Gustatory Neural Coding in the Cortex of the Alert Cynomolgus Macaque: The Quality of Bitterness

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Scott, Thomas R., Barbara K. Giza, and Jianqun Yan. Gustatory neural coding in the cortex of the alert cynomolgus macaque: the quality of bitterness. J. Neurophysiol. 81: 60±71, 1999. We sought to define the gustatory neural representation in primates for stimuli that humans describe as predominantly bitter. Thus we analyzed the responses of single neurons from the insular cortex of two alert, male cynomolgus macaques in response to the oral application of four basic taste stimuli (glucose, NaCl, HCl, and quinine HCl) and fruit juice, and to a series of 15 other chemicals to which humans ascribe a bitter component. Gustatory neurons occupied a volume of 109 mm³ across an area of 4.0 mm in the anteroposterior plane, 4.4 mm in the mediolateral, and 6.2 mm in the dorsoventral. Taste cells represented 161 (8.6%) of the 1881 neurons tested for chemical sensitivity. Fifty of these could be monitored throughout the delivery of the entire stimulus series, and their responses constitute the data of this study. The mean spontaneous discharge rate of the cortical gustatory cells was 3.2 ± 3.3 spikes/s (range = 0.2–17.7 spikes/s). The mean breadth-of-tuning coefficient was a moderate 0.77 ± 0.15 (range = 0.25–0.99). Forty-eight neurons responded to taste stimuli with excitation, and two responded with inhibition. Forty-one of the 50 neurons were able to be classified into one of four functional types based on their responses to the four basic stimuli used here. These were sugar (n = 22), salt (n = 7), acid (n = 7), and quinine (n = 5). A two-dimensional space was generated from correlations among the response profiles elicited by the stimuli array. The 16 bitter chemicals formed a coherent group that was most closely related to HCl, moderately to NaCl, and bore no relationship with glucose. Within the bitter stimuli, six formed a subgroup that was most separated from all nonbitter chemicals: quinine HCl, phenylthiocarbamide, propylthiouracil, caffeine, theophylline, and phenylalanine. Humans describe these stimuli as rather purely bitter. Of the remaining 10 bitter compounds, 4 were on the fringe of the bitter group leading to NaCl: MgCl₂, CaCl₂, NH₄Cl, and arginine. Humans characterize these as bitter-salty. Three were on the fringe leading to HCl: urea, cysteine and vitamin B₁. Humans call these bitter-sour. The remaining three (nicotine, histidine, and vitamin B₂) occupied the center of the bitter group. Taste quality, inferred from the position of each stimulus in the space, correlated well with human descriptions of the same stimuli, reinforcing the value of the macaque as a neural model for human gustation.

INTRODUCTION

A fundamental purpose of the taste system is to distinguish nutrients from toxins. Some 10% of plants contain toxic alkaloids, and toxic glycosides are still more common (Kingsbury 1964). Those among our predecessors who rejected these chemicals would have been favored to pass this response on to their progeny. Hence it is not surprising that toxicity is related to behavioral rejection among species of every phylum and to an aversive experience that humans call bitterness (Garcia and Hankins 1975).

Across mammalian orders, sensitivity to bitterness, as defined by behavioral rejection of quinine HCl, is directly proportional to susceptibility to toxicosis (Glendinning 1994). Carnivores encounter few alkaloids, are highly vulnerable to their effects, and are sensitive to the taste of quinine at 10⁻⁵ M (Carpenter 1956). Omnivores, including rodents and primates, experience more alkaloids and have quinine thresholds an order of magnitude higher (Glaser 1980; Patton and Ruch 1944). Herbivores inevitably ingest a wider range of alkaloids, develop mechanisms for tolerating them, and are another order of magnitude less sensitive to quinine (Goatcher and Church 1970; Randall et al. 1978).

Rejection of a stimulus is directly proportional to its oral LD₅₀ (unpublished observations). Naïve rats, fluid deprived for 23 h, were offered 32 chemicals in short-exposure tests where postigestive effects would be negligible. Despite having neither experience nor consequences to guide them, these moderately deprived rats decreased their licking as oral LD₅₀ of chemicals declined through 8,000 mg/kg and rejected stimuli with increasing resolve as more toxic chemicals were presented (the correlation of licks vs. LD₅₀ = 0.77; P < 0.001). This native capacity to assess the toxicity of chemicals is also evident in humans. In a psychophysical study, Schiffman and Erickson (1971) reported a correlation of +0.86 between subjective assessment of poisonousness and actual toxicity. Therefore sensitivity to toxins is inherent and ubiquitous among animals, and rejection is related to the degree of toxicity.

The bitterness that marks toxicity is not uniform. McBurney et al. (1972) showed that adaptation to quinine HCl in humans decreased the bitterness of Q₂SO₄, caffeine (CAF), and sucrose-octaeacetate but not the bitter components of urea (UR), MgSO₄, CaCl₂ (CAL), KCl, or phenylthiocarbamide (PTC), implying the existence of more than one receptor mechanism for the bitter quality. The authors concluded from these cross-adaptation data that the predominantly bitter compounds they used could be divided into at least three classes: 1) quinine, caffeine and sucrose-octaeacetate, 2) urea and MgSO₄, and 3) PTC and KNO₃. Moreover, the term bitterness itself contains variations captured by humans in the adjectives poisonous, nauseous, strangling, obnoxious, mineral, and repulsive (Schiffman and Dackis 1975).

Here we explore the range of variation within the bitter category by means of electrophysiological recordings from...
the taste cortex of alert macaques. The primary taste cortex in the macaque is located in the frontal operculum and adjoining anterior insula (Pritchard et al. 1986; Scott et al. 1986, 1991). Recordings from this area reveal a small but consistent subset of neurons responsive to chemical stimulation of the oral cavity. This paper is the eighth in a series describing gustatory neural coding in the cortex of the alert macaque monkey.

METHODS

Subjects

The subjects were two male cynomolgus monkeys (Macaca fascicularis) weighing 4.2–5.3 kg during the course of data collection. They were maintained on pellet food and tap water, which was available ad libitum in their home cages. Room lights were on from 0600 to 2200 h. Each monkey was adapted to a primate chair where he was reinforced with fruit and fruit juice for several weeks before surgery.

Surgery

Full sterile precautions were observed throughout surgery. Each monkey was sedated with ketamine hydrochloride (10 mg/kg im) and anesthetized to a surgical level with pentobarbital sodium (30–40 mg/kg im). The depth of anesthesia was monitored by frequent testing for the presence of flexion reflexes and heightened muscle tonus, which, if found, warranted a supplemental dose of barbiturate. Atropine (0.10 mg/kg im) was administered to prevent excessive mucous secretions and salivation; glycine was applied ophthalmically to prevent drying of the eyes. Respiratory rate was monitored throughout surgery, and core temperature was maintained at 34–36°C. The monkey was shaved and placed in a Kopf stereotaxic instrument. A 30-mm-diam stainless steel ring, to which a microdrive could be fitted during recording sessions, was placed on the skull at the anteroposterior level of the insular cortex and was centered 5-mm lateral to the midline to permit ready access to the insula in one hemisphere. The ring was fixed in place with dental acrylic.

To ensure full stability and support of the monkey’s head during recording sessions, two stainless steel tubes (12-mm OD, 7-mm ID, 6 cm long), through which horizontal support bars could be inserted during recording, were cemented to the skull cap in front of and behind the ring. The skull was left intact except for anchor pieces and a 2-mm-diam hole that was drilled in a promising location over the gustatory target area. Nitrofurazone (0.2% topical) and penicillin G-benzathine and G-procaine (100,000 U/kg im) were used for several days as a prophylaxis against postoperative infection.

Electrophysiological recording

SESSIONS. After a postoperative recovery period of 10 days, daily recording sessions were initiated. Each monkey was transferred from his home cage to the primate chair, where his head was supported as described previously. The monkey was otherwise free to move and normally adopted a relaxed sitting position. Adjustments were made to the chair to provide maximum support according to each monkey’s size and shape, and the chair was dedicated to that subject’s use for the duration of his tenure. The monkey’s comfort was continually attended to, and fruits and nuts were offered intermittently throughout the recording session.

ELECTRODES, POSITIONING AND PENETRATION. Glass-insulated tungsten electrodes with pencil-shaped tips of 2–5 μm (impedance of 1–2 MΩ at 1 kHz) were used to isolate the activity of individual cells in insular cortex. Electrodes were systematically positioned on each track with the use of a Kopf X-Y positioner attached to the stainless steel ring. The dura mater was anesthetized with lidocaine hydrochloride (0.20 ml of 4% Xylocaine), and a sterile stainless steel guide tube (0.5-mm OD) was passed through it. The sterile electrode was then lowered through the guide tube to a predetermined depth 5- to 10-mm dorsal to the insula, from which it was advanced with a Trent-Wells hydraulic microdrive and chronic adapter system.

RECORDING SYSTEM. Conventional electrophysiological recording techniques were used for differential amplification, display, and recording of the neural signal. Action potentials of a single cell were identified by consistency of amplitude and waveform. Neural data, voice commentary, and onset marker signal for the stimulus delivery system were stored on a four-channel TEAC tape recorder for off-line analysis.

Stimuli and stimulus delivery

Deionized water and 20 sapid chemicals were used in this study. These comprised fruit juice, glucose, NaCl, HCl, and 16 stimuli that humans describe as predominantly bitter. All stimuli, their chemical formulas, abbreviations used to identify them in this paper, their concentrations, and molecular weights are listed in Table 1. Fruit juice [Ocean Spray cranraspberry juice (CR)] was used as an effective search stimulus, possessing a complex and highly palatable taste quality. However, every neuron whose activity could be isolated was also tested by application of the four basic stimuli to ensure that there was not a bias toward identifying only cells associated with appealing tastes.

All stimuli were dissolved in deionized water and semirandomly applied at room temperature. Concentrations were based on the results of previous electrophysiological (Scott et al. 1986, 1991) and psychophysical (Hall et al. 1975; McBurney et al. 1972; Schiffman and Dackis 1975) studies. Stimuli were delivered through a hand-held syringe in quantities of 1.0 ml. Manual delivery was used in the alert monkey to permit stimulation of a large and nearly constant receptive field throughout a recording session despite different mouth and tongue positions adopted by the monkeys as the palatability of the taste solutions varied. Each stimulus was followed by 2–5 ml of deionized water as a rinse and a rest period of 30–120 s. If there were indications that either the behavioral (licking, chewing, or facial expressions) or neural activity had not returned to prestimulus levels, the water rinse was reapplied, and the rest period was extended.

The responsiveness of each neuron to visual and tactile stimulation and to motor activity was also assessed. Recordings were made as the syringe containing fruit juice was exposed to the monkey at a distance of 60 cm for 3 s and then brought toward his mouth over a 3-s period. Spontaneous actions (chewing, gaping, and tongue extension) were noted and correlated with neural activity. In addition, the teeth, tongue, gingivae, palate, and lips were stroked with a cotton-tipped applicator to assess the cell’s sensitivity to tactile stimulation.

Data analysis

An IBM computer counted action potentials for 3 s before and 5 s after stimulus application and performed basic statistics. The number of net spikes per second (gross minus spontaneous) over 5 s provided data for derived analyses, which included calculations of interneuronal and interstimulus Pearson product-moment correlation coefficients. From these, stimuli were placed in a two-dimensional (2-D) “taste space” by application of a multidimensional scaling routine based on the Guttman-Lingoes program (Guttman 1968). Correlations among the sensitivity profiles of neurons
TABLE 1. Listing of the 21 chemicals used as stimuli in this study, with their concentrations, molecular weights, chemical formulas, chemical categories, and the abbreviations by which they are referred in this paper

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Molar Concentration</th>
<th>Chemical</th>
<th>Molecular Weight, daltons</th>
<th>Formula</th>
<th>Category</th>
</tr>
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<tbody>
<tr>
<td>W</td>
<td></td>
<td>Deionized water</td>
<td>18</td>
<td>H₂O</td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>20%</td>
<td>Cranberry–raspberry juice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>1.0</td>
<td>Glucose</td>
<td>180</td>
<td>CH₉(OH(CH₂OH)₄CHO</td>
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<tr>
<td>N</td>
<td>0.3</td>
<td>Sodium chloride</td>
<td>58</td>
<td>NaCl</td>
<td></td>
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<tr>
<td>H</td>
<td>0.01</td>
<td>Hydrochloric acid</td>
<td>36</td>
<td>HCl</td>
<td></td>
</tr>
<tr>
<td>Q</td>
<td>0.001</td>
<td>Quinine HCl</td>
<td>361</td>
<td>C₁₀N₀₂O₂</td>
<td>Alkaloid</td>
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<tr>
<td>PTC</td>
<td>0.001</td>
<td>Phenylthiocarbamide</td>
<td>152</td>
<td>C₇H₇N₂S</td>
<td>Carbamine</td>
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<tr>
<td>PROP</td>
<td>0.5</td>
<td>Propylthiouracil</td>
<td>170</td>
<td>C₇H₁₀N₂OS</td>
<td>Pyrimidine</td>
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<td>CAF</td>
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<td>PHE</td>
<td>0.1</td>
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<td>CaCl₂</td>
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<tr>
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<td>Thiamine HCl</td>
<td>95</td>
<td>C₃H₇NO₂HCl</td>
<td>Vitamin</td>
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<tr>
<td>MG</td>
<td>0.3</td>
<td>Magnesium chloride</td>
<td>180</td>
<td>MgCl₂</td>
<td>Salt</td>
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<tr>
<td>THE</td>
<td>0.01</td>
<td>Theophylline</td>
<td>174</td>
<td>C₇H₈N₂O₂</td>
<td>Amino acid</td>
</tr>
<tr>
<td>ARG</td>
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<td>Arginine</td>
<td>60</td>
<td>CH₉N₂O</td>
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<tr>
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<td>Urea</td>
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<td>C₇H₀N₂O₂</td>
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<tr>
<td>HIS</td>
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<td>1-Histidine</td>
<td>53</td>
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<td>Salt</td>
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<tr>
<td>NI</td>
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<td>C₉H₈N₂</td>
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<tr>
<td>CYS</td>
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<td>Cysteine</td>
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<td>C₇H₈NO₂S·HCl·H₂O</td>
<td>Amino acid</td>
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<tr>
<td>B₂</td>
<td>0.3</td>
<td>Riboflavin</td>
<td>376</td>
<td>C₂H₆N₂O₆</td>
<td>Vitamin</td>
</tr>
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</table>

served as the basis for separating the taste cells into subgroups, by use of the cluster analysis program of Wishart (1978).

Location of recording sites

The position of each recording site was determined in two ways. First, after each track, X-ray photographs were taken from frontal and lateral perspectives. Recording sites could be reconstructed to within 0.25 mm by reference to skull landmarks. Second, in the final session, electrolytic lesions were made through the recording electrode (50 μA for 50 s, electrode negative). At the end of the experiments, the monkeys were sedated with ketamine hydrochloride (10 mg/kg im) and then administered a lethal dose of pentobarbital sodium. They were immediately perfused transcardially with physiological saline (0.9%) followed by 10% formalin in physiological saline solution. The brains were removed and placed in 30% sucrose-10% formalin solution for several days, after which 50- to 100-μm frozen serial sections were cut and stained with neutral red (Fig. 1).

RESULTS

Characteristics of insular cortex

Data were collected during 135 recording sessions (46 from the 1st monkey, 89 from the 2nd) over a period of 8 mo. Taste cells were isolated from 89 recording tracks in the insula. They constituted 161 (8.6%) of the 1881 neurons whose activity was isolated and tested for taste sensitivity with five sapid solutions (cranberries and the 4 basic stimuli). They were scattered over a volume of 109 mm³ from 1.0-mm anterior to 3.0-mm posterior to the anterior clinoid process of the sphenoid bone, from 13.5- to 17.9-mm lateral to the midline, and from 6.2-mm dorsal to the lateral sulcus down to the sulcus. Of these 161, the evoked activity of 50 taste cells was followed through at least one complete stimulus series, and their responses compose the data of this study. Another 468 (24.9%) neurons responded during mouth movements, although it could not be determined whether this represented motor, sensory, or proprioceptive activity; 77 (4.1%) neurons responded to light touch of the tongue, 13 (0.7%) were activated by the sight of the syringes and 3 (0.2%) responded during extension of the tongue. Sensitivities of the remaining 1159 (61.6%) neurons could not be determined from the stimuli applied.

Functional characteristics of taste cells

SPONTANEOUS ACTIVITY AND RESPONSE CRITERION. These 50 taste neurons had a mean spontaneous rate of 3.2 ± 3.0 (SD) spikes/s with a range of 0.2 to 17.7 spikes/s. The low rate of spontaneous activity combined with the possibility that nongustatory influences may impinge on these cells made the danger of false-positives high. Therefore we maintained the rather strict response criterion established in previous papers: spontaneous rate ± 2.33 (P < 0.01, one-tailed) sustained through the 5-s period after stimulus application.

RELIABILITY OF RESPONSES. Application of the complete stimulus series required 50–60 min. If isolation of the neuron’s activity remained uncompromised at the end of this period, we reapplied each stimulus to assess the reliability of the response. A quantitative measure of reliability can be determined by calculating the Pearson product-moment correlation coefficient between two columns of responses, one from each application of the same stimulus while recording from a given neuron. If all responses were identical, the coefficient would be +1.00. We performed this analysis on the basis of 544 reapplications that included all 50 neurons and 20 stimuli. The resulting coefficient was +0.82, indicating a moderate degree of stability across the entire range of physiological and methodological factors that can affect the spike count.

EXCITATION AND INHIBITION. Evoked activity in the gustatory cortex is primarily excitatory. With 50 taste cells and
20 sapid stimuli, there were 1,000 stimulus–neuron interactions. Of these, 395 (39.5%) satisfied the criterion for excitation, 20 (2.0%) for inhibition, and 585 (58.5%) evoked no response. However, 32 (64%) of the taste neurons were excluded from the possibility of giving an inhibitory response; their spontaneous activity levels were sufficiently low and SDs were sufficiently large that $2.33 \times SD$ exceeded spontaneous rate. Therefore even a total cessation of responding during the 5-s recording period could not satisfy the criterion for inhibition. Rather, nearly all inhibitory responses, 17 of 20, came from two cells with high and constant (i.e., small SD) spontaneous activity. The neuron with the highest spontaneous rate responded with inhibition to every stimulus and reached response criterion for 13. Thus inhibition does exist as a response option for cortical gustatory neurons in the macaque but is quite uncommon.

**BREADTH OF TUNING.** The accepted metric for evaluating a neuron's breadth of sensitivity across the basic taste stimuli is the entropy coefficient introduced by Smith and Travers (1979). The proportion of a cell's total response that is given to each of the four prototypical stimuli is expressed as a coefficient that ranges from 0.00 (exclusive sensitivity to 1 stimulus) to 1.00 (equal response to all 4). The mean breadth-of-tuning metric for the 50 taste cells in this study (with the absolute value of negative responses) was $0.77 \pm 0.15$ (range = 0.25–0.99), near the mean of 0.70 from the first seven studies of this series.

**GUSTATORY NEURON TYPES.** The mean net response to each stimulus is shown in Fig. 2. As is typical, 1.0 M glucose elicited the largest response, and water elicited the smallest response. The bitter stimuli were moderately effective overall, with 0.3 M arginine (ARG), 0.3 M vitamin B₁, and 0.3 M NH₄Cl evoking the greatest activity and 0.5 M urea evoking the least.

Perhaps more salient is a determination of how those responses were distributed across neurons. The issue of the existence of gustatory neuron types relates to the basic composition of the taste system. Are all cells generated from a limited number of templates, each replicated many times to create a discrete set of neurons, or does each cell possess a unique profile of responsiveness, filling its place along a continuum of chemical sensitivity? To address this, we determined the distribution of activity profiles among the 50 taste cells in this data set to determine whether a tendency toward recurring profiles emerged.

We defined the activity profile of a neuron by its responses to the four prototypical stimuli. We then calculated the Pearson correlation between each pair of profiles ($n = 50 \times 49/2 = 1,225$ coefficients) to create a matrix of functional similarity among the 50 cells. Finally, we subjected this matrix to a cluster analysis (Wishart 1978) to reveal possible groupings of response profiles.

The results of the cluster analysis appear as a dendrogram in Fig. 3. Neurons are numbered in the order in which they
were isolated. Pairs of cells were connected at the level of correlation between their response profiles, and groups were fused at the mean level between their constituent members in an iterative process that continued until all neurons in the sample were interconnected. Beneath each cell is labeled the prototypical stimulus that evoked its largest response, followed by any other tastant that elicited >80% of that maximum. Analyses of variance (ANOVAs) were used to determine significant differences among the prospective subgroups. Then post hoc comparisons were performed on the responses to the defining stimulus of each subgroup (e.g., NaCl for the N-cells) to ensure that the activity from cells within the subgroup was significantly different from that of neurons beyond it.

Of the 50 neurons in Fig. 3, 41 were associated with four statistically coherent groups that could be identified according to their best stimulus as (left to right) glucose-oriented (cells 31–14, n = 22), sodium-oriented (cells 43 and 44, n = 7), acid-oriented (cells 27–6, n = 7), and quinine-oriented (cells 35–3, n = 5). The remaining nine neurons (cells 29–40) composed a loose cluster with sensitivity to all four stimuli. Their response profiles were not sufficiently coherent to be recognized as a group.

Among the glucose-oriented neurons (hereafter “S-cells” [sugar]), 13 (cells 31–30 in Fig. 3) had profiles that were nearly identical, with all correlations above +0.92. Although a direct comparison between stimulus and neuronal profiles is not proper, the fact that this is higher than the +0.82 reliability coefficient in this study implies that these cells were so functionally similar that we could not discriminate among them. Another nine neurons were sufficiently similar to be included with this group. Therefore, 22 of these 50 taste neurons in macaque insular cortex were devoted to the recognition of sugar.

The sodium (“N-cells” [natrium]), acid (“H-cells” [hydrogen]), and quinine (“Q-cells”) groups did not total as many neurons as did the S-cells alone. However, each was statistically coherent and, with the exception of neurons 43 and 19, dominated by sensitivity to one basic taste quality.

As in other analyses of neuron types in the macaque, S- and N-cells were positively related ($r = +0.36$), as were H- and Q-cells ($r = +0.43$). However, these two supergroups, one representing generally appetitive (S and N), the other aversive tastes (H and Q) were negatively correlated with one another ($r = -0.46$).

The mean profile of each cell group across all 21 stimuli is shown in Fig. 4. Bitter stimuli, except for quinine, are ordered according to the response magnitude they elicited from Q-cells. The 22 S-cells responded well to glucose (hence their assignation) and to cranraspberry juice but poorly to all other stimuli. They were clearly not much involved in coding for predominantly bitter chemicals. N-cells responded best to NaCl but nearly as well to 0.3 M ARG (a “salty” amino acid) and a half-dozen others. H-cells responded quite well to all stimuli except cranraspberry juice, glucose, and NaCl, whereas Q-cells were more selective, responding strongly to certain bitter chemicals [PTC, propylthiouracil (PROP), and caffeine] and not at all to others [nicotine, cysteine (CYS), and vitamin B$_2$].

We addressed the issue of the degree to which the responses of the four groups were distinct from one another by comparing the distribution of evoked activity within a group to those of the other three groups for each stimulus. Thus we could determine which stimuli evoked significantly different responses from the various neuronal groups (Table 2). Separate ANOVAs revealed a significant effect of the neural group for water, glucose, NaCl, and HCl and for NH$_4$Cl, ARG, B$_1$, B$_2$, caffeine, CaCl$_2$, CYS, histidine, nicotine, phenylalanine, PROP, PTC, quinine, theophylline, and urea [$F(1, 25) > 4.534; P < 0.04$] but not for cranraspberry juice or MgCl$_2$ [$F(1, 25) < 2.225; P > 0.10$]. The most frequent distinction was between responses from S- and from H-cells, which were significantly different to all but 3 of the 21 chemicals.

Response to bitter stimuli: across-neuron analysis

The profile of activity evoked by each stimulus across the 50 neurons was generated, and the correlation between that
and every other profile was calculated \( n = 21 \) stimuli (including water); \( 21 \times 20 / 2 = 210 \) profiles. The resulting matrix of correlations served as the basis for a multidimensional space in which the proximity among stimuli was proportional to the degree of similarity among their profiles. We will first describe the broad relationship between the nonbitter basic stimuli and the 16 bitter compounds and then turn to a more detailed analysis of the latter.

**RELATIONSHIP OF BASIC STIMULI TO BITTER COMPOUNDS.** A 2-D representation of the relative similarities among all 20 sapid stimuli and water is shown in Fig. 5. A measure of the correlational level at which each stimulus or group of stimuli were affiliated with others is displayed as a stimulus dendrogram in Fig. 6. The dendrogram has the advantage of providing a quantitative description of the relationship among stimuli, in that the correlations among the profiles they elicited may be read directly from the ordinate. However, the dendrogram does not reveal the nature of the differences among stimuli, either within or between clusters. For this, one must refer to the relative positions of stimuli within the 2-D space. Therefore the two displays offer complementary information on the relationships among the taste qualities of the stimuli used in this study.

Both the space and dendrogram show a relationship among the basic stimuli that is typical in many respects. Glucose and quinine \( r = +0.04 \) occupy opposite ends of the dominant horizontal dimension. HCl is between and rather close both to water \( r = +0.84 \) and quinine \( r = +0.53 \), as is usually the case. The predominantly sweet taste of cranraspberry juice is represented near that of glucose \( r = +0.60 \).

The 16 bitter stimuli formed a diffuse group at the opposite end of the space from glucose. Its composition will be analyzed. As a group, they had the most distant relationship with glucose, with a mean correlation of \( +0.04 \) (range = \( -0.09 \) to \( +0.21 \), \( n = 16 \)). They were closer to NaCl, with a mean correlation of \( +0.40 \) (range = \( +0.28 \) to \( +0.58 \), \( n = 16 \)), and closer still to HCl, at \( +0.52 \) (range = \( +0.36 \) to \( +0.62 \), \( n = 16 \)). These relationships are manifested in the stimulus dendrogram, where the 16 bitter stimuli, although not tightly bound to one another, still form a coherent cluster that is joined first by water and HCl and finally by cranraspberry juice, glucose, and NaCl, which are themselves only poorly related.

**RELATIONSHIP AMONG BITTER STIMULI.** The 16 bitter compounds elicited response profiles that were moderately well
intercorrelated. The mean correlation coefficient between all pairs of bitter-evoked profiles was +0.67 [range = +0.39 (PTC vs. NH₄Cl) to +0.89 (caffeine vs. phenylalanine), \( n = 120 \). Eleven of the 120 correlations were greater than +0.80, near the reliability coefficient of +0.82 that should be considered the limit of resolution for this data set.

Within the bitter group, the profiles of PTC, PROP, phenylalanine, theophylline, and caffeine were characterized by correlations that were (1) nonexistent (mean = −0.02) with glucose, (2) low (mean = +0.34) with NaCl, (3) moderate (mean = +0.53) with HCl, and (4) high (mean = +0.74) with quinine. These five occupied the right side of the space and formed a coherent cluster closely related to quinine in the dendrogram. A different profile, common to CaCl₂, MgCl₂, NH₄Cl, ARG, histidine, CYS, nicotine, and B₂ was characterized by correlations that were (1) low (mean = +0.09) with glucose, (2) higher (mean = +0.46) with NaCl, (3) moderate (mean = +0.49) with HCl, and (4) lower (mean = +0.48) with quinine. As the last three mean correlations require, this group as a whole was nearly equidistant from NaCl, HCl, and quinine, implying a

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>G vs. N</th>
<th>G vs. H</th>
<th>G vs. Q</th>
<th>N vs. H</th>
<th>N vs. Q</th>
<th>H vs. Q</th>
</tr>
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<tbody>
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See Table 1 for definitions.
complex bitter-salty-sour quality. In addition to tending toward NaCl (MgCl₂, NH₄Cl, CaCl₂, and ARG) or HCl (CYS and vitamin B₁) in the space, these stimuli formed a second cluster within the bitter group in the dendrogram. Urea had the highest correlation with HCl (+0.62) and water (+0.50) among the bitter stimuli, edged away from the others toward HCl and water in the space, and was marginalized in the dendrogram.

**DISCUSSION**

The basic features of gustatory activity from this data set were in accord with those reported earlier. The proportion of taste-responsive neurons in the insula was slightly >5%; the spontaneous rate of taste cells was ~3 spikes/s; nearly all responses were excitatory; neurons were moderately broadly tuned across the basic stimuli; net responses to the basic stimuli across all taste neurons ranged from ~2 (quinine) to ~5 (glucose) spikes/s; most taste cells could be assigned to statistically distinct groups according to their response profiles to the basic stimuli.

The reliability coefficient in this study ($r = +0.82$) was below those seen in previous data sets from this lab ($r = +0.89$ to +0.94). We attribute that to the predominantly aversive quality of the stimulus series and the attempts of subjects to limit the area over which these stimuli spread. Their variable success in this effort may have caused differences in the receptor surface over which stimuli made contact. This source of variability is not addressed by implanting
Physicochemical attributes of stimuli as indicators of taste quality

Salty and sour stimuli are chemically homogeneous. Those that are sweet and bitter are heterogeneous. The array of bitter stimuli used here comprises a wide variety of chemical structures and attributes that can be related to the neural representation of taste quality.

The three bitter salts (MgCl$_2$, NH$_4$Cl, and CaCl$_2$) generated similar profiles (mean $r = +0.69$, $n = 3$) and, in the space, were positioned on the fringe of the bitter group that extended toward NaCl. All share the common properties of low molecular weight and a chloride anion.

The four stimuli that contain sulfur (PROP, PTC, CYS, and B$_1$) were separable into two pairs. PROP and PTC ($r = +0.83$) were highly similar, and CYS and B$_1$ ($r = +0.69$) were moderately so, but the mean correlation across the two pairs ($r = +0.56$, $n = 4$) was rather low. PROP and PTC share an N–C = S linkage that elicits a rather pure and intense bitterness in a majority of humans at low concentrations, although about one-third of subjects require a hundredfold concentration increase to experience the same taste (Kalman 1971). This bimodal distribution of sensitivity is a genetically conferred double recessive trait (Blakeslee 1932; Blakeslee and Salmon 1935; Fox 1932) and was related to sensitivity to other bitter chemicals (Bartoshuk 1979; Bartoshuk et al. 1988; Gent and Bartoshuk 1983; Hall et al. 1975; Mela 1989). This created considerable interest in PTC, but concerns about its toxicity and odor caused a shift to PROP as a chemical of similar structure and taste quality (Lawless 1980). CYS and B$_1$ may have been brought together not so much by their sulfur atoms as by the HCl radical each contains that took them toward the edge of the bitter group that faced HCl.

Profiles generated by the two xanthines (caffeine and theophylline) were tightly correlated ($r = +0.85$). Both were moderately similar to that of QHCl (mean $r = +0.75$, $n = 2$) and of nicotine (mean $r = +0.67$, $n = 2$) but in opposite directions such that the latter two were quite disparate ($r = +0.50$). Thus the four alkaloids spanned a range of qualities.

Despite encompassing a variety of taste qualities in humans, the four amino acids (CYS, HIS, ARG, and PHE) evoked well-correlated profiles (mean $r = +0.73$, $n = 6$). CYS, the only sulfurous amino acid, was the most disparate of the four.

The physical properties of molecular weight (Iwasaki et al. 1985; Noble 1994) and hydrophobicity (Gardner 1978; Schiffman et al. 1994; Tamura et al. 1990; Tan et al. 1993) were correlated with the degree of bitterness reported by human subjects. Molecular weight was not related to the organization of bitter stimuli in the space or dendrogram. The six chemicals toward the right of the space and composing the left subgroup of bitter stimuli in the dendrogram, those described by humans as most purely bitter, have a mean molecular weight of 204 daltons. The 10 toward the left of the bitter cluster in the space and composing the right subgroup of bitter stimuli in the dendrogram, described by humans as bitter-salty or bitter-sour, are nearly the same at 170 daltons. The correlation between molecular weight and position on the horizontal dimension of the space was 0.14 and between weight and position on the vertical dimension was $-0.39$ (both nonsignificant).

Hydrophobicity, however, was related to the degree of bitterness of the stimuli used here. The most common measure of hydrophobicity is log $P$, or the logarithm of the partition coefficient between octanol and water (Murray et al. 1975). A series of chemicals, beginning with olive oil, was used to mimic the characteristics of cell membranes. Octanol has become the preferred surrogate for the cell membrane because its structural features most closely resemble those of the lipid bilayer. Thus the proportion of a solution that partitions into the octanol fraction in an octanol-water mixture is a reasonable index of hydrophobicity. We were able to find log $P$ for 10 of the 16 bitter compounds. Measures do not exist for the three bitter salts, which are highly soluble in water and so would have log $P$’s approaching $-2.0$, nor could we find those for B$_1$, PROP, or CYS. For the remaining bitter chemicals, log $P$ ranged from $-0.20$ for B$_2$ to $+1.01$ for PTC (Hansch and Leo 1979). The correlation between log $P$ and position on the horizontal dimension of the taste space was $+0.65$ ($P < 0.025$, one-tailed). Thus the capacity of these solutions to penetrate cell membranes was significantly related to their degree of bitterness.

Toxicity as a measure of taste quality

We opened this paper with the statement that the taste system is responsible for separating nutrients from toxins. The mechanism for such separation in the present data set would be the distribution of chemicals along the horizontal axis of the taste space according to their toxicities. Measures of toxicity are available (MDL Information Systems 1997; U.S. Department of Health, Education and Welfare 1981). However, they are not well coordinated, varying by route of administration (oral, intragastric, intraperitoneal, intramuscular, intravenous, subcutaneous, topical, or inhalant), by measured effect (lowest toxic, 50% toxic, lowest lethal, 50% lethal), and by subject (man, woman, child, macaque, other primate, dog, rabbit, rat, mouse, general mammal, and nonmammal). We required that the route of administration be oral because taste is a relevant variable; we accepted only lowest reported lethal dose (LD$_{50}$) to have a definite outcome because measures of perceived toxicity were idiosyncratic to the experimenter; however, to gather sufficient data for a meaningful analysis, we accepted data from five mammals: macaque, human, rat, mouse, and dog in that order of preference (i.e., the subject in which the electrophysiology was performed, followed by omnivores of progressively greater phylogenetic distance, and finally a carnivore). We were able to gather LD$_{50}$ figures for 19 of 20 sapid
stimuli, excepting only cranberry juice. The correlation between LD₅₀ and position of the horizontal dimension of the taste space was +0.77 ($P < 0.001$). It seems the taste system performs a physiological risk—benefit analysis and attributes greater bitterness to increasingly toxic chemicals.

**Relationship between the taste of bitter stimuli and those of other basic qualities**

Bitter stimuli formed a distinct group in the stimulus space and dendrogram, with some of its members reaching out toward the positions of NaCl or HCl. There was no positive relationship between bitter stimuli and glucose. Indeed, with quinine and glucose at opposite extremes of the taste space, and bitterness and sweetness at opposite ends of the human gustatory experience, the only controversy is whether they are fully independent of one another or are mutually suppressive. Hellekant and Ninomiya (1994), recording from single fibers of the chimp chorda tympani nerve, found no interaction between responses to sucrose and quinine; each stimulus drove activity in its fiber type and left fibers of the other type unaffected. The correlation we report here between profiles generated by glucose and quinine across all taste cells, +0.04, supports the contention that there is no relationship, positive or negative, between them. Moreover, S-cells as a group gave almost exactly zero ($-0.2$ spikes/s) net response to quinine (Fig. 4), as did Q-cells ($-0.5$ spikes/s) to glucose. This is similar to the results reported in the other papers of this series. The issue of whether there is mutual suppression is left open, however, by the fact that 15 of the 22 S-cells gave small, nonsignificant negative responses to quinine, and 4 of the 5 Q-cells did the same to glucose. Our strict response criterion may have excluded activity that signals meaningful suppression to the monkey.

Schiffman et al. (1994) reported that detection and recognition thresholds for quinine and other bitter chemicals were elevated and that all concentrations tested were perceived as less intense when natural or artificial sweeteners were mixed with the bitter stimuli. This suggestion of suppression is reinforced by the electrophysiological data of Smith et al. (1994) from the hamster parabrachial nucleus that sucrose and quinine mutually inhibit the activity elicited by the other. The mechanism is proposed to be a GABAergic local inhibitory network in the rostral nucleus of the solitary tract that permits the independent peripheral pathways to influence one another when they converge on that nucleus. It was also demonstrated by Contreras et al. (1995) that the tastes of sucrose and quinine (plus 3 other bitter compounds) offset one another in licking experiments with rats, i.e., the lick rate is dependent on the sucrose:quinine ratio in the mixture. However, this result does not distinguish between a sensory neural interaction between sucrose and quinine versus the existence of two parallel reflex circuits driving competing behaviors. Thus the question of whether sweet and bitter chemicals are mutually suppressive in the CNS or merely independent is not resolved.

In contrast, it seems clear that acids and bitter stimuli have a positive relationship. Cummings and Kinnamon (1992) reported that citric acid and quinine block the same potassium channels on the taste receptors of Necturus, although by different mechanisms that may permit their discrimination. Moreover, the electrophysiological responses evoked by acids and quinine show a reasonably high degree of similarity, whether in rodent (Giza and Scott 1991) or primate (Plata-Salamán et al. 1995), of which the correlation in this paper ($+0.53$) is representative. McBurney et al. (1972) showed that adaptation of human subjects to the purely sour quality of citric acid decreased the perceived bitterness of quinine, implying an overlap of receptor or central coding mechanisms. That implication is reinforced by several reports that the terms ‘‘sour’’ and ‘‘bitter’’ are often confused, particularly by males (Gregson and Baker 1973; McAuliffe and Meiselman 1974; Meiselman and Dzendolet 1967; O’Mahony et al. 1979; Robinson 1970).

**Role of Q-cells in discriminating among bitter stimuli**

The role of neuronal subtypes in coding taste qualities was the subject of controversy for decades. In this study there were only five cells categorized as quinine oriented in the cluster analysis. We performed the multidimensional analysis of relative stimulus quality a second time with Q-cells removed to determine the effects of losing this specific segment of the neural code.

The relationship between bitter chemicals and other stimuli did not change appreciably when the contributions of these five cells was lost. The mean correlation between glucose and the 16 bitter chemicals was +0.04 with all cells and +0.05 without Q-cells. That between cranberry juice and bitter stimuli increased only from +0.22 to +0.23, between NaCl and bitter chemicals from +0.40 to +0.42, and between HCl and bitter from +0.52 to +0.53 (all nonsignificant).

The relationship among bitter stimuli, however, collapsed when Q-cells were removed. The mean correlation among all bitter stimuli rose from +0.67 to +0.72 ($r = 9.26; P < 0.001; df = 119$), and the mean distance between pairs of bitter stimuli in the multidimensional space decreased by 38% ($r = 3.34; P < 0.005; df = 119$). Therefore, without the contribution of the 10% of cells that are quinine oriented, discrimination among bitter stimuli is compromised. Q-cells are not critical to distinguishing between bitter and nonbitter. They are integral to serving the more subtle distinctions within the basic category of bitterness.

**Relationship between monkey electrophysiology and human psychophysics**

We know of no comprehensive investigation of the relationship among bitter stimuli in humans to which our results may be compared directly. Therefore the relationship between neural activity in the macaque and human perception must be gleaned from a number of sources.

The bitter stimuli in our array were divisible into two clusters, represented in the dendrogram as two subgroups. On the left are six chemicals that humans describe as almost exclusively bitter. Quinine is the bitter prototype. Phenylalanine is described by humans as sharp, metallic, and bitter and is located immediately beside the position that represents pure bitterness in a psychophysically derived multidimensional space (Schiffman and Dackis 1975). Caffeine is described as almost exclusively bitter (McBurney et al. 1972),
whereas PROP (Hall et al. 1975), PTC (McBurney et al. 1972), and theophylline (Merck Index 1976) are characterized as purely bitter. Overall, the similarity of these six chemicals in the dendrogram and their positions in the space, away from the three other basic stimuli and at the opposite extreme from the position of glucose, are appropriate.

On the right of the bitter group in the dendrogram are 10 chemicals that humans report as having mixed qualities, bitter-salty or bitter-sour, still usually dominated by bitter. This group may be divided further by referring to the space, where they compose the left side of the bitter cluster. Those four that humans describe as bitter-salty [ARG (Schiffman and Dackis 1975), MgCl2 (Pfaffmann 1959; von Skramlik 1926), NH4Cl (Frings 1948), and CaCl2 (Fabian and Blum 1943; von Skramlik 1926)] are at the top, toward the position of NaCl. The mean correlation between the profiles of these four chemicals and that of NaCl is +0.45 (n = 4), identical to their mean correlation with the profile evoked by QHCl. The three that humans call bitter-sour [urea (Kalmus 1971), CYS, and B2 (Schiffman and Dackis 1975)] are at the bottom, toward the position of HCl. Urea was described as more sour than bitter by human subjects (McBurney et al. 1972); accordingly, its profile correlates better with that of HCl (+0.62) than with that of QHCl (+0.57), although differences so slight are not likely to be meaningful. Histidine, characterized as bitter-salty-sour (Schiffman and Dackis 1975), is appropriately between these two subgroups. The correlations between the profile generated by HIS and those of NaCl, HCl, and QHCl were +0.53, +0.47, and +0.57, respectively, implying nearly equal parts of saltiness, sourness, and bitterness.

Only nicotine (Moncrieff 1951) and B2 (Schiffman and Dackis 1975) are not where human reports would predict, which would be among the group of more purely bitter chemicals on the right of the space. Nicotine had nearly equal correlations (≈ +0.70) with all bitter stimuli except QHCl and PTC (≈ +0.50). These two coefficients drove it out of the pure bitter group in the space to the center of the bitter-salty, bitter-sour complex. The same was true for vitamin B2, which was forced away from the more purely bitter stimuli by low correlations with QHCl, PTC and PROP (all approximately +0.50). It should be noted that the report of a strong bitter taste for B2 is from subjects who had purified powder placed directly on their tongues (Schiffman and Dackis 1975) as opposed to our relatively mild concentration of 0.3 M. Still, the reasons for the difference in presumed responses of the human and monkey to these two stimuli are not determined.

Comparisons with human data that cross the two groups of bitter stimuli in the dendrogram are not common. The clearest dichotomy is between the responses to PTC and urea, described as rather purely bitter (PTC) as opposed to sour-bitter (urea) (McBurney et al. 1972). The correlation between their profiles in our study was only +0.48, the lowest against any bitter stimulus for urea, and the second lowest (after NH4Cl) for PTC. A distinction was also made between QHCl and urea, based on the findings that adaptation to the former does not suppress sensitivity to the latter (McBurney et al. 1972) and also that, although quinine thresholds rise with age, those to urea do not (Cowart et al. 1994). Both imply that QHCl and urea are transduced at different receptor sites. Thus the rather low correlation between them in our study (+0.56) is appropriate.

**Conclusions**

With this paper, we complete the basic analysis of responses in the primary taste cortex of the macaque. Studies included measures of thresholds and concentration-response functions (Scott et al. 1991), a broad measure of taste quality (Smith-Swintosky et al. 1991), responses to amino acids (Plata-Salaman et al. 1992) and to mixtures of the basic taste stimuli (Plata-Salaman et al. 1996), and intensive investigations of the domains of each of the four basic qualities (Plata-Salaman et al. 1993, 1995; Scott et al. 1994). As in this paper, there are minor anomalies between human reports and monkey electrophysiological responses, but the only consistent difference was the weaker response to acids in macaques than the psychophysical data would predict, a difference supported by the insensitivity shown by macaques to acids in a behavioral study (Pritchard et al. 1994). Wherever else we were able to make human–monkey comparisons, and the analyses presented in this paper are the least thorough because of the paucity of psychophysical data on bitter tastes, the match was nearly perfect. Thus the macaque would appear to serve as a reliable neural model for human gustation.

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RESPONSES TO BITTER STIMULI IN MONKEY CORTEX


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