12. The following oligonucleotides served as probes: 5′-CAAAGCGCTGGTCTTGTCCTTACTTCCGGAGTCCTTGCT-3′ (oligo A-flip, complementary to sequences encoding residues 771 to 783 of the mature GluR-A flip polypeptide); 5′-CAAAGCGCTGGTCTTGTCCTTGGAGTCACCTCCCCC-3′ (oligo A-flop, complementary to residues 772 to 783 of GluR-A flop); 5′-GAGGGCACTGGTCTTTTCCTTACTTCCCGAGTCCTTGGC-3′ (oligo B-flip, complementary to residues 775 to 787 of GluR-B flip); 5′-GAGGGCACTGGTCTTTTCCTTGGAATCACCTCCCCC-3′ (oligo B-flop, complementary to residues 776 to 787 of GluR-B flop); 5′-TAGAGCACTGGTCTTGTCCTTACTCCCGGAGTCCTT-3′ (oligo C-flip, complementary to residues 780 to 791 of GluR-C flip); 5′-TAGAGCACTGGTCTTGTCCTTGGAGTCACCGCCCCC-3′ (oligo C-flop, complementary to residues 780 to 791 of GluR-C flop); 5′-CAAGGCACTCGTCTTGTCCTTGCTTCCCGAGTCCTT-3′ (oligo D-flip, complementary to residues 777 to 788 of GluR-D flip); 5′-CAAGGCACTCGTCTTGTCCTTGGAGTCACCTCCCCC-3′ (oligo D-flop, complementary to residues 777 to 788 of GluR-D flop). These oligonucleotides incorporated the maximum number of nucleotide differences between the flip and flop versions on one hand and between the four receptor sequences on the other. Due to the high degree of sequence conservation in and around the region affected by the alternative splice, these oligonucleotides, optimized in length versus sequence substitutions, differed in as few as three nucleotides (oligos D-flop and A-flop). Hence, high stringency conditions were employed, resulting in a lower signal than that achieved with the pan probes. At this stringency, the distinction between flop and flip sequences was clear. Some cross-hybridization of a given flop (or flip) probe to other flop (or flip) mRNAs cannot be excluded, but was often negligible as indicated, for example, by the absence of a signal for GluR-D flip mRNA in all brain regions but cerebellum. The probe D-flip (36 nt) differs from A- and B-flip in five, and from C-flip in six, positions. In situ hybridization was as described [W. Wisden, B. J. Morris, S. P. Hunt, in *Molecular Neurobiology—A Practical Approach*, J. Chad and H. Wheal, Eds. (IRL Press, Oxford, in press), vol. 2]. Oligonucleotides were 3′ end-labeled with terminal deoxynucleotidyl transferase (Bethesda Research Laboratories) and α[$^{35}$S] dATP (1200 Ci/mmol, New England Nuclear) at a 30:1 molar ratio of labeled nucleotide to oligonucleotide. Sections were hybridized overnight at 42°C with probe (1 pg/μl) in 50% formamide, 4× standard saline citrate (SSC), and 10% dextran sulfate and then washed in 0.1× SSC at 60°C for 20 min before dehydration and apposition to Kodak XAR-5 film for 1 week. After film exposure, sections were dipped in Kodak emulsion and exposed for 4 weeks at 4°C, developed in Kodak D19 developer, and counterstained with thionin before viewing through a Zeiss Axioplan microscope. Structures were identified according to I. G. Paxinos and C. Watson [*The Rat Brain in Stereotaxic Coordinates* (Academic Press, Sydney, ed. 2, 1986)] and S. A. Bayer [in *The Rat Nervous System*, G. Paxinos, Ed. (Academic Press, Sydney, 1985) vol. 1, pp. 335–352].
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22. Single-letter abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
23. Male BALB/c mouse liver DNA was partially digested with Mbo I, fractionated on a 10 to 40% sucrose gradient, and DNA fragments of 16 to 20 kbp were pooled. Cohesive Mbo I termini were partially filled in with dAMP and dGMP with Klenow enzyme (Boehringer Mannheim). This DNA was ligated to λ vector that had been digested with Xho I and partially filled in with dTMP and dCMP. Packaged ligation products were used to infect *Escherichia coli* strain P2 392, and a library of 4.4 × $10^6$ recombinant phage was obtained. This library was screened with 5′ end-labeled oligonucleotides B-flip and B-flop (*12*), as well as with an oligonucleotide (5′-CACTCTCGATGCCATATACGTTGTAACCTTCCTTATAAGTTGC-3′) complementary to sequences encoding residues 546–859 (ATYKEGYNVYGIES) of the mature GluR-B polypeptide (*4*). Four independent, overlapping clones were isolated, mapped, and sequenced across the specified region.
24. P. Werner constructed the rat brain cDNA libraries.
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# Coding Channels in the Taste System of the Rat

THOMAS R. SCOTT AND BARBARA K. GIZA

**Basic taste qualities are thought to be perceived independently, yet discrete neural coding channels have not been demonstrated in the central nervous system. The response profiles of taste cells in the nucleus tractus solitarius (NTS) of the rat were categorized into four groups, and the effects of amiloride, a passive sodium channel blocker, on each were determined. NTS neurons that responded specifically to sodium chloride (NaCl) or to NaCl and sugars were suppressed by amiloride; those broadly sensitive to salts, acids, and bitter stimuli were unaffected. Moreover, the response profile evoked by NaCl lost its distinctiveness after treatment with amiloride, becoming similar to those evoked by acids and quinine. Receptors that respond to sodium must relay their information through independent coding channels to identifiable subgroups of NTS neurons, the activity of which is responsible for the perception of saltiness.**

GUSTATORY RECEPTORS THROUGHOUT the oral cavity form synapses on peripheral neurons whose axons transmit taste-evoked activity to second-order cells in the NTS of the medulla. It is not known if the taste system is composed of information channels, each with a specific coding responsibility, or if all neurons contribute to every afferent signal. Most psychophysicists contend that the basic taste qualities are perceived independently (*1*). Despite a partitioning of peripheral and central nervous system taste cells based on several criteria, however (*2, 3*), electrophysiologists have not demonstrated a corresponding independence among subgroups of neurons (*4*).

We have adopted a strategy that appears to provide a resolution. We have identified presumed subgroups of cells by their distinct response profiles. Then we have altered the significance of a particular chemical to the rat in order to determine if the resulting accommodation in the function of the taste system is distributed among all cells or is restricted to the subgroup with sensitivities that are most relevant to address the new condition.

Three applications of this approach indicate that the taste system is indeed organized into a discrete number of independent coding channels. First, we created a conditioned taste aversion to sodium saccharin and monitored its neural effects on second-order taste cells in the NTS (*5*). Only cells with "sweet" response profiles were affected by this experience. The change in activity was appropriate to address the new situation: the neural response to formerly appetitive sodium saccharin took on a profile more like that of quinine, providing a neural basis for the behavioral rejection that rats show to sodium saccharin after the conditioning procedure.

Next, we altered the rat's physiological need by $Na^+$ deprivation, generating an intense $Na^+$ appetite (*6*). The response to

Department of Psychology, University of Delaware, Newark, DE 19716.

NaCl in the NTS shifted: neurons with $Na^+$-oriented profiles fell nearly silent, while those with sugar-oriented profiles responded more briskly. The resulting response to NaCl assumed the character of the codes for highly appetitive sugars. Thus two groups of neurons, identifiable a priori according to their response profiles, reacted independently and in opposite directions to reveal a plausible neural mechanism for the $Na^+$ appetite.

In the present experiment, we used amiloride to interfere with a specific transduction event on the surface of the tongue and monitored the consequences in the NTS. Amiloride is an inhibitor of passive $Na^+$ transport. Its application to the tongue (i) blocks short-circuit current across lingual epithelium (*7*) and inward $Na^+$ current across taste receptor membranes (*8*); (ii) suppresses activity to $Na^+$ and $Li^+$ salts in the whole chorda tympani nerve (*7, 9, 10*) and a subset of single fibers (*11*); and (iii) reduces the perception of saltiness in humans (*12*).

Twenty-seven female Wistar rats were prepared for electrophysiological recording under general anesthesia (100 mg of Ketaset per kilogram of body weight followed by 48 mg of chloral hydrate) (*13*). Respiration, heart rate, and body temperature were maintained within normal ranges. Action potentials of 36 single second-order neurons in the NTS were isolated with micropipettes, then amplified, filtered, displayed, and stored by conventional techniques (*5, 6*). A total of 15 stimuli were used (Table 1). The emphasis was on $Na^+$-$Li^+$ salts, but each of the other basic tastes was represented, as were complex combinations of the basic tastes (for example, sodium saccharin) and stimuli that evoke unique taste perceptions [monosodium glutamate (MSG) and Polycose]. The tastant solution (5 ml at room temperature) was sprayed across the tongue and palate at a rate of 2 ml/s. Gustatory-evoked activity was recorded for 5 s after stimulus onset. The tastant was then rinsed from the mouth with distilled water and 90 s was permitted to elapse before the next application to prevent adaptation.

**Table 1.** The stimulus array.

| Stimulus | Abbreviation | Human subjective taste quality (*19*) |
|---|---|---|
| 0.01 M NaCl | N1 | Weak sweet, salty |
| 0.03 M NaCl | N2 | Salty |
| 0.10 M NaCl | N3 | Salty |
| 0.30 M NaCl | N4 | Salty |
| 0.10 M LiCl | L | Salty |
| 0.50 M sucrose | S | Sweet |
| 1.00 M glucose | G | Sweet |
| 0.03 M sodium saccharin | Sa | Salty-sweet |
| 0.01 M HCl | H | Sour |
| 0.01 M citric acid | Ci | Sour |
| 0.01 M quinine hydrochloride | Q | Bitter |
| 0.10 M KCl | K | Salty-bitter |
| 0.10 M $CaCl_2$ | Ca | Bitter |
| 0.10 M MSG | M | Meatlike |
| 0.20 M Polycose | P | Bland |

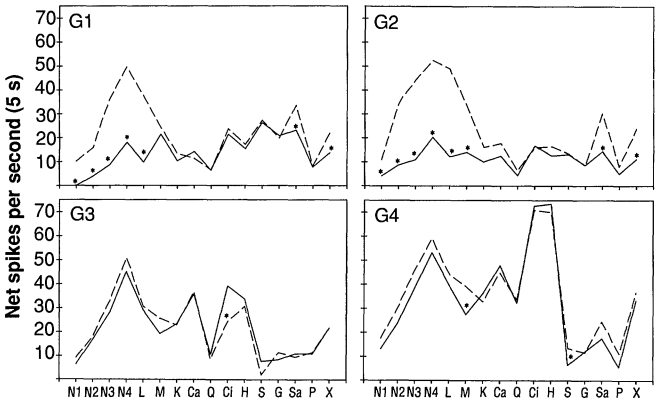


**Fig. 1.** Mean response profiles of each group of NTS neurons to the stimulus array before (dashed line) and after (solid line) lingual application of amiloride. The spontaneous activity of each cell (mean, 11.7 spikes per second before amiloride; 10.4 spikes per second after amiloride) has been subtracted from its total response. Abbreviations are from Table 1. X, mean response to all stimuli. Values for *n*: G1, 9; G2, 10; G3, 8; G4, 9. $*P < 0.05$. SEMs for all significant comparisons ranged from 1.48 to 13.15, with a median of 4.53 spikes per second.

After the initial stimulus series, 180 ml of 0.5 mM amiloride was sprayed into the mouth at a rate of 1.0 ml/s for 3 min. This was followed by a 30-s rest and reapplication of the stimulus series. During the period after amiloride administration, all procedures for stimulus application were identical to those used earlier, except that the amiloride solution was used for rinses between taste stimuli (*14*).

The NTS neurons could be categorized into four groups (Fig. 1) on the basis of their response profiles to the entire stimulus array before the application of amiloride (*15*). Group 1 (salt-sweet) cells showed high sensitivity to NaCl and LiCl plus responsiveness to stimuli that humans describe as sweet (*16*). Group 2 (salt) neurons responded only to NaCl and LiCl. Group 3 (salt-acid) cells were sensitive to NaCl and LiCl but also responded well to acids and to $CaCl_2$. Group 4 cells (acid-salt-bitter) responded well to NaCl and LiCl but showed exceptional sensitivity to the acids and a side band of responsiveness to stimuli that humans perceive as bitter. Thus, although the mean sensitivity to NaCl and LiCl was roughly equal in the four groups, their full profiles permitted statistically reliable discriminations among them.

Amiloride suppressed salt responses in groups 1 and 2 but did not affect those in groups 3 and 4. The sensitivity of cells in group 1 to the four concentrations of NaCl declined by a mean of 79%, to 0.1 M LiCl by 74%, and to 0.03 M sodium saccharin by 30% (*17*) (Fig. 1, G1). Their responses to all other stimuli were unaffected. Group 2 cells (Fig. 1, G2) showed a mean decline of 68% to NaCl, 75% to LiCl, 58% to 0.1 M MSG, and 52% to sodium saccharin. In contrast,

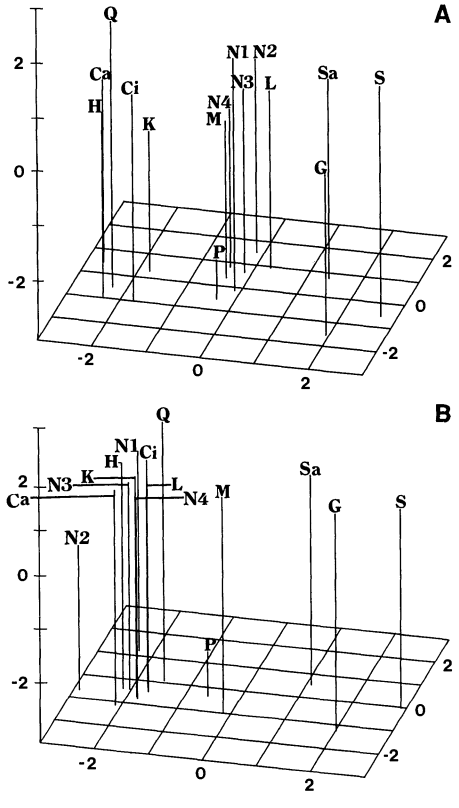


**Fig. 2.** Multidimensional spaces showing the relative similarity of stimulus profiles before (**A**) and after (**B**) amiloride. Configurations represent the optimal solutions computed by the Guttman-Lingoes model (*20*), from a matrix of correlations between each pair of stimulus profiles. The higher the correlation between two profiles of activity, the closer the stimuli that evoked those profiles are situated in the space. The stimulus locations in (A) are typical of those in a normally functioning rat taste system. After amiloride application (B), all $Na^+$-bearing stimuli except MSG-generated profiles similar to those of acids and bitter chemicals. The axes of the spaces are labeled in arbitrary units.

the salt sensitivity of cells in groups 3 and 4 was unaffected by amiloride (Fig. 1, G3 and G4), despite the fact that the response to NaCl and LiCl before amiloride was similar in all groups. Thus, the broad response profiles of NTS neurons allow prediction of the sensitivity of each cell to interference with the transduction mechanism for $Na^+$ and $Li^+$.

The taste quality of NaCl and LiCl is distinctly altered by amiloride, as revealed by changes in the response profiles these salts evoke from the neural sample. Multidimensional scaling, based on the relative similarity among the neural response profiles elicited by all stimuli, shows that NaCl and LiCl occupy their normal central position between stimuli that humans perceive as sweet (to the right) and those that are sour and bitter (left) in the unadulterated taste system (Fig. 2A). With amiloride application, salts move to the sour-bitter extreme, leaving only MSG in the position they formerly occupied (Fig. 2B). If NaCl and LiCl elicit the perception of "saltiness" in the normally functioning taste system, then this quality has been lost with amiloride. Therefore, with the $Na^+$-$Li^+$ response suppressed in group 1 and group 2 neurons, saltiness is eliminated, although approximately 50% of the total activity evoked by these salts remains. Saltiness may be coded exclusively by neurons that emphasize $Na^+$ and $Li^+$ responsiveness and exclude sensitivity to other nonsweet stimuli (groups 1 and 2). These cells appear to be served by receptors that use a passive transport channel (susceptible to amiloride blockade) as a transduction mechanism, as opposed to receptors innervating those neurons that respond broadly to nonsweet chemicals, including NaCl and LiCl. These latter receptors, and the NTS neurons they serve, appear to be responding only to the halogen anion of the NaCl molecule (*10*).

Our results show that (i) the effects of amiloride on responsiveness to NaCl and LiCl are profound in some NTS cells and negligible in others; (ii) the response profile of any neuron permits a prediction of which result will obtain for that cell; and (iii) with the responses of groups 1 and 2 neurons selectively suppressed by amiloride, the neural profiles elicited by NaCl and LiCl lose their distinctive character.

Amiloride causes increased membrane resistance and a 20-mV hyperpolarization in 25% of taste receptors, thus reducing, but not eliminating, the excitability of these cells (*18*). Accordingly, in our NTS recordings from group 1 and group 2 cells, intensity-response functions to NaCl after amiloride paralleled those of the unadulterated system, although reduced by some 75%.

Moreover, if a subset of receptors is hyperpolarized by amiloride, it should be less responsive to any stimulus, regardless of the transduction mechanism by which it is depolarized. Because the responses to chemicals that do not contain $Na^+$ or $Li^+$ remained unaffected in the NTS, these stimuli must not be transduced by receptors that have amiloride-sensitive channels. This logic implies a thorough segregation of transduction processes by receptor cell type combined with a predictable link between receptors and taste neurons in the NTS. Group 1 cells might receive meaningful innervation only from $Na^+$ and sugar receptors such that amiloride treatment leaves them with just a response to sugars. Group 2 neurons receive input exclusively from $Na^+$ receptors and are rendered unresponsive by amiloride, save for residual $Na^+$-induced activity and system noise. Cells in groups 3 and 4 receive input from neither $Na^+$ nor sugar receptors but rather from those that transduce acids as well as a variety of compounds that have the common property of bitterness. The segregation of function that is maintained from receptor to the central nervous system implies the existence of discrete information channels in the taste system.

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15. See (*3*) for a full description of the statistical procedures used to partition neurons.
16. Our method of stimulus application does not elicit robust responses to sweet stimuli. Thus, although the responses to sugars and sodium saccharin from group 1 cells are not impressive in absolute spike rate, they are sufficient to characterize these neurons as the group with sugar-sensitive profiles.
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