CHANGES IN GUSTATORY NERVE DISCHARGES WITH SODIUM DEFICIENCY: A SINGLE UNIT ANALYSIS

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ABSTRACT

CHANGES IN GUSTATORY NERVE DISCHARGES WITH SODIUM DEFICIENCY: A SINGLE UNIT ANALYSIS

By

Robert John Contreras

The sensory system of taste is the interface between the internal milieu and external stimulating environment. It is the final arbiter between what gets accepted or rejected. But taste is not necessarily a passive receptacle for information from the external environment; it may be active and participate in processing information. For example, the sensory system of taste may be affected by the changing state of the organism induced by cyclic processes of hunger and thirst, electrolyte balance, caloric deficiency, hormonal regulation, etc. As a consequence, subtleties in the actual taste or hedonic quality of an ingestant may be altered concomitant with fluctuations in the internal milieu. Thus, information about the physiological condition of the organism is transmitted to the gustatory system to determine the relative desirability of the external stimulus.

Sodium appetite is one classic example of a state dependent phenomenon that influences intake. In sodium need, many mammals have an innate preference for salt that is specific to the sodium ion. Taste factors have been implicated in having an important role in controlling the appetite for sodium. But the relevance or nature of

taste control over sodium appetite has not been resolved. The neural mechanisms of taste that may underly sodium appetite have always been studied at a gross, multifiber level. Analysis of sodium coding, though, has never gotten as far as the single unit level. A single unit analysis may exhibit differences between Na-deprived animals and normal controls that would have been masked, otherwise, by studying whole nerve responses. The purpose of this investigation, then, was to study the effects of sodium deficiency on the behavior of single taste fibers to chemical stimulation of the tongue.

The experimental group received a Na-deficient diet for 10 days; matched controls received the same diet but with 1% NaCl added. Responses from 42 chorda tympani taste neurons, 21 per group, were analyzed in terms of (1) their sensitivity to moderately intense stimuli; (2) their change (or lack of) in response rate over time; and (3) their across-fiber patterns of activity.

Analysis revealed that control fibers may be more sensitive to NaCl than fibers from the Na-deprived group. That is, .1 M NaCl, a stimulus of moderate intensity, stimulates control fibers to a significantly greater degree (more impulses in 10 sec). It was proposed that NaCl must elicit a critical total number of impulses in the taste nerve before the rat's intake of NaCl would cease. As a result, it would take a Na-deprived rat a greater amount of time to adapt to the taste of a salt stimulus than a normal control. This gustatory mechanism would permit greater than normal intake of substances containing sodium under conditions of sodium deficiency.

Further analysis revealed that taste stimuli elicited significantly different patterns of activity across the fibers of Na-deprived rats than across control fibers. This result suggests that there are subtle differences in the sensations that a rat, in sodium need, receives from certain taste stimuli. It was pointed out that the animal's behavior in sodium need will be more likely guided by the "saltiness" rather than the "sweetness," "bitterness," or "sourness" of a food substance because of altered taste sensations.

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By

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INTRODUCTION

The nature of taste control over sodium intake has been a subject of controversy for almost 40 years. The most prominent theory of this period was proposed by Richter (1939). He suggested that sodium deficiency made the taste receptors more sensitive to salt stimulation. This conclusion arose from the observation that the preference threshold for salt in a two-bottle preference test with water was decreased considerably by sodium deficiency (Richter, 1939; Bare, 1949). Richter's notions were challenged by psychophysical (Carr, 1952; Harriman & Macleod, 1953) and electrophysiological (Pfaffmann & Bare, 1950; Nachman & Pfaffmann, 1963) experiments, however, since they demonstrated that detection thresholds for NaCl were the same in adrenalectomized and normal rats.

Even if Richter's theory were correct in asserting that thresholds were lowered, it does not explain why a sodium deficient rat consumes more sodium than a normal rat. An increase in gustatory sensitivity only increases the availability of sodium to the rat. It does not insure that a rat will consume more salt to meet its physiological needs. The problem is to explain why a Na-deprived rat increases its NaCl intake across a wide range of concentrations (Nachman & Cole, 1971). Some mechanism is needed that permits greater than normal intake when the animal's "sodium reservoir"

(Stricker & Wold, 1966) has been depleted. One possibility is a float-valve type of mechanism which is time dependent like the process of adaptation. The valve shuts off intake once the reservoir has been filled. Thus, if emphasis is shifted from when to start drinking (detection threshold) to when to stop drinking (adaptation) saline, then different experimental questions arise. Although the shut-off mechanism for NaCl drinking would depend, in part, upon post-ingestional factors, some aspect of NaCl intake may be controlled by gustation. For example, a Na-deprived rat may take longer to adapt (as opposed to having lower detection thresholds) to the taste of a salt stimulus than a normal control.

The rat's drinking behavior is characterized by being intermittent because it drinks in bursts, pauses, and drinks again. To test the premise of differential adaptation, the temporal patterns of intake in Na-deprived rats and normal rats were compared (Contreras & Hatton, in press). Analyses included burst duration and interdrink interval. In the complex situation of a two-bottle preference test, drinking time was measured to determine how long the rat stayed with one solution. In agreement with the adaptation model, Na-deprived rats had shorter interdrink intervals and longer drinking times than normal rats. Burst duration was not correlated with an increase in NaCl preference. These changes in NaCl acceptability following sodium deficiency were attributed primarily to taste factors because the differences were apparent in the first ten minutes of the test period when post-ingestional influences were at a minimum. And the same results were obtained with and without the confounding factor of

thirst, a factor which has been present in all previous studies (Nachman, 1962; Nachman & Pfaffmann, 1963; Smith, Stricker & Morrison, 1969).

Gustatory adaptation as it concerns sodium appetite may occur centrally or peripherally. Although there were not threshold changes (Carr, 1952; Harriman & MacLeod, 1953; Pfaffmann & Bare, 1950; Nachman & Pfaffmann, 1963), there might be changes in gustatory adaptation that occur at the peripheral level due to sodium deficiency. As a result, peripheral mediation cannot be rejected until all aspects of gustatory coding have been studied.

In a physiological vein, Bradley's (1973) study substantiated the notion that the changed salt preference in sodium deficiency may be a result of a changed neural response at the receptor level. He showed that the chemistry of the blood bathing the taste receptors influenced the afferent gustatory input. And since adrenalectomized rats have lower plasma sodium levels and higher plasma potassium levels (Jalowiec & Stricker, 1973) than intact controls, a mechanism is thus provided for state changes (sodium deficiency) to influence taste at a peripheral rather than at a central level (Bradley, 1973).

From the above discussion it is clear that the relevance or nature of taste control over sodium appetite has not been resolved and continues to be a promising research topic. Sodium appetite is an ideal motivational phenomenon suitable for experimental analysis of internal-external environment interaction. The appetite for sodium can be induced quite readily by a variety of methods (Wolf, McGovern & Dicara, 1974). It is not dependent on any learning factors for its

induction (Nachman & Cole, 1971). And the taste of sodium salts can be distinguished from the taste of nonsodium salts (Nachman, 1962). Thus, it appears that there is an innate neural system specific to the sodium ion that is used by a Na-deficient animal to discriminate sodium solutions from nonsodium solutions (Nachman & Cole, 1971). The present study was designed to determine whether sodium deficiency affects the behavior of single taste neurons. This approach bears directly on the sensory processing of taste. Therefore, two prominent theories that attempt to account for the coding of taste quality will be briefly reviewed.

Labelled Line Versus Across-Fiber Pattern

A dispute presently exists on how the gustatory system operates to code quality information. Researchers in the field have parcelled themselves into two major camps--those that believe the so-called "labelled line" theory versus those that subscribe to the "across-fiber pattern" theory. Although this dichotomy is not hard and fast, research has been directed at supporting or disproving one of the two theories. The split in ideology occurs when discussing the relative importance a single taste neuron has in transmitting information about quality to the CNS. The labelled line theory attributes more importance to the behavior of a single taste neuron than does the across-fiber pattern theory.

The across-fiber pattern theory was introduced by Pfaffmann (1959) and was subsequently formalized by Erickson *et al.* (1965). This theory asserts that taste quality information is coded by a pattern

of activity across several nerve fibers. The degree to which different sapid solutions elicit similar patterns of activity determines their degree of taste similarity. This theory originated because mammalian taste fibers are nonspecific in their sensitivity to chemical stimulation (Sato, 1971). A particular fiber will generally respond to two or more of the stimulus classes of salt, sweet, sour, and bitter. Furthermore, not only do first order neurons respond nonspecifically, but so do taste receptor cells (Kimura & Beidler, 1961).

If across-fiber patterns represent the form in which taste quality is encoded, then the more similar the pattern of two stimuli the more difficult would be a discrimination between these two stimuli. Stated another way, stimuli which generate similar patterns should taste somewhat alike. Evidence to support this notion has been obtained in the rat. Potassium chloride and NH_4Cl produce similar patterns of neural activity (Erickson, 1963) that are different from the patterns of LiCl and NaCl, which are strikingly similar (Erickson et al., 1965). Hence, a rat should find it difficult to discriminate between KCl and NH_4Cl but not between NaCl NH_4Cl. It was found (Erickson, 1963) that rats trained to avoid drinking NH_4Cl avoided drinking KCl to a much greater degree than NaCl. Similarly, rats trained to avoid KCl generalized to NH_4Cl to a greater degree than to NaCl. Rats trained to avoid NaCl exhibited a slight effect on the avoidance of both NH_4Cl and KCl. Further supporting evidence was reported by Nachman (1963). He showed that avoidance learning to the toxic effects of LiCl generalized to a greater extent to NaCl than to either KCl or NH_4CL. These aforementioned experiments support a

concept that gustatory quality may be determined by the pattern of activity across several neurons. This theory runs into minor difficulty, however, because analysis of across-fiber patterns does not include the first 300 msec of the neural response which is used by the rat for coding taste quality (Bernard, in press, b). A rat can reject an aversive stimulus by licking 5 ul for as little as 50 msec (Halpern & Tapper, 1971). This short period for behavioral taste recognition has been supported electrophysiologically by recording whole nerve responses to lick-duration stimuli (Halpern & Marowitz, 1973).

The labelled line viewpoint, historically, is the oldest explanation of taste quality coding that originated at the end of the 19th century (Pfaffmann, 1974a). This classical view assumes that the sense of taste is composed of four primary taste receptors corresponding to the sensations of sour, salty, bitter, and sweet. A corollary to this notion is that there are specific fiber types corresponding to the primary receptors. These fiber types retain their independence by relaying information about a single taste quality. The compelling data of multiple sensitivity found in papillae, receptor cells, and first order neurons, however, caused many investigators to abandon labelled line in favor of across-fiber pattern theory, except for researchers like von Békésy (1964, 1966) and Bernard (1971). Von Békésy reported that localized chemical (Békésy, 1966) or electrical stimulation (Békésy, 1964) of the tongue elicited pure taste sensations of sweet, sour, salty, or bitter depending on the papillae stimulated, the concentration of the chemical stimulus or the parameters of

current. Accordingly, von Békésy re-established the classical theory of taste, now known as the neo-Müllerian view (Bernard, 1971). He argued that there were specific receptors and nerve pathways corresponding to the four basic tastes. He also suggested that neural patterns are necessary to code nuances in the complex tastes of natural substances. For instance, various sugars (e.g., sucrose, glucose, and fructose, etc.) each of which tastes sweet, can nonetheless be distinguished from one another. In an attempt to find the physiological underpinnings of von Békésy's psychophysical results, Bernard (1971) studied the responses of single nerve fibers. Quantitative analysis showed a greater overall sensitivity to one of the many stimuli which could activate them. It was argued that taste neurons respond "best" (i.e., with a higher frequency of response) to one of four basic stimuli and that this "physiological" specificity did not require an across-fiber pattern approach (Wang & Bernard, 1970). This is not a convincing argument unless this "best" response in taste neurons can be related to behavior. Erickson (1968) pointed out that the difference between labelled line and across-fiber pattern theories was not whether there is specificity or maximal sensitivity but whether there were a few taste primaries or an indefinite number of them. In a conciliatory tone, Bernard suggests that both specific receptor-fiber and neural pattern theories are necessary to explain the coding of taste stimuli (Bernard, in press, b). There are neuron types corresponding to the basic taste qualities and neural patterns which code distinctions within a stimulus class.

Although across-fiber pattern theory has been the most popular of the two, momentum has shifted and Pfaffmann, the initiator of the across-fiber pattern theory, now subscribes to a labelled line point of view. The reason for this shift in thought is based on work obtained from the squirrel monkey (Pfaffmann, 1974b). It was shown by a two-bottle preference test (sugar versus water) that sucrose was preferred over water at a weaker concentration than either fructose or glucose. And further, fructose was a stronger stimulus than glucose. At equimolar concentrations, however, fructose produced a magnitude of response that was greater than sucrose or glucose in the total chorda tympani nerve. But the sugars should produce the same order of effectiveness by both behavioral and physiological methods if it is assumed that the magnitude of the chorda tympani response to sugar stimulation underlies behavioral preference. This disagreement between preference and electrophysiological data was resolved when sugar sensitivity in single fibers was examined in the squirrel monkey (Pfaffmann, 1974b). It was found that fibers which responded best to salt also gave a good response to fructose that was stronger than the responses to either sucrose or glucose. Sucrose-best fibers, on the other hand, showed an order of effectiveness of sucrose > fructose > glucose. In these fibers where sucrose was a more effective stimulus than fructose, the response to salt was minimal. Accordingly, the behavior of single fibers instead of whole nerve responses more accurately corresponds to preference behavior. These electrophysiological data support a labelled line point of view for two reasons. First, in all the units studied there was never an exception to the

order of responsivity. In sucrose-best fibers fructose was always second in responsivity, followed by glucose, and last were the salts. Secondly, the order of sensitivity in sucrose-best fibers paralleled the order in which the sugars were preferred. Thus, it appears that a taste fiber can be accurately classified by its best response and that the sucrose-best fibers and not the other (salt best) sugar sensitive fibers are correlated with behavior.

Further support for the concept of best taste in classifying individual taste fibers comes from the work of Frank (in press). Using the best frequency analysis she demonstrated that rat glossopharyngeal taste fibers show a higher incidence of quinine-best responses (over 35%) than rat chorda tympani fibers (less than 5%). These data correspond with the earlier observation that the back of the tongue and the glossopharyngeal nerve are more responsive to bitter tasting stimuli (Bernard, in press, a).

Research has been devoted to resolving the physiological mechanism of taste that underlies sodium appetite. Analysis, though, has never gotten as far as the single unit level. A single unit analysis may exhibit differences between Na-deficient animals and normal controls that would have been masked, otherwise, by studying whole nerve responses, as happened when Pfaffmann and his associates tried to determine the physiological basis of sugar preferences.

Analysis of single unit data indicating that Na-deprived rats encode saltiness differently than normal controls could support either across-fiber pattern theory, labelled line theory, or both. According to the across-fiber pattern view, saltiness would be represented by

the pattern of activity across many fibers. If the particular pattern of activity elicited by NaCl stimulation were altered in sodium deficiency, then this would suggest that NaCl might taste different for Na-deprived rats. For instance, NaCl might taste sweeter, less sour, less bitter or some combination of the three to a deficient animal.

On the other hand, a "salt-best" system in the rat may be important in determining NaCl preference. Changes in the milieu interne due to sodium deficiency may affect this system such that an increase in NaCl preference is reflected solely in the activity of salt-best fibers. In this case, sodium deficiency may be shown to modify the temporal pattern (adaptation) of activity elicited by NaCl stimulation of only one receptor class--the salt-best class. This notion is credible because on a different problem, Kow and Pfaff (1973/1974) have shown that the hormone estrogen can modify receptor sensitivity of one class of tactile endings of the perineal region of the female rat. But the only tactile endings that were affected are those from the small guard hairs. Thus, the selective effects in receptors may be selective as to receptor classes.

The purpose of this investigation, then, is to determine whether single chorda tympani fibers code taste stimuli differently in Na-deprived rats than in normal controls. Contrary to the accepted view of central mediation (Carr, 1952; Harriman & MacLeod, 1953; Pfaffmann & Bare, 1950; Nachman & Pfaffmann, 1963), it is believed that some aspect of the taste control over sodium appetite occurs at a peripheral level. And peripheral mediation may become evident with the study of the behavior of single taste fibers.

METHOD

Subjects

The animals were 36 male albino rats of the Holtzman strain, and between 90 to 120 days old. They were individually housed in standard Acme Metal Products metabolism cages and had food and distilled water present at all times. The colony room was kept on a 14-10 hr light-dark cycle for the duration of the experiment. They were fed a powdered, sodium deficient test diet (supplemented with 1% NaCl, Nutritional Biochemicals Test Diet). Each rat was adapted to this diet for at least seven days, after which it was either put into a control or a Na-deprived group. The animals of the Na-deprived group were fed a sodium-free diet for a 10 day period before being used for chorda tympani recording. On the day of recording each animal of both groups was weighed and a urine specimen was collected and stored for future analysis of sodium concentration by flame photometry. This latter physiological measure was used to verify diet conditions.

Chorda Tympani Recording

Preparatory to surgery, urethane was injected intraperitoneally at a dosage of 1.5 g per kg of body weight. Supplementary doses of 0.0025 g were occasionally given to maintain a uniform level of anesthetization. The trachea was cannulated to facilitate respiration. Body temperature was monitored and maintained between 36°-38°C with a

variable temperature heating coil that was wrapped around the subject's body. The corners of the mouth and the lower incisors were severed to permit greater access to the tongue. One end of a six inch piece of thread was sutured to the epithelium on the bottom surface of the tongue. The animal was placed in a headclamp (Hosko, 1972) and the tongue was withdrawn from the mouth by pulling the loose end of thread and securing it to the table top with tape. From the side surface of the cranium, the chorda tympanic branch of the seventh cranial nerve was dissected free from the overlying tissues, bones, and blood vessels.[1] The dissection required a binocular microscope which was set between 10 and 30 power. The right chorda tympani nerve was isolated (approximately 5 mm in length) from the point where it joins the lingual nerve to its exit from the bulla, where it was cut. The nerve's sheath was peeled away with a pair of fine dissecting forceps and small strands of fibers were separated from the nerve trunk. Neural activity was recorded from the fibers of the chorda tympani by placing these small strands over a fine stainless steel electrode. A fiber was judged to be active (1) if it discharged spontaneously, or (2) if it did not discharge spontaneously but responded to chemical stimulation of the tongue. This procedure minimized the possibility of recording from a limited population of fibers, i.e., only those fibers that had a spontaneous rate of discharge.

A gravity flow system delivered the solutions at a rate of 50 ml per minute to the entire dorsal surface of the anterior portion

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[1]Thanks are due to Dr. Michael B. Wang of Temple University, who taught me the surgical technique for exposing the chorda tympani nerve.

of the tongue. One of two solutions (stimulus or rinse) continuously bathed the tongue, eliminating the influence of saliva. A five-channel switch box controlled five solenoid valves that were independently linked above to separate 1000 ml jugs and terminated below into a glass spigot by tubing. The spigot was anchored with clamps over the tongue for chemical stimulation. Of the five 1000 ml jugs, four were filled with one of the following solutions: .1 M NaCl, .01 N HCl, .02 M quinine HCl, and .5 M sucrose. The remaining jug was filled with distilled water. These concentrations were chosen on the basis of a power function relationship between the average number of impulses discharged during 5-10 sec after stimulation in chorda tympani fibers of rats and hamsters and the concentration of four basic gustatory stimuli (Sato, 1971). The solutions were equated to the response strength of .1 M NaCl, a standard solution in taste research. All solutions were made as molar concentrations and were kept at room temperature. The switch box that controlled solution delivery also controlled the internal calibration signal of the oscilloscope. This signal was used to distinguish between stimulus presentation and rinse conditions. Thus, activation of one of the stimulus channels by pushing one of the switches automatically closed the water channel and opened a stimulus channel, and simultaneously triggered the calibration signal of the oscilloscope. Thus, the onset of the calibration signal indicated when a stimulus channel was activated by pushing a switch, not when the solution actually contacted the tongue. This calibration signal remained on until the water channel was re-activated. This arrangement enabled the experimenter to

switch rapidly from one solution to the other and to control the duration of the stimulus and rinse conditions.

Provided the responses of an active fiber could be reliably identified, based on the size and shape of the impulses with oscilloscope and audio monitor displays, stimulation was continued according to a prescribed schedule. Whenever possible, the stimuli were presented three times each; this varied, however, depending upon the length of time the responses of that fiber were identifiable. The order of stimulus presentation was arbitrary and was not necessarily the same for each fiber. A 30 sec water rinse separated stimulus presentations.

Responses were recorded on magnetic tape for future "off-line" processing. Photographs were taken from the oscilloscope traces of every chorda tympani fiber's response to water and the four taste stimuli. These photographic records were used to determine whether an individual neuron could be functionally isolated. Based on whether one or more fibers could be isolated from the chorda tympani nerve, 19 out of 36 rat preparations were considered successful. Nine of these 19 preparations were from control animals. The specificity of each fiber's response to one of four taste stimuli was determined by counting the number of impulses elicited in 10 sec of stimulation in fibers that could be clearly identified. This 10 sec response was used because the time-course of the response varies across fibers and stimuli. For instance, a fiber may respond with a transient burst of activity for the first second and decline to a sustained, steady rate during the remaining part of the stimulation period. In other

instances, response latencies may be as long as three or four seconds, and be characterized with not having a transient response--the response rate increases as the stimulation period progresses. The point at which a response started was estimated from the photographs and was usually associated with an abrupt change in on-going activity. A spontaneous response rate was defined as the mean rate during the two sec preceding each stimulus onset averaged over all stimulus presentations. And when the number of impulses discharged in certain fibers during chemical stimulation exceeded the mean + 2 X S.D. of the spontaneous firing rate, they were judged to respond to the given chemical. The temporal characteristics of the impulse train were analyzed by a LINC computer. Frequency-time histograms, which represent the distribution of spikes in time, were computed for periods before, during and after stimulus application.

RESULTS

The Distribution of Response Across Fibers

Differences were found in the sensitivity of chorda tympani fibers according to (a) whether animals were Na-deprived or not, and (b) which of four classes of taste stimuli were employed. For example, the responses to each of the four stimuli (.1 M NaCl, .5 M sucrose, .01 N HCl, and .02 M quinine) are not restricted to separate sets of rat chorda tympani fibers, nor to limited ranges of frequencies. More correctly, the responses to each of these stimuli vary considerably in frequency (number of impulses elicited in 10 sec). The fibers for the two groups of rats are rank ordered four different times according to the frequency of the responses to each stimulus. Figure 1 presents these distributions of ordered response frequency.

The best stimuli are NaCl and HCl; i.e., they stimulate a greater number of fibers to a greater degree than the other two basic tastes. For example, NaCl elicits at least 20 impulses during the 10 sec stimulation period in 38 of the 42 fibers from both Na-deprived and control groups. Hydrochloric acid does in 22, quinine in 20, and sucrose in 11. The sensitivities to .5 M sucrose, .01 N HCl, and .02 M quinine appear to be the same for the two groups of rats when the fibers' responses are ordered according to frequency.

Fig. 1.--The distribution of responses of 21 fibers in Na-deprived rats and of 21 fibers in controls to .5 M sucrose, .02 M quinine, .01 N HCl, and .1 M NaCl rank ordered four different times according to the size of the responses to each stimulus. Response size refers to the number of impulses elicited in 10 sec of stimulation. A dot above a bar indicates whether a particular fiber is a sucrose-best responder (top left), a quinine-best responder (top right), a HCl-best responder (lower left), or a NaCl-best responder (lower right). The chief difference between fibers from Na-deprived animals and controls is in responses to NaCl (lower right), where "high salt-responders" (> 75 impulses/10 sec) in control animals are more sensitive ($p \leq .05$ Kolmogorov-Smirnov 2 sample test).

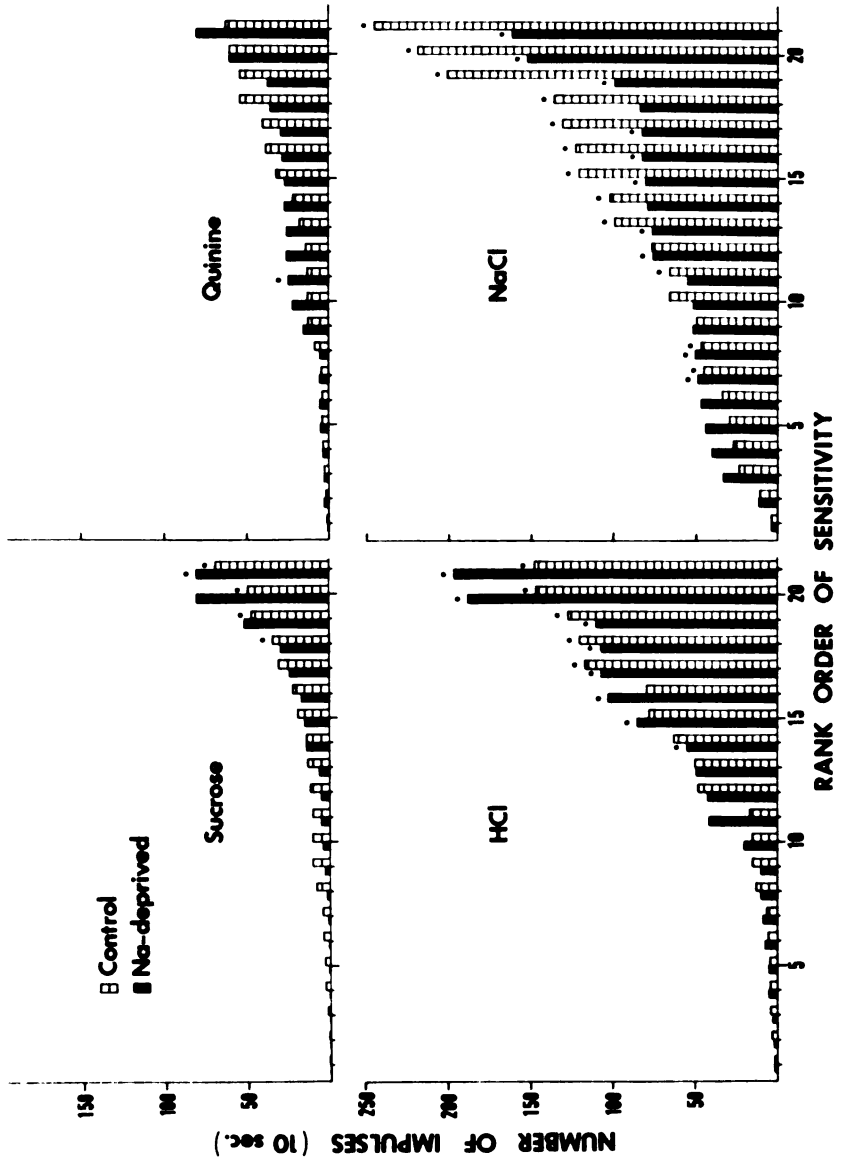
Control
No-deprived
Sucrose
Quinine
HCl
NaCl
NUMBER OF IMPULSES (10 sec.)
RANK ORDER OF SENSITIVITY

Figure 1

On the other hand, the control group appears to be more sensitive to NaCl than the Na-deprived group. That is, .1 M NaCl, a stimulus of moderate intensity, stimulates control fibers to a greater degree than fibers obtained from Na-deprived rats. Control fibers that are high salt-responders (fibers 12-21, $\geq$ 75 impulses per 10 sec) have stronger responses to NaCl than do the corresponding fibers from Na-deprived rats ($p < .05$; Kolmogorov-Smirnov two-sample test; one-tailed test). A one-tailed Kolmogorov-Smirnov test is used to decide whether or not the values of the population from which one of the samples was drawn are stochastically larger than the values of the population from which the other sample was drawn (Siegel, 1956). It is noteworthy that most fibers from either group which are high salt responders are also salt-best fibers. As determined by the Kolmogorov-Smirnov test, the responses to NaCl in salt-best fibers from controls are not, however, significantly stronger than the corresponding responses in Na-deprived rats. Also, the 10 sec response to NaCl stimulation is divided into the number of impulses elicited in the first second, in the first 5 sec, and in the last 5 sec of the response in all fibers and ordered according to response size (see Figure 2). In each of these distributions, control fibers that are high salt responders (fibers 12-21) have significantly stronger responses to NaCl than do the corresponding fibers from Na-deprived rats (Kolmogorov-Smirnov two sample test). The data suggest that NaCl elicits stronger responses in control fibers than in fibers from Na-deprived rats; and these results are apparent in both the transient and steady state phases of the response.

Fig. 2.--The responses of 21 fibers in Na-deprived rats and of 21 fibers in controls are ranked ordered according to the number of impulses elicited in the first second, first 5 sec, and last 5 sec by .1 M NaCl.

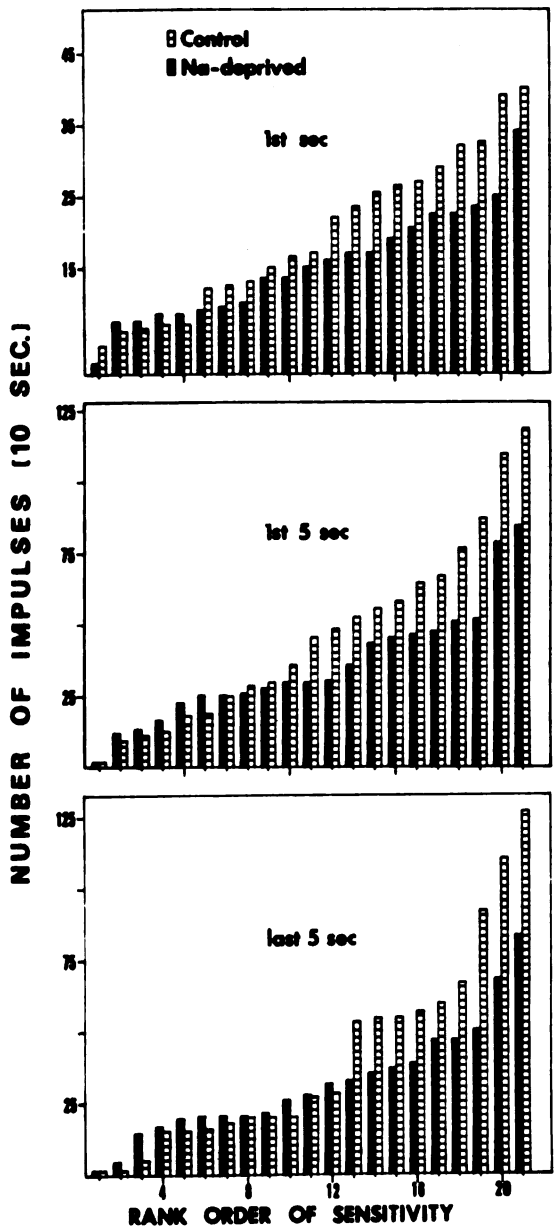
Control
Na-deprived
1st sec
1st 5 sec
last 5 sec
NUMBER OF IMPULSES (10 SEC.)
RANK ORDER OF SENSITIVITY
4
8
12
16
20

Figure 2

Although the relative ordering according to response size for all fibers may be the same in both the transient and steady state phases of the response, there still may be differences in the relative change in response rate over the 10 sec response interval between the two groups of animals. This is so because a ranking based upon response size does not say anything about distance between individual points on a graph.

Qualitative analysis of the time course of impulses in single fibers to NaCl stimulation shows two types of response categories: (1) a rapid burst of activity (transient) of 1 to 2 sec duration, followed by a rapid decrement to a steady state of activity for the remaining portion of the stimulation period; and (2) a sustained, steady rate of activity for the duration of the stimulation period with no initial transient response. This finding confirms an earlier report (Fishman, 1957), and examples of the two types of responses are shown in Figures 3 and 4. Most fibers from both groups of rats had type I responses to NaCl stimulation. Although the control group had proportionally more type I responses than the Na-deprived group, this difference was not significant (a test for differences in proportions, Bruning and Kintz, 1968). Because most single unit responses to NaCl stimulation peak early and then decline in rate, one measure of adaptation over the 10 sec response interval is determined by dividing the number of impulses elicited in the last second of response (steady state) by the number of impulses elicited in the first second (transient). Thus, if there is complete adaptation, this ratio will approach zero; but if there is little or no adaptation this ratio will

Fig. 3.--The numbers of impulses in successive 250 msec period are plotted against time. The top graph illustrates the response pattern of a type 1 fiber and the bottom graph of a type 2 fiber. It is worthwhile to point out that they may not be different types of fibers at all concentrations. A type 2 fiber may become a type 1 fiber at higher test concentrations. The solid line (stimulus trace) below each graph represents the 10 sec period of stimulation. The beginning of the stimulus trace indicates when a stimulus channel is activated by pushing a switch, not when the solution actually contacts the tongue.

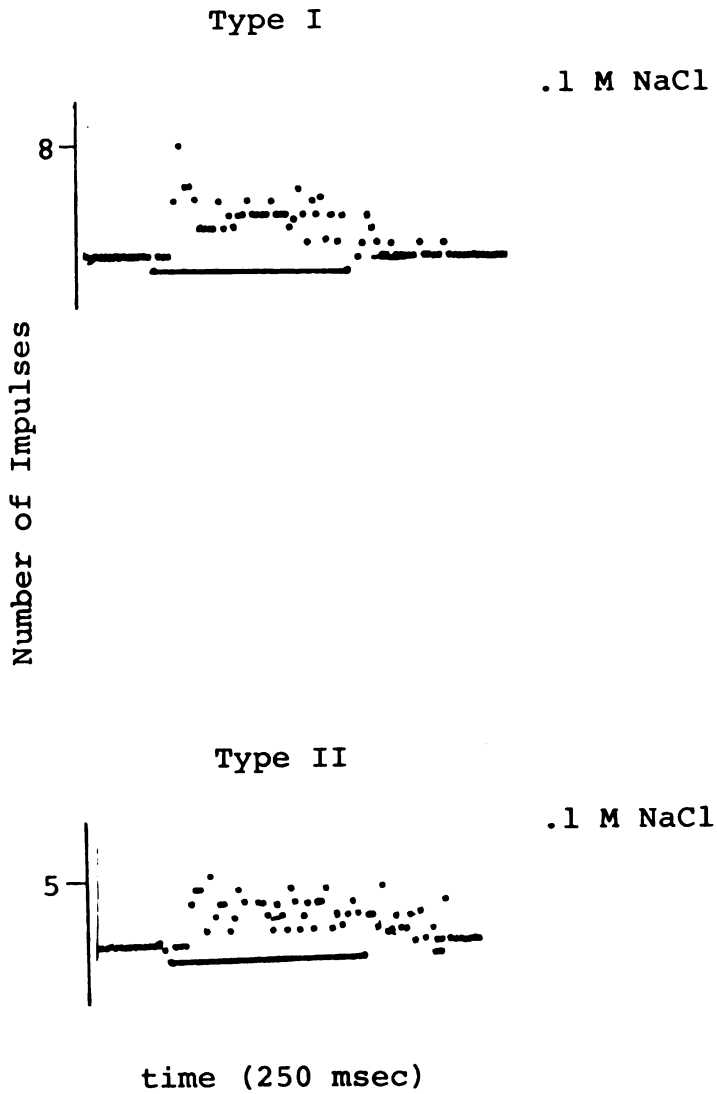
Type I
.1 M NaCl
8
Number of Impulses
Type II
.1 M NaCl
5
time (250 msec)

Figure 3

Fig. 4.--A cumulative frequency distribution of the number of impulses elicited in the first 3 sec of two different fibers' response to NaCl stimulation. The straight line represents the average number of impulses elicited in the last 5 sec (steady state) of each fiber's response. The type II fiber does not deviate very much from the straight line, but the type I fiber does, especially in the first second of the response. When the responses of all fibers are plotted in this manner, the transient or peak responses primarily occur within the first second.

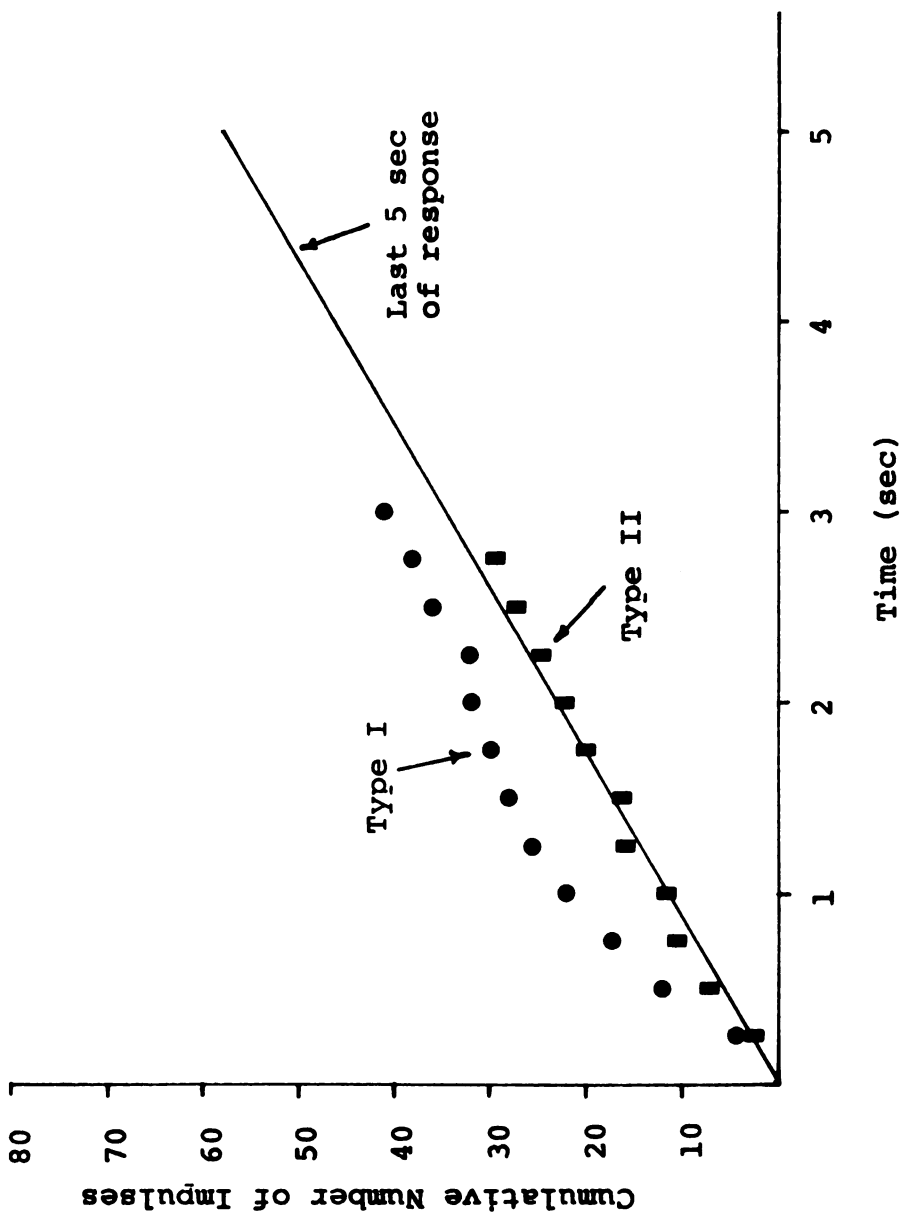
Cumulative Number of Impulses
80
70
60
50
40
30
20
10
1
2
3
4
5
Time (sec)
Last 5 sec
of response
Type I
Type II

Figure 4

approach the value of one. The average adaptation ratios across all fibers, and across all salt-best fibers in control animals are, however, not significantly different from those in Na-deprived animals.

Spontaneous Activity

Taste fibers often show spontaneous activity before stimuli are applied to the tongue. Generally, the spontaneous response is much smaller than the response to chemical stimulation of the tongue. In two fibers from Na-deprived rats, however, the spontaneous response is a significant proportion of the NaCl response. The spontaneous firing rate is significantly more variable in the Na-deprived group (test for homogeneity of variance, $p < .001$). The mean spontaneous response is .324 $\pm$ .055 impulses per sec for controls and .438 $\pm$ .187 impulses per sec in the Na-deprived group. The median spontaneous response is .269 impulses per sec for controls and .125 impulses per sec in the Na-deprived group, but this difference is not significant ($x^2 = 2.386$, two tailed, $.20 < p < .10$, median test, Siegel, 1956). The spontaneous firing rate is 0 for five fibers in the Na-deprived group, but is 0 for only 1 fiber in controls. Because most sucrose and quinine responses are small, the spontaneous response is usually a significant proportion of the sucrose and quinine responses. Furthermore, rank-difference correlations across fibers between spontaneous response size with the responses to NaCl ($r = +.21$), responses to HCl ($r = +.004$), responses to sucrose ($r = +.25$), and the responses to quinine ($r = +.22$) are not significantly correlated in fibers obtained from control rats. Spontaneous response size is statistically correlated with responses to HCl ($r = +.51$, $.02 < p <$

.01), with the responses to sucrose ($r = +.63$, $p < .001$), with the responses to quinine ($r = +.74$, $p < .001$), but not with the responses to NaCl ($r = -.03$) in Na-deprived rats. These high positive correlations are, however, not significantly different from those obtained from control fibers (test for difference between independent correlations, Bruning & Kintz, 1968). The significant correlations that are obtained in fibers from Na-deprived subjects indicate that there is some tendency for fibers that respond better to quinine, or to a less extent to sucrose or acid, to have larger spontaneous responses; but the size of the response to NaCl is not at all related to the spontaneous response size.

Across-Fiber Correlations

Across-fiber correlations between the amounts of responses to the basic tastes across all 21 control fibers and across all 21 fibers in Na-deprived rats are presented in Table 1. Spearman rank-difference correlations are used, and are measures of how similarly different pairs of stimuli order the fibers' responses. In control fibers a negative correlation coefficient is obtained between the responses to sucrose and the responses to each of the other three stimuli. Positive correlation coefficients are obtained between the responses to NaCl, HCl, and quinine. Only the response to HCl and quinine are positively correlated ($p < .001$, t test). In fibers from Na-deprived rats, however, the correlation coefficients between the responses to sucrose with the responses to each of the other three tastes are positive. The responses to HCl and quinine are positively

Table 1.--Rank difference correlations between responses to the basic tastes.

	NaCl	HCl	Quinine
		Controls	
Sucrose	-.387	-.194	-.070
NaCl	--	+.312	+.431
HCl	--	--	+.903*
		Na-deprived	
Sucrose	+.140	+.413	+.606*
NaCl	--	+.122	+.015
HCl	--	--	+.793*

N = 21 per group, * indicates a significant correlation.

correlated ($p < .001$, t test), as well as the responses to sucrose and quinine ($p < .01$, t test).

The correlation values found in Table 1 are plotted into taste profiles for control animals (top row) and for Na-deprived animals (bottom row) in Figure 5. These taste profiles for each of the basic tastes are very different, but the taste profiles obtained from Na-deprived rats do not match those from controls.

Random Distribution of Sensitivities to Four Tastes

Many chorda tympani fibers show a spontaneous firing rate. Hence, when the number of impulses discharged in certain units during chemical stimulation exceed the mean + 2 x S.D. of the spontaneous firing rate, they are judged to respond to the given chemical. Using this criterion, the proportion of fibers responding to the four stimuli for both groups of animals are presented in Table 2. For both

Fig. 5.--Correlation taste profiles across the four taste stimuli: 0.5 M sucrose (S), 0.1 M NaCl (N), 0.01 N HCl (H), and 0.02 M quinine hydrochloride (Q) for the responses of 21 fibers from controls (top row) and 21 fibers from the Na-deprived group (bottom row). The r's plotted are Spearman rank-difference correlation coefficients expressing how similarly different pairs of stimuli order the fibers' responses. The correlation for sucrose and "sucrose" and each other stimulus and itself are assumed to be +1.00.

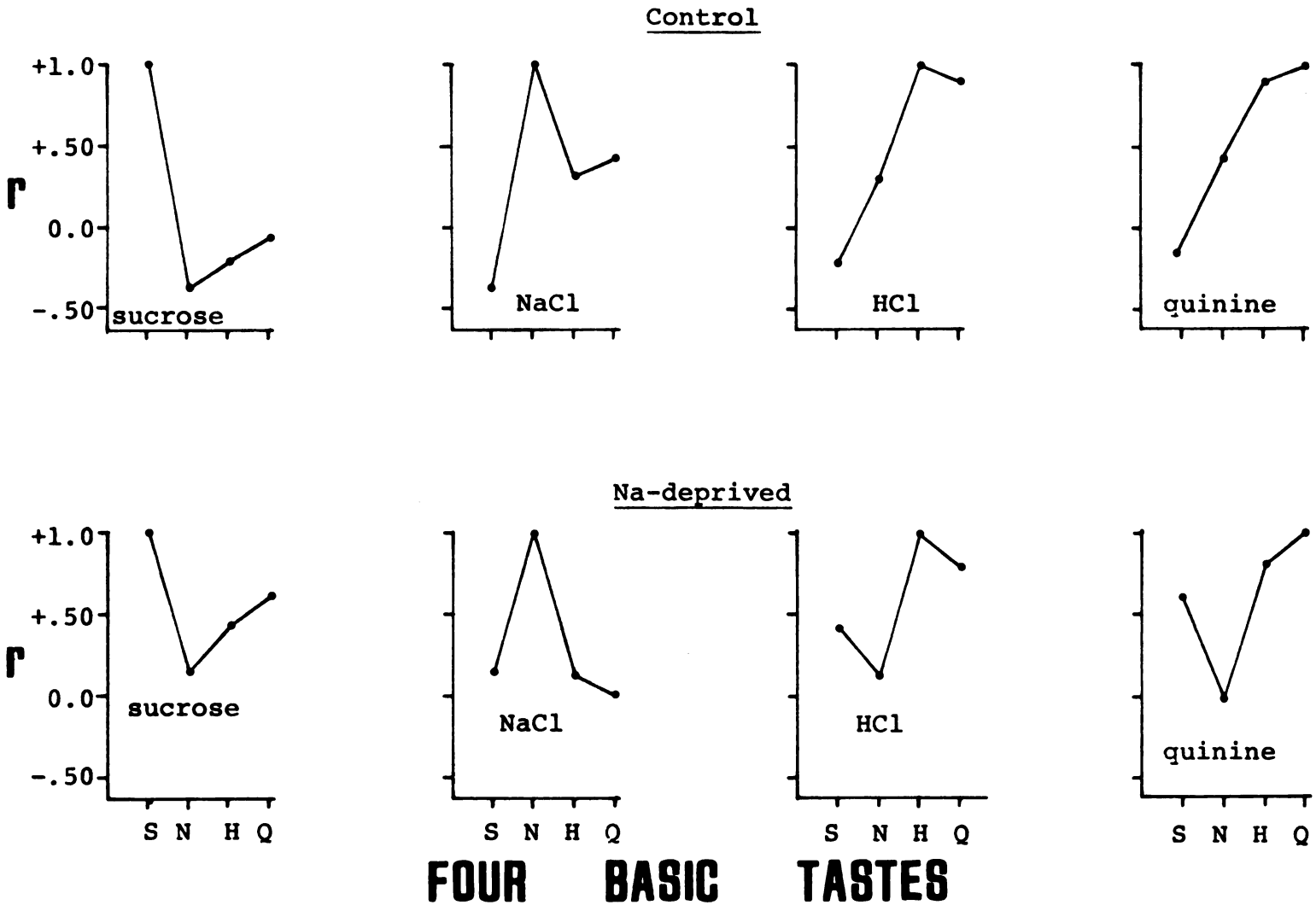
Control
+1.0
+.50
r
0.0
-.50
sucrose
NaCl
HCl
quinine
Na-deprived
+1.0
+.50
r
0.0
-.50
sucrose
NaCl
HCl
quinine
S N H Q
S N H Q
S N H Q
S N H Q
FOUR BASIC TASTES

Figure 5

Table 2.--The proportion of fibers that respond to the four basic tastes.

Stimuli	21 units (controls)	21 units (Na-deprived)
.1 M NaCl	20/21 (0.952)	19/21 (0.905)
.5 M Sucrose	9/21 (0.428)	11/21 (0.524)
.01 N HCl	16/21 (0.762)	16/21 (0.762)
.02 M Quinine	15/21 (0.714)	16/21 (0.762)

groups of animals the major portion of the units respond to NaCl, and decreasingly less to HCl, to quinine, and to sucrose. The discrepancies in the proportions between the groups are slight, although more units respond to sucrose in Na-deprived rats.

Assuming that the probability of response to each of the four basic taste stimuli in chorda tympani fibers is independent of its responsiveness to the other three stimuli, as others have done (Frank and Pfaffmann, 1968; Ogawa, Sato, Yamashita, 1968; Frank, 1973), one can calculate the expected frequencies of units responding to a combination of the four stimuli. For example, a fiber's response to sucrose is independent of that same fiber's response to NaCl if the probability that a NaCl response will occur is not influenced by whether a sucrose response has or has not occurred. Using this notion of independence the probabilities of responses to the four stimuli can be estimated by the proportions of fibers in a sample which respond to each of the stimuli (see Table 2). Hence, the probability of obtaining responses to any pair of these stimuli are given by the product of the probabilities of obtaining responses to each stimulus

pair. The observed and predicted numbers of single fibers responding to each of the six combinations of two tastes for both control and Na-deprived animals are given in Table 3. For both groups of rats there are slight differences between the predicted and observed number of fibers responding to the six possible stimulus pairs. The discrepancies between predicted and observed numbers are, however, somewhat larger for the Na-deprived group especially when sucrose is involved. The largest discrepancy for both groups, though, comes from the HCl-quinine stimulus pair. Table 3 also indicates the probabilities of independent occurrence between pairs of stimuli as determined with Fisher's exact probability test (Siegel, 1956). Each of these values is the sum of the probabilities for all outcomes, including the more extreme cases that occur in either direction. These probabilities indicate that, although the observed-predicted differences are not very large, some of these differences would rarely occur by chance if the stimuli did have independent effects. The responses to HCl and quinine are, therefore, not independent of one another, but occur concomitantly.

More discrepancies arise when the predicted numbers of fibers which should respond to zero, one, two, three, or four of the stimuli are compared with the observed numbers. The predicted numbers are calculated from the multiplication and addition laws of probability, using the proportion of fibers which respond to each of the basic tastes (see Table 2) as reasonable estimates of the probabilities that any fiber sampled would respond to the stimuli. The predicted and observed values for both groups of animals are given in Table 4.

Table 3.--Numbers of single fibers responding to each combination of two tastes.

	Observed	Predicted	Probability of Independent Occurrence
	Controls		
Sucrose, NaCl	8	8.6	.381
Sucrose, HCl	7	6.8	.540
Sucrose, Quinine	6	6.4	.523
NaCl, HCl	16	15.2	.238
NaCl, Quinine	15	14.3	.286
HCl, Quinine	15	11.4	.0003
	Na-deprived		
Sucrose, NaCl	11	10.0	.450
Sucrose, HCl	10	8.4	.217
Sucrose, Quinine	10	8.4	.217
NaCl, HCl	16	14.5	.200
NaCl, Quinine	15	14.5	.800
HCl, Quinine	15	12.2	.013

Table 4.--Numbers of single fibers responding to 0, 1, 2, 3, or 4 tastes.

Responses	Observed	Predicted
	Controls	
0	0.0	0.0
1	4.0	1.0
2	1.0	5.5
3	10.0	9.8
4	6.0	4.6
	Na-deprived	
0	1	.1
1	2	.9
2	3	4.8
3	5	9.5
4	10	5.8

The largest deviations of theory from observation are in the number of fibers which respond to one taste and the number which respond to two tastes when controls are considered. There are too many responses to only one taste and too few responses to two tastes. For the Na-deprived group, there are too many fibers that respond to all four tastes but too few fibers that respond to only three tastes.

Specificities of Individual Fibers

Figures 6 and 7 present response profiles of 26 different chorda tympani fibers which are most sensitive to NaCl (A), HCl (B), sucrose (C), or quinine (D) in control and Na-deprived animals, respectively. Out of a total of 21 control fibers, 12 respond best to NaCl, 5 best to HCl, 4 best to sucrose, and there are no fibers that respond best to quinine. Out of 21 fibers in Na-deprived rats,

Fig. 6.--Response profiles for the basic tastes: 0.5 M sucrose (S), 0.1 M NaCl (N), .01 N HCl (H), and .02 M quinine hydrochloride (Q), of 14 chorda tympani fibers from control animals. The profiles are for fibers which respond most to NaCl (A), HCl (B), and sucrose (C).

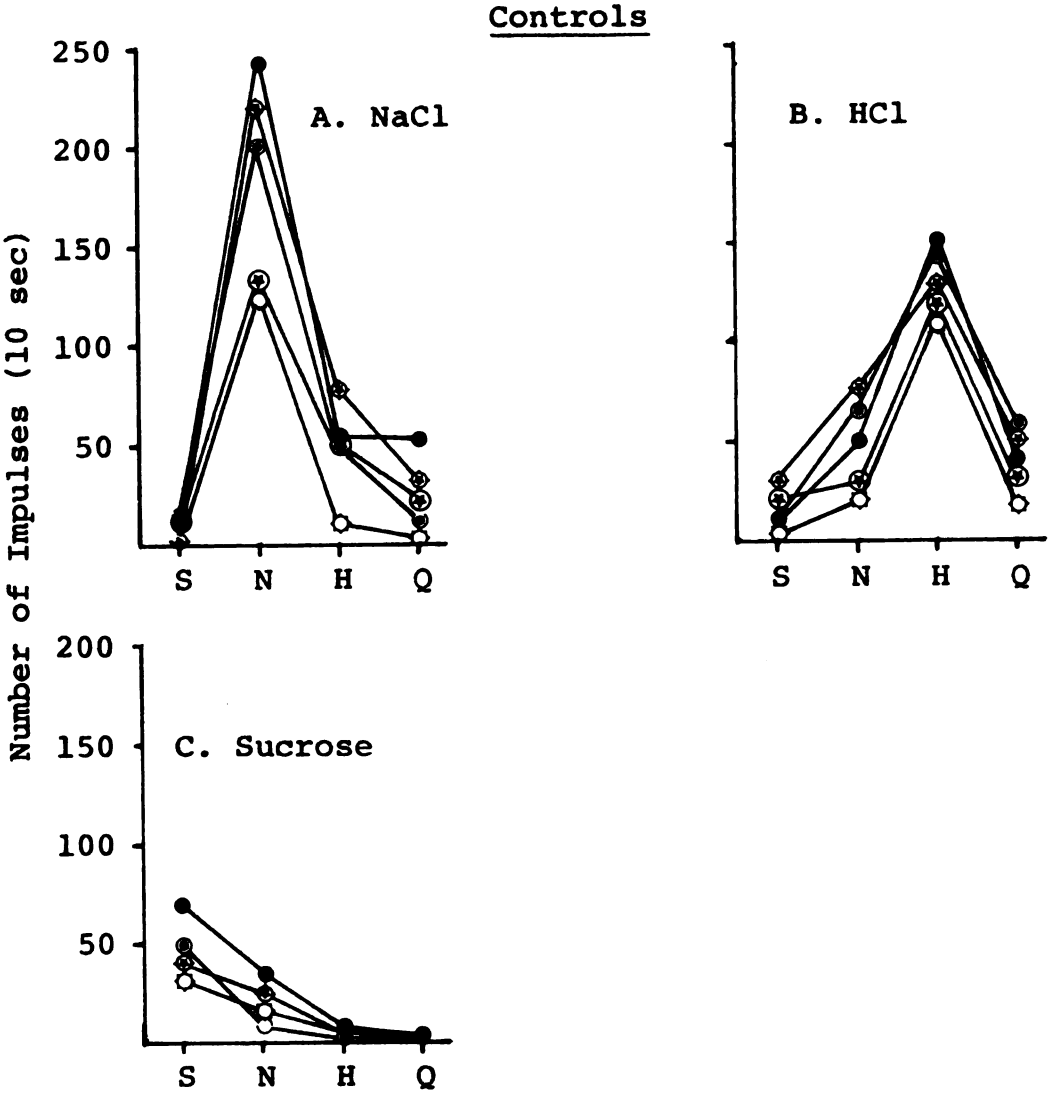
Controls
A. NaCl
B. HCl
C. Sucrose
Number of Impulses (10 sec)
250
200
150
100
50
S
N
H
Q
FOUR BASIC TASTES

Figure 6

Fig. 7.--Response profiles for the basic tastes: 0.5 M sucrose (S), 0.1 M NaCl (N), .01 N HCl (H), and 0.02 M quinine hydrochloride (Q), of 12 chorda tympani fibers from Na-deprived rats. The profiles are for fibers which respond most to NaCl (A), HCl (B), sucrose (C), and quinine hydrochloride (D).

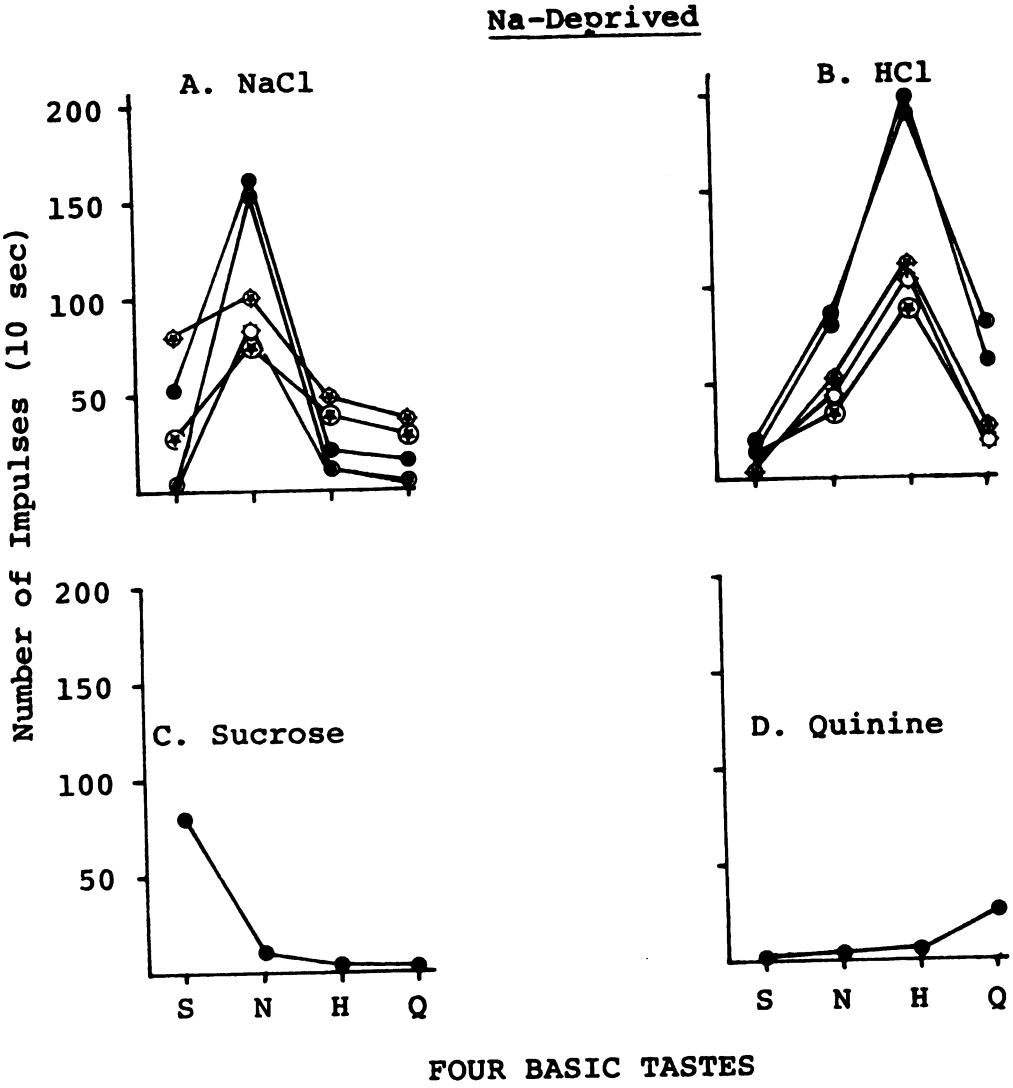
Na-Deprived
A. NaCl
B. HCl
C. Sucrose
D. Quinine
Number of Impulses (10 sec)
200
150
100
50
S
N
H
Q
FOUR BASIC TASTES

Figure 7

10 respond best to NaCl, 8 best to HCl, 1 best to sucrose, 1 best to quinine, and 1 fiber does not respond at all. All the fibers that respond best to HCl (5) or to sucrose (4) in control subjects are graphically represented in Figure 6. Five fibers that respond best to NaCl in control subjects, and five that respond best to NaCl and five that respond best to HCl in Na-deprived subjects are also represented in Figures 6 and 7. The response profiles of these fibers are representative of each category.

In control animals, none of the five fibers most sensitive to NaCl are very sensitive to sucrose (Panel A, Figure 6). The extent to which these salt-best fibers are specific varies. For example, the percentage of the second-best response varies from 4 to 64. If the salt-best fiber responds to more than one taste, the second-best response is always to HCl, the third-best response to quinine, and the fourth-best to sucrose. Of the fibers that respond best to sucrose, none are very sensitive to HCl or quinine. All sucrose-best fibers respond second-best to NaCl and the NaCl response varies from 7% to 52% of the sucrose response. Acid-best fibers usually give good responses to NaCl and quinine but weak responses to sucrose. Four out of five acid-best fibers respond second-best to NaCl, but the other fiber responds second-best to quinine. Not one fiber is a quinine-best responder.

In Na-deprived animals, unlike controls, two of the five fibers most sensitive to NaCl are very sensitive to sucrose (Panel A, Figure 7). Their average response to sucrose is 57% of the size of the NaCl response; the response to sucrose of the other NaCl-best fibers is

only 5%. These two salt-best fibers respond second-best to sucrose, third-best to HCl and fourth-best to quinine. The characteristics of the remaining salt-best fibers are much like those of control salt-best fibers. That is, the second-best response is to HCl, third-best to quinine, and fourth-best to sucrose. One salt-best fiber responds second-best to quinine, third-best to HCl and fourth-best to sucrose. There is only one fiber that responds best to sucrose. It responds second-best to NaCl, but does not respond to HCl or quinine. All acid-best fibers respond second-best to NaCl, third-best to quinine, and very little to sucrose. There is one fiber that responds best to quinine. This fiber does not respond to the other three tastes.

In Figure 8 are mean response profiles of all fibers sampled. The second, third, and fourth largest responses are expressed as percentages of the best response in each fiber. And these percentages are averaged for all fibers in each best-stimulus category for both groups of animals. No mean profiles for quinine-best fibers in control animals, for quinine-best and sucrose-best fibers in Na-deprived animals are plotted because there are too few fibers in these categories.

In control animals, the NaCl-best group of fibers is the most specific because it has the smallest second, third, and fourth-best responses. This is also true for the NaCl-best group of fibers in Na-deprived animals. But the mean response profiles of the NaCl-best fibers are not the same for the two groups of animals. The mean relative response to sucrose in NaCl-best fibers is greater in the Na-deprived group than in controls. Thus, both the response profiles

Fig. 8.--Mean relative response profiles of rat chorda tympani fibers across the four basic stimuli: .5 M sucrose (S), .1 M NaCl, .01 N HCl, and .02 M quinine hydrochloride (Q). The fibers from control animals were divided into those which responded best to sucrose (n=4), NaCl (n=12), or HCl (n=5). The fibers from Na-deprived animals were divided into those which responded best to NaCl (n=10) or HCl (n=8). These profiles show what might be considered a quasi-generalization gradient.

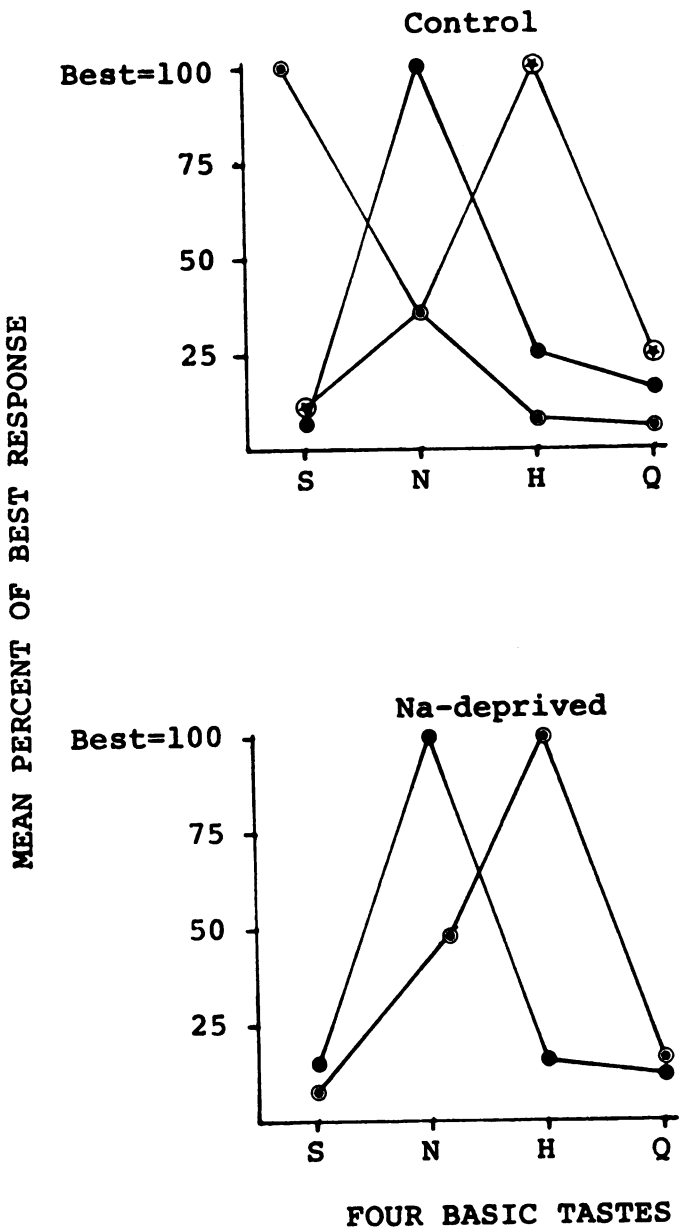
Control
Best=100
75
50
25
S
N
H
Q
Na-deprived
Best=100
75
50
25
S
N
H
Q
MEAN PERCENT OF BEST RESPONSE
FOUR BASIC TASTES

Figure 8

of individual NaCl-best fibers, and the mean response profiles of NaCl-best groups of fibers show a greater sensitivity to sucrose in Na-deprived rats. Also, the mean relative responses to HCl and quinine are smaller in the Na-deprived group. In acid-best fibers, the mean relative response to NaCl is greater in the Na-deprived group.

Chorda Tympani Responses

Figures 9 and 10 are composed of photographs of two different fibers' responses to .1 M NaCl, .5 M sucrose, .01 N HCl, and .02 M quinine. The fiber in Figure 9 responds to .1 M NaCl but does not respond to the other three chemicals. In other words, it is relatively specific in its sensitivity to chemical stimulation. The fiber in Figure 10, on the other hand, responds to all four stimuli, but responds "best" to NaCl and less to the other three chemicals. Accordingly, this unit is nonspecific in its sensitivity to chemical stimulation.

Body Metabolism

Table 5 shows the values of urine Na, urine K, and body weight for control and Na-deprived animals.

Table 5.--Mean (± S. E.) total urinary sodium and potassium excreted, and body weight values for Na-deprived and control rats.

	Na-deprived	Control
Urine Na (mEq./day)	.071 ± .005	2.694 ± .224
Urine K (mEq./day)	3.391 ± .203	3.565 ± .317
Body weight (g)	440.4 ± 15.9	456.8 ± 9.4

Fig. 9.--Photographs of oscilloscope tracings of one rat chorda tympani fiber's response to .1 M NaCl, .5 M sucrose, .01 N HCl, and .02 M quinine. The solid line (stimulus trace) below each response represents the time during the 10 sec stimulation period. Any activity that appears before the stimulus trace is part of the fiber's spontaneous response. The beginning of the stimulus trace indicates when a stimulus channel is activated by pushing a switch, not when the solution actually contacts the tongue. This fiber is relatively specific in its sensitivity to chemical stimulation.

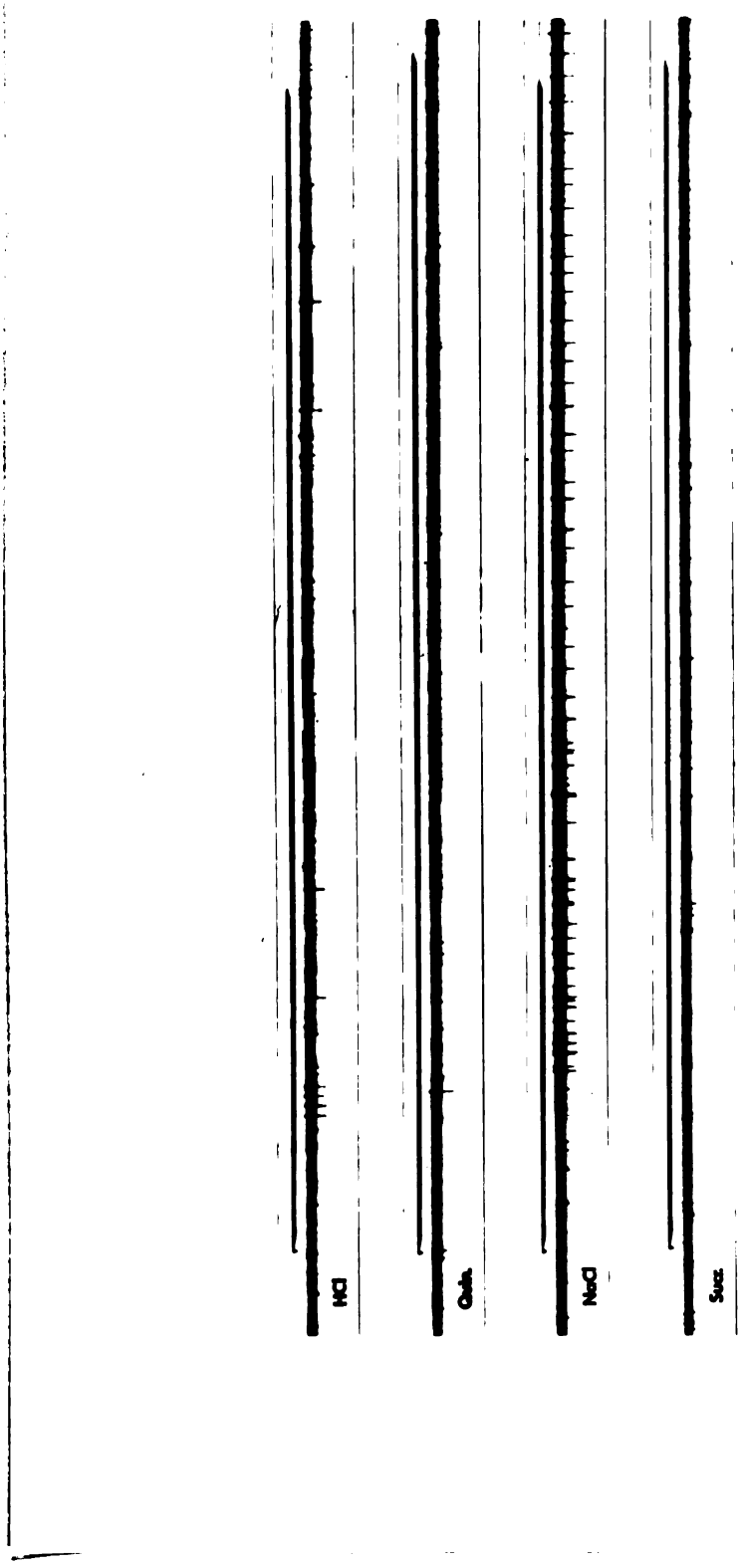

Figure 9

Fig. 10.--Photographs of oscilloscope tracing of one rat chorda tympani fiber's response to .1 M NaCl, .5 M sucrose, .01 N HCl, and .02 M quinine. This fiber is nonspecific in its sensitivity to chemical stimulation.

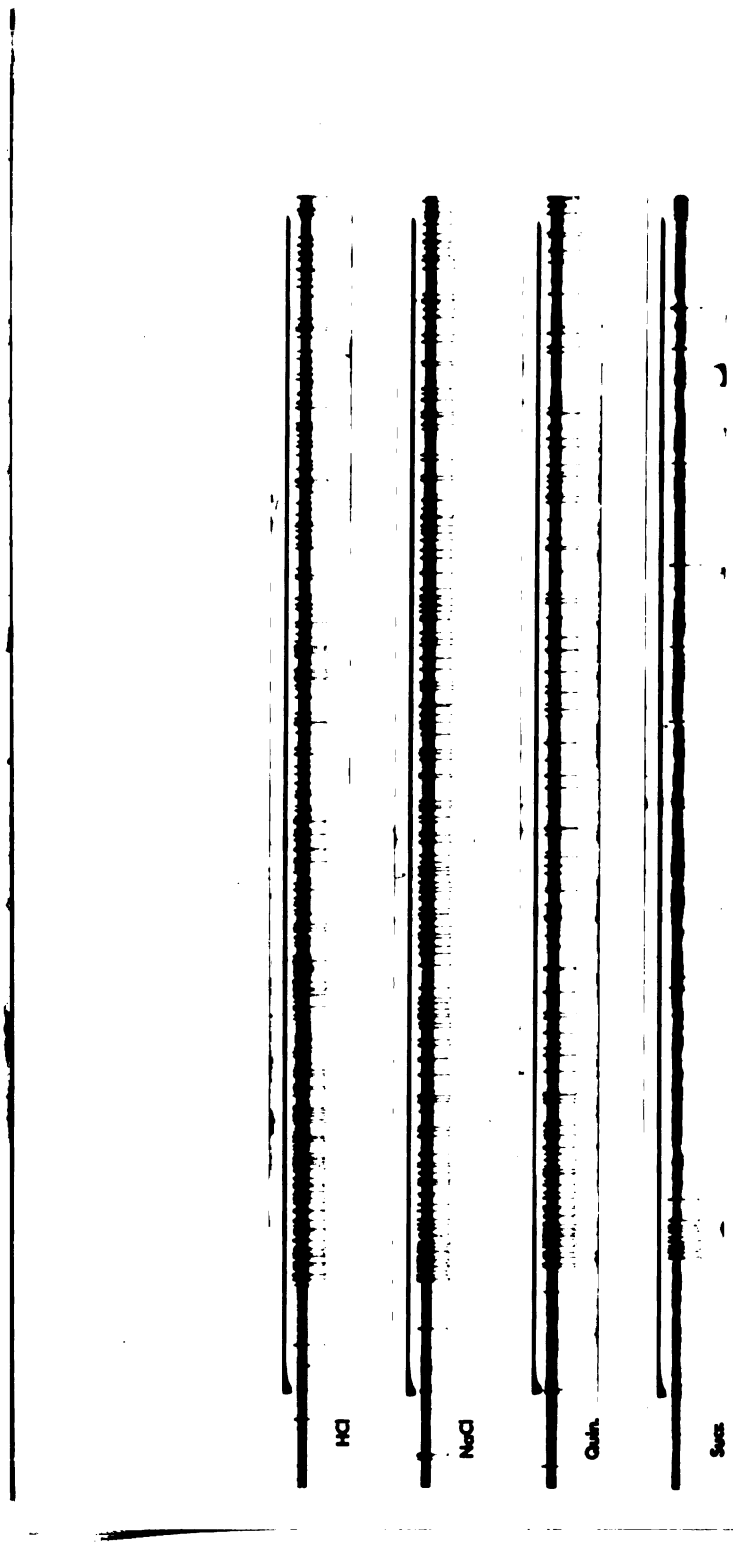

Figure 10

The results (mean $\pm$ S. E.) contained in Table 5 indicate that rats fed a sodium-free diet excrete significantly less urinary sodium than control animals (t = 13.05, df = 18, $p < .001$). There are no differences, however, in urine potassium and in body weight between the two groups of animals.

DISCUSSION

The data obtained in this investigation support the hypothesis that some aspect of the taste control over sodium appetite occurs at a peripheral level. The study of the behavior of single taste fibers unmasked differences in the neural processing of gustatory information between Na-deprived and control rats that were not apparent with the study of whole nerve responses (Bare & Pfaffmann, 1950; Nachman & Pfaffmann, 1963). The behavior of single taste fibers from Na-deprived rats were different from the behavior of control fibers in the following ways: (1) They were significantly less sensitive to a particular supra-threshold concentration of NaCl exemplified by a smaller range or distribution of response frequency; (2) the responses to the four taste stimuli were more strongly correlated with their spontaneous response frequency; and (3) they had significantly different patterns of activity across fibers.

Body Metabolism

The classic research on sodium appetite and lowered detection thresholds (Carr, 1953; Harriman & MacLeod, 1953; Pfaffmann & Bare, 1950) was done on the adrenalectomized rat. Because adrenalectomy interrupts much more than sodium balance a sodium-free diet was used to induce sodium deficiency. Small amounts of urinary sodium in rats are a consequence of being fed a diet without sodium (Contreras &

Hatton, in press). The amount of urine sodium output is, therefore, an especially good measure to distinguish between rats that are fed a sodium-free diet from rats that are fed a normal diet. Before meaningful comparisons are made between the behaviors of two groups of fibers, it is necessary to show that these fibers do, indeed, come from independent populations of rats. In a previous report (Nachman & Pfaffmann, 1963) the investigators neglected to verify treatment conditions within the same animals that were used for electrophysiological study. To this end, urine analysis showed that Na-deprived rats excreted significantly smaller amounts of sodium than did controls.

Distribution of Chorda Tympani Responses

Only one concentration of NaCl, HCl, sucrose, and quinine hydrochloride is used to represent the four taste qualities of salt, sour, sweet, and bitter now described in man. These test stimuli are of moderate intensity quite within the range that a rat might ordinarily taste in real life. But the effectiveness of these stimuli at one intensity level varies in the sizes of the responses they evoke in different chorda tympani fibers. Some fibers respond strongly and others are weak responders. Many chorda tympani fibers respond strongly to both NaCl and HCl; very few respond to both sucrose and quinine (Pfaffmann, 1955). This is because the anterior two-thirds of tongue, which the chorda tympani nerve innervates, is highly sensitive to NaCl and HCl, but poorly sensitive to sucrose and quinine (Pfaffmann, Fisher, & Frank, 1965). The results of this investigation are in agreement with the above findings. At test concentrations, NaCl and HCl elicit stronger responses than do sucrose and quinine.

Unexpectedly, though, the range of responses to NaCl stimulation is reduced in fibers from Na-deprived rats. The maximum level of response to NaCl is higher for control fibers. This indicates that although chorda tympani fibers are differentially sensitive to the four taste stimuli for both groups, control fibers are more sensitive to NaCl stimulation than fibers from Na-deprived rats.

What significance does this have for the behaving animal? It might suggest that the reason Na-deprived rats increase their intake of high concentrations of NaCl solution is because they are less, not more, sensitive to the NaCl taste.

Recently, Morrison (1974) was critical of the psychophysical (Carr, 1952; Harriman & MacLeod, 1953) and electrophysiological (Bare & Pfaffmann, 1950; Nachman & Pfaffmann, 1963) experiments that measured absolute taste thresholds in the rat. In the first place, Morrison argues, there is no consistent definition of threshold that these experiments adhere to. And secondly, the response criterion is not the same for all experiments. Morrison (1974) describes unpublished observations using signal detection theory and methods that have shown that adrenalectomized rats have a decreased sensitivity to supra-threshold concentrations of NaCl. This finding matches the electro-physiological results of this investigation.

Morrison's data is compatible with the finding that the NaCl drinking pattern of a Na-deprived rat had shorter interdrink intervals and longer drinking times than that of a normal rat (Contreras & Hatton, in press). Contreras and Hatton (in press) used .4 M NaCl as the test solution, a concentration that normal controls find aversive.

The finding that Na-deprived animals are less sensitive to NaCl might account for their preference for the higher concentrations (Morrison, 1974). These rats would drink hypertonic saline more frequently and for longer periods of time. Morrison's notion of decreased sensitivity in sodium deficiency cannot explain why adrenalectomized rats show a preference for NaCl at low concentrations where normal controls show unconcern. Contreras and Hatton (in press) proposed that Na-deprived rats take longer to adapt to the taste of a salt stimulus than controls. Intuitively, this notion can account for the increased consumption of NaCl at all concentrations. In other words, it is not a question of detection, but of adaptation--when to stop drinking saline.

The present study demonstrated that the total number of impulses elicited by NaCl was significantly fewer in fibers from Na-deprived rats. It appears that Na-deprived rats may be getting a "weaker" signal from sodium than do control rats. A model to account for the increased consumption of NaCl in sodium deficiency was offered by Dr. John I. Johnson. It is that NaCl must elicit a critical total number of impulses in the taste nerve before the rat's intake of NaCl would cease. It would take the taste nerve of a Na-deprived rat more time to accumulate this critical number of impulses. This is consistent with the previous proposal (Contreras & Hatton, in press) which states that a Na-deprived rat may take longer to adapt to the taste of a salt stimulus than a normal control. This gustatory mechanism would permit greater than normal intake of substances containing sodium under conditions of sodium deficiency.

Anecdotal evidence to support the adaptation proposal comes from personal observations. This investigator has observed that people who smoke tend to salt their foods more heavily than nonsmokers. Since smoking damages taste cells, smokers' taste acuity is weaker than nonsmokers. Smokers probably get a weaker taste signal from table salt (NaCl) than nonsmokers. Thus, smokers may take longer to adapt to the taste of a salt stimulus. The higher incidence of heart disease in smokers may partly rest on their peculiar eating habits. That is, greater than normal salt consumption may contribute to higher blood pressure.

Spontaneous Activity

By simultaneously manipulating the chemistry of the blood bathing the taste receptors and recording from the chorda tympani nerve, Bradley (1973) demonstrated that when NaCl concentration in the blood was reduced, the level of spontaneous activity in the nerve was decreased. Consequently, Bradley suggested that a large measure of the spontaneous activity in the chorda tympani was due to the constant stimulation of intravascular receptors by elements in the blood. Since the level of sodium in the blood drops in adrenalectomy (Jalowiec & Stricker, 1973), the level of spontaneous activity of the chorda tympani nerve may be the neural mechanism that underlies the appetite for sodium. In other words, the need for sodium and the level of spontaneous activity are inversely related. A reduction in the level of spontaneous activity indicates that there is a need for sodium. Conversely, an increase in the level of spontaneous activity indicates a return to homeostasis.

In disagreement with Bradley's proposal, it was found in the present investigation that the levels of spontaneous activity in fibers from control and Na-deprived rats were not significantly different from each other.[2] It had been shown that intact rats maintained on a sodium-free diet, unlike adrenalectomized rats, did not have lower levels of plasma sodium (Contreras & Hatton, in press). As a result, hyponatremia could not be the stimulus for altering spontaneous activity. But this does not preclude other blood-borne factors from having an effect on spontaneous activity. For instance, rats that are maintained on a sodium-free diet have higher plasma levels of aldosterone than controls (Marusic & Mulrow, 1967).

Across-Fiber Correlations

Each of the correlation taste profiles (Figure 5) for the four taste stimuli are different from each other. Some are more different than others. In control fibers, for example, the correlation taste profile for HCl is very similar to that for quinine. This implies that these two stimuli stimulate, to some extent, the same sets of fibers in the chorda tympani nerve. The positive correlation between the responses to HCl and quinine indicate a more frequent occurrence of larger responses to these two stimuli together in the same fibers, and the less frequent occurrence of fibers which respond strongly to one, but not at all to the other stimulus. This conclusion is supported by the finding that the responses to HCl and

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[2]There were no significant differences in median spontaneous rate and in the correlations between the size of the responses to the four stimuli and spontaneous response size.

quinine are not independent (Fisher's exact probability test) of one another, but occur concomitantly. The correlation profiles for HCl and quinine are also very similar to one another in Na-deprived rats as well, although these profiles are very different from control profiles. The profiles for sucrose, NaCl, and HCl in control fibers are clearly different from one another, suggesting that they might taste more different to a rat than would HCl and quinine at moderate intensities. This inference is credible because stimuli of like quality produce very similar correlation profiles (Frank, 1973).

The four stimuli (S, N, H, & Q) on the X-axis of the correlation taste profiles (see Figure 5) are hedonically ordered from behavioral preference to rejection. These four stimuli are considered quality archetypes of the four basic tastes known to man (Ogawa, Sato, & Yamashita, 1968; Frank, 1973). Accordingly, quinine and HCl are located on the negative end of the scale and are usually rejected. Sucrose is located on the positive end and is usually accepted. Located in the middle, NaCl is somewhere between the quinine-HCl pair and sucrose in hedonic value.

The neural basis for taste discrimination depends upon differences in the relative amounts of activity across many neurons (Erickson, Doetsch, & Marshall, 1965). As a consequence, any differences in neural input may be measured by the correlation between the amounts of activity across several fibers. If across-fiber patterns represent the form in which taste quality is encoded then the more similar the correlation profiles of two stimuli the more difficult would be a discrimination between these two stimuli. Conversely,

stimuli which generate dissimilar correlation taste profiles should have dissimilar tastes. This theory may provide a basis to account for differences in the processing of gustatory information between Na-deprived and control rats.

The correlation taste profiles that are generated by sucrose, HCl, and quinine in control fibers are quite different from those that are generated by these same stimuli in fibers from Na-deprived rats. Thus, the taste sensations that a Na-deprived rat receives from sucrose, HCl, of quinine may be altogether different than the sensations that a control rat receives. Because of sodium deprivation, there is a greater tendency for sucrose, HCl, and quinine to stimulate strongly the same sets of fibers (all are positively correlated with each other. See Table 1 and Figure 5). In addition, the correlation profiles for these three stimuli have approximately the same "salt" component. According to across-fiber pattern theory, then, sucrose, HCl, and quinine taste more alike after sodium deprivation. But the correlation taste profile that these three stimuli share in Na-deprived rats is unlike the NaCl, sucrose, HCl, or quinine profiles that are characteristic of control fibers. Thus, this taste sensation that a Na-deprived rat gets from these stimuli may be unique. That is not to say that a rat can no longer recognize the "sweet" taste of sucrose, the "sour" taste of HCl, or the "bitter" taste of quinine. Recognition is not the issue. These taste stimuli do not elicit pure taste sensations. The altered taste sensations that a Na-deprived rat gets from these stimuli are subtle and may be important in affecting the rat's preference behavior. If we accept the slogan "molecules

for motivation" (Pfaffmann, 1965) then we believe that the actual taste and the hedonic quality of that taste are inseparable.

Speculatively, these changes in taste may have an adaptive value for a Na-deprived animal to satisfy its need for sodium. The primary concern for a Na-deprived rat is to select between foods or liquids that have sodium in them from those that do not. As a result, this animal may be more apt to pass over "good-tasting" substances (sucrose) if they do not contain sodium, and less apt to refuse "bad-tasting" substances (sour, bitter, hypertonic saline) because they do contain sodium. Since sucrose, HCl, and quinine, the archetypes for sweet, sour, and bitter, do not taste the same after sodium deprivation, their positive and negative hedonic values may also be different, probably weakened. Thus, the likelihood that the animals' behavior will be guided by the "saltiness" rather than by the "sweetness," "sourness," or "bitterness" of the substance will be increased.

Specificities of Individual Fibers

There is a high degree of variability in the size of the responses to the four stimuli (see Figures 3-6). And when the responses across all fibers are divided into those which respond best to each of the four stimuli, there is also wide variability in the size of the best response. Among all this variability, there is still order and consistency. This order and consistency is apparent after perusing the graphs in Figure 6.

When the four stimuli are ordered along the X-axis (sucrose, NaCl, HCl, quinine), most fibers have response profiles with one peak, as others have shown (Frank, 1973; Frank, 1974; Pfaffmann, 1974). In

control animals, there are fibers that peak (respond best) at sucrose, NaCl, and HCl. But by knowing that a fiber peaks or responds best to one of these stimuli allows prediction of the relative effectiveness of the other three stimuli. For example, a fiber that responds best to NaCl, always responds second-best to HCl, third-best to quinine, and fourth best to sucrose at the concentration chosen. It is also interesting to note that the stimuli that are scaled nearest the peak (best stimulus) usually have an effective order that is greater than stimuli away from the peak. For example, NaCl is more effective than HCl in sucrose-best fibers, but NaCl is more effective than sucrose in HCl-best fibers. On the dimension (behavioral preference-rejection) which these stimuli are scaled, it seems that NaCl is nearer to HCl than to sucrose, since HCl is more effective than sucrose in NaCl-best fibers. To make these comparisons in distance along the dimension of behavioral preference it is assumed that each peak (best response) defines a labelled line cluster (Pfaffmann, 1974). And further, the tuning curves are symmetrical around each peak.

Response profiles from Na-deprived rats, with some exceptions, show order and consistency, too (see Figure 13). For example, fibers that respond-best to HCl always respond to the other three stimuli with the same relative order of effectiveness as do control fibers. These fibers respond second-best to NaCl, third-best to quinine, and fourth-best to sucrose. The single sucrose-best fiber responds in the same manner as do control sucrose-best fibers. One fiber out of a combined total of 42 responds best to quinine. Fibers especially tuned to bitter are not found in chorda tympani fibers (Frank, in

press). It is noteworthy that this quinine-best fiber, which is on the "negative" end of the hedonic scale, responds to the other three stimuli in an order of effectiveness that is opposite to that for sucrose which is on the "positive" end.

The response profiles from Na-deprived rats are, in some cases, at variance with the profiles from controls. The mean response profile of NaCl-best fibers indicates that sucrose is as effective a stimulus as HCl. This would suggest that NaCl is just as near to sucrose as it is to HCl on the hedonic dimension. But has NaCl, as a result of sodium deprivation, moved closer to sucrose, sucrose closer to NaCl, or both? Since the mean relative response to HCl in NaCl-best fibers is smaller in fibers from Na-deprived rats than in controls, this suggests that NaCl may have moved toward sucrose. Perhaps after sodium deprivation the positive hedonic value of NaCl has increased. That is, NaCl may taste "better" to a Na-deprived than to a normal control.

This explanation would be compatible with labelled line theory since it implies a change in the relative location of the "tuning curves" for the four taste stimuli. Thus, for NaCl to taste better, labelled line theory would predict that NaCl-best fibers would have stronger responses to sucrose but weaker responses to HCl. Furthermore, since NaCl has moved closer to sucrose, sucrose-best fibers should have stronger responses to NaCl. This would seem appropriate since the labelled line significance of sucrose-best fibers is to transmit "sweet" information. Hence, for NaCl to taste "better" or

"sweeter" it would have to elicit stronger responses in the "sweet" line.

Although there are three fewer sucrose-best fibers in Na-deprived rats than in controls, the range or distribution of responses to sucrose are the same for the two groups (see Figure 3). In control animals, the fibers that respond the most to sucrose are sucrose-best fibers. But in Na-deprived animals, the fibers that respond the most to sucrose are NaCl-best fibers, which may suggest that NaCl tastes "better." There are too few fibers to suggest that NaCl is a more effective stimulus in sucrose-best fibers from Na-deprived animals than controls.

Qualifications

It should again be emphasized that only one concentration of each of the four stimuli was used in this experiment. Stimulus concentration was an important part of every finding that was uncovered. The distribution of response functions and across-fiber patterns for the four stimuli would have different characteristics under different stimulus intensities. The division into best-stimulus categories also depended upon concentration. In this regard, it was assumed that taste fibers increase in response size as stimulus concentration was increased. Most fibers do increase in response size as intensities are increased (Frank, 1973); there are, however, some notable exceptions (Ogawa, Sato, & Yamashita, 1969; Wang & Bernard, 1969). No doubt, variation in stimulus intensity should be studied, and in the future may provide more insight into elucidating the neural mechanisms of sodium appetite. The primary task of this investigation, however, was

to show that Na-deprived rats process gustatory information differently than controls. To that end, test stimuli of moderate intensity and in the range that a rat might ordinarily taste in real life were used.

Sample size is also an important factor to be noted. In some instances, there were not enough fibers within each best-stimulus category in order to make adequate comparisons. Nevertheless, it was gratifying to find that the numbers of fibers within each best-stimulus category reflected the sensitivity of the nerve. There were several salt-best and acid-best fibers, but few sucrose-best and quinine-best fibers, in both groups of rats.

Also, the changes that were obtained in gustatory nerve discharges may be a result of the interaction between sodium deficiency and the anesthetic. This investigator is not, however, aware of any studies that imply such an interaction. It may be important in the future to study gustatory nerve discharges in Na-deficient rats using a different anesthetic than the one used in this study.

In summary, the data from single taste fibers indicate that Na-deprived rats: (1) may be less sensitive to NaCl stimulation, and (2) may get different sensations from the four taste stimuli than control rats. These changes in the sensory system increase the likelihood that a rat will consume more sodium. Thus, biologically meaningful stimuli are highlighted at the peripheral level, enabling the organism to make appropriate decisions.

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APPENDICES

APPENDIX A

APPARATUS

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Equipment and supplies

1. Individual metabolism cages with base, Acme Metal Products.
2. 100 ml graduated cylinders, M.S.U. Stores.
3. Flame photometer #143, Instrumentation Laboratories.
4. Stereozoom dissecting microscope, Nikon.
5. Preamplifiers, 122, Textronix.
6. Power supply, 125, Textronix.
7. Oscilloscope, 502A, dual-beam, Textronix.
8. Tape recorder, 1028, Magnecord.
9. Electronic counter, 5321B, Hewlett Packard.
10. Printout counter, Digitron.
11. Audiomonitor, AM5, Grass Instruments.
12. Thermistor thermometer, Yellow Springs Instruments.
13. Micromanipulator, Narishige.
14. Magnetic tape, Scotch, 1.5 mil acetate, 1/4" x 2500".
15. LINC computer, Digital Equipment Corp.
16. Teletype, Teletype Corporation.
17. Oscilloscope, 561A, Textronix.
18. Plotter, MFE .815 Plotamatic.

19. Oscilloscope, 565 dual-beam, Textonix.
20. Micromanipulator, David Kopf Instruments.
21. Power supply, Power Mate Corporation.
22. 24v regulated power supply, homemade by G. Connors.
23. 5 channel stimulus delivery system, homemade by G. Connors.
24. Window discriminator, Model 120, W-P Instruments, Inc.
25. Kymograph Camera, Grass Instruments.

APPENDIX B

RAW DATA

APPENDIX B

RAW DATA

Table 6.--Raw Data--Neural Responses.

	Rat	Number of Impulses (10 sec)	Spontaneous (1 sec)
		Control	
1.	A-7, 174-434	N=101.67, H=15.8, Q=14.2, S=4.4	.286
2.	A-7, 749-1038	N=122.6, H=78.2, Q=60.0, S=14.0	.375
3.	A-7, 719-796	H=127.33, N=77.33, Q=53.67, S=31.0	.289
4.	A-7, 0-116	N=130.5, H=5.5, Q=2.5, S=2.0	.357
5.	A-7, 719-796	N=65.5, H=4.5, Q=3.5, S=.5	.000
6.	A-7, 272-365	S=47.0, N=27.0, H=3.5, Q=3.0	.486
7.	F-7, 874-945	N=245, H=63, Q=62.5, S=12.0	.875
8.	F-7, 773-780	H=117.0, N=23.0, Q=17.5, S=3.5	.214
9.	F-7, 563-675	N=135.5, H=50.0, Q=22.5, S=11.5	.688
10.	C-7, 635-724	N=200.5, H=48.0, Q=12.5, S=13.0	.208
11.	C-5, 400-489	S=68.0, N=33.5, H=4.0, Q=2.0	.063
12.	A-6, 902-977	N=98.5, H=7.0, Q=4.5, S=1.0	.025
13.	C-5, 661-733	H=146.5, N=65.5, Q=54.0, S=11.5	.225
14.	B-2, 839-914	S=48.5, N=3.5, H=1.5, Q=1.5	.363
15.	A-3, 728-895	S=33.67, N=11.0, H=4.33, Q=3.67	.042

Table 6.--Continued.

	Rat	Number of Impulses (10 sec)	Spontaneous (1 sec)
16.	C-9, 882-948	N=47, H=15, Q=13, S=4.5	.313
17.	C-9, 460-533	H=147.5, N=49.5, Q=40, S=11.5	.214
18.	F-13, 1010-1100	N=218, H=79, Q=32, S=3	.100
19.	F-13, 947-1008	N=45, H=14.5, Q=8.5, S=7.0	.571
20.	F-13, 947-1008	N=121.0, S=19.0, H=13.0, Q=13.0	.875
21.	A-7, 749-829	H=120.0, Q=38, N=28.5, S=32	.250
		Na-deprived	
1.	C-4, 225-322	H=187.5, N=83.0, Q=80.0, S=17.0	.250
2.	C-8, 787-867	N=77.0, H=7.0, Q=2.5, S=1.0	.000
3.	A-5, 743-833	H=196.5, N=79.0, Q=60.0, S=14.0	.500
4.	C-4, 420-507	H=107.0, N=44.0, Q=27.0, S=5.0	.214
5.	C-6, 0-120	H=107.0, N=54.0, Q=25.5, S=5.0	.063
6.	C-8, 195-302	N=82.5, H=5.0, Q=2.5, S=3.5	.000
7.	C-8, 195-302	S=80.0, N=11.5, Q=4.5, H=2.5	.125
8.	C-6, 901-973	N=98.5, S=80.0, H=49.0, Q=36.5	.750
9.	C-6, 1118-1213	H=55.0, N=40.0, Q=28.5, S=6.5	.125
10.	C-6, 1118-1213	H=110.5, N=51.0, Q=25.5, S=4.5	.188
11.	F-4, 96-190	N=80.0, H=42.0, Q=35.5, S=24.0	2.786
12.	C-3, 858-926	N=161.5, S=51.5, H=20.0, Q=15.0	.071
13.	F-4, 294-378	N=48.5, H=1.50, Q=1.0, S=0.0	.125
14.	C-1, 281-370	H=85.0, N=33.0, Q=28.0, S=15.0	.375
15.	F-6, 1061-1118	N=50.0, Q=5.0, H=2.0, S=0.0	.000

Table 6.--Continued.

Rat	Number of Impulses (10 sec)	Spontaneous (1 sec)
16. F-6, 887-950	N=82.5, H=8.0, Q=3.0, S=1.5	.000
17. C-1, 281-323	N=152.0, H=10.0, Q=5.0, S=2.0	.250
18. F-12, 1021-1078	H=102.5, N=51.0, Q=22.0, S=1.0	.125
19. F-12, 955-1007	N=76.0, H=10.0, Q=5.0, S=0.0	.000
20. F-12, 882-945	Q=24.5, H=5.0, N=3.5, S=.5	.125
21. B-3, 321-407	N=47.0, H=42.5, Q=27, S=29	3.125

Table 7.--Raw Data--Body Metabolism.

Rat	Body Weight (g)	Urine Na (mEq./day)	Urine K (mEq./day)
		Controls	
1. B-2	481	2.100	2.460
2. C-9	473	3.024	4.284
3. F-13	449	3.056	3.984
4. F-7	423	1.976	2.691
5. C-7	417	2.413	3.154
6. A-6	456	2.520	3.346
7. A-3	487	2.052	2.508
8. A-7	492	4.048	4.976
9. C-5	433	3.060	4.680
		Na-deprived	
1. B-3	417	.056	3.276
2. C-4	366	.098	3.626
3. C-8	424	.040	2.688
4. C-6	446	.090	4.560
5. A-5	378	.077	3.399
6. C-3	484	.088	3.960
7. F-4	477	.000	1.968
8. F-12	427	.076	3.990
9. C-1	450	.063	3.297
10. F-6	535	.076	3.382