Peripheral Interactions among Single Papilla Inputs to Gustatory Nerve Fibers

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ABSTRACT Recordings were made from single fibers of the rat chorda tympani nerve while the peripheral receptor fields were mapped using a stimulator developed to stimulate single fungiform papillae which in the rat contain a solitary taste bud. The results indicate that several fungiform papillae may supply input to a single fiber, and the most sensitive papilla of these provided, on the average, about one-half of the response of that fiber to stimulation of the entire tongue. The magnitude of the response to each concentration of stimulus and the shape of the concentration-response curves differ among papillae innervated by the same fiber. If one of the papillae supplying input to the fiber was stimulated individually with NaCl solution, application of this stimulus to the tongue surface surrounding the isolated papilla resulted in enhancement of the fiber response. If the papilla was stimulated with NaCl and potassium benzoate solution was applied to the surround, a depression of the response occurred. The excitatory input of the cationic stimuli and the depressing influence of the anionic stimuli interacted to determine the resultant steady-state impulse frequency of the single afferent fiber. A hypothetical model involving the summation of generator currents along the unmyelinated terminals of the single afferent neuron is presented as a speculative explanation of the integration of inputs from several receptors innervated by the same single fiber.

INTRODUCTION

Degeneration experiments (Guth, 1957; Whiteside, 1927) have led to the deduction that gustatory nerve fibers overlap in their receptor fields; that is, a single taste receptor organ may be innervated by more than one nerve fiber, while one nerve fiber may innervate several taste receptor organs. Though an anatomical precedent for multiple innervation has existed for some time, the physiological significance of the overlap has not been extensively investigated.
Rapuzzi and Casella (1965) electrically stimulated single fungiform papillae in the frog and recorded antidromically propagated action potentials in papillae which were located nearby. This study confirmed that nerves overlap on nearby receptors, but it did not show whether the overlapping fibers responded to chemical stimulation. They concluded that the average number of branches going to different papillae from each fiber was between five and six for the frog. Since they did not use chemical stimuli, they were unable to identify the peripheral fields of chemosensory fibers.

Filin and Esakov (1968) observed that action potentials could be propagated antidromically in gustatory fiber branches in the frog by chemical stimulation of adjacent regions of the tongue. They showed that electrical stimulation of nerve fiber branches could depress the sensitivity of the receptor for a period of 5 min and that the depression continued for about 10 min.

In the rat each of the bilateral chorda tympani nerves innervates the fungiform papillae on the homolateral half of the anterior two-thirds of the tongue. A recent study (Beidler, 1969) using the electron microscope, has shown quantitatively that nerve fibers branch profusely in and below the taste bud to form the presumed terminals of the afferent fibers. The present investigation examines the peripheral fields of single chorda tympani fibers by chemical stimulation of single fungiform papillae. Consolidation of the electrophysiological data with the anatomical architecture of the receptor presents a concept of the functional organization of the peripheral nerve fiber unit; i.e., a single afferent nerve fiber and the attending receptors supplying input to it.

Fig. 1 shows the dorsal surface of the rat tongue covered uniformly with the slender and pointed filiform papillae which contain no taste buds. Scattered within this forest of filiform papillae in a punctate dispersion are the shorter but more robust fungiform papillae. Fish, Malone, and Richter (1944), using 103 domestic Norway rats, found that each rat bore from 114 to 221 of the fungiform papillae with a mean of 178 per tongue. The fungiform papilla in the rat contains a single taste bud located on its apical surface as seen in the cross-section of the papilla (insert A, Fig. 1). The tip of the taste bud can be seen protruding slightly from the crater in the top of the papilla shown in the scanning electron micrograph in insert B of Fig. 1 (from Beidler, 1969). Thus, stimulation of a single rat fungiform papilla is equivalent to stimulation of a single taste bud, so that it should be possible to study the relationship between single nerve fiber responses and chemical stimulation of single taste buds.

The antagonistic effects of the anion and cation in stimulation of the rat taste receptor were pointed out by Beidler (1954). He showed that as the chain length of a series of organic anions increased, the anion exerted a progressively greater inhibitory action on the stimulating effect of the cation. According to Beidler (1967), "Since cation binding to taste receptor mem-
branes is excitatory and anion inhibitory, the net magnitude of the taste response may depend on the relative number of cationic to anionic membrane sites available to the taste stimulus as well as their relative binding constants for a given stimulus. For the rat, sodium has been shown to be a potent cationic stimulus and chloride a relatively weaker anionic stimulus. Potassium, on the other hand, is a relatively less effective cationic stimulus, while the benzoate anion seems to be effective in reducing excitation produced by the cation in the lower concentration ranges. Thus, the combination of sodium chloride as a potent excitatory stimulus and potassium benzoate as a potent inhibitory stimulus came from earlier experiments on this animal (Beidler, 1961). These stimuli were utilized in this study to test the ability of the fiber

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1 Beidler, L. M. 1967. Anion influences on taste. In Olfaction and Taste. T. Hayashi, editor. Pergamon Press, New York. p. 524.
to integrate the excitatory and inhibitory events which occurred at different membrane sites.

A preliminary presentation of the results of this investigation (Beidler, 1969) showed that the responses evoked in a single fiber by stimulation of an isolated papilla with NaCl solution could be modified by stimulation of surrounding papillae. Enhancement and depression are described quantitatively in this report for a population of single nerve fibers and their associated taste buds. A speculative explanation of the neurophysiological mechanism is offered through which the integration of the simultaneous inputs to the fiber is accomplished.

METHODS

Adult male and female Sprague-Dawley rats were used in these experiments. The animals were anesthetized with intraperitoneal injections of sodium pentobarbital (Diabutal) at a dose of 30 mg per kg of body weight. The chorda tympani nerve was exposed by the lateral approach (Cohen et al., 1955) and divided into thin strands with dissecting needles. The thin strands were placed on a platinum-iridium electrode fixed in a glass tube and supported by a micromanipulator. Prior to placement of the strands of nerve on the electrodes, the tongue was extended from the mouth and fixed to a small Plexiglass platform by a dissecting pin through the contralateral side of the tongue as shown in Fig. 1. A bilateral section of the hypoglossal nerves was performed to insure the immobility of the tongue. Dilute methylene blue solution was applied to the tongue surface to enhance the visual contrast between the fungiform and filiform papillae so that individual fungiform papillae could be easily identified under the magnification of the dissecting microscope.

The spike potentials from the nerve fibers were amplified by a Grass P-5 RC preamplifier, the output of which was simultaneously displayed on a Tektronix 502 oscilloscope and recorded on magnetic tape by a Webcor tape recorder. Subsequently, the action potentials were played back through the oscilloscope and photographed by a Grass C4K kymograph camera.

The single papilla stimulator shown in Fig. 2 was mounted on a micromanipulator and the innermost chamber placed directly over a single fungiform papilla with visual surveillance through the dissecting microscope. Four different solutions (three stimuli and distilled water rinse) were alternately flowed over the isolated papilla by introducing them into the inner chamber of the stimulator. The solutions were contained in reservoirs consisting of 50 cc disposable syringes connected through a valve to the manifold of the stimulator by polyethylene tubing. A rotary valve allowed one or none of the four solutions to flow through the stimulator at any given time. The tubes remained full of fluid so that when the valve was opened to one of the solutions, the fluid flowed into the manifold pushing the remaining liquid ahead of it to flow over the papilla. The dead volume of the stimulator was \(2 \times 10^{-3}\) cc and the flow rate through the stimulator was 0.044 ml/sec when it was placed over one of the papillae. When the valve was changed from water rinse to NaCl, the latency for the onset of stimulus was about 0.1 sec.
A vacuum line with a fluid trap was applied to the annular chamber surrounding the central stimulus chamber. This suction held the stimulator against the tongue and evacuated the solution after it flowed over the isolated papilla. The negative pressure applied over this region was between 0.05 and 0.1 atmosphere. When the central chamber of the stimulator was properly placed over a single papilla, the papilla was isolated from any stimulus solution which was applied liberally over the dorsum of the tongue; likewise, any solution introduced into the stimulator did not leak onto nearby papillae outside the circumference of the stimulator.

Recordings of single or few fiber preparations in which individual fiber responses
were discriminable were obtained by recording from the finely divided nerve strands. First, the receptor field of these fibers was determined roughly by applying 0.1 molar NaCl to an area of the tongue of several square millimeters with a small wisp of cotton saturated with the stimulus solution. In this manner the receptor field of the fiber was limited to one-eighth or one-tenth of the tongue surface innervated by the chorda tympani nerve bundle. Papillae within the roughly determined area were then stimulated individually with the single papilla stimulator.

In order to map the papillae which contributed input to the afferent nerve fiber, the papillae located within the roughly determined area were stimulated individually with a single concentration of NaCl (Baker, reagent grade), usually 0.3 molar. After one of the papillae was selected for study, its response to several concentrations of sodium chloride, in most cases 0.1, 0.3, and 1.0 molar solutions, was recorded. Each stimulation period lasted for 20 sec and was interspersed with periods of equal duration in which single distilled water at room temperature was flowed over the papilla. The magnitudes of the responses were compared with the response of the same nerve fiber to a single 2 ml application of the stimulus applied with a medicine dropper over the entire tongue surface. The stimuli were applied for 20 sec periods and the tongue was washed with a single application of distilled water which remained on the tongue for 20 sec until the subsequent stimulus was applied. For stimulation of the single papilla, the number of nerve impulses produced in the 4 sec interval from the 6th through the 9th sec following stimulus onset was used as a representative measure of the steady-state response.

In a different series of stimulations about 2 ml of stimulus solution was applied with a medicine dropper to the area of the tongue surrounding the isolated papilla, and the response frequency of the nerve fiber was investigated as a function of the concentration and identity of the surround stimulus. The steady-state response to stimulation of both the single papilla and the surround was measured by the impulses during the 2nd through the 5th sec following the application of stimulus to the surround. The steady-state response frequency of the fiber (impulses during the 6th through the 9th sec following the onset of stimulation) produced by stimulation of the isolated papilla alone with a given concentration of NaCl at a constant flow rate served as the control. The steady-state response to stimulation of both the single papilla and the surround was compared to the response of the fiber to stimulation of the single papilla alone.

The stimulation sequence proceeded as follows: (a) stimulation of the single isolated papilla via the stimulator was followed 10 sec later by (b) application of a second stimulus solution to the exposed tongue surface surrounding the isolated papilla; (c) the surround stimulus was rinsed with distilled water after 10 sec of application; and 30 sec following the onset of stimulation to the isolated papilla (10 sec after the surround was rinsed), stimulation of the single papilla was stopped and distilled water was flowed through the stimulator over the isolated papilla for 30 sec. In each stimulation cycle the single papilla was stimulated for 30 sec and rinsed with distilled water for an equal amount of time. During the middle 10 sec of stimulus application to the isolated papilla a second stimulus was applied to the area surrounding the isolated papilla. It is the effect produced by stimulation of the surround on the ongoing
steady-state response of a single nerve fiber responding to a continuous flow of NaCl solution over the isolated papilla which is to be quantified. For the enhancement series the surround stimuli consisted of NaCl solutions in 0.01, 0.1, 0.3, and 1.0 molar concentrations. In the depression series 0.003, 0.01, 0.03, 0.1, 0.3, and 0.5 molar potassium benzoate solutions were the surround stimuli.

RESULTS

The responses of 31 single chorda tympani nerve fibers from 27 rats are contained in this report. The data were collected from 15-60 min of continuous recording from a single fiber while from 1 to 12 fungiform papillae were individually stimulated. The response of a single fiber to stimulation of a single papilla (Fig. 3) is qualitatively the same as the response of the fiber to stimulation of the entire tongue which has been extensively studied (Cohen et al., 1955; Pfaffmann, 1955; Fishman, 1957; and others). The response is initiated with a phasic burst of impulses of variable frequency and duration up to 2 sec. Following the initial burst of activity the nerve responds to the continuous stimulation of the flow-through stimulator with a maintained frequency of impulses during 30 sec which represents the longest stimulation period used in these experiments. The interspike intervals are variable as seen in Fig. 3, while the average frequencies over one or more seconds of activity approach constancy. In Fig. 3 for 0.1 m NaCl stimulation, the numbers of impulses per second in the first 15 sec are as follows: 32, 22, 19, 17, 13, 9, 27, 19, 13, 14, 23, 17, 21, 22, and 14 (mean 2nd-15th sec = 17.9 ± sd 4.8). This maintained level of activity is referred to as the steady state.

Prior to NaCl stimulation (Fig. 3) the papilla was continuously washed.
with distilled water, and at the termination of the stimulation period the distilled water wash was reinstated. The background activity of the fiber in the prestimulation period was typically less than five impulses in the 10 sec preceding stimulation. The wash of the stimulus from the papilla and the

![Graph](image)

**Figure 4.** Concentration-response curves and locations of active papillae. The concentration of NaCl applied to the single papilla is indicated on the abscissa and the steady-state response frequency is plotted on the ordinate. Where responses were obtained for more than one papilla per single nerve fiber, the symbol denoting the position of the papilla corresponds with the curve for that papilla.
subsequent return of the fiber to its prestimulation level required less than 10 sec of the 30 sec wash period.

Concentration-Response Functions

The concentration-response curves for 24 single papillae innervated by 18 single nerve fibers are shown in Fig. 4. In 15 single fiber preparations the concentration series was performed on only one papilla; and on two, three, and four papillae, respectively, in three preparations. The responses of 22 individual papillae increased with an increase in concentration from 0.1 to 0.3 M NaCl, one remained about the same (within 10%), and one decreased. As the NaCl concentration was raised from 0.3 to 1.0 molar, 13 increased, 7 remained about the same, and 4 decreased.

The response of the four fibers that decreased (4 C, 4 E, 4 O, 4 R) at 1.0 molar NaCl stimulation began with an initial burst of high frequency which gave rise to a steady-state frequency lower than the steady-state response to 0.3 M NaCl. This phenomenon was referred to by Pfaffmann (1955) as “overload.” If the strong stimulus was applied for short intervals the effect was reversible, but the responses of the fiber to subsequent stimulation of the papilla with lower NaCl concentrations were diminished if the strong stimulus were not rinsed within 30 sec. This type of response was exhibited by some papillae and not by others innervated by a single nerve fiber (4 E, 4 O).

The Single Papilla vs. the Entire Tongue

In Fig. 5 the response of eight nerve fibers to stimulation of single papillae is compared with the response to stimulation of the entire tongue. The papilla which was chosen gave the largest response of the papillae examined in a rough mapping procedure. In general, the response of the fiber was greater for stimulation of the entire tongue than for the single papilla, while some fibers (Fig. 5 A) appeared to receive all of their input from a single papilla.

The mean frequency of the eight fibers at each stimulus concentration is plotted for stimulation of single papillae and the entire tongue (Fig. 5 I). The shape of the stimulus-response curve is about the same for stimulation of the single papilla and the entire tongue; however, the average response of these fibers to stimulation of the entire tongue was about twice as large for 0.1, 0.3, and 1.0 molar NaCl as the response of the single papilla.

Single Fiber Mapping

Extensive mapping of the peripheral fields of single fibers was accomplished by application of the single papilla stimulator to individual fungiform papillae in the area determined by the rough mapping procedure. The mapped papillae were located about halfway between the tip and the back of the
tongue due to the close proximity of adjacent papillae on the tip, the curved surface on the tip of the tongue which hindered firm application of the stimulator, and obstruction at the posterior of the tongue by the fauces. About one-half the fibers dissected at random from the nerve were excluded from single papilla stimulation due to inaccessibility.

![Graphs showing concentration-response curves from stimulation of a single papilla (solid circles) and the entire tongue (open circles) for the same nerve fiber.]

Figure 5. Concentration-response curves from stimulation of a single papilla (solid circles) and the entire tongue (open circles) for the same nerve fiber.

Of nine single fibers investigated in this way, four fibers responded to the stimulation of one papilla, two responded to two papillae, two to three papillae, and one responded to the stimulation of four papillae. For two of the fibers which responded to only a single active papilla, stimulation of the entire tongue produced a response of the same magnitude as that of the single papilla (as in Fig. 5 A) which corroborates that these two fibers received the majority of their input from a single papilla.

A scanning electron micrograph at low magnification of a region of the rat tongue found by the rough mapping procedure to produce impulse
activity in a single nerve fiber is shown in Fig. 6. The single papilla stimulator was placed over each of the papillae in the array and the response of the single fiber was recorded. Papillae numbered 5 and 9 were slightly out of the field of the micrograph but produced no discernible response in the nerve fiber. Papilla number 8 was actually two single papillae; however, they were stimulated simultaneously and produced no response in the fiber. The nerve impulse activity produced by stimulation of the four papillae responding to the same concentration of NaCl is shown in Fig. 7. Stimulation of the other six papillae in the array produced no fiber response. In order of the decreasing magnitude of the steady-state response to 0.3 M NaCl: \( P.1 > P.4 > P.2 > P.3 \).
The receptor field of the fiber illustrated in Fig. 6 is exemplary of those which responded to the stimulation of more than a single papilla. The active papillae contributing to the response of the fiber were found to lie within an area of 25 mm$^2$ by the rough mapping procedure. The active papillae were juxtaposed and no inactive papillae were found between two active ones. When one papilla exhibited a higher steady-state response than the others, as in this example, it was usually located near the center of the array of the active papillae. Shrinkage due to dehydration for the scanning electron microscope reduced the surface lengths in the micrograph by 50–60% from measurements of the living tissue.

Enhancement

Fig. 8 illustrates the enhancement produced by stimulation of the surround with NaCl solution of increasing concentration while the single papilla was stimulated with 0.3 m NaCl. The control frequencies for 4 sec (2nd-5th) prior to stimulation of the surrounds were 43, 46, 39, and 48 (imp./4 sec) reading from top to bottom. The enhancement was initiated with a phasic response of about 1 sec duration followed by an elevated steady state. Concentrations of 0.03, 0.1, 0.3, and 1.0 m NaCl applied to the surround produced enhanced responses of 47, 75, 106, and 136 (imp./4 sec) for the 2nd-5th sec following stimulus onset. When the surround was washed with distilled water, the fiber
Peripheral Interactions among Single Papilla Inputs

FIGURE 8. Enhancement of the single fiber response to stimulation of a single isolated papilla by application of NaCl to the surround. 0.3 molar NaCl was applied to the single papilla via the stimulator for 30 sec in each of the four stimulation cycles. Subsequently, NaCl was applied to the surround (underline) in the four concentrations shown beneath the records and rinsed off 10 sec later. Time mark = 2 sec.

returned to the steady state produced by stimulation of the single papilla alone (39, 36, 42, and 48 impulses for the 2nd-5th sec following rinse).

The enhanced responses of 9 papillae innervated by 9 different nerve fibers plotted in Fig. 9 illustrate the salient features of the experimental population of 19 fibers on which the enhancement procedure was performed. The concentration of NaCl in the surround stimulus is plotted on the abscissa, and the frequency of nerve impulses is represented on the ordinate. The control responses to stimulation of the single papilla alone are indicated by the solid circles, and their mean values are denoted by the dashed horizontal line. The concentration of NaCl used to stimulate the single papilla is indicated at the right. The open circles mark the response frequency of the fiber to stimulation of the single papilla and the surround during the 2nd through 5th sec following application of the surround stimulus. Responses plotted on the ordinate result from application of water to the surround. The range of enhancement shown in 9 B, 9 C, 9 H, and 9 I is three- to fourfold compared to 9 D and 9 G where the responses were nearly the same as that of the single papilla alone. In 9 E only a small enhancement occurred with lower concentrations of surround stimulus and no systematic increase with increasing
concentration. In 9 A the response of the fiber was diminished below the average steady-state control; however, the diminution was not greater than the range of control responses. 5 of 19 fibers failed to show enhancement as in 9 D, 9 G, and 9 A when their associated single papillae were stimulated, and corroborative evidence suggested that 2 of these received input from only 1 single papilla.

In order to compare the relative enhancement throughout the population of fibers in this study, an enhancement ratio was computed by dividing the 4 sec response following stimulus application to the surround by the 4 sec response during the control period. This computation is averaged in Table I for 28 enhancement series carried out on 22 papillae innervated by 18 nerve fibers. The concentration of NaCl applied to the isolated papilla is shown on the left, and the concentration of the surround stimulus is shown above the
TABLE I

AVERAGE ENHANCEMENT RATIOS FOR 29 ADDITION SERIES

| Concentration of NaCl applied to single papilla | Concentration of NaCl applied to the surround | Total No. of series |
|---------------------------------------------|---------------------------------------------|-------------------|
| 0.1                                        | 0.01                                        | 1.228             | 0.1966 | 2.809 | 7 |
| 0.3                                        | 0.1                                         | 1.385             | 1.859  | 2.487 | 2.580 | 17 |
| 1.0                                        | 0.3                                         | 1.680             | 1.785  | 3.065 | 2.968 | 5 |

Frequency distribution of enhancement ratios for 0.3 m NaCl applied to the single papilla

Enhancement ratio

| Concentration of NaCl applied to surround | 0.001 | 1.001 | 2.001 | 3.001 | 4.001 | 5.001 | 6.001 | Mean ratio |
|-----------------------------------------|-------|-------|-------|-------|-------|-------|-------|-----------|
| No. of series                           | 0.01  | 1.00  | 2.00  | 3.00  | 4.00  | 5.00  | 6.00  | 11.000    |
| 7                                       | 3     | 3     | 0     | 1     | 0     | 0     | 0     | 1.385     |
| 17                                      | 3     | 10    | 1     | 1     | 0     | 2     | 0     | 1.859     |
| 17                                      | 0.3   | 3     | 7     | 4     | 0     | 2     | 0     | 2.487     |
| 17                                      | 1.0   | 2     | 7     | 4     | 1     | 1     | 1     | 2.580     |

average ratios. Enhancement would yield a ratio greater than the control value 1.000. In the lower portion of the table is a frequency distribution to illustrate the spread of the ratios for 0.3 m NaCl as the single papilla stimulus. Table I reveals that increasing the concentration of the surround stimulus produced a greater enhancement of the response to a maximum of about three times the control response. The range of the enhancement ratios for the 0.3 m NaCl (to the single papilla) series was from 0.664 to 11.000.

Depression

When potassium benzoate instead of NaCl was applied to the surround of an isolated papilla stimulated with NaCl, a depression of the single papilla response resulted (Fig. 10). The control responses for the 4 sec period prior to surround stimulation were 35, 33, 42, 44, 41, and 49 (mean 40.7 ± 5.1), respectively, reading from top to bottom. Beginning with 0.003 m potassium benzoate no depression was evident with a response of 36 impulses/4 sec (2nd-5th sec following surround stimulation). For potassium benzoate concentrations of 0.01 and 0.03 molar the responses were 31 and 27 impulses/4 sec, respectively. Upon application of potassium benzoate to the surround the response was initiated with a transient cessation of activity followed by a maintained response lower than the control frequency. The maximum depression (17 imp./4 sec) occurred at 0.1 m K benzoate, while the responses to 0.3 and 0.5 m potassium benzoate were 24 and 23 impulses/4 sec, respectively. When the surround was washed with distilled water, there was a period of
from 1 to 2 sec in which the frequency was greater than that for control levels. The period is referred to as "postdepression overshoot" and was followed by a return of the frequency to its prestimulation level.

The potassium benzoate depression response was investigated in a population of 20 papillae innervated by 16 nerve fibers. Fig. 11 shows the response curves (open circles) for nine single papillae innervated by nine different single fibers. The control responses are indicated by the solid circles, and the dashed horizontal line indicates the mean of the control responses. The concentration of NaCl used to stimulate the single papilla is indicated on the right, and the concentrations of potassium benzoate are plotted on a log scale on the abscissa. Fig. 11 illustrates that the concentration of potassium benzoate...
benzoate required to elicit depression varied from 0.01 molar (11 G, 11 H) to 0.1 molar (11 B, 11 I), and the degree of depression produced by any given concentration of the stimulus varied considerably among preparations. Examples 11 A and 11 H showed little increment of depression with increasing potassium benzoate concentrations from 0.01 to 0.3 molar, while 11 C, 11 D, and 11 F demonstrated a decrement proportional to the stimulus concentration. The maximum degree of depression varied from nearly total abolition of the single fiber response (11 D, 11 E, 11 G) to a more modest reduction of 20–30% of the control frequency (11 A, 11 H). Those fibers which reached a maximum depression at 0.1 or 0.3 molar potassium benzoate usually exhibited decreasing depression when the concentration was increased (11 A, 11 E, 11 G). Example 11 F illustrates the reproducibility of the response on a single preparation.
The mean response for the population was computed through a depression ratio as in the enhancement series. The response during the 2nd through the 5th sec following potassium benzoate application to the surround was divided by the response of the single papilla alone during the 2nd through the 5th sec preceding surround stimulation. The average depression ratios are tabulated in the upper portion of Table II, while the lower portion of the table contains a frequency distribution of the depression ratios for 0.3 molar NaCl applied to the single papilla stimulus. At concentrations of potassium benzoate below 0.03 molar, depression was equivocal; however, depression increased with stimulus concentration from 0.03 molar to a maximum depression at 0.3 molar. The depression produced by 0.5 molar potassium benzoate was less than for 0.3 molar.

Each of the enhancement or depression series contained three to five presentations of NaCl to the single papilla followed by a different concentration of either NaCl or potassium benzoate to the surround. The reproducibility of the steady-state control response (impulses during the 6th through the 9th sec) in a single fiber to stimulation of a single papilla was computed for 19 papillae innervated by 15 single fibers. The intermediate stimulus concentration, 0.3 molar NaCl, was chosen because it was used in more preparations than the 0.1 or 1.0 molar concentrations. In 127 presentations of 0.3 molar NaCl...
NaCl to the single papilla in 30 series, the control frequencies of a series varied, on the average, 11.3% from the mean control frequency of the series.

\textit{Sodium Chloride and Potassium Benzoate Mixtures}

Depression resulting from the application of potassium benzoate to the surround was compared with the response obtained by mixing the sodium chloride and potassium benzoate and applying the mixture to the single papilla alone. The responses of five fibers are compared in Table III. The control and depression responses are measured by the number of impulses in the 2nd through the 5th sec preceding and succeeding the application of potassium benzoate to the surround, respectively. The responses to the mixture are measured as the number of impulses in the 2nd through the 5th sec following application of the mixture to the single papilla through the stimulator. The NaCl concentration indicates the stimulus applied to the single papilla in the depression series and the concentration in the mixture. Since the single papilla stimulator would hold four solutions, two mixtures of sodium

\begin{table}
\centering
\begin{tabular}{ccccccc}
\hline
 & & & & & & \\
Fiber & Control & |0.1| & |0.3| & Sodium chloride | Potassium benzoate & \\
 & & concentration & & concentration & & concentration \\
\hline
1 & 47 & 13 & 0.1 & 20 & \\
 & 49 & 7 & 0.1 & 5 & \\
2 & 62 & 40 & 0.1 & 27 & 18 & \\
 & 57 & 30 & 0.1 & 18 & \\
3 & 56 & 18 & 0.1 & 63 & 39 & \\
 & 49 & 12 & 0.1 & 43 & \\
 & 65 & 13 & 0.1 & 51 & \\
 & 59 & 14 & 0.1 & 39 & \\
 & 61 & 23 & 0.1 & \\
 & 59 & 24 & 0.1 & \\
4 & 34 & 15 & 0.3 & 27 & 16 & \\
 & 41 & 10 & 0.3 & 16 & \\
5 & 45 & 31 & 0.3 & 50 & 36 & \\
 & 44 & 34 & 0.3 & 28 & \\
 & & & 0.3 & 51 & \\
\hline
\end{tabular}
\caption{COMPARISON BETWEEN SEPARATION AND MIXTURE OF NaCl AND POTASSIUM BENZOATE IN DEPRESSION}
\end{table}
chloride and potassium benzoate were used in addition to sodium chloride alone and distilled water for a rinse. Table III shows that 0.1 molar potassium benzoate stimulation of the surround produced a greater depression than the mixture on the single papilla in four preparations (1, 3, 4, 5), while the mixture produced a greater depression in one case (2). For 0.3 molar potassium benzoate, the mixture produced a greater depression in one fiber (2), about the same depression in two fibers (1, 5), while the separated stimuli produced a greater depression in two fibers (3, 4). Fiber 3 is the most graphic example of separation of the stimuli which produced a greater depression than the mixture on the single papilla.

The response of the entire chorda tympani nerve to sodium chloride and potassium benzoate mixtures using the summated response (Beidler, 1961) is shown in Fig. 12. The stimulus solution contained 0.1 or 0.3 molar NaCl and various concentrations of potassium benzoate. The mixtures were flowed through a flow chamber attached to the anterior portion of the tongue for 15 sec followed by a 15 sec distilled water rinse. Subsequent stimuli were presented at 60 sec intervals. Control stimulations with NaCl alone were interspersed among the applications of the mixture to the tongue. Depression

![Figure 12](image-url)

Figure 12. Summated responses of the whole chorda tympani nerve to stimulation of the tongue with sodium chloride, potassium benzoate, and mixtures of the two compounds. In the two series at the top of the figure sodium chloride was applied by itself and then in combination with potassium benzoate. The concentrations shown for the mixtures are the final concentrations of the stimuli in the mixtures. The gain of the recorder was adjusted to nearly maximum deflection. Below, concentration series are shown for sodium chloride and potassium benzoate applied independently. The tongue was adapted to 0.01 molar sodium chloride for the potassium benzoate series, and the gain setting remained constant for the continuous record shown at the bottom. The first of two marks above the numbers denoting the potassium benzoate concentrations indicates the application of potassium benzoate, while the second indicates its rinse.
ratios were computed by dividing the height of the response obtained from the mixture by the height of the response to NaCl alone for two complete series (Table IV). Little depression was produced by 0.01 molar potassium benzoate in the mixture, though a progressive depression occurred from 0.03 to 0.3 molar potassium benzoate. Less depression was produced by 0.5 molar than by 0.3 molar potassium benzoate.

In the lower portion of Fig. 12 the integrated response of the nerve is illustrated for concentration series of NaCl and potassium benzoate. The potassium benzoate series was conducted with the tongue adapted to 0.01 molar NaCl. The first of two small marks above the numbers denoting the K benzoate concentrations indicates the application of the solution; and the second, the washing of the solution from the tongue with 0.01 molar NaCl. The depression was sustained until the tongue was washed. The magnitude of the depression increased from 0.01 to 0.1 molar potassium benzoate, little depression was produced by 0.3 molar, and the response to 0.5 molar potassium benzoate was strictly increasing. When the potassium benzoate was washed from the tongue, there was an overshoot which increased in magnitude with increasing concentration of the stimulus. Sodium chloride was applied following the benzoate series to show the relative magnitude of the responses.

**DISCUSSION**

The anatomical basis of the multiple taste bud input to the single chorda tympani fiber in the rat is probably branching of the fiber in the tongue. The present study revealed that several single papillae contributed input to a single fiber, and, on the average, only one-half of the maximum input from NaCl stimulation came from the most sensitive papilla. The maximum steady-state response of the fiber to stimulation of the entire tongue represented the upper limit of the enhanced response of the single papilla. From studies in the frog which has about three times as many fungiform papillae as the rat Rapuzzi and Casella (1965) concluded that connected papillae were coupled by two to four interconnecting nerve fibers. The peripheral input from a
single taste bud to the central nervous system is probably carried through several nerve fibers; thus, the receptors within a taste bud and in neighboring taste buds which are connected might constitute a functional subset of the entire receptor population. Therefore, the identification of the "unit" of neural input as the response of a single nerve fiber or single taste bud in the light of the interaction between these elements is probably too simple.

The concept of cationic excitation and anionic inhibition in the rat taste system has been extended by the demonstration that the potent excitatory and inhibitory events can be spatially separated within the receptor field and still be integrated into the fiber response. In the frog, antidromic action potentials electrically produced in one branch of a gustatory fiber have been shown to invade another branch of the fiber, and it is suggested that they inhibit afferent activity arising from that branch (Filin and Esakov, 1968). It seems plausible that the antidromic feedback may be responsible for the phenomenon referred to as "overload" which is produced by relatively strong stimuli, and may also account for the poststimulatory depression reported for gustatory receptors by Hellekant (1968). Depression resulted from potassium benzoate at concentrations between 0.01 and 0.03 molar applied to the surround of a papilla stimulated with NaCl, but the concentration at which potassium benzoate stimulated impulse production in the fiber was between 0.1 and 0.3 molar. It is difficult to explain the depression by K benzoate in terms of antidromic depression of the receptors or by collision of impulses at a branching point of the fiber since the effect occurs below the concentration which produced action potentials.

Mixing NaCl and potassium benzoate and applying the mixture to the single isolated papilla did not produce as large a depression effect as stimulation of the single papilla with NaCl and the surround with K benzoate. The smaller depression resulting from the mixture may be due to competition among the cations and anions for the respective receptor sites. This result also suggests that the surround depression did not result from stimulus leaking into the stimulator to the isolated papilla. If a dilute surround stimulus leaked into the stimulator it would dilute the stimulus to the single papilla and diminish the response, but stimulation of the surround with a less concentrated stimulus produced an enhanced response. The isolated papilla was bathed with the stimulus at a rate which replaced the volume of the stimulator every 0.1 sec. The surround stimulus at atmospheric pressure would have to pass the outside evacuating chamber at −0.05 to −0.1 atmosphere pressure and then diffuse against the positive pressure of the fluid bathing the papilla in order to reach it. This does not seem likely.

The single nerve fiber ascends the fungiform papilla, loses its myelin sheath near the base of the taste bud, and gives up unmyelinated nerve branches. For the purpose of speculation on a model for the mechanism of integration
of the peripheral inputs, it is assumed that some of these branches innervate the taste bud while others descend the papilla to course in the nerve plexus beneath the epithelium to nearby papillae which they ascend to innervate the taste bud. Fig. 13 schematizes a myelinated nerve fiber which branches into two unmyelinated terminals each innervating a taste bud in a different fungiform papilla. The illustrated condition is for the application of NaCl to single papilla A and potassium benzoate to papilla B. If the NaCl causes a depolarization of the axon terminals at A, and K benzoate causes hyperpolarization of the terminals at B, then ionic current (broken lines) will tend to flow toward C from region B, and toward A from region C. If spike potentials are generated at the first node of Ranvier (D) when that region is depolarized to threshold, it would be assumed that the membrane potentials at D would be dependent on the direction and magnitude of current flow in nearby regions of the nerve membrane including region C. The net effect at C would depend on the events at A and B, and would determine the frequency of spike potentials generated and propagated toward the central nervous system.

The potassium benzoate depression of the ongoing response of a single papilla to NaCl stimulation is explicable in terms of the model as the case when the degree of depolarization of the prenodal membrane by NaCl is diminished by the current influx from branches stimulated by potassium benzoate. The enhancement by NaCl of the single papilla response could be explained as the result of augmentation of the depolarization of the prenodal membrane by additional NaCl-stimulated branches. The postdepression overshoot following the wash of potassium benzoate from the surround might be the result of a transient current sink created by the rapid return of the hyperpolarized membrane toward its resting potential.
Could decremental currents traverse the distances between papillae and remain large enough to produce an effect at a distant site of impulse production? The membrane parameters of these elements have not been measured, but in the unmyelinated terminal segment of the pacinian corpuscle nearly 40% of the generator potential remains at a distance of 0.8 mm from the point of stimulation (Loewenstein, 1965). Extrapolating from the curve of Loewenstein (his Fig. 1), one might expect 20% of the generator to remain at a distance of 1.3 mm from the point of stimulation. If the generator currents from five parallel branches were synchronized in time at a hypothetical common branching point, their additive effect might produce a transmembrane potential difference comparable to that at the point of origin.

The inhibitory nature of potassium benzoate at lower concentrations and its decreasing effect at higher concentrations suggest that the anionic and cationic mechanisms are sensitive at different concentration ranges. At concentrations from 0.01 to 0.1 molar the benzoate anion seems to predominate while at higher concentrations the cation exerts a greater effect. The potency of other stimuli in which the organic anion might dominate the response at low concentrations is illustrated by the synthetic sweeteners, salts of cyclohexane sulfamic acid (cyclamate), and orthobenzenosulfamide (saccharine); as well as the bitter alkaloids, quinine and strychnine.

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A preliminary report of experiments by Drs. M. K. Frank and M. Wang of The Rockefeller University who also mapped the peripheral field of single chorda tympani fibers in the rat by stimulation of single fungiform papillae was brought to my attention by Dr. C. Pfaffmann (Pfaffmann, C. Physiological and behavioural processes of the sense of taste. Ciba Found. Symp. Taste and Smell in Vertebrates. 1970. (31). Frank and Wang found from one to nine papillae innervated by a single fiber with an average of 4.5 papillae per fiber.

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