



Taste Responses in the Nucleus of the Solitary Tract in Saccharin-preferring and Saccharin-averse Rats

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Abstract

A minority of rats consistently reject the taste of sodium saccharin at concentrations that the majority find palatable. We chose rats that selected either water (WP), or 0.03 M NaSaccharin (SP) in two-bottle preference tests and monitored single unit responses to a range of taste qualities in the nucleus of the solitary tract. WP rats gave significantly greater responses to Na/Li salts and QHCl. Their responses to sugars were equal to those from SP rats. Total activity to NaSaccharin did not differ between the two groups, but its distribution across the three identified neuron types did. The response was skewed from one in which sugar (S) and sodium salt (N) participated nearly equally (SP) to one dominated by the activity of N cells and nearly devoid of an S cell contribution (WP rats). Accordingly, the response profile for NaSaccharin was correlated nearly as well with those of the sugars (+ 0.60) as with the Na/Li salts (+ 0.73) in SP rats, but was reshaped in WP rats to be nearly identical with those of the salts (+ 0.85) and unlike sugars (+ 0.30). In their heightened sensitivity to stimuli that humans call salty and bitter, and in their rejection of the complex taste of NaSaccharin, WP rats showed many of the characteristics of human tasters of PTC/PROP. *Chem. Senses* 21: 147–157, 1996.

Introduction

Saccharin is a palatable substance for most rats. Those given saccharin drink more fluid than those rats offered only water (Hammer, 1967; Gilbert and Sherman, 1970; Sclafani and Nissenbaum, 1985), and they prefer saccharin to water in two-bottle tests. Saccharin serves as an effective reinforcer of operant behavior (Smith and Capretta, 1956; Collier, 1962) and can be used in a classical conditioning paradigm to establish a taste preference (Fanselow and Birk, 1982). There is considerable evidence in rats that saccharin evokes similar electrophysiological and behavioral activity to that of sugars. Thus, if 'sweetness' typifies the perception of sugars, saccharin tastes predominantly sweet (Thompson and Mayer, 1959; Ganchrow and Erickson, 1970; Hsaio and Tuntland, 1971; Mook, 1974; Woods and Bernstein, 1980;

Stewart and Krafczek, 1988). However, it is more complex than that, having a substantial aversive component that increases with rising concentration (Ganchrow and Erickson, 1970; Morrison and Jessup, 1977).

While most rats prefer saccharin to water at low concentrations, a minority show the opposite preference (Nachman, 1957, 1959; Collier, 1962; Young and Madsen, 1963; Mook, 1974; Dess, 1991, 1993). Estimates of the incidence of saccharin-averse rats across a distribution of sex and strains have ranged from 5% (Dess, 1993), through 7% (Collier cited in Dess 1993) to 19% (Nachman, 1959) when saccharin concentrations were approximately 0.01 M and the upper limit for saccharin acceptance was 30–50%. Nachman (1959) demonstrated the genetic contribution to taste preference by

selectively breeding rats that preferred water to saccharin. Water-preferring parents produced 50% saccharin-averse offspring by the second (F2) generation. Significant differences between the saccharin- and water-preferring groups emerged at a concentration of 0.01 M. The rejection of saccharin at low concentrations in the water-bred line suggests that these rats may have been more sensitive to saccharin's aversive component.

Dess (1991, 1993) has initiated a more extensive breeding program to evaluate the genetic contribution of various taste qualities to differences in saccharin preference. By the third generation, she found significant differences between amounts of various tastants consumed by Oxy Low-Sacc and Oxy High-Sacc rats. Low-Sacc rats showed a reduced acceptance of saccharin and quinine, but not of sucrose. These results support the notion that the genetically based differences in saccharin preference arise from the aversive as well as appetitive properties of this stimulus. The literature offers no reports of sucrose-averse rats (Dess, 1993).

The fact that saccharin preference has a genetic component is not surprising in view of the genetic link that has been well established for other tastants. The threshold concentrations at which humans taste the bitter substances phenylthiocarbamide (PTC) and 6-*n*-propylthiouracil (PROP) are bimodally distributed with the means of the two distributions separated by slightly more than one log unit (Looy and Weingarten, 1992). This difference may result from segregation at a single autosomal locus (Fox, 1932; Snyder, 1932; Blakeslee and Salmon, 1935) though it is expressed at multiple receptor sites (Miller and Bartoshuk, 1991). Nontasters—those with high thresholds—carry two recessive genes, while tasters are either heterozygous or homozygous for the dominant gene (Whissell-Buechy, 1990). Results from comparative studies of humans, apes and rodents (Fischer *et al.*, 1939; Richter and Clisby, 1941; Harris and Kalmus, 1949; Kaplan *et al.*, 1967; Klein and DeFries, 1970) suggest that these polymorphisms are maintained across a range of species. Early data revealed differences between PTC/PROP tasters and nontasters only to bitter compounds (Blakeslee, 1932; Salmon and Blakeslee, 1935; Harris, 1953; Hall *et al.*, 1975). More recently, Bartoshuk has reported heightened sensitivity to the sweetness of sugars, saccharin and neohesperidin dihydrochalcone among PROP tasters (Bartoshuk, 1979; Gent and Bartoshuk, 1983; Bartoshuk *et al.*, 1988; Marks *et al.*, 1988). Saccharin also tastes significantly more bitter to tasters than to nontasters of PROP. These results led Bartoshuk to suggest that

a heightened sensitivity to bitterness might be related to the reduced acceptance of saccharin in water-preferring rats (Bartoshuk, 1979). Such individual differences would have important consequences for studies of the hedonic value of saccharin as well as for generalization studies based on conditioned taste aversion where saccharin serves as the conditioned stimulus.

Taste stimuli generate afferent signals that carry quality-intensity information in a spatial (across neurons) and temporal code. The behavioral results described above imply that a genetic distinction is responsible for individual differences in this code. In the present experiment we evaluated whether rats with pronounced preferences for saccharin or for water would also show differences in the afferent code for saccharin and for a range of taste qualities at the level of the solitary tract.

Materials and methods

Subjects

A total of 47 female Wistar rats ranging from 300–487 g, maintained on *ad libitum* food and H₂O, were used as subjects for the electrophysiological recordings. Prior to recording, rats were given simultaneous access to water and 0.03 M NaSaccharin for a period of at least 7 days. The saccharin ion confers qualities that humans describe as sweetness and bitterness, while Na elicits saltiness. Thus the NaSaccharin molecule generates a complex of qualities with both appetitive and aversive components. The concentration selected was well above the point of divergence in preference between saccharin-preferring and water-preferring (i.e. saccharin-averse) rats as reported by Nachman (1959). Positions of the fluid bottles were changed randomly on a daily basis to control for side preferences. Bottles were weighed daily, and intakes of both fluids were calculated and compared. Total intakes of NaSaccharin and water were averaged across the final 7 days of preference testing. Rats that demonstrated a preference of >75% for either NaSaccharin (SP) or water (WP) were used as subjects for the electrophysiological recordings. The percent of total fluid taken as NaSaccharin for each of the 27 rats defined as NaSaccharin-preferring (SP) and the 20 defined as water-preferring (WP) is shown in Figure 1. To arrive at this sample, 166 rats were screened and many more qualified as SP than were used. Mean (\pm SD) body weights (378.0 \pm 45.5 g for SP and 366.4 \pm 41.1 g for WP rats) did not differ significantly between groups.

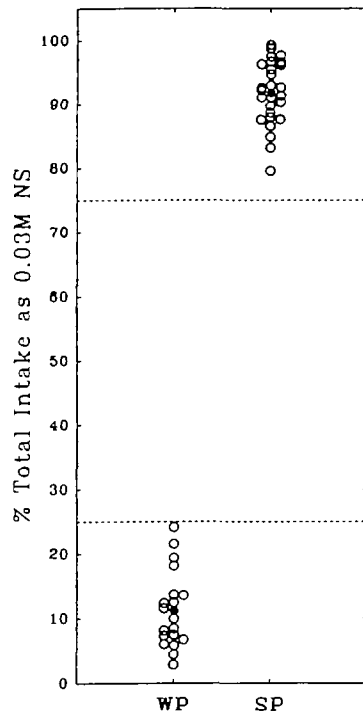


Figure 1 Seven-day means of 0.03 M NaSaccharin as a proportion of total fluid in rats selected as subjects for electrophysiological recording. Individual rats are represented by open circles. Mean values for each group are represented by filled circles. Dashed lines indicate criterion levels for selection for recording.

Surgical procedure

Surgical levels of anesthesia were induced by an intramuscular injection of 100 mg/kg Ketaset followed after 10 min by 48 mg chloral hydrate, which was subsequently administered as needed. A tracheotomy was performed to permit tracheal clearing and, if needed, artificial respiration (Harvard respirator). An esophageal fistula was implanted to avoid chemical and mechanical post-ingestive effects during gustatory stimulation of the oral cavity, pharynx and esophagus. The rat was mounted in a non-traumatic head holder and a slender length of perforated vinyl tubing through which a fine stimulus spray could be administered to the entire oral cavity, was placed in its mouth and secured. The rat's heart rate was monitored and the interbeat interval maintained at about 180 ms through adjustments in respiration rate and depth of anesthesia. Rectal temperature was maintained between 35 and 37°C.

Portions of the occipital and parietal bones were removed and the cerebellum was aspirated to expose the surface of the medulla.

Table 1 The stimulus array

Stimulus	Abbreviation
0.03 M NaCl	N1
0.10 M NaCl	N2
0.10 M LiCl	L
0.10 M MSG	M
0.10 M KCl	K
0.10 M CaCl ₂	Ca
0.01 M QHCl	Q
0.01 M HCl	H
0.01 M Citric acid	Ci
1.00 M Glucose	G
0.5 M Sucrose	S
0.03 M Sodium Saccharin	NS
0.20 M Polycose	P

Recording

Activity from single neurons was isolated by micropipettes (i.d. = 1.0 μm ; $z = 5\text{--}10\text{ M}\Omega$) filled with 1.6 M Kcitrate. Initial coordinates used to locate the NTS were 2.7 mm anterior to the obex, 1.7 mm lateral to the midline and 1.0 mm ventral to the surface of the medulla. The electrode was advanced in 50 μm steps and neural activity tested until robust gustatory responses were encountered. Action potentials were identified by consistency of waveform, spike amplitude and the requirement that no two spikes occur within an interval of less than 1.5 ms. Single unit potentials were amplified, filtered and displayed on an oscilloscope using conventional recording techniques. A direct-coupled four-channel tape recorder was used to preserve unit activity, the onset marker pulse and a voice commentary for later analysis off-line.

Stimuli and stimulus delivery

Thirteen stimuli were employed (see Table 1). NaSaccharin, sugars, salts, acids and quinine hydrochloride were represented, as were MSG and Polycose. All solutions were prepared in distilled water except for sucrose and glucose to which 5% tap water was added to ensure adequate conductivity to activate a stimulus onset marker.

Five ml of solution was sprayed over the tongue at a rate of 2 ml/s. Gustatory-evoked activity was recorded for 5 s following stimulus onset. The moment of stimulus contact with the tongue was marked by a TTL logic device that

passed 11 nA current through the rat (Chang and Scott, 1984), an amount two orders of magnitude below the threshold for electric taste (Bujas, 1971; Pfaffmann and Pritchard, 1980). Each tastant was followed by a 20-ml DH₂O rinse and by a minimum rest period of 60 s. Stimuli were presented at intervals of no less than 90 s to prevent possible adaptation effects. Additional rinses and rest periods were occasionally required for baseline activity levels to be re-established. Stimuli were presented in a quasi-random order with the stipulation that chemicals representing similar taste qualities not be applied consecutively. If the neuron remained well isolated at the end of the series, stimuli were reapplied to obtain a measure of response reliability.

Data analysis

Independent analyses of the data obtained from the SP and WP rats were performed off-line using an IBM-AT computer. Spontaneous activity was subtracted from the evoked response to yield net spikes/s for the 5-s post-stimulus period. These counts were used to calculate response magnitude and breadth of tuning (BOT) (Smith and Travers, 1979) across all cells in the SP and WP groups. BOT provides an index of the distribution of a cell's response across the four prototypical stimuli. It is calculated according to the formula

$$H = -k \sum_{i=1}^n p_i \log p_i$$

where H = breadth of tuning coefficient; k = a scaling constant, which is 1.661 for four stimuli; p_i = the proportion of the neuron's response devoted to any one stimulus. The value of H ranges from 0.00 for a neuron that responds to only one of the prototypical stimuli, to 1.00 when the response is equal to all four.

Derived analyses, including calculation of interstimulus and interneuronal correlation matrices, multidimensional scaling (Guttman-Lingoes algorithm), univariate F tests and *post-hoc* comparisons were all performed with the SYSTAT package (Wilkinson, 1988). Hierarchical cluster analyses, used to identify subgroups of cells with similar functional properties, were conducted using the Clustan routine (Wishart, 1987) on a Zenith 386 computer. Summary statistics, including calculation of mean spontaneous rate, breadth of tuning and evoked rates were obtained for each neuronal cluster as they had been for the entire population of cells and were compared across the SP and WP groups. Finally, net spikes for each of fifty consecutive 100-ms bins were averaged across units to give post-stimulus time histograms (PSTH's) for NaSaccharin. Values for the first 20 bins (2 s)

were chosen to represent the phasic response component, and were compared across SP and WP groups using independent t -tests. All chemicals were used in each analysis, with the exception of cluster analysis and calculation of breadth of tuning, where the basic stimuli were used (see below).

Results

Summary of findings

The taste systems of WP rats were more responsive than those of SP rats. WP rats showed significantly greater activity than did SP rats when responses were averaged across all stimuli. As shown in Figure 2, this effect resulted from larger responses in WP rats to the sodium and lithium salts, MSG, KCl and quinine. The difference between groups was also apparent in subtypes of NTS taste cells. Neurons could be categorized into groups based on their response profiles to 0.5 M sucrose, 0.1 M NaCl, 0.01 M citric acid and 0.01 M QHCl (see 'Division into neuron types' below). There were no differences between the responses of S (sugar) cells of WP and SP rats to any stimulus. N (salt) neurons gave significantly larger responses to NaCl, LiCl, citric acid and QHCl in WP rats. Similarly H (acid) cells showed greater activity to acids, to salty and bitter salts and to QHCl. Thus, the subtypes of taste neurons that have primary responsibility for signaling Na/Li salts, acids and bitter stimuli were more responsive in WP (NaSaccharin-averse) rats.

The profile of activity evoked by NaSaccharin across neurons in SP rats was well correlated with those of Na/Li salts and nearly as well with those of sugars. In WP rats, the correlations between NaSaccharin and salts were higher still, while those with sugars were low. This implies that NaSaccharin has very little sugar-like component to WP rats.

Response criterion and spontaneous activity

The criterion for classification as a taste cell was an evoked response to at least three stimuli that exceeded 1.65 SD ($P < 0.05$, one-tailed because inhibitory responses have been encountered only rarely in prior recordings from rat NTS) from the mean spontaneous rate for that cell, sustained for 5 s. There was a total of 1703 stimulus–neuron interactions (131 neurons \times 13 stimuli). Of these, 1558 (91.5%) exceeded the criterion for excitation, 6 (0.3%) satisfied the criterion for inhibition and 139 (8.2%) gave no response. Mean (\pm SD) spontaneous rate across all cells for SP rats was 14.2 ± 14.8 spikes/s, a value that did not differ significantly from the mean of 11.0 ± 9.7 in WP rats [$F(1,129) = 2.0$;

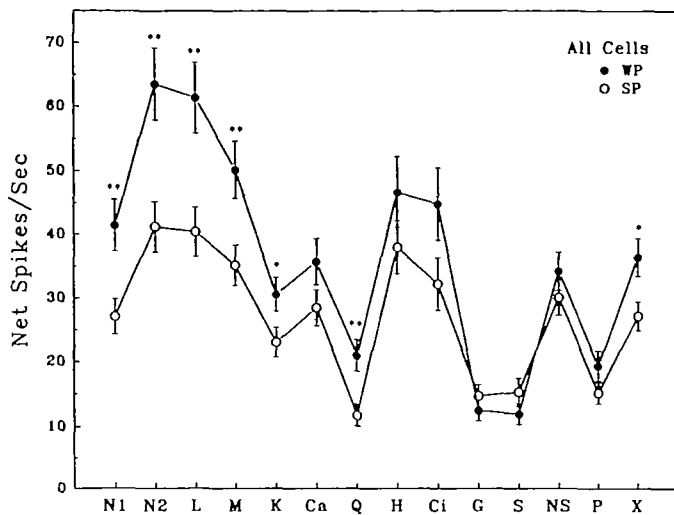


Figure 2 Mean response profiles across the stimulus array in SP (open circles) and WP (closed circles) rats. The responses of all neurons (SP = 71; WP = 60) are included in the calculation of the mean. Abbreviations are from Table 1. X, mean response across all stimuli. *, $P < 0.05$; **, $P < 0.01$.

$P > 0.1$]. Nor did mean spontaneous rates differ between groups within any of the subtypes of neurons ($P > 0.20$ for all comparisons).

Evoked activity

Response reliability

Application of the complete stimulus series required unequivocal isolation of a neuron's responses for ~30 min. If stability remained uncompromised, we began to reapply stimuli to assess the reliability of our recordings. A quantitative measure of reliability was derived by organizing responses to the first application of a stimulus in one column, and to the second in another, then calculating the Pearson correlation coefficient between the two columns. We performed this analysis on the basis of 104 stimulus reapplications—eight for each stimulus—taken equally from the SP and WP groups. The resulting correlations were +0.94 for both SP and WP rats. Because 45 min had typically elapsed between applications of a particular stimulus, this finding indicates a high degree of stability throughout the system, from stimulus delivery, to the rat's physiological condition, to the identification of spikes from a single neuron.

Breadth of responsiveness

NTS cells in each group of rats showed typically broad responsiveness across the basic stimuli as indexed by the breadth of tuning metric (BOT). The mean BOT was 0.82 ± 0.13 in SP and 0.80 ± 0.11 in WP rats ($\chi^2 = 5.08$; $P > 0.2$).

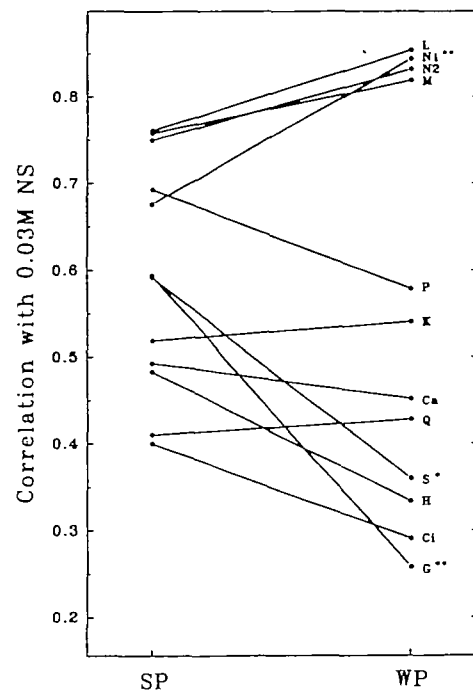


Figure 3 Correlations between the profile evoked by 0.03 M NaSaccharin and those of each of the remaining stimuli in SP and WP rats. *, $P < 0.05$; **, $P < 0.01$.

Relationship between NaSaccharin preference and overall response rate

Activity was measured in 71 neurons in the SP group and 60 neurons in the WP group. Taste cells of WP rats were more responsive across all stimuli (Figure 2) ($F [1,129] = 6.5$; $P < 0.025$). This was a result of the WP rats' greater sensitivity to 0.03 M NaCl, 0.1 M NaCl, 0.1 M LiCl, 0.1 M MSG, 0.01 M QHCl ($P < 0.01$ for all comparisons) and to 0.1 M KCl ($P < 0.04$).

Taste quality

The net (total minus spontaneous) discharges evoked by each neuron in 5 s constitutes the 71-point (SP rats) and 60-point (WP rats) response profile for each of the 13 stimuli. The correlation coefficient between any pair of profiles is taken as an index of the relative similarity of the two taste qualities they represent. In Figure 3 we have plotted the correlations between the profile generated by 0.03 M NaSaccharin and those of the 12 other stimuli in both SP (left column) and WP rats. The profile representing NaSaccharin in WP rats was more similar to that for 0.03 M NaCl [$r = +0.68$ (SP) and $+0.84$ (WP); $P < 0.01$] and to the artificial profile generated from the mean response of each neuron to NaCl and LiCl [$r = +0.75$ (SP) and $+0.86$ (WP); $P < 0.05$; not shown in Figure 3]. Conversely, NaSaccharin's

profile was more discrepant from those of the sugars in WP subjects. The correlation between profiles for NaSaccharin and sucrose declined from +0.59 to +0.36 ($P < 0.05$); between NaSaccharin and glucose, from +0.59 to +0.26 ($P < 0.01$); between NaSaccharin and the artificial profile generated from each neuron's mean response to the two sugars, from +0.65 to +0.35 ($P < 0.01$; not shown). Thus the quality evoked by NaSaccharin was more closely related to those of the Na/Li salts in WP rats, and more distant from those of sugars. The taste of 0.03 M NaSaccharin, which was nearly as similar to those of sugars as to salts in SP rats, became allied with Na/Li salts and had little

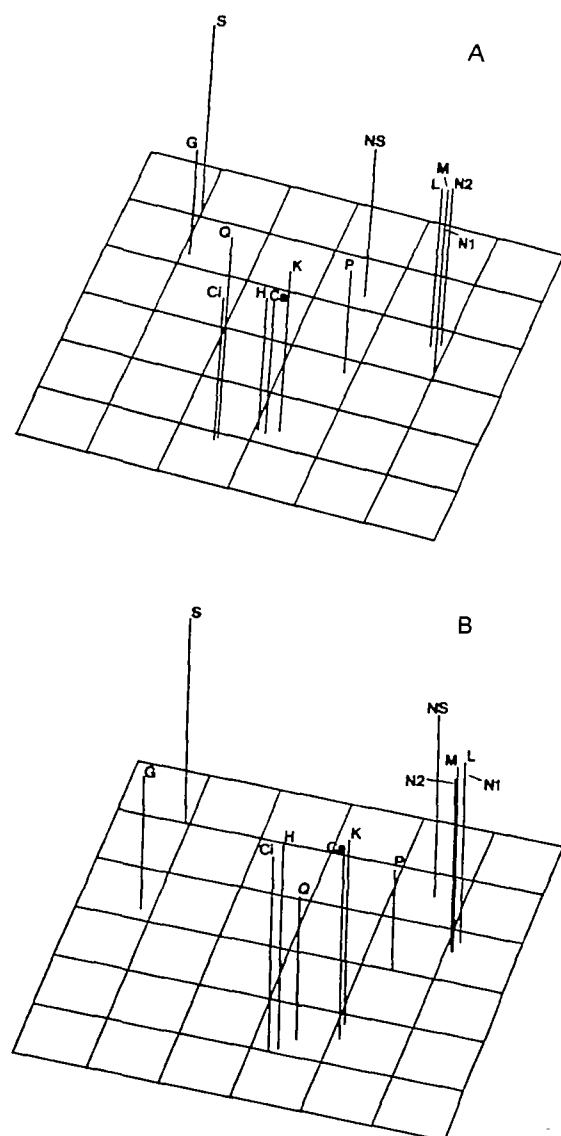


Figure 4 Three-dimensional spaces representing the relative similarities of response profiles evoked by each stimulus in (A) SP and (B) WP rats. Abbreviations are from Table 1.

relationship to sugar in WP rats. The relationship of NaSaccharin to KCl, CaCl₂ and QHCl was not significantly different across groups.

The matrix of correlations between all pairs of stimulus profiles ($n = 13 \times 12/2 = 78$) may be used to create a multidimensional space in which the relative similarity of stimulus quality is proportional to proximity. Such a space, derived from neural responses from SP rats, is shown in Figure 4A. In it may be seen a clear separation among stimuli possessing different taste qualities (as described by humans), with sugars, Na/Li salts, and acids–quinine–bitter salts forming three discrete groups. NaSaccharin, although toward the salts, is distinct from them, as is Polycose. In Figure 4B is shown the space generated from responses in WP rats. It is similar to that obtained from SP rats with the exception of NaSaccharin, whose position is now unequivocally with the salts. Thus the spaces demonstrate that 0.03 M NaSaccharin generates a profile similar to those of Na/Li salts and largely devoid of similarity to sugars in WP rats.

Division into neuron types

There are many morphological and functional criteria by which neurons may be categorized (Boudreau and Alev, 1973; Frank *et al.*, 1983; Whitehead, 1986; Davis and Jang, 1988; Travers, 1988). One such index for neuronal classification is the response profile of a cell. The net discharges evoked by the basic taste stimuli (0.5 M sucrose, 0.1 M NaCl, 0.01 M citric acid and 0.01 M QHCl) in 5 s constituted the response profile for each of the 71 SP and 60 WP neurons. Correlation coefficients were calculated between all pairs of profiles ($n = 71 \times 70/2 = 2485$ coefficients in SP, and $n = 60 \times 59/2 = 1770$ in WP rats), providing a matrix of relative functional similarity among all cells in each group. This matrix was subjected to a cluster analysis (Wishart, 1987) which suggested that most neurons may be organized into three distinct clusters, i.e. neuronal subtypes. Analysis of variance and *post-hoc* comparisons confirmed that each subtype was composed of neurons with response profiles significantly different from those of the other two subtypes, allowing them to be treated independently in further analyses. S cells (SP: $n = 15$; WP: $n = 4$) gave robust responses to sugars and also responded moderately to salts and acids. N cells (SP: $n = 23$; WP: $n = 24$) responded almost exclusively to salts. The third subtype, H cells (SP: $n = 28$; WP: $n = 20$) was characterized by greatest responses to the acids but large responses to salts as well. In WP rats a fourth cluster ($n = 8$) existed between the S and N subtypes. They, and other cells from

either the SP ($n = 5$) or WP ($n = 4$) groups which had profiles that correlated poorly with all others, were not included in the following analyses.

Comparison of neuronal subtypes between groups

The mean response profile of the cells within each cluster is shown in Figures 5A–C for SP and WP rats. Responses from S cells (Figure 5A) did not differ between SP and WP rats, either overall or for any individual stimulus ($P > 0.20$ for all comparisons).

The responses of N cells (Figure 5B) were significantly greater in the WP group to Na/Li (N1, N2, L) salts, citric acid [$F(1,45) > 4.14$; $P < 0.05$ for all comparisons] and QHCl [$F(1,45) = 9.233$; $P < 0.005$].

H cells in WP rats gave significantly larger responses overall than those in SP rats [$F(1,46) = 7.61$; $P < 0.01$].

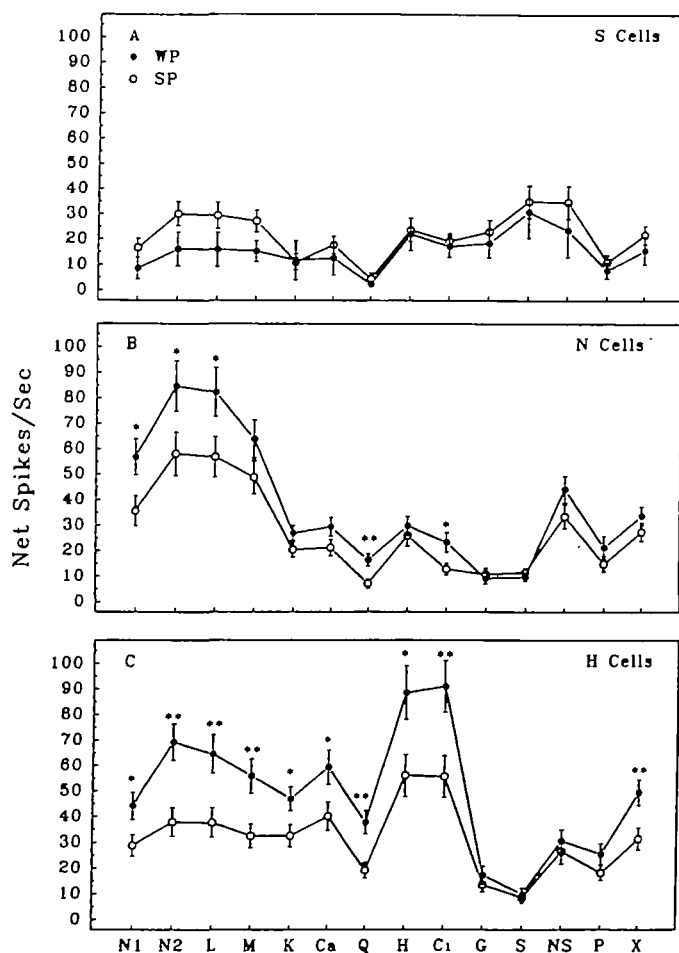


Figure 5 Mean response profiles of each type of neuron in SP (open circles) and WP (filled circles) rats. (A) S cells (sugar-sensitive); (B) N cells (salt-sensitive); (C) H cells (acid and quinine-sensitive). Abbreviations are from Table 1. X, mean across all stimuli. *, $P < 0.05$; **, $P < 0.01$.

Specifically, responses from H cells were higher to the salty (N1, N2, L, M) and bitter (K, Ca) salts as well as to acids (H, Cl) and Q than those in SP rats [$F(1,46) > 4.94$; $P < 0.05$ for all comparisons].

Net spike rate evoked by NaSaccharin did not differ significantly between SP and WP rats across all neurons or within any subtype. However, there were differences in the proportion of the total activity contributed by each neuronal subtype.

Subtypes of taste cells have been hypothesized to serve as coding channels, each bearing primary responsibility for signaling one of the basic taste qualities. We calculated the mean total response to the four basic stimuli in SP (100 spikes/s) and WP (141 spikes/s) rats, and found that the latter are significantly more responsive ($t = 2.84$; $P < 0.01$, Table 2A). Thus the taste system of a water-preferring rat is more sensitive across a representative range of stimuli than that of a rat that prefers NaSaccharin. We then determined the proportion of that response that was carried by cells in each of the three neuronal subtypes. Through this analysis, we are assessing the combined effect of the response rate within a subtype and the number of cells belonging to that subtype. Sodium cells contributed a larger proportion of the total response in WP rats, at the expense of sugar cells. N cells were responsible for 38% of the total activity evoked by the four basic stimuli in WP, versus 29% in SP rats. Conversely, S cells contributed just 3% of the total activity in WP rats, versus 19% in SP rats. H cells, which are broadly responsive to all non-sugars, gave equal shares (49 versus 48%) in each group.

The same calculation for the response to NaSaccharin

Table 2A

	Total resp/cell to four basic stimuli (spikes/s)	Distribution of the response (%)			
		S cells	N cells	H cells	Other
SP	100	19	29	48	5
WP	141	3	38	49	10

Table 2B

	Mean resp/cell to 0.03 M NS (spikes/s)	Distribution of the response (%)			
		S cells	N cells	H cells	Other
SP	30	24	36	35	5
WP	34	5	52	30	14

alone revealed a similar shift in the proportions contributed by the subgroups (Table 2B). Responses of N cells contributed 52% of the total activity evoked by NaSaccharin in WP but only 36% in SP rats. This was offset by the contributions of S cells, which composed just 5% of the NaSaccharin response in WP rats versus 24% in the SP group. This accounts for the higher correlations between profiles for NaSaccharin and Na/Li salts in WP rats, and the loss of similarity between NaSaccharin and the sugars.

Temporal analysis

The correlations among stimulus profiles described above are based on the total response of each cell, summed over the 5-s post-stimulus recording period. Thus the time course over which the response develops is not considered. To analyze temporal properties, the responses to NaSaccharin were divided into fifty 100-ms bins and these were averaged across neurons for a given stimulus. This represents the temporal response profile or post-stimulus time histogram (PSTH) for NaSaccharin. The similar response across all neurons to NaSaccharin in SP and WP rats was the result of significant offsetting differences in the phasic responses of S and N cells (Figure 6). S cells gave a smaller and sharper phasic response to NaSaccharin in WP rats, $t = 3.94$, $P < 0.001$ (Figure 6B), while N cells showed greater

phasic activity, $t = 2.35$, $P < 0.025$ (Figure 6C). Since N cells are much more common, the net effect was the marginally larger response to NaSaccharin in WP rats seen in Figure 2 and Table 2B.

Thus, there are indications in both the spatial (across neurons) and temporal aspects of the code that the signal for NaSaccharin is different between SP and WP rats. In WP rats, NaSaccharin evoked a smaller proportion of its total activity from S cells. This difference resulted from a combination of having fewer S cells in the WP group and their smaller phasic responses. At the same time, WP rats had a higher proportion of the NaSaccharin response carried through N cells, throwing the weight of the response toward a putative salt channel.

Discussion

Responses to NaSaccharin

In this experiment, we have examined the taste codes that underlie differences in NaSaccharin preference. The results may provide a neural counterpart to the differential palatability of 0.03 M NaSaccharin expressed by SP and WP rats in two-bottle preference tests.

In rats that preferred NaSaccharin, the profile it elicited was intermediate between those evoked by sugars and by

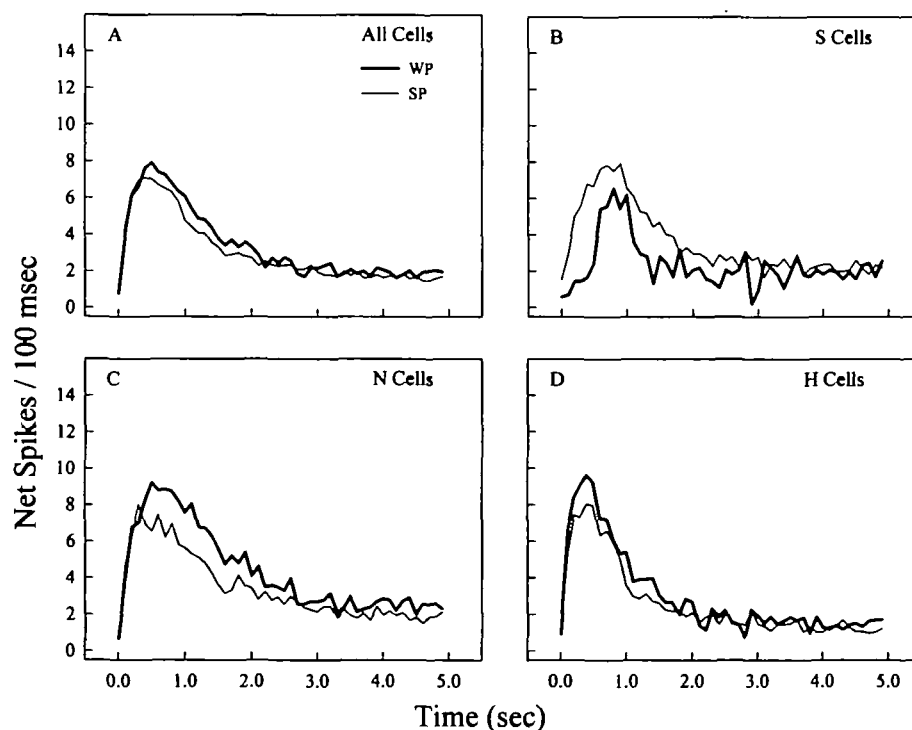


Figure 6 Post-stimulus time histograms for 0.03 M NaSaccharin across all cells (A) and in the S (B), N (C) and H (D) subtypes. Phasic responses (first 2 s) were significantly higher in WP rats in N ($t = 2.35$, $P < 0.025$) and lower in S cells ($t = -3.94$, $P < 0.001$).

Na/Li salts, as shown by individual correlations (Figure 3) and the resulting positions of these stimuli in a multidimensional space (Figure 4A). In WP rats, however, NaSaccharin evoked a response profile that was correlated more highly with salts and less with sugars (Figure 3), resulting in its close alignment with Na/Li salts in the space (Figure 4B). This is the typical location for intense 0.25 M NaSaccharin (Nolan, 1992), a stimulus that is avoided by nearly all rats, including those that prefer NaSaccharin at lower concentrations (Nachman, 1959). The implication is that 0.03 M NaSaccharin has a taste quality dominated by the sodium component to which WP rats are so sensitive, an outcome that occurs only at higher (unacceptable) concentrations in an unselected sample of rats, most of which would be NaSaccharin-preferring.

Where does this dissociation from sugars and affiliation with Na/Li salts originate? Measured across all cells and over the full 5 s recording period, the response to NaSaccharin was only marginally greater in WP rats (Figure 2). Thus, it does not derive simply from a heightened sensitivity to NaSaccharin. When neurons were divided into subtypes, this small increase was shown to be the result of offsetting (and still non-significant) tendencies: in WP rats, S cells gave smaller and N cells larger responses to NaSaccharin (Figure 5). Then, an analysis of the time course of this activity revealed that these tendencies were attributable to significant differences in the phasic (2 s) portions of the S and N cell responses (Figures 6B and C). Therefore, in WP rats, 0.03 M NaSaccharin elicited a smaller and briefer phasic response from S cells, and a more pronounced response from N cells (Table 2). Consequently, the profile elicited by NaSaccharin in WP rats was more closely related to those of the Na/Li salts, and more removed from those of the sugars (Figures 3 and 4).

Thus, behavioral differences toward NaSaccharin revealed disparities in its neural code that resulted from differences in the phasic responses in subsets of neurons. This reinforces the conclusion of Halpern and Tapper (1971) and others of the importance of the phasic response in determining taste quality and palatability.

WP rats were also significantly more responsive to QHCl (Figure 2), and this difference was most apparent in the H cells that are presumed to play a major role in conveying the 'bitter' quality (Figure 5C). While this implies a heightened sensitivity to the 'bitter' component of NaSaccharin as a basis for its rejection, such a result was not found. NaSaccharin evoked only marginally greater responses across all neurons and from H cells in particular, and its profile was

not more highly correlated with that of QHCl (Figure 3). It has been suggested that WP rats are more sensitive to the bitter component of NaSaccharin (Bartoshuk, 1979; Dess, 1991, 1993). Our results do not offer support for this specific suggestion, but rather that the lower level of palatability derives from a combination of reduced similarity to sugars and a greater affinity with Na/Li salts.

Responses to other stimuli

Although water-preferring rats were selected only on the basis of their responses to NaSaccharin, they proved to be highly sensitive tasters in general. Their mean response across the stimulus array was significantly higher than the responses of SP rats, an advantage that accumulated from greater activity to all stimuli except the two sugars (Figure 2). An inference can be drawn about the extent of this increased sensitivity from the responses given to the two concentrations of NaCl: WP rats gave almost precisely the same magnitude response to 0.03 M NaCl (41.4 spikes/s) as SP rats did to 0.10 M (41.2 spikes/s). The implication is that sodium sensitivity, at least, is more than tripled in WP rats. The proportional difference to quinine was even greater than to the Na salts, though the comparison across concentrations is not available.

In their saccharin preferences and exaggerated neural responsiveness to quinine, WP subjects were similar to ovariectomized rats (DiLorenzo and Monroe, 1990). These animals gave larger responses to quinine in the parabrachial nucleus than did intact females, and they also avoided saccharin (Marks, 1974).

Water-preferring rats and PTC/PROP-tasting humans

Saccharin acceptance is genetically determined in rats (Nachman, 1959; Dess, 1991, 1993). There is a related phenomenon in humans: sensitivity to phenylthiocarbamide (PTC) and 6-*n*-propylthiouracil (PROP). Tasters of PTC/PROP reject saccharin, characterizing it as 'more aversive' and in particular 'more bitter' than non-tasters (Bartoshuk, 1979; Marks *et al.*, 1988). Accordingly, they are more sensitive to quinine (Gent and Bartoshuk, 1983; Leach and Noble, 1986, but also see Hall *et al.*, 1975; Frank and Korchmar, 1985; Bartoshuk *et al.*, 1988). They also show heightened perception to sugars (Gent and Bartoshuk, 1983; Marks *et al.*, 1988) and NaCl (Gent and Bartoshuk, 1983), i.e. to all basic taste stimuli tested. While WP rats and human tasters share several characteristics of taste sensitivity, they differ in two respects: WP rats offer no evidence for greater 'bitterness'

in the neural code for NaSaccharin (as indexed by the correlation between profiles for NaSaccharin and QHCl), nor for high responsiveness to sugars. The PTC/PROP tasting status of WP and SP rats has not been tested.

Conclusion

The assumption that all rats of a species are equivalent for the purpose of studying taste is clearly not supported. Just

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