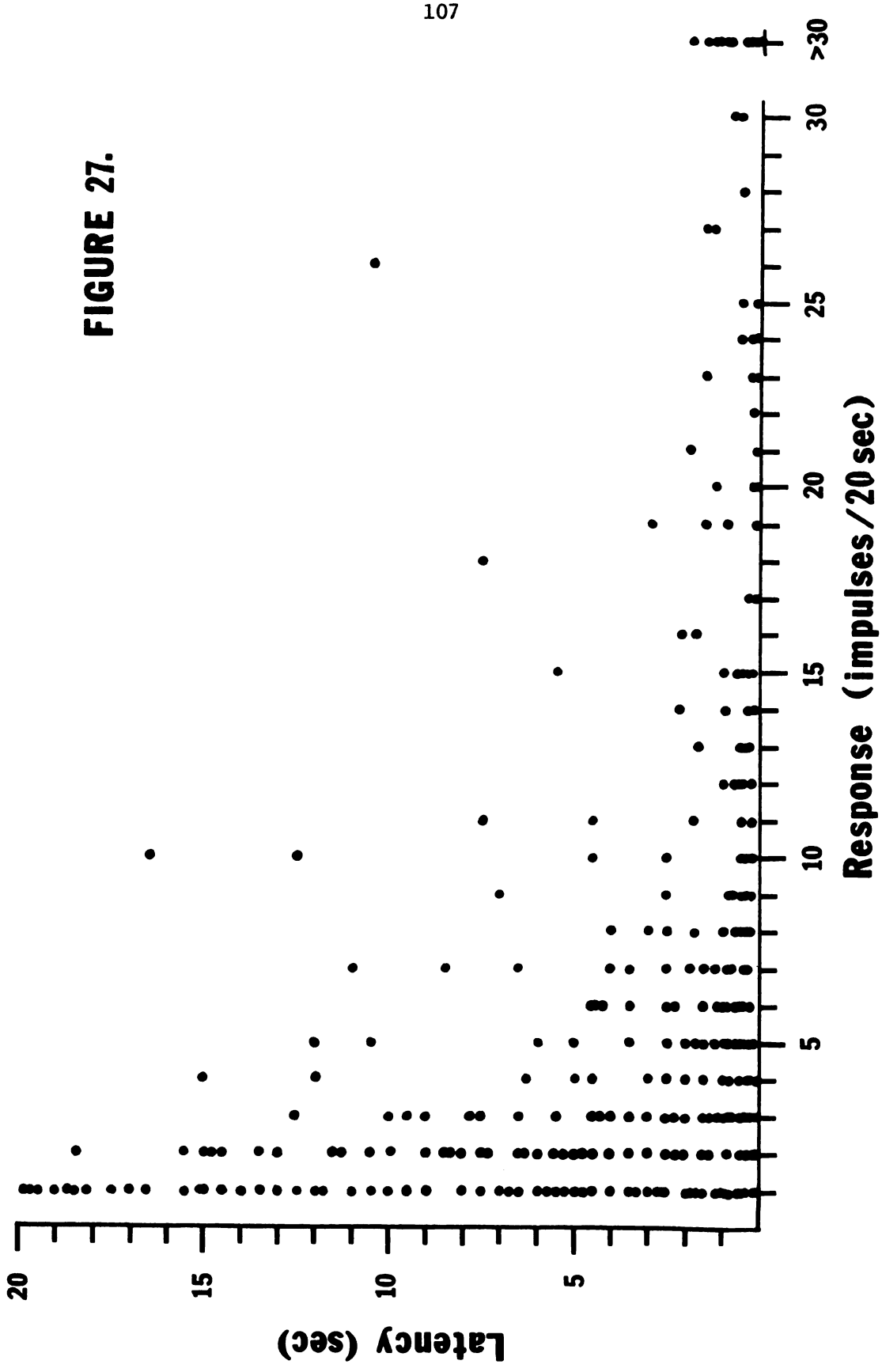

Latency (sec)
20
15
10
5
Response (impulses/20 sec)
5
10
15
20
25
30
>30

FIGURE 27.

Table II. Distribution of SR and latency functions.

						Total SR
	-SR -L	-SR OL	-SR +L	-SR IL		
	0	0	12	4		16
	OSR -L	OSR OL	OSR +L	OSR IL		
	2	4	2	4		12
	+SR -L	+SR OL	+SR +L	+SR IL		
	21	1	0	17		39
	ISR -L	ISR OL	ISR +L	ISR IL		
	7	2	6	44		59
Total L	30	7	20	69	/	126
	No Response =					29
						155

Above are the numbers of SR and latency (L) functions observed during the experiments on 29 gustatory neurons. Classification of the functions was determined for a complete test series (e.g., as +SR) or for partial series when two forms were combined over the whole series (e.g., -SR combined with +SR). The symbols +, 0, -, and I refer to the slopes of the functions as determined graphically (positive, zero, negative, and indeterminate). The two gustatory units with high level spontaneous activity are not included in this distribution and are considered separately in the text. The dashed line separates those pairs of SR=latency functions that are graphically defined from those that have an indeterminate relation to concentration.

the latencies declined regularly from several seconds (for responses to the weakest stimulus) to several hundred milliseconds or less as concentration increased. Figure 22B shows one such curve. Notice that graphical extension of latency to its most extreme value does not greatly alter the curve (20 seconds = no response). Other examples of negative latency are seen in Appendix IV, Figure IV-5.

The opposite form, a positive latency function, was also observed. As seen in Figure 23B, the latency increased regularly from 50 msec for the most dilute solution, to several seconds, to no response with increasing concentration. Other examples are in Appendix IV, Figure 43. A combined slope function also occurred, in which positive and negative latencies combined to form a bell-shaped function, with the maximum represented by one or more responses of infinite latency (see Figure 24, B and D and Appendix IV, Figure 44).

Unexpectedly, several examples were found in which latency remained unchanged with concentration, i.e., functions with zero slope. For these curves, individual latencies varied slightly about an average value with no discernible trend to positive or negative over the 3.5 Log concentration range. Figures 22D and Appendix IV, Figure IV-5 are examples of the zero slope latency function. For many of these, the mean latency value was 500 msec. Exact statistical determination of the slope was not possible.

The rinse responses had two regular types of latency function. Most had zero slope. The other type had a positive latency function for the rinse responses to sucrose.

Latency functions were not calculated for the suppressed activity responses or for the rinse responses of the units with high level spontaneous activity. In these cases, the exact onset of either response was ill-defined, especially for low concentrations where depression was mildest. The onset of depression was not an immediate drop to the average lower level of activity but rather a gradual decline, which showed in the photographic records as an increase in the duration of the interspike intervals. Without interval analysis, the onset of suppression could not be accurately determined.

Combined SR-Latency Functions

It was possible to clearly define both SR and latency functions for a test series in many gustatory neurons (42 of 126 functions in Table II) (see Figures 22, 23, and 24). Figure 22 A and B shows the most typical combination, a positive SR function with a negative latency function. For these, greatest stimulus concentration resulted in the largest gustatory response occurring with the shortest latency. Weaker concentrations resulted in smaller gustatory responses with latency of seconds, or caused no response (equivalent to a 20 sec latency). The exact opposite SR-latency functions were also observed (Figure 23). The largest responses had the shortest latencies at the weakest concentrations. Notice that in both combinations, response magnitude and latency are inversely related. U-shaped SR functions were usually found with bell-shaped latency curves. This can be seen in Figure 24. Minimum responses had maximum latencies.

Three types of latency function accompanied SR curves of zero slope: 1) a zero slope latency function, in which a response of relatively invariable magnitude occurred at the same time after stimulus onset (e.g., 500 milliseconds) over the 3.5 Log concentration range, 2) zero slope SR functions also occurred with negative latency functions, and 3) positive latency functions occurred with zero sloped SR functions. These were the least common, occurring only twice each.

Thus a family of taste responses in which each type is defined by combined latency and SR functions was developed for gustatory neurons. These are diagrammed in Figure 28. The three blocks at the top represent three successively greater concentrations of a stimulus, separated by a continuous distilled water rinse. Each type of response is presented in a separate row with vertical lines representing action potentials. Figure 28A shows the most typical type of response in which greater responses occurred with shorter latency as concentration increased ("+R, -L"). In B the response is the inverse of A, i.e., the greatest responses occurred with the shortest latencies at the weakest concentrations ("-R, +L"). In C, D, and E responses of unchanging magnitude occurred over the range of concentrations with response latencies that shortened ("OR, -L"), remained constant ("OR, OL"), or lengthened with greater stimulus strength ("OR, +L"). In F and G rinse responses occurred at the same time after water rinse onset ("Rinse, OL") or at progressively longer latencies as stimulus concentration increased ("Rinse, +L"). Finally, for the units with relatively high level of spontaneous activity, responses of depressed activity occurred (H, I) in which the degree of depression increased ("+R") or decreased

Figure 28. Gustatory responses defined by SR and/or latency function. Three tests of stimuli of successively greater concentration, separated by distilled water rinse, are represented by blocks 1-3. A family of nine different gustatory responses was defined by specific combinations of their latency and SR functions. These are illustrated by the rows of vertical lines which represent action potentials.

A-E. Responses of increased activity on stimulus presentation. +R, -R, and OR are the slopes of the SR functions and +L, -L, and OL the slopes of their latency functions. These occurred in the combinations shown in the figure. (See Table II for their distribution among the tests.)

F and G are rinse responses which occurred with constant latency (OL) or latency that increased with greater concentration (+L).

H and I are responses of depressed activity. These were observed mainly from the units with high level prestimulus activity. These also occurred in combination with a rinse response on application of the post-stimulus distilled water rinse (represented in the figure by the closer spacing of lines after the tests).

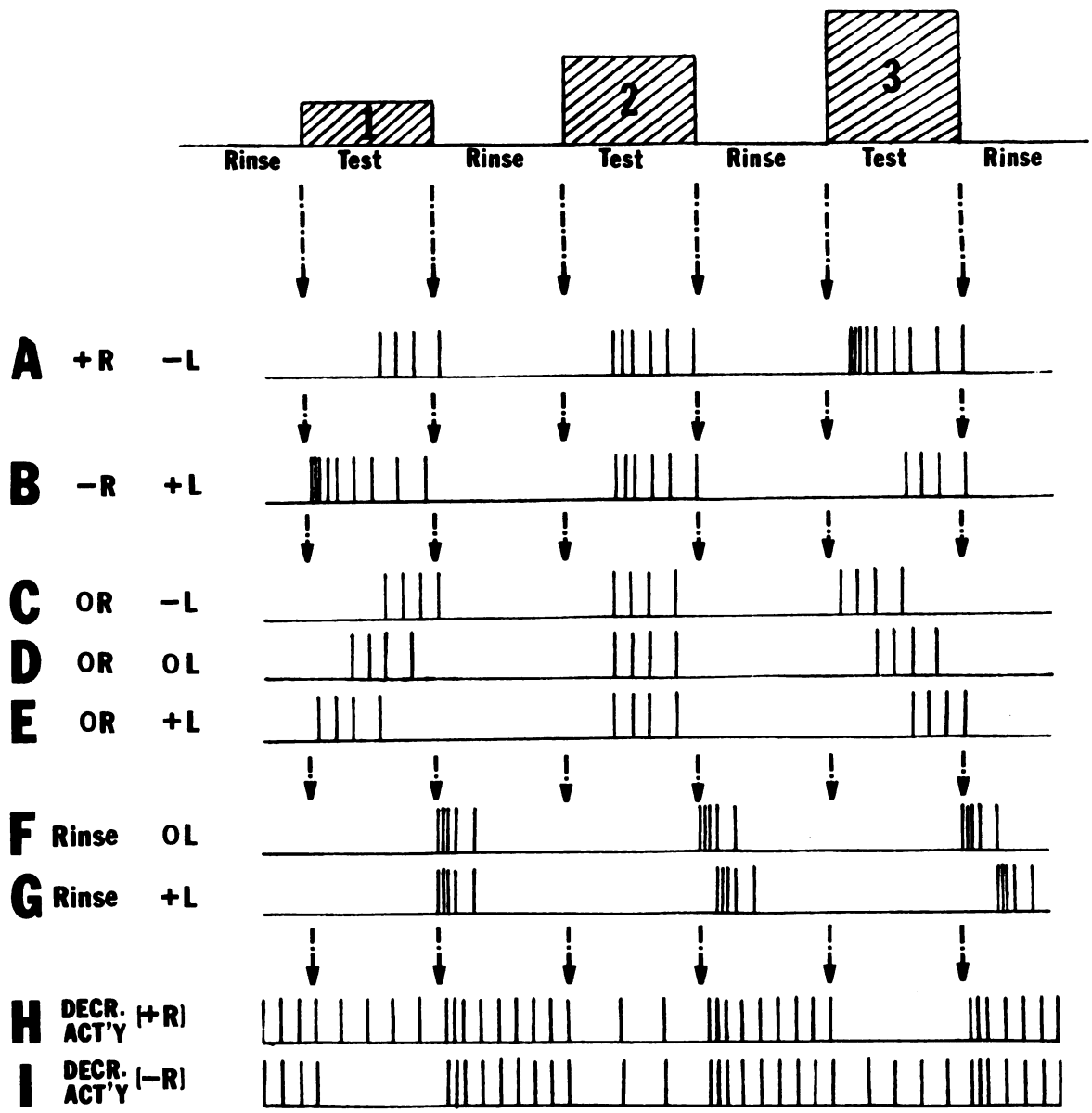

1
2
3
Rinse
Test
Rinse
Test
Rinse
Test
Rinse
A +R −L
B −R +L
C OR −L
D OR OL
E OR +L
F Rinse OL
G Rinse +L
H DECR. ACT'Y (+R)
I DECR. ACT'Y (−R)

FIGURE 28.

("-R") with greater stimulus strength. These two forms frequently occurred at opposite ends of the concentration range and each was followed by a rinse response.

Individual gustatory neurons exhibited a variety of the responses described above. Typical examples are shown in Figure 29. Unit 17.1, for example, was a less versatile unit. It exhibited low spontaneous activity levels, no suppressed activity nor rinse responses. Its strongest response occurred to HCl, for which it formed a U-shaped SR function and bell-shaped latency function. It responded to sucrose with only one or two action potentials with a positive latency function. It was not responsive to NaCl and only weakly so to quinine. Unit 15.2 gave greatest responses to NaCl. For the HCl stimulation series it responded with a negative SR function. The responses to quinine and sucrose do not conform to a regular SR function but are above the spontaneous activity levels. None of its latency functions is clearly determinate. Unit 21.1 responded with relatively constant SR and latency functions to HCl at all concentrations. To quinine stimulation its magnitude function was less regular while its latency function stabilized to constancy in the last half series at greatest concentrations. To sucrose, its responses resembled complete activity suppression though its pre-stimulus activity was too low for such decreases to be obvious. Its NaCl responses were relatively weak and indeterminate for both latency and SR functions. Finally, unit 29.2, one of the two units with high level spontaneous activity responded positively and strongly, but irregularly, to the HCl series, after initial activity

Figure 29. Combined SR functions to the four primary stimuli for several neurons. Both regular and indeterminate SR functions occurred for four different neurons. (H = HCl, N = NaCl, Q = quinine hydrochloride, S = sucrose, and Sr = rinse responses after sucrose tests for unit 21.1) 17.1, 15.2, and 21.1 were units with low level pre-stimulus activity of 0.268, 1.594, and 0.210 impulses/5 sec averaged for the four tests (not shown). The responses for unit 29.2, a neuron with high level spontaneous activity, were determined by subtracting the 20 second pre-stimulus discharge from the 20 second test response. The latency functions (not shown) in many cases served to further define the unit's responses (as described in the text).

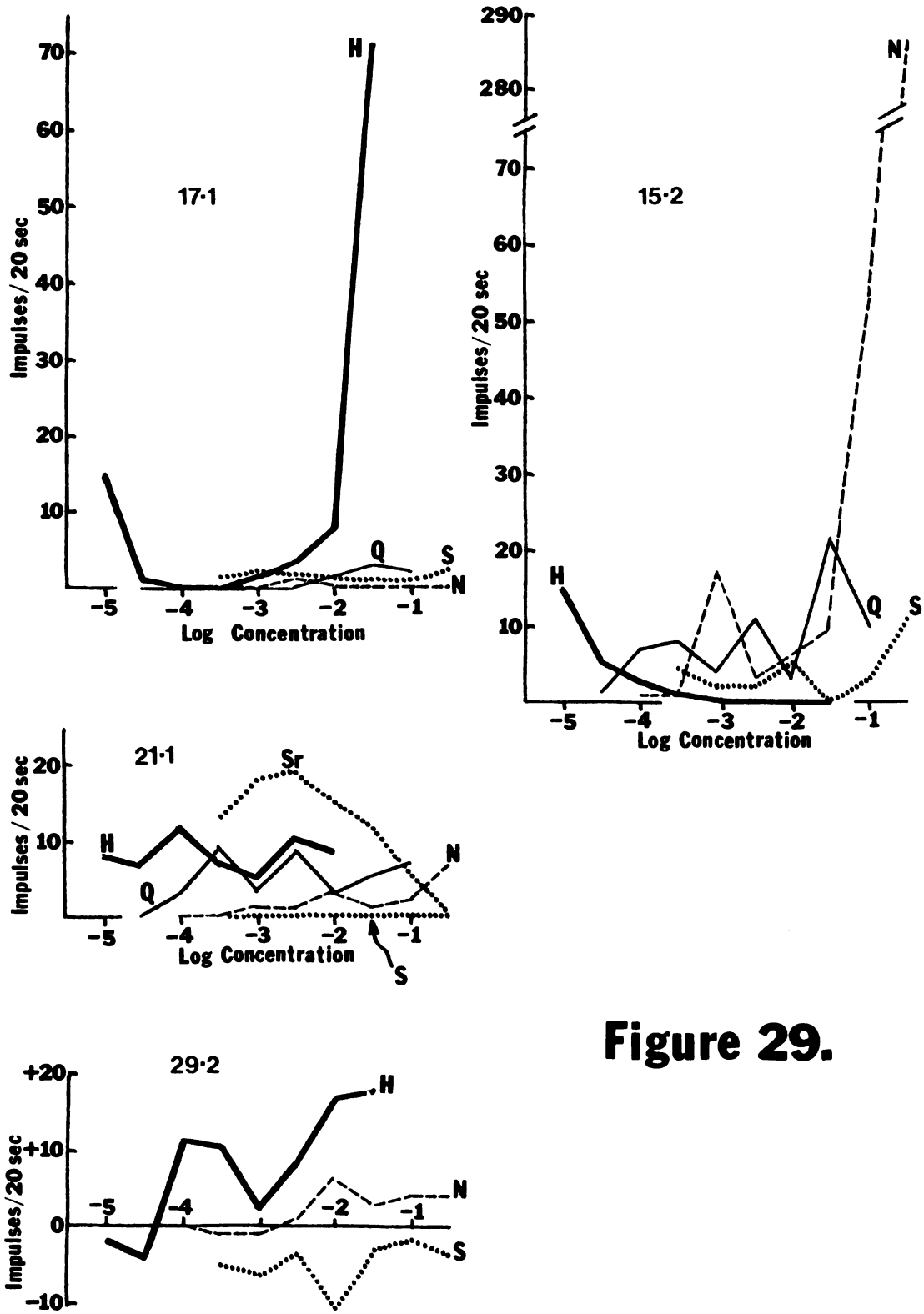

17·1
H
Q
S
N
Impulses / 20 sec
70
60
50
40
30
20
10
-5
-4
-3
-2
-1
Log Concentration
15·2
290
280
70
60
50
40
30
20
10
21·1
Sr
20
10
29·2
+20
+10
0
-10

Figure 29.

depression for the two lowest concentrations. To sucrose, its spontaneous activity was consistently depressed but to varying degrees. To NaCl it showed initially, no response or weak depression until the test concentrations above the whole nerve threshold for which its SR function stabilized to constancy. As mentioned previously, its latency functions were not obvious without interval analysis.

Mechanical and Thermal Responses

All target neurons (identified during the experiment as responsive to gustatory stimuli) exhibited tactile responses. Nonetheless, in all experiments, gustatory stimuli were tested even when tactile responses were absent. No neurons responded to gustatory and not mechanical stimulation. However, the possibility of non-mechanosensitive gustatory units must remain open, some chemosensitive neurons with smaller amplitude action potentials were discovered during computer data processing, for which tactile responsiveness had not been adequately tested during the experiment.

Fourteen out of 20 gustatory neurons responded to lingual cooling. A majority of the sensitive fibers responded to cooling both from room temperature to 2-4^{o} C and from hot water (50-60^{o} C) to room temperature (21-23^{o} C). Most units responded best to one of the two cooling modes. Of the thermally sensitive gustatory units tested with both, 6 to 11 responded more to 2-4^{o} C after room temperature water rinse, 4 to 11 responded best to room temperature rinse after hot water, while one showed equal responsiveness to both cooling modes. Six units responded to the application of the hot water itself as well as to either or both

modes of cooling. The largest responses to cooling occurred after hot water rinse (38.7 impulses/20 seconds) followed by cold stimulation (23.1 impulses/20 seconds) and then hot responses (4.625 impulses/20 seconds). In most cases higher instantaneous frequencies were observed on the photographic records than were revealed by the 20 second average. Figure 30 (A-C) shows a unit whose thermal responsiveness is greater in the direction of cooling from room temperature. Figure 30D shows a second cold sensitive neuron which responded to the application of hot water. This fiber responded best to cooling to room temperature from hot water rinse.

Figure 30. Thermal responses of gustatory neurons.

A-C are the thermal responses from three successive tests for one neuron. A is the response to cold water (4° C after room temperature distilled water, 22° C). In B, the neuron gave no response to hot water (49° C) after room temperature rinse. C, room temperature rinse after the previous application of hot water causes a response of lesser magnitude with a longer latency than in A.

D. Some neurons responded to the hot water stimulus (55° C) as is shown for a second neuron.

Arrows coincide with the onset of the stimulus cycle.

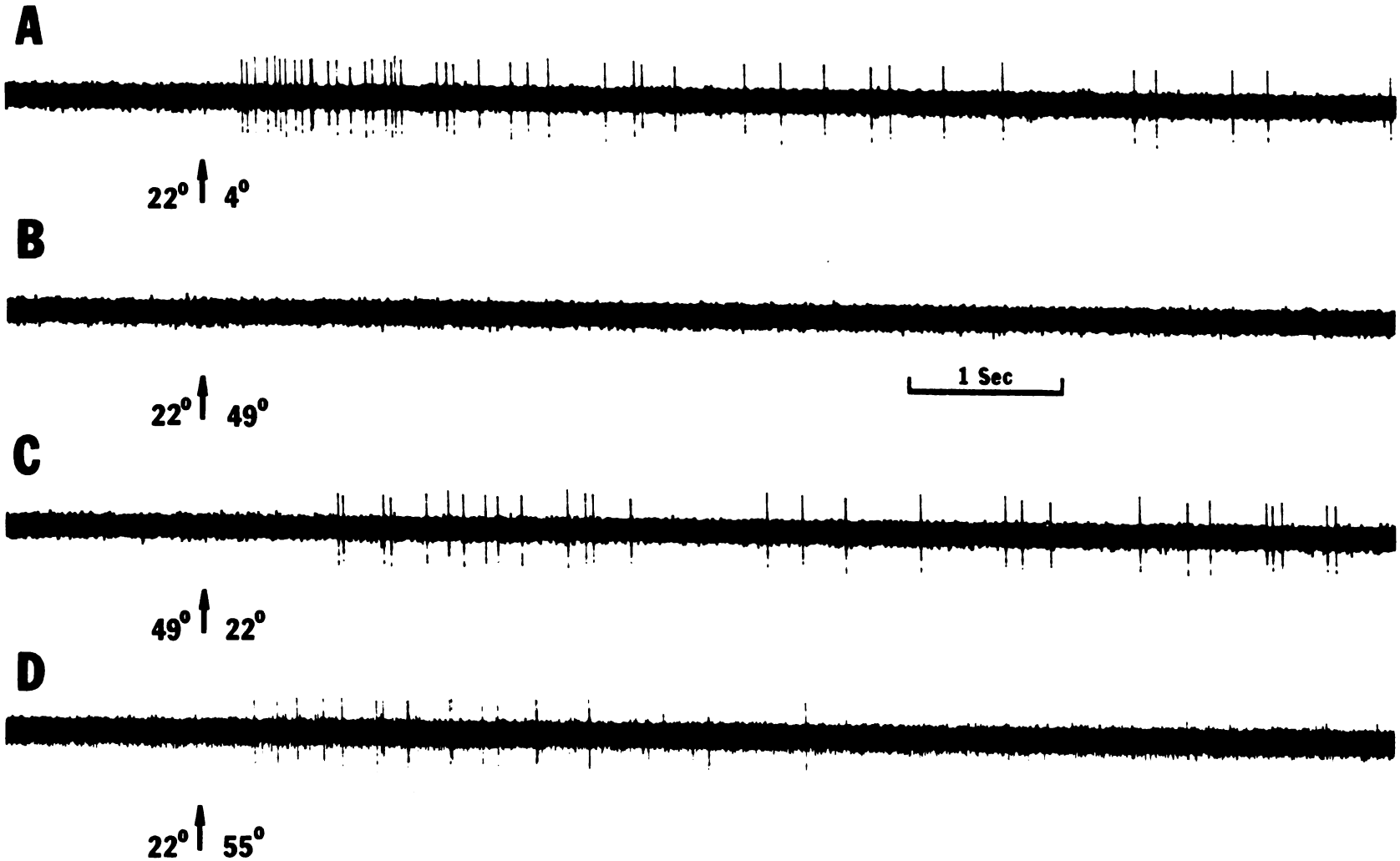


FIGURE 30.

VI. DISCUSSION

Mechanical and Thermal Sensitivity of the Gustatory Neurons

The mechanical and thermal sensitivity of the mud puppy glossopharyngeal neurons paralleled that of the frog, both classes of stimulus being effective for most of the fibers. The thermal sensitivity was not identical among all fibers. The gustatory neurons may possess varying thermal response functions since many responded more greatly to one of the modes of cooling. The form of the thermal functions could not be determined using only the two broadly defined thermal stimuli.

The Expanded Gustatory Response

Considerations of Stimulus-Response (SR) Functions. The systematic investigation of the SR functions of the gustatory neurons of Necturus has revealed the type that have been observed in the past: 1) the classical SR function with a direct relation between stimulus concentration and response magnitude, 2) SR functions with an inverse relation between concentration and response, 3) SR functions with constant response magnitude with increasing stimulus concentration, and 4) SR functions with curves of both positive and negative slopes, combined as U-shapes and bell-shapes. The less common SR functions, such as those with zero or negatively sloped curves and U-shaped curves,

were more greatly represented among Necturus gustatory neurons than has been reported for other species. The U-shaped form may have been more frequently observed because of the testing of an extended range of stimulus concentrations, 1.5-2.0 Log M below the whole nerve threshold. The minimum responses of the U-shaped curves frequently occurred near the whole nerve threshold. The negatively sloping portion of the curve was often confined to the subthreshold range of concentrations.

The complete definition of the SR function for any of the four stimuli was not attained for the neurons. This would have involved the testing of several greater dilutions of the stimulus, approaching pure distilled water. The minimum dilutions were at least two Log units above this concentration (e.g., -5 Log N was the least concentrated solution of HCl tested and -7 Log N is equivalent to neutral pH). Moreover, the U-shaped functions often had responses from the most dilute stimuli that were much greater than the criterion water-adapted activity levels. For unit 17.1 (Figure 24A) the response to -5 Log N HCl was 17 impulses/20 sec. If solutions between -7 and -5 Log N HCl were tested, it could be expected that they would have risen from the pre-stimulus activity level of 1 impulse/5 sec to attain that observed magnitude.

The role of the SR functions was found to be incomplete in defining the taste response when considered alone. Other aspects of the taste response were revealed in specific relation to the SR functions.

Considerations of Latency Functions. Alone, the latency functions were as varied and complex as the SR functions. Many types were

observed across the sample population of neurons. Latency-concentration curves were found with zero slope and positive slope, as well as with the inverse relation between stimulus concentration and latency found by T. Sato (1976) and Ogawa et al. (1974). However, the latency functions were found in specific relation to the SR functions of the neurons.

Examination of Table II for the units with defined latency and SR functions (within the dashed line) shows missing combinations of latency and SR functions. For example, the +SR function was never observed with a zero or positively sloped latency function. This might have reflected sampling limitations only. On the other hand it served to emphasize that the latency function and SR function of the neurons were joined in specific combinations. Similarly, the reverse combination of -SR, + latency function was found to be nearly the sole combination for neurons which responded to stimuli of greater concentration with smaller magnitude responses.

The most revealing combination of latency and SR function was found for responses that were previously considered to represent the absence of a taste response, the zero sloped SR functions. Combined with constant latency, the neuron fired with the same magnitude after the same delay to all concentrations of the stimulus. This function potentially conveys information about stimulus onset. Moreover, this particular combination of functions in any neuron was seen for only one stimulus among those tested. This therefore represented information about the onset of a particular stimulus for that neuron.

In a similar manner, the latency and SR functions were found to combine specifically, to form five disparate gustatory responses.

The zero sloped SR function was also found with positive latency functions for some units, and with negative latency functions for others. Both of these combinations could convey information about the concentration of the stimulus in a novel manner, by varying latency as concentration increases. This is functional redundancy in the sense that many neurons relay intensity information by response magnitude (the + or - SR function). However, it is novel in that the information can be relayed with constant activity and with activity of a lesser amount.

Varied Response Forms. The various forms of the taste response, i.e., increased activity or depressed activity on stimulus presentation, or rinse responses, have also been demonstrated previously in other species as considered in the Literature Review. However, in the mud puppy taste neurons, the several response forms were clearly seen in the same neuron. One of the units with high level spontaneous activity epitomized this variety of response form. The unit's responses included increasing activity to HCl and NaCl as well as depressed activity to sucrose tests which were followed by rinse responses. These then, when common to a variety of neurons, and all arising from the same unit could be seen as distinct classes of responses. The depressed activity responses formed U-shaped SR functions with positive and negative slope. These were considered separately to define two additional types of gustatory response: decreased activity with a positive SR function, and decreased activity with a negative SR function. The rinse responses were seen to combine with either positive or negative latency functions and defined two additional types of gustatory response.

Taken together, SR functions, latency functions, and forms of the gustatory responses defined nine different types of response (Figure 28). The individual parameters joined in specific combinations and thus more completely defined the gustatory responses of the glossopharyngeal neurons of Necturus.

Multiple Chemosensitivity of the Taste Neurons

Many units exhibited multiple sensitivity within any one type of the nine responses, e.g., unit 28.2 gave increased activity responses with positive SR and negative latency functions to both quinine hydrochloride and HCl. On the other hand, few fibers were truly monogustatory, i.e., by responding with only one form of response and one type of SR and latency function to one chemical and being non-responsive to the other stimuli. For several neurons both multiple sensitivity and chemo-specificity were found. In this case the neurons displayed one of the nine response types to two or three of the stimuli but gave a distinctly different type of response to one other stimulus. The complete specificity to all four stimuli by distinct types of response to the four stimuli was not often observed (for only one of the 31 gustatory neurons). The relation more commonly observed was for the neuron to respond with less complete response chemospecificity. The unique feature observed with these fibers though, is that any degree of specificity can be conveyed by the different forms of response of a neuron.

In part the lack of more complete definition may have arisen with the complex or indeterminate forms of the latency and SR functions.

These forms are an enigma. They were found for 59 SR functions and 69 latency functions (of 126 SR functions and 126 latency functions). They might be simply considered to be complex functions occurring under the regular schedule of stimulation. However, they might also reflect an error in establishing the particular stimulation protocol, that too little inter-trial rinsing may have disallowed the system's return to baseline conditions. In this case it must follow that single neurons have quite different adaptation properties, for the schedule resulted in the complete definition of SR and latency functions for some neurons, and that the adaptation properties must vary among stimuli, since some neurons displayed a regular function to one stimulus and not to another.

Considerations of the Origins of the Taste Response

Considering the physical parameter of stimulus diffusion, only the positive SR function and negative latency function should be expected. The greatest stimulus concentrations should diffuse most rapidly through surface barriers such as mucus or saliva and arrive in greatest concentration at the receptor interface to most rapidly initiate the response.

A very different explanation is required for the reverse combination of functions, -SR, +latency, in which greater stimulus concentrations cause smaller, more delayed responses. This is even more apparent for the non-monotonic, U-shaped and bell-shaped latency functions. For these, the neuron responded equally and with nearly the same latency to extreme stimulus concentrations which were often more than two Log units apart. The classical model assumes that the action

of the stimulus molecule on the taste system is invariably excitatory, a consideration belied by the demonstration in several taste fibers of the depression of activity by chemical stimulation. It was also shown in these experiments that depression is dependent on stimulus concentration. These neuronal functions can be explained by postulating a concentration-dependent inhibitory action of the stimulus molecules. The exact form of inhibition is not suggested by these experiments. It may be by the diverse action of stimulus molecules at disparate receptor sites of the receptor cells of the taste buds as suggested by Ogawa et al. (1969). On the other hand it could involve more complex relations between many elements of the taste system, actions from other cells of the taste buds or from interactions between the neurons.

Single Unit Activity and the Whole Nerve Response

Given the varied forms of the SR and latency functions, it is not obvious that all of those sampled in the experiments could contribute to the responses of the whole nerve. For example, the SR functions of the whole glossopharyngeal nerve of Necturus are all regularly increasing functions for the four primary stimuli. To test this concept, the responses of the single neurons were summed for each test and then expressed as relative response SR functions as described in the Literature Review. All the individual responses sum to the SR functions shown in Figure 31. The curves for the four stimuli are comparably related to those from the whole nerve's responses (Figure 6). This parallels the findings of Ganchrow and Erickson (1970) who summed the

Figure 31. Summed SR functions of the four primary stimuli for 29 gustatory neurons of Necturus. These can be compared with the summated whole nerve SR functions (Figure 6). Responses to each stimulus concentration are expressed as a percent of the response to 0.1 M NaCl. Baseline activity was determined from the 5 sec pre-stimulus activity for all tests (increased 4X). The inter-quartile range of the water baseline is shown from the ordinate (arrows). The summed responses form regularly increasing SR functions despite their widely varying individual forms. The summed HCl (H) and sucrose (S) SR functions are in close parallel to the whole nerve functions. NaCl (N) and quinine hydrochloride (Q) differ in their slopes and thresholds from the whole nerve functions but are in the same relation to the HCl and sucrose functions.

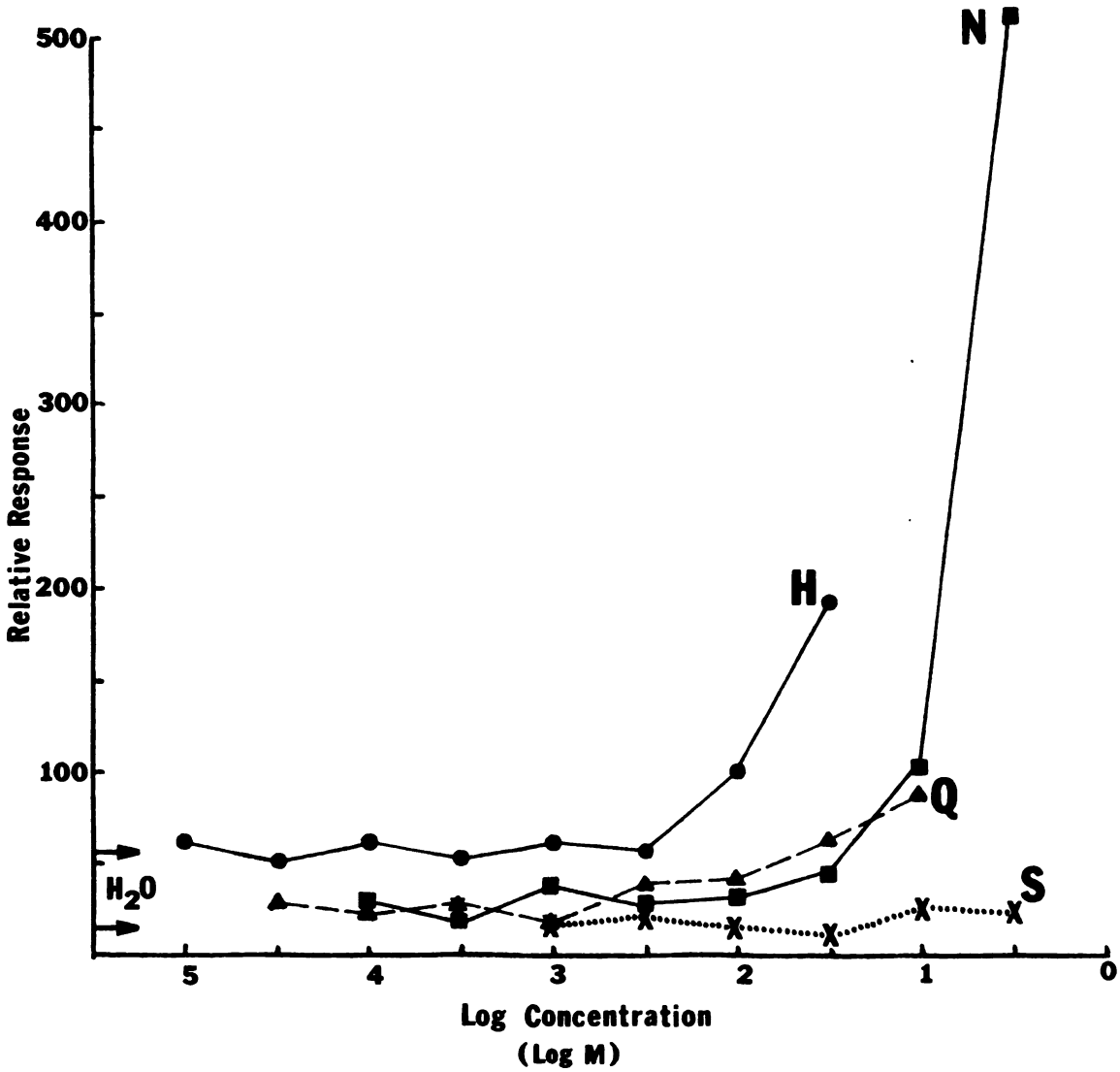

500
400
300
200
100
Relative Response
N
H
Q
S
H_2O
5
4
3
2
1
0
Log Concentration
(Log M)

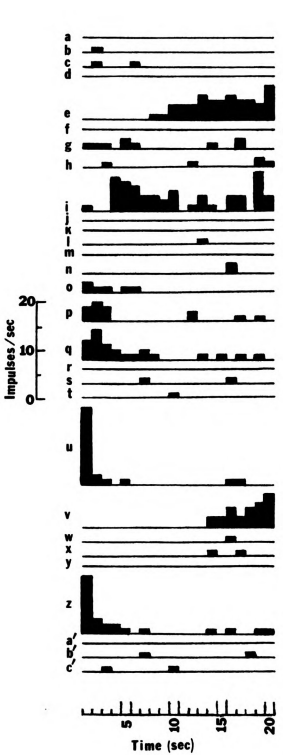
Figure 31.

individual responses of rat chorda tympani neurons forming the regularly increasing functions of the whole rat chorda. Thus, though an SR function with positive slope is observed for the whole nerve, quite varied activity from the many neurons can be hidden within the population's responses.

Similarly, various response latencies underlie the response of the whole nerve, which for Necturus is a consistently phasic response (see Figure 5). To compare the varied onset and activity patterns of the units with the phasic response of the whole nerve, the activity of each neuron in the sample (of 29 neurons) was first averaged over succeeding one second intervals for their twenty second taste response to 0.1 M NaCl. The bar graphs showing the individual response patterns and latencies of the neurons are shown in Figure 32. The activity of all neurons was then added for each second of the response. The net activity occurring in the sample population over succeeding one second intervals is shown in the graph to the right. This summed activity is seen to form a large initial phasic response paralleling the whole nerve response. The varied latency functions found in the experiment are not inconsistent with the population response of the whole nerve. These observations also reveal the severe limitations of whole nerve studies in defining the mechanisms underlying the taste system.

Similar limitations can now be easily seen for studies of the taste system using only single concentrations of stimulus. The response magnitude of the single neuron at any one concentration may represent the maximum, minimum or any intermediate locus of the neuron's SR

Figure 32. Summed response patterns for 29 gustatory neurons of Necturus. The responses of the individual neurons to a twenty second test of 0.1 M NaCl are shown to the left (lines a through z and a' through c'). Despite their varied latencies and response patterns, the summed response pattern (to the right) parallels the whole nerve response pattern, both having a large initial phasic response. (Compare with Figure 5.)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b
c
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k
l
m
n
o
p
q
r
s
t
u
v
w
x
y
z
z'
Impulses/sec
20
10
0
5
10
15
20
Time (sec)

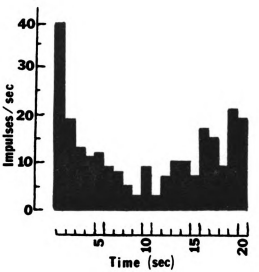
Impulses/sec
40
30
20
10
0
5
10
15
20
Time (sec)

Figure 32.

function for that particular chemical. Furthermore, without latency information, the response detected with one concentration may seem to be of insignificant magnitude and all the while its precise delay may have carried the information that sucrose is on the tongue.

VII. SUMMARY

The individual gustatory neurons of the mud puppy responded with a variety of stimulus-response (SR) and latency functions to the four primary stimuli, HCl, NaCl, quinine hydrochloride, and sucrose: 1. SR functions with curves of positive, negative, or zero slope. 2. SR functions with combined positive and negative slopes to form U-shaped or bell-shaped curves. 3. Latency functions with curves of positive, negative, and zero slopes. 4. Latency functions with combined positive and negative slopes to form bell-shaped curves. 5. SR and latency functions with complex slopes. Several different response forms were seen: 1) increasing impulse discharge with stimulus presentation, 2) decreasing activity on stimulation, and 3) rinse responses. The parameters of the form of the response, the SR function, and the latency function were not randomly associated but were seen in specific combinations that defined nine types of gustatory response:

1. Responses of increased activity with positive SR and negative latency functions.

2. Responses of increased activity with negative SR and positive latency functions.

3. Responses of increased activity with both SR and latency functions of constant slope, perhaps signalling stimulus onset.

4. and 5. Responses of increased activity with constant SR functions but with positive or negative latency functions, representing a unique form of response representation of stimulus concentration—by the latency parameter.

6. Rinse responses with constant latency.

7. Rinse responses with positive latency functions.

8. Depressed activity responses with positive SR functions.

9. Depressed activity responses with negative SR functions.

The neurons were seen to respond with more than one of the nine types of response and in some cases with specific types of response for different stimuli. Thus while most neurons exhibited multiple sensitivity to the four primary taste stimuli, they also showed some degree of chemo-specificity. The varied forms of the SR and latency functions were seen to require an underlying mechanism involving inhibition by the stimulus molecules.

Despite the variety of SR and latency functions, the responses of the sample neuronal population summed to a net regularly increasing SR function for each of the stimuli, the form of the whole nerve SR function. Similarly, the various latencies and subsequent activity patterns added over time to give a net phasic response, the form of the summated taste response of the whole glossopharyngeal nerve of Necturus. This showed that a wide variety of SR functions and response latencies can underlie the whole nerve SR function and response and suggests that the functions and responses seen in the sample population of Necturus gustatory neurons may not be unrepresentative.

VIII. RECOMMENDATIONS FOR FUTURE STUDY

1. It is not known whether the central nervous system of Necturus integrates the latency of the taste response. That must be decided by experiments that seek to define the responses and interrelationships of higher order neurons of the taste system.

2. The experiments performed in this study should be repeated to ascertain the persistence of the variety of nine types of taste response found for the mud puppy. Protocol should be altered to record pre-stimulus activity over longer periods so that a more accurate criterion of background spontaneous activity can be used to evaluate the responses.

3. The experiments might be best conducted on a different species since the responses and levels of spontaneous activity were far lower for Necturus than other animals. The largest response observed for any neuron was 344 impulses/20 sec or 17.2 Hz while to the same stimulus, 0.3 M NaCl, rat chorda tympani neurons gave responses of 70, 30, and 35 Hz (Figure 10A, from Pfaffmann, 1955).

4. In its present form, the enumerator program, SAM-COUNT requires separate analysis runs if different lengths of time are desired for test counts (e.g., for the first 5 sec vs the full 20 sec test count). The program should be altered to analyze and store the activity occurring

in each successive second of the rinse and test periods. These should be stored on magnetic tape so that the operator can easily determine the activity in periods of any duration by simple recall and summation of the activity in each second.

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APPENDICES

APPENDIX I

OPERATION OF FREDSAM

OPERATION OF FREDSAM

FREDSAM, an acronym for "frequency distribution of spike amplitude", is a computer program that identifies the number and distribution of action potentials according to their diphasic amplitude. The major indication of a neuron's isolation with extracellular recording is the uniformity of action potential amplitude. Computer analysis was required since most experimental records had several active neurons with superimposed electrical noise from recording and playback equipment to add variance to the spikes' amplitudes (Figure 33). Furthermore, the neurons of the mudpuppy's taste system usually responded with a low average frequency (< 1 Hz), too few spikes to contrast visually on the oscilloscope. FREDSAM measures all recorded potentials--action potentials, baseline fluctuations, and any artifacts (e.g., filtered EKGs, EMGs, static discharge) and displays these as a histogram showing the distributions of the diphasic amplitudes of the waveform.

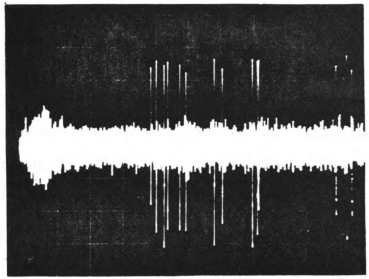
The diphasic amplitude was measured in contrast to the usual analyses which determine the size of only one phase of the action potentials since the cumulative effects of electrical noise altered both phases of diphasic action potentials but not necessarily equally nor coincidentally. Potentials were measured above and below a balanced null potential, established between the playback equipment and

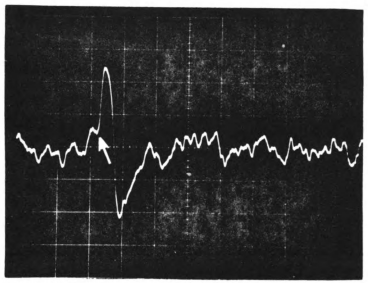
Figure 33. Action potential alterations by background electrical noise.

A. 14 action potentials with variable amplitude are displayed at 200 msec per division.

B. Action potential #12 of A., viewed at 2 msec per division. The cumulative effects of the irregular baseline can be seen as: 1) the initial rise of the negative (upward) phase starts from a point above the zero potential centerline (white arrow) and 2) the altered appearance of the positive (downward) phase of the action potential.

C. Action potential #6 of A. at 2 msec per cm. Neither positive nor negative phase of the action potential's waveform are greatly altered. This action potential rises from the centerline in contrast to #12.





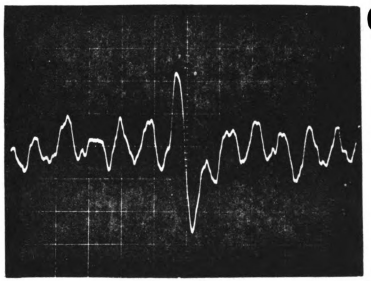


FIGURE 33.

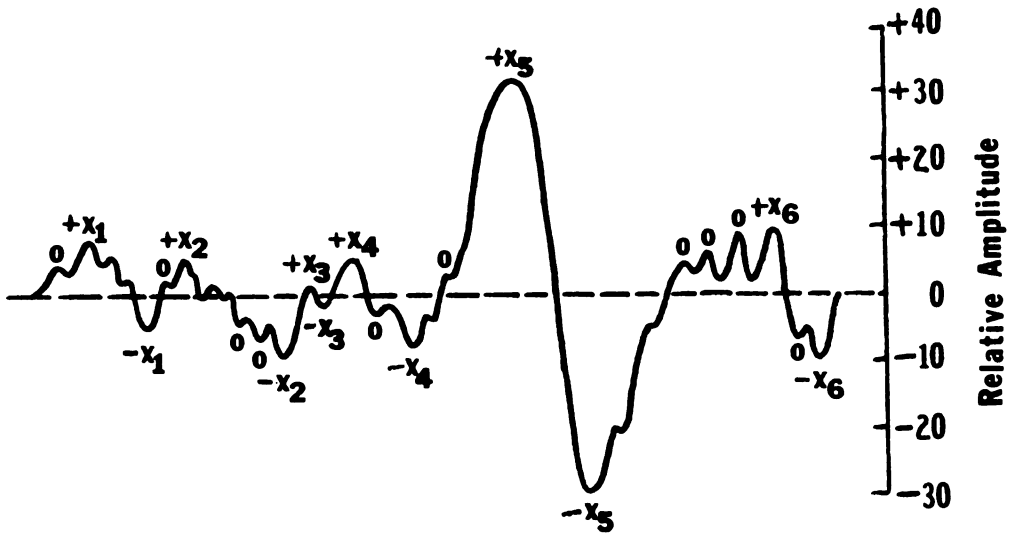
computer, by the following procedure. On the signal to start (an electric pulse delivered by the operator or the timer pulse recorded on the second tape channel) the computer tracked the signal, rapidly sampling its height above (or below) baseline. Each sample of successively greater amplitude was saved as the present maximum. When a new maximum was found, the previous maximal value was discarded. When the signal encountered zero baseline, the computer paused to detect whether the signal was passing to the region of opposite sign. If the signal did proceed across, the previous maximum was temporarily saved. In the same manner, the signal's greatest deflection of the opposite sign was also retained. When the computer detected a new movement across the baseline, the previous maximum positive and negative values were summed (with respect to their absolute value) and the total was recorded as the value of the diphasic amplitude of the signal (see Figure 34). The temporary maxima were discarded and sampling continued. Sampling proceeded at one of two different rates: 1) when tracking a new maximum and comparing it to a previous maximum the rate was slower (20 KHz) than when, 2) encountering no new maximum and sampling was at 25 KHz. Therefore, for a diphasic action potential of 2 msec duration (both phases) 45 samples were evaluated to find its total amplitude. All signals, whether nerve impulses, the irregular background electrical noise, or artifacts were evaluated in this same manner, i.e., were assigned a diphasic amplitude value regardless of their waveform. Data collection was terminated when a second electrical pulse was delivered by the operator or the data tape's command channel.

Figure 34. Diphasic amplitude measurement and display.

Above. The computer follows the signal trace (left to right) rapidly sampling its amplitude above and below zero potential centerline (dashed line is zero amplitude). Maxima are temporarily saved (o) or recorded for computation (-X, +X) if no greater are encountered before the signal passes the centerline.

Center. The amplitudes of succeeding pairs of positive and negative maxima are added together to determine the absolute amplitude (Yn) of the diphasic waveform.

Below. The FREDSAM display of the diphasic amplitudes of the above waveforms. Baseline potentials (Y_1-Y_4, Y_6) are nearest the origin. The signals of equal amplitude (Y_1 and Y_4) are entered in the same bin (12). Notice that all signals, regardless of waveform are evaluated by their greatest positive and negative maximum.



Computations:

	$\lvert+X_n\rvert + \lvert-X_n\rvert = Y_n$		
X_1	+8	−4	12
X_2	+5	−9	15
X_3	+1	−2	3
X_4	+6	−6	12
X_5	+32	−29	61
X_6	+10	−10	20

FREDSAM Display

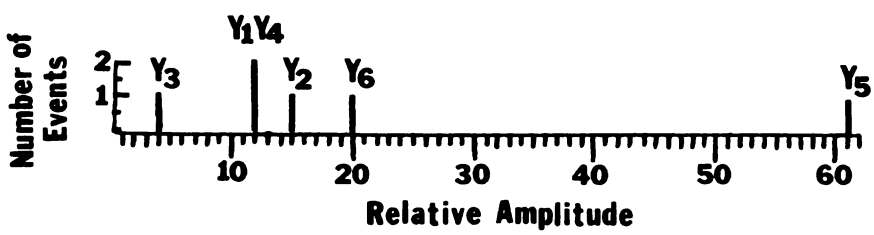


FIGURE 34.

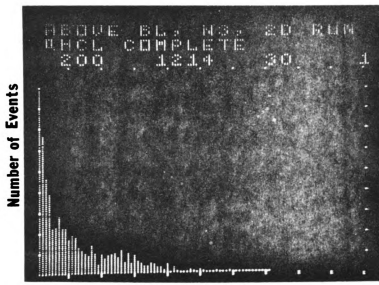
Data values were organized by frequency of occurrence (the number of events) within each amplitude category and were displayed as a population histogram (see Figure 14, "Methods" section). Each vertical line (or bin) of the histogram represents the number of events of a particular amplitude. The width of each amplitude bin is set by the operator at 1 to 999 relative amplitude units. The value of the amplitude unit is arbitrary and depends on the amplification of the input signal. To calibrate this relative value in μV, a signal (sine-wave) of known amplitude (20 μV) was processed and displayed with a histogram using bins of the smallest possible amplitude unit (1 relative unit per bin) (see Figure 16, Methods section). The peak in the amplitude histogram of the calibration signal then identified the calibration factor, e.g., 20 μV = 63 computer amplitude units. The maximum resolution of FREDSAM depended on the playback amplification but averaged 100 divisions for a 50 μV diphasic potential or 0.5 μV.

When a record had more than one active neuron with distinct or well-isolated action potentials, the histogram contained discrete peaks corresponding to each neuron to the right of the initial peak, which represented the baseline potentials. The histogram of poorly isolated neurons did not have well-defined peaks and appeared instead as a large initial baseline peak with a positively skewed tail. Neurons of low activity had histograms with an irregular array of amplitudes (Figure 35). Both cases were rejected from further analysis since no active neuron could be clearly defined. Therefore, FREDSAM was employed to judge an experiment's acceptability when completed or to direct further dissection during the experiment.

Figure 35. FREDSAM displays of units with poor isolation, low activity.

Upper. To the right of the baseline population, no distinct maximum is resolved among this large number of potentials (1214) indicating that the record contains poorly isolated neurons.

Lower. For this record, only 88 action potentials (above the baseline population) occurred in 26 of 31 responses analyzed by FREDSAM. No maximum was resolvable and the experiment was eliminated from further analysis.


ABOVE BL, N3, 2D RUN
UHCL COMPLETE
200 . 1214 . 30 . 1
Number of Events
Relative Amplitude

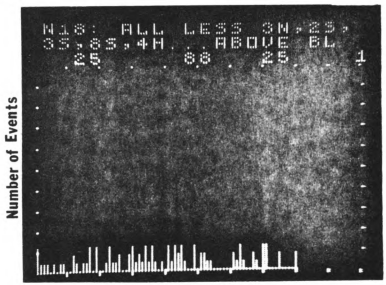

N18: ALL LESS 3N,2S,
3S,8S,4H. ABOVE BL
.25 . 88 . 25 . 1
Number of Events
Relative Amplitude

FIGURE 35.

Our current version of FREDSAM offers several options to the operator: 1. The amplitude option, already mentioned, was used to check the persistence of maxima and minima defining the various populations by displaying their histograms with bins of different amplitude (1, 2, and then 3 relative amplitude units). 2. The total range of amplitudes displayed could be limited (from the minimal display of a single amplitude to the maximum of all amplitudes showing). This option was used continually to eliminate the omnipresent large baseline population from the display. A second useful application of this option was to display only the range of amplitudes defining a single neuron. The corresponding total number of events could then be read from the legend (see Figure 14, 2nd numerical value). 3. The ordinate could be scaled to various maximal number of events, from 25 to 6400. 4. Any single histogram could be recorded on magnetic tape. 5. Similarly, the data of any FREDSAM analysis could be permanently recorded and any histogram then reconstructed on recall. This option was used with all experiments, even those rejected for poor isolation or low responsiveness.

APPENDIX II

OPERATION AND EVALUATION OF SAM-COUNT

OPERATION AND EVALUATION OF SAM-COUNT

SAM-COUNT is a computer program that counts the action potentials occurring in test and rinse periods, accepting only those impulses within the amplitude window established by FREDSAM as the limits defining a single nerve cell.

FREDSAM and SAM-COUNT computed the diphasic amplitude of the recorded signals according to the same algorithm (Appendix I, Figure 34). SAM-COUNT enumerated impulses in three different ways depending on the period to be analyzed. 1. For full test counts, SAM-Count started tracking and counting at the recorded onset of the timer pulse and continued until the arrival of the second timer pulse. 2. For post-stimulus rinses, tracking and counting started similarly but continued beyond the second timer pulse until a pre-set interval had elapsed. 3. Pre-test counts proceeded in a more complex manner. (The desired count was from a period of pre-set length occurring before a stimulus onset signal.) The method of evaluation and rejection used in this procedure required more analysis time than the direct count procedures (1 and 2 above).

To evaluate SAM-COUNT, two procedures were used. The first analyzed the program's reproducibility. Repeated counts were taken of the same test and rinses using the three modes of operation (full count, pre-test count, and post-test count). The standard errors of the mean counts were calculated (Table III).

Table III. SAM-COUNT reproducibility.

Mode	Mean A.P. Count	Range	Number Runs	S.E.
Full period count (20 sec)	30.821	30-31	28	0.075
Partial count, pre-test (20 sec)	47.773	47-49	21	0.115
Partial count, post-test (20 sec)	48.107	48-49	28	0.061

The variability can be ascribed to: 1) the computer's sampling rate (22-25 KHz) in determining diphasic amplitude, or 2) to variability in the triggering of enumeration by the command pulse (which would in turn effect the reading of each amplitude value), or 3) to noise in the playback equipment. The larger standard error of the pre-test mode was expected given its more complex operation.

The second procedure was to compare action potential counts determined by photographic analysis and by SAM-COUNT. Twenty-one tests were chosen randomly and the counts were taken for 5 sec pre-test, 20 sec test, and 5 sec post-test (using a 5X hand magnifier with scale in 10 mil divisions from the photograph of the responses). These were compared to the SAM-COUNT evaluation for the same periods. (In Table IV, 15 of the 63 counts can be seen to differ.) The product-moment correlation coefficient was calculated for each mode (Sokal and Rohlf, 1969). Correlation between the photograph and computer analysis is high ($r = 0.969$ for full test counts, 0.931 for pre-test counts, 0.989 for post-test counts). Furthermore, the product-moment correlation coefficients were all found to be highly significant ($p < 0.0005$) using

Table IV. Enumeration product-moment correlation.

Neural Unit	Test	Number A.P's Computer			Number A.P's Photograph		
		Pre-	Full	Post-	Pre-	Full	Post-
14-1	0.0003 M NaCl	0	6	2	0	6	2
14-1	0.0003 M sucrose	0	2	3	0	2	3
15-1	0.0003 M quinine	0	4	1	0	7	1
15-1	0.0003 M sucrose	0	0	0	0	0	0
15-2	0.001 M NaCl	6	13	1	10	17	1
15-2	0.03 N HCl	0	3	0	3	2	0
15-2	0.03 M NaCl	1	5	1	1	10	1
15-2	0.1 M quinine	1	8	0	2	10	0
19-1'	0.03 N HCl	1	8	6	1	9	6
19-2	0.0003 N HCl	0	0	1	0	1	0
23-1	0.3 M NaCl	0	14	0	0	14	0
23-2	0.0003 M quinine	0	3	0	0	3	0
23-2	0.03 M NaCl	1	3	0	1	3	0
23-3	0.1 M NaCl	1	24	1	1	24	1
23-3	0.0001 M NaCl	0	6	0	0	6	0
24-3	0.00003 M quinine	1	2	0	1	2	0
27-1	0.003 N HCl	1	2	1	1	2	1
27-3	0.01 M sucrose	0	0	0	1	1	0
28-1	0.003 M quinine	0	2	0	0	3	0
28-2	0.003 N HCl	0	1	0	0	0	0
30-1	0.03 M sucrose	1	3	2	1	3	2

(Discrepant photographic counts are underlined.)

	Pre-	Full	Post-
r	0.931	0.969	0.989
t_{test}	11.118	17.096	29.145
$t_{critical}$ ($p=0.0005, \nu=19$)	3.883	3.883	3.883

the t-test for the significance of correlation coefficients (Sokal and Rohlf, 1969).

All experimental enumerations determined by SAM-COUNT were reviewed and altered 1) to eliminate artifact potentials identified in the photographs, 2) to adjust test count action potentials occurring before stimulus delivery, and 3) to adjust post-test counts occurring before rinse delivery. Other discrepancies in the two enumerations usually were with greater counts resolved by photographic analysis. This is ascribed to the less precise determination of amplitude allowed with the hand lens and scale and the computer counts were usually accepted.

APPENDIX III

EVALUATION OF DELIVERY TIME MEASUREMENTS

EVALUATION OF DELIVERY TIME MEASUREMENTS

The delivery time was measured using a fluid switch circuit described in the "Methods" section. The flow of 0.1 M NaCl served to close the circuit between leads placed in the stimulus reservoir of the stimulus delivery system and on the tongue (Figure 18, "Methods"). The oscilloscope trace, triggered on the initiation of stimulus delivery registered the arrival of solution at the tongue by a steady deflection from the change of potential when the circuit was completed.

To evaluate this method of detecting delivery time, we compared the fluid switch to an independent measure using the flow of colored saline detectable by a phototransistor. A fiber-optic light source illuminated a small region of a flexible plastic tube which was connected to the output of the stimulus delivery system (see Figure 36 and Figure 17 of the "Methods" section). Distilled water from the rinse reservoir preceded the flow of green saline (25 ml green food color/ liter 0.1 M NaCl, U. S. Certified Food Color, 2.5% in propylene glycol and water, The Kroger Company, Cincinnati, Ohio). A silicone phototransistor detected the passage of green saline through the illuminated tube. The non-insulated tip of the "lingual" lead of the fluid circuit was inserted through the wall of the tubing into the illuminated region and also detected the saline flow. The phototransistor (Archer, No. 276-130, Radio Shack, Fort Worth, Texas) was placed above the ocular of

Figure 36. Dual determination of delivery time. The flow of green saline following the flow of distilled water through a flexible, transparent tube is detected by two methods:

1) the standard measurement using the saline to close a circuit to the lead, $\underline{L}_2$, and

2) detection of the green color by a phototransistor placed above the ocular of a microscope which views the illuminated region about $\underline{L}_2$.

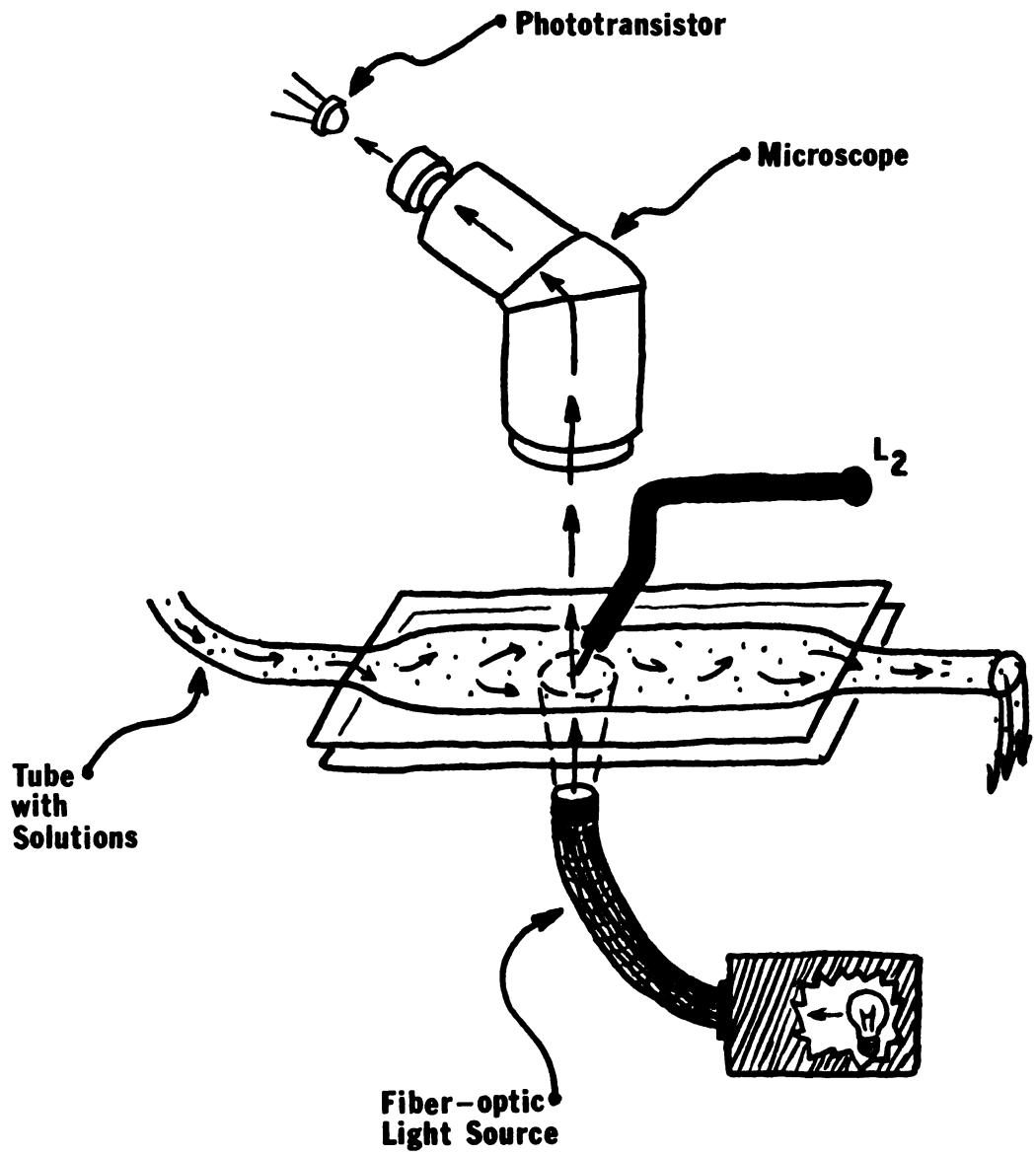

Phototransistor
Microscope
L_2
Tube
with
Solutions
Fiber-optic
Light Source

FIGURE 36.

a dissecting microscope which viewed the tip of the fluid switch circuit's lead in the illuminated region of the tubing at 40X magnification. The flexible tubing was flattened between two glass slides to decrease the dispersal of light from the fiber-optic light source. As can be seen in Figure 37 (bottom traces, above and below) both systems detected the arrival of green saline at the illuminated region. Detection times for the phototransistor (800 msec) and fluid switch (770 msec) were nearly equal. The controls, the flow of distilled water and stopping the water flow affected neither system.

Repeated measures of the delivery time using the fluid switch alone revealed its variability (mean delivery time of 650 msec $\pm$ 2.622 for 12 measurements). This small standard error occurred when measured without an animal present. In situ, only two measurements of delivery time were taken with each experiment, insufficient replication to calculate any variance statistics. The in situ measurements, showed a range of differences between the two determinations (0-26 msec). This indicates that the variance in the experimental measurements may be less than 26 msec.

The effects of concentration of the saline used in the circuit to detect delivery time are shown in Figure 38. The slope of the developing potential varied with concentration but the onset remained the same. Because of this lack of effect on concentration on the delivery time determinations, only 0.1 M NaCl was used for the experimental measurements.

Figure 37. Comparison of phototransistor and fluid switch determinations of delivery time.

Traces 1 (above and below). 1st Control. The initial flow of distilled water is terminated (no green saline in stimulus reservoir) affecting neither determination.

Traces 2 (above and below). 2nd Control. The flow of distilled water followed by distilled water from the stimulus reservoir cannot be detected by phototransistor nor is the fluid switch circuit completed.

Traces 3 (above and below). When green saline (food color in 0.1 M NaCl) flows from the stimulus reservoir, the phototransistor detects the change in illumination (downsweep, above) and the fluid switch circuit's completion is registered by the observed change in potential (upsweep, below).

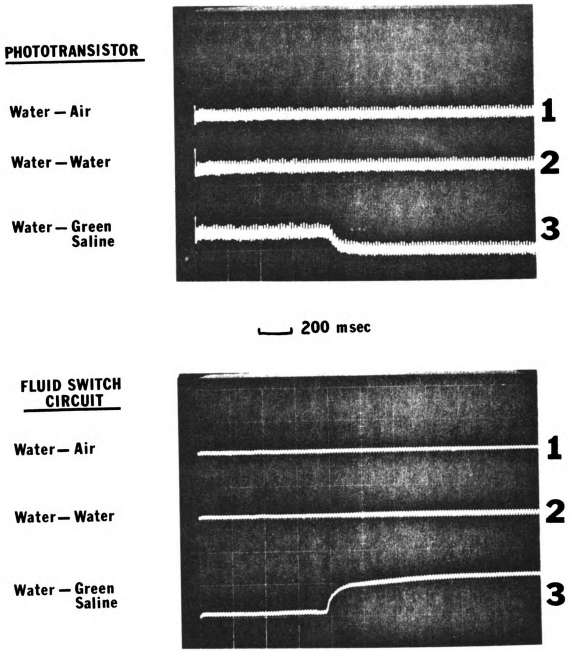

PHOTOTRANSISTOR
Water — Air
Water — Water
Water — Green Saline
1
2
3
200 msec
FLUID SWITCH CIRCUIT
Water — Air
Water — Water
Water — Green Saline
1
2
3

FIGURE 37.

Figure 38. Effects of saline concentration on delivery time measurement. The delivery time determination is made with different concentrations of NaCl and distilled water. The slope of the developing potential is altered but the delivery time measurement is unaffected. Only 0.1 M NaCl was used in the experiments.

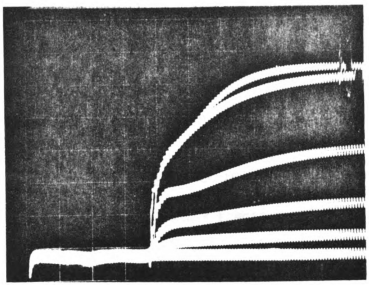


FIGURE 38.

APPENDIX IV

SR AND LATENCY FUNCTIONS

Figure 39. Complex SR and latency functions. Several of the SR and latency functions were of such complex form that graphical analysis resolved no regular slope for part or all of the concentration series. These are shown for the SR functions (above) and the latency functions (below). The symbols of Figure 22 apply (i = impulses). Notice the expanded ordinate for the quinine latency of unit 24.4 (to 0.5 seconds).

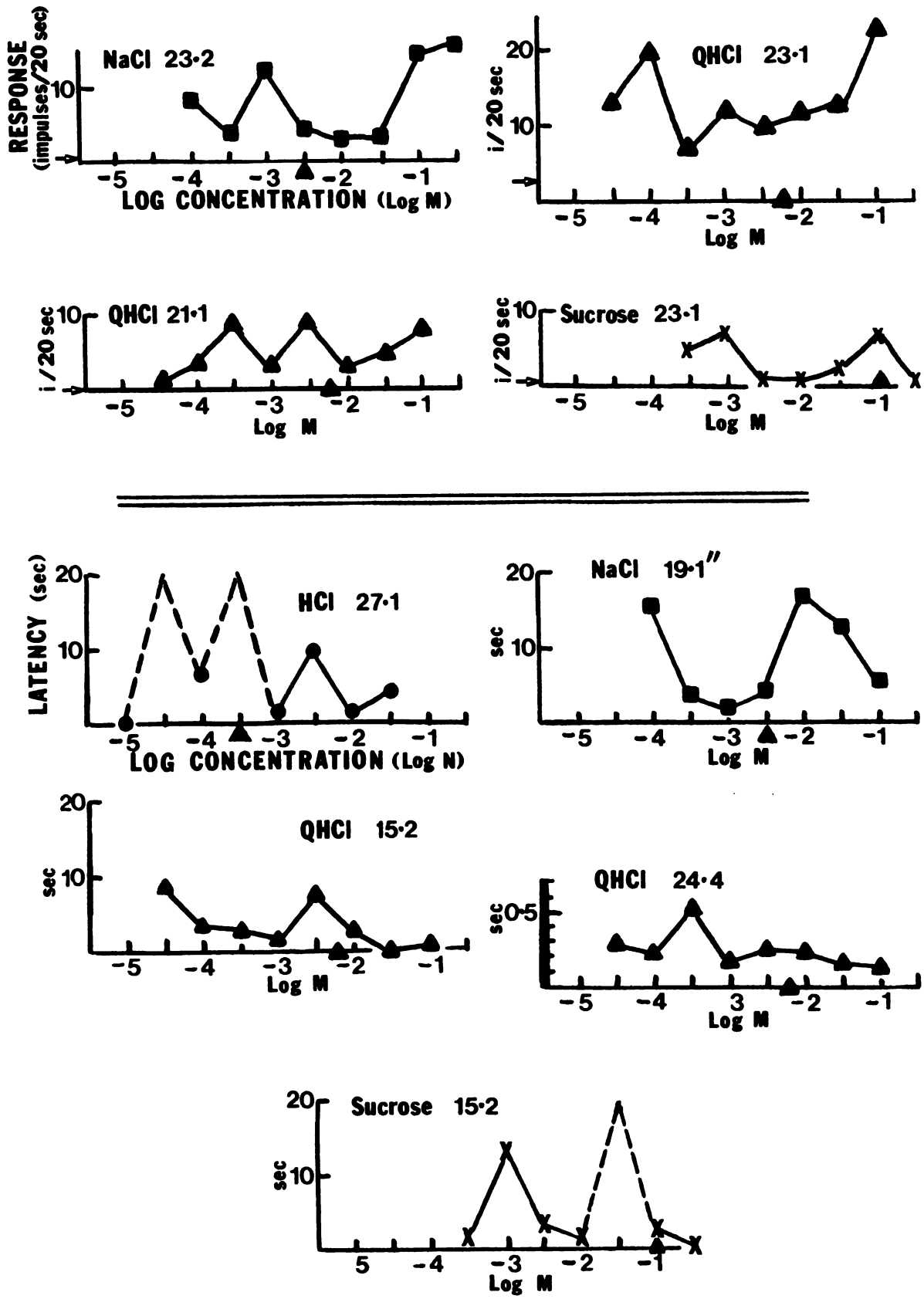

RESPONSE (impulses/20 sec)
NaCl 23·2
LOG CONCENTRATION (Log M)
QHCl 23·1
i/20 sec
Log M
QHCl 21·1
Sucrose 23·1
LATENCY (sec)
HCl 27·1
LOG CONCENTRATION (Log N)
NaCl 19·1"
sec
QHCl 15·2
QHCl 24·4
Sucrose 15·2

FIGURE 39.

Figure 40. Positive SR functions. These are shown for seven neurons (with two functions for unit 13.2). The same symbols as in Figure 22 apply here (i = impulses). Notice the expanded ordinate for the NaCl test series of unit 13.2.

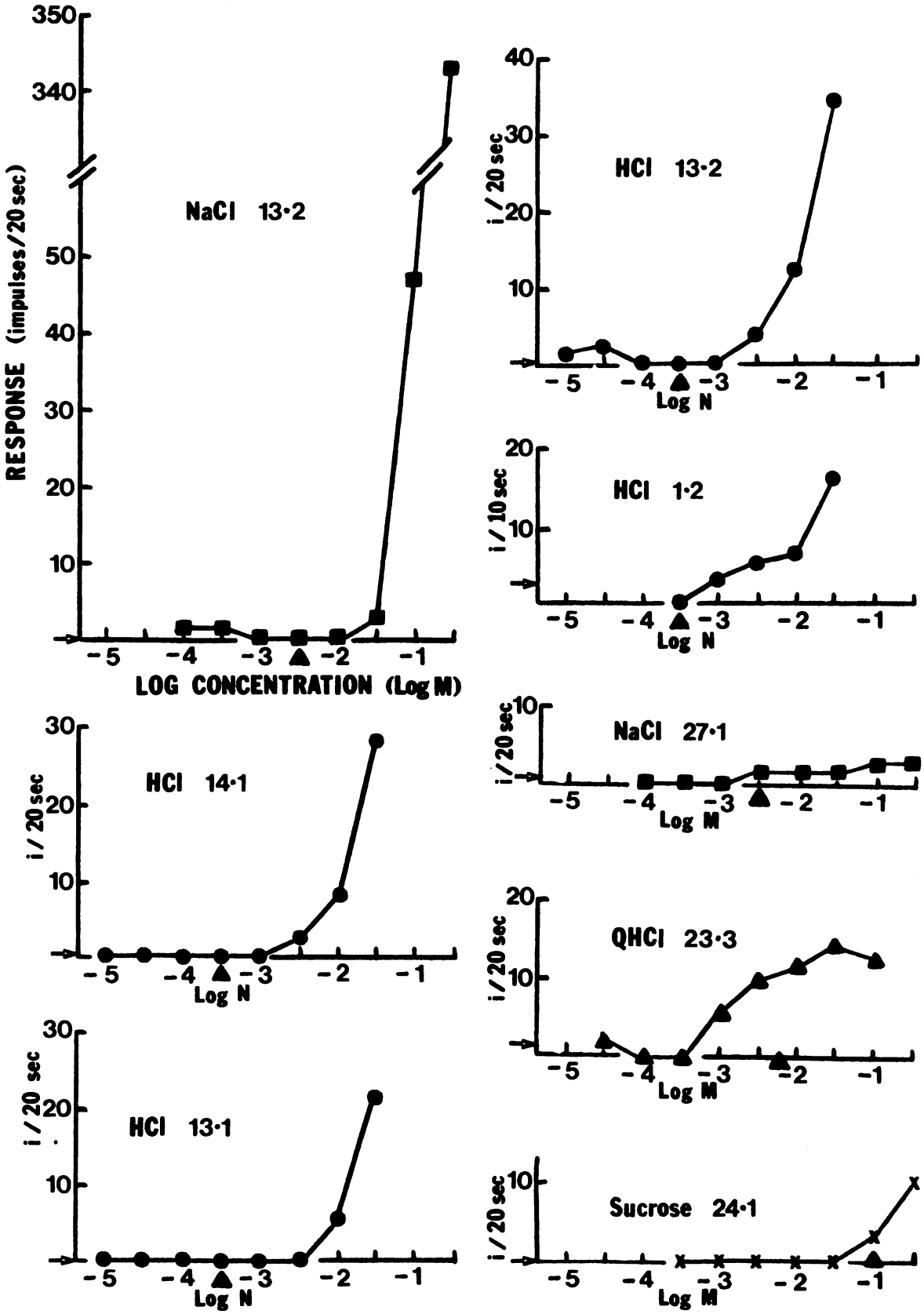

RESPONSE (impulses/20 sec)
NaCl 13·2
LOG CONCENTRATION (Log M)
HCl 13·2
i / 20 sec
Log N
HCl 1·2
i / 10 sec
Log N
HCl 14·1
i / 20 sec
Log N
NaCl 27·1
i / 20 sec
Log M
QHCl 23·3
i / 20 sec
Log M
HCl 13·1
i / 20 sec
Log N
Sucrose 24·1
i / 20 sec
Log M

FIGURE 40.

Figure 41. SR functions with zero and negative slope. These are shown above and below in the figure. Symbols are those of Figure 22 (i = impulses). The NaCl test series for unit 1.2 was for only ten second tests as shown on the ordinate. The pre-stimulus activity for the quinine series of unit 15.1 was averaged for twenty seconds (C at the origin).

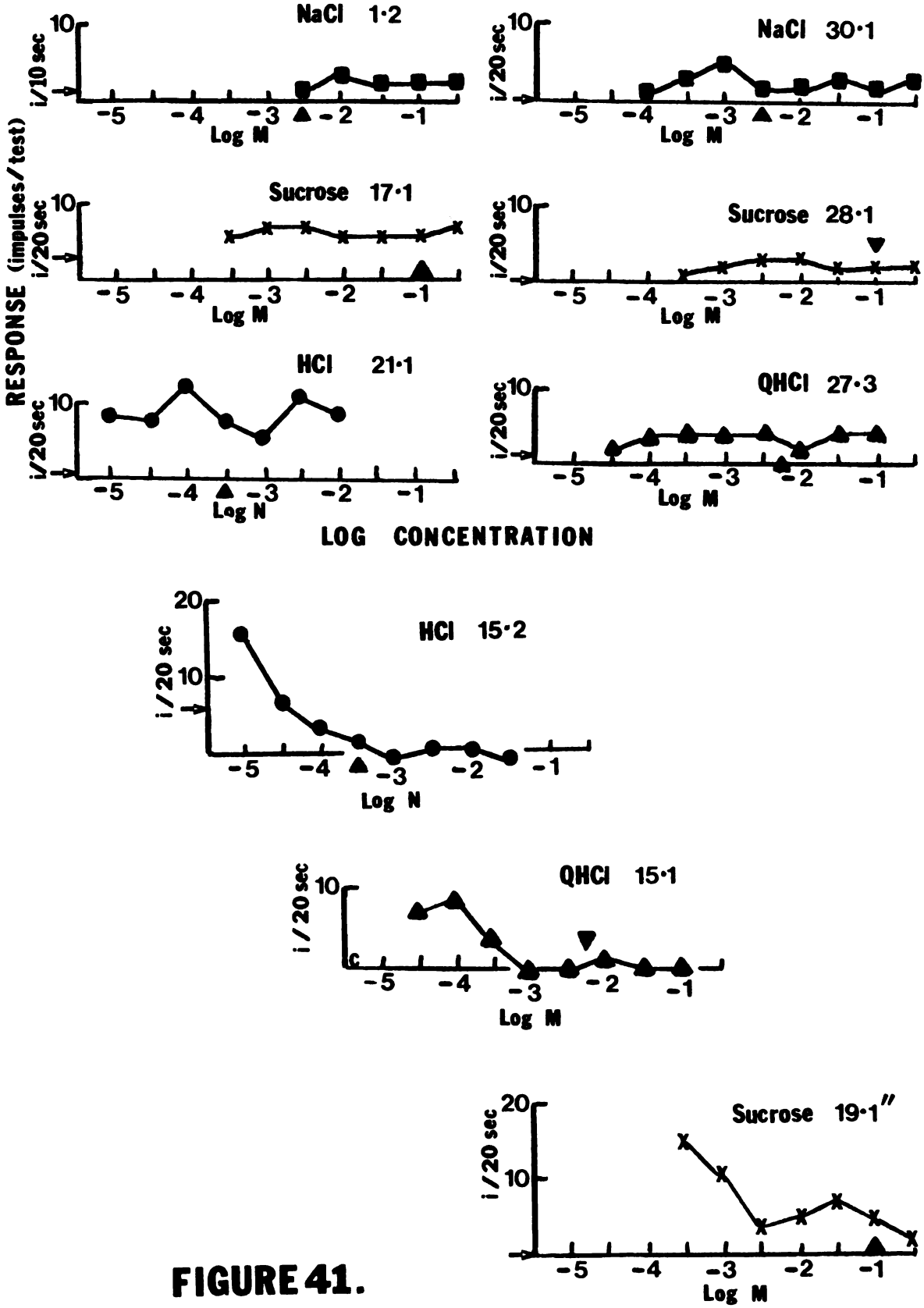

NaCl 1·2
NaCl 30·1
Sucrose 17·1
Sucrose 28·1
HCl 21·1
QHCl 27·3
RESPONSE (impulses/test)
i/10 sec
i/20 sec
10
-5 -4 -3 -2 -1
Log M
Log N
LOG CONCENTRATION
HCl 15·2
20
i/20 sec
QHCl 15·1
Sucrose 19·1"

FIGURE 41.

Figure 42. U-shaped and bell-shaped SR functions. These are shown for six units. Symbols are those of Figure 22 (i = impulses). C at the origin of the graph for unit 29.1 refers to its 20 second average pre-stimulus activity determinations.

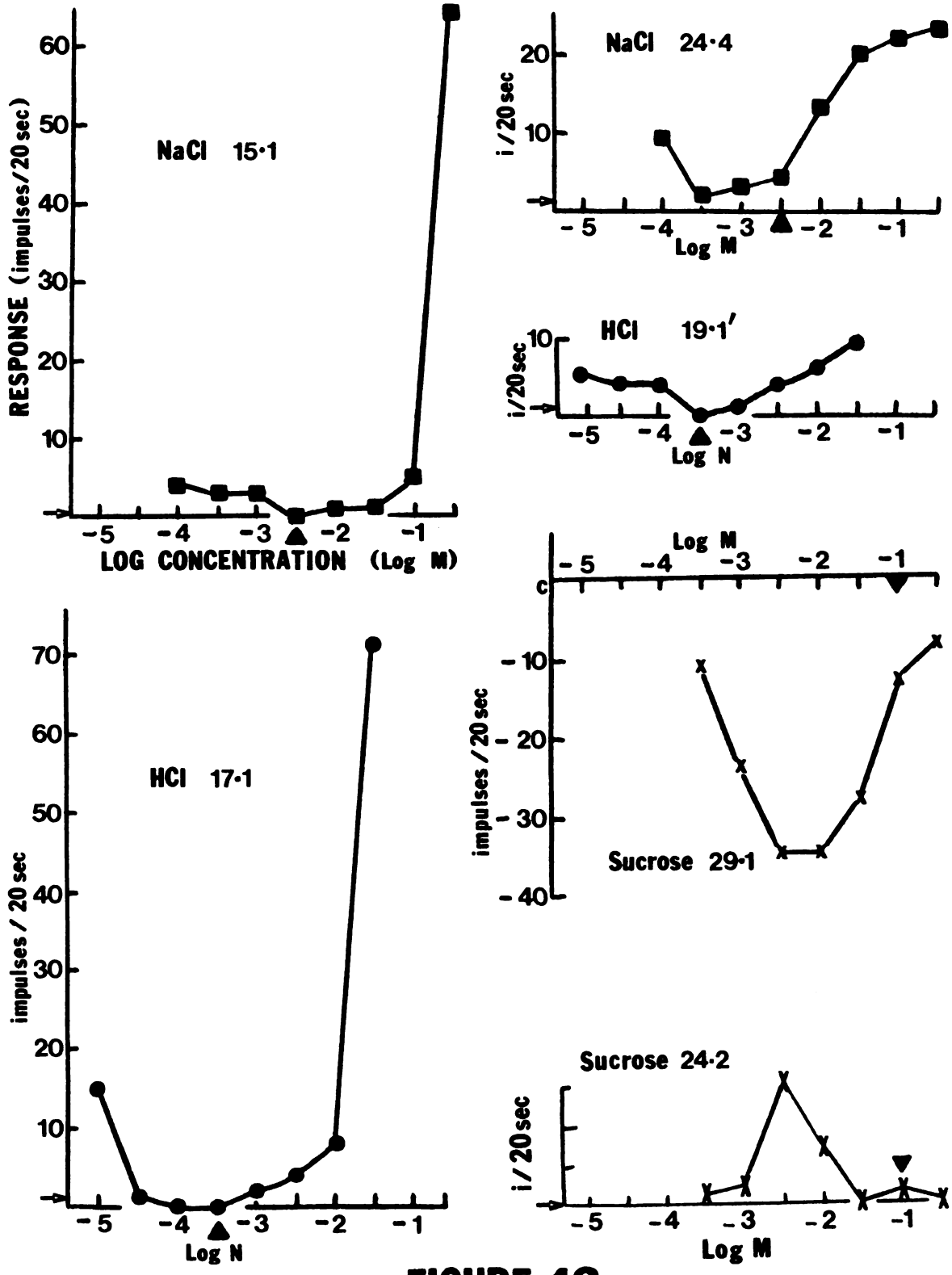

NaCl 15·1
RESPONSE (impulses/20 sec)
LOG CONCENTRATION (Log M)
NaCl 24·4
i/20 sec
Log M
HCl 19·1′
i/20 sec
Log N
HCl 17·1
impulses / 20 sec
Log N
Log M
impulses / 20 sec
Sucrose 29·1
Sucrose 24·2
i/20 sec
Log M

FIGURE 42.

Figure 43. Latency functions with negative, zero, and positive slope. These are shown for nine units. The ordinate for unit 23.3 HCl series has been expanded at the inset. Symbols are those of Figure 22.

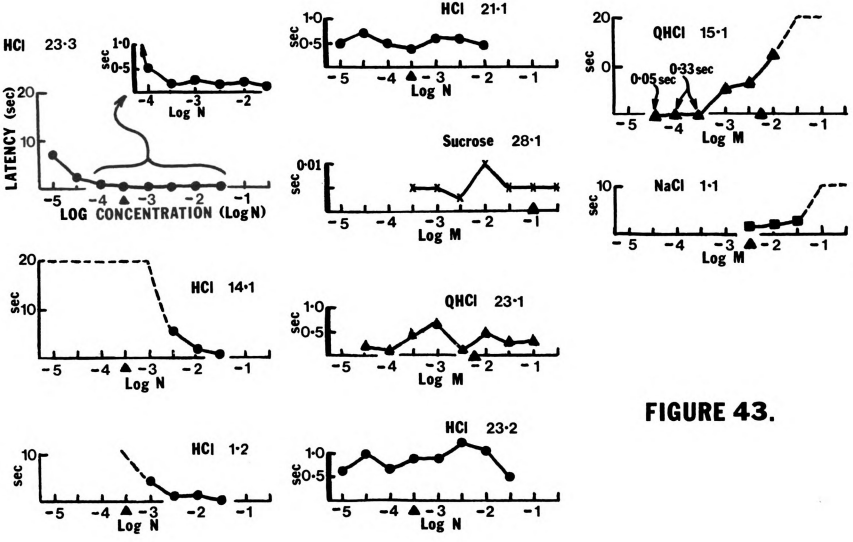

HCl 23·3
LATENCY (sec)
LOG CONCENTRATION (Log N)
Log N
HCl 21·1
Sucrose 28·1
Log M
QHCl 15·1
0·05 sec
0·33 sec
NaCl 1·1
HCl 14·1
QHCl 23·1
HCl 1·2
HCl 23·2
sec

FIGURE 43.

Figure 44. Latency functions with positive and negative slopes combined. These are shown for five units with the standard symbols of Figure 22. Notice the expanded ordinates for units 23.2 and 27.2 as insets with each.

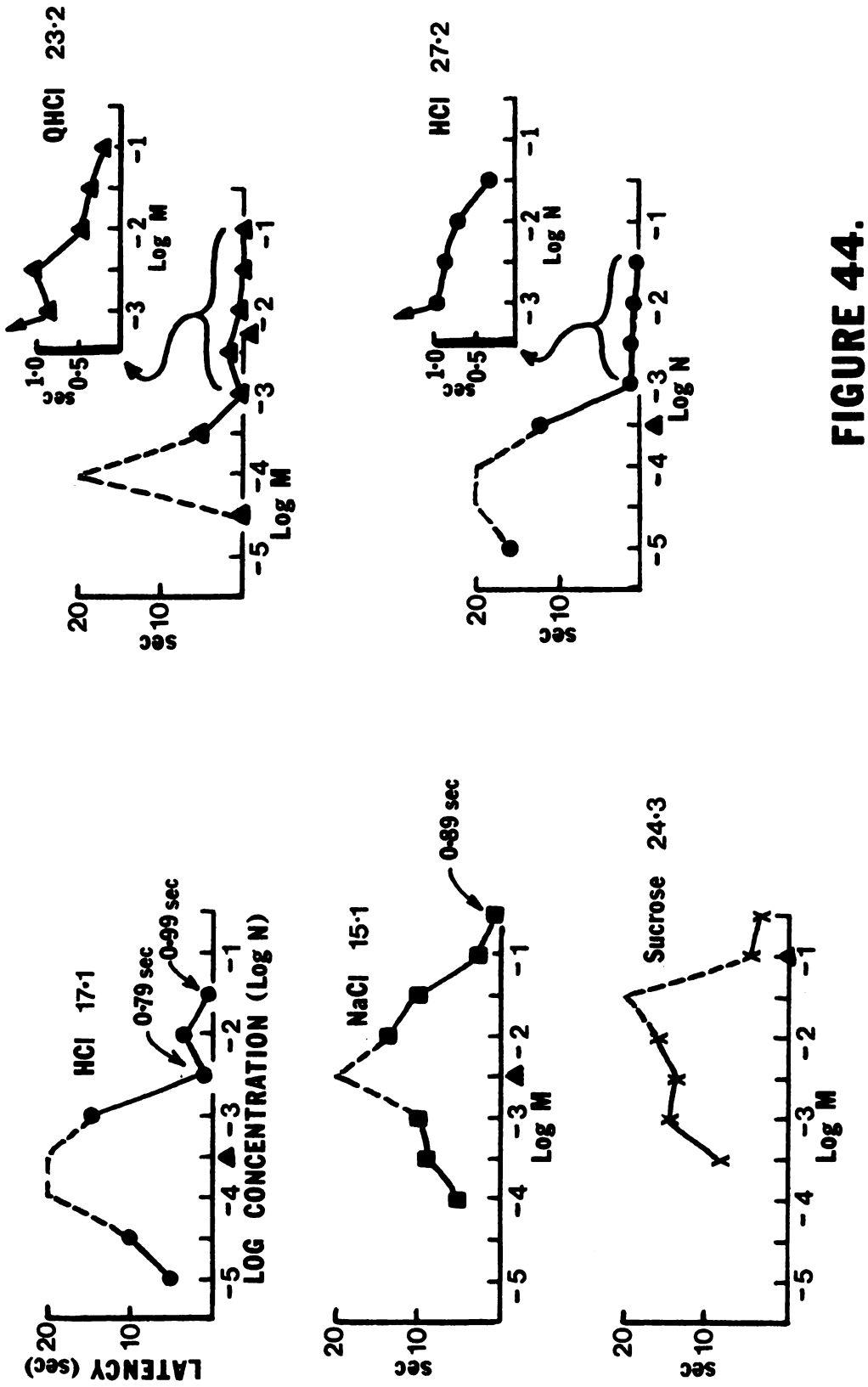

QHCl 23·2
HCl 27·2
HCl 17·1
NaCl 15·1
Sucrose 24·3
0·79 sec
0·99 sec
0·89 sec
LATENCY (sec)
LOG CONCENTRATION (Log N)
Log M
Log N
sec

FIGURE 44.