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Gustatory Responses in the Frontal Opercular Cortex of the Alert Cynomolgus Monkey

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SUMMARY AND CONCLUSIONS

1. The responses of 165 single taste neurons in the anterior operculum of the alert cynomolgus monkey were analyzed. Chemicals were deionized water, blackcurrant juice, and the four basic taste stimuli: glucose, NaCl, HCl, and quinine HCl.

2. Taste-evoked responses could be recorded from an opercular region that measured ~4.0 mm in its anteroposterior extent, 2.0 mm mediolaterally, and 3.0 mm dorsoventrally. Within this area, taste-responsive neurons were sparsely distributed such that multiunit activity was rarely encountered and neuronal isolation was readily achieved.

3. Intensity-response functions were determined for nine cells. In each case, the lowest concentration of the dynamic response range conformed well to human electrophysiological and psychophysical thresholds for the basic taste stimuli.

4. There was some evidence of chemotopic organization. Cells that responded best to glucose tended to be distributed toward the anterior operculum, whereas most acid-sensitive neurons were located more posteriorly. The proportion of cells responding best to NaCl peaked in the middle of the area, whereas quinine sensitivity was rather evenly distributed throughout.

5. Opercular neurons in the monkey showed moderate breadth of sensitivity compared with taste cells of other species and at other synaptic levels. A breadth-of-tuning coefficient was calculated for each neuron. This is a metric that can range from 0.0 for a cell that responds specifically to only one of the four basic stimuli to 1.0 for one that responds equally to all four stimuli. The mean coefficient for 165 cells in the operculum was 0.67 (range = 0.12–0.99).

6. Efforts were made to determine whether neurons could be divided into a discrete number of types, as defined by their responsiveness to the stimulus array used here. It was concluded that most taste cells may be assigned to a small number of groups, each of which is statistically independent of the others, but within which the constituent neurons are not identical.

7. An analysis of taste quality indicated that the sweet and salty stimuli evoked patterns of activity that were significantly intercorrelated. Similarly, patterns representing HCl, quinine HCl, and water were related.

INTRODUCTION

In the monkey, taste input realizes its cortical representation in the frontal operculum and anterior insula. The opercular cortex was implicated in gustatory function by Bornstein (5, 6) who observed ageusias in a dozen human patients with bullet wounds in this area. Patton (31), Ruch and Patton (38), and Bagshaw and Pribram (1) performed lesions in the same region of the rhesus monkey and noted a reliable, if temporary, elevation of taste thresholds. Benjamin and Emmers (4) and Benjamin and Burton (3) stimulated the peripheral taste nerves and recorded evoked potentials both on the lateral convexity of the postcentral gyrus and, with slightly longer latency, in the underlying operculum. Burton and Benjamin (7) interpreted this latter region as the pure taste area. The boundaries of the cortical gustatory area in the macaque were most recently delimited by Morse et al. (26) and Pritchard et al. (35) using the autoradiographic antero-

grade fiber-tracing technique. Infusing tritiated amino acids into the thalamic taste area, they labeled fibers that terminated in anterior opercular and insular cortices. These regions have been shown to be cytoarchitectonically distinct by Jones and Burton (16), Mesulam and Mufson (23, 28), Roberts and Akert (36), and Sanides (39, 40).

Sudakov et al. (46) have recorded single-neuron activity in the frontal operculum and insula in response to chemical stimulation of the tongue. Of 946 cells tested for gustatory sensitivity, only 33 (3.5%) gave responses, 30 of them excitatory. Each neuron responded to more than one of the three stimuli (NaCl, sucrose, milk) employed.

The identification of the opercular cortex as a taste receptive area in the monkey warrants a thorough analysis of its functional characteristics. The present paper offers a report on taste-evoked activity from single neurons in the frontal operculum of the alert monkey.

METHODS

Subjects

The subjects were two male cynomolgus monkeys (*Macaca fascicularis*) weighing 3.8–4.0 kg during the course of data collection.

Surgery

Full sterile precautions were observed throughout surgery. Each monkey was sedated with an intramuscular injection of ketamine and anesthetized to a surgical level with intravenous thiopentone sodium (50 mg/ml). The depth of anesthesia was monitored by frequently testing for the presence of the leg flexion reflex, which, if found, warranted a supplemental administration of anesthetic. Atropine (0.1 ml/kg) was administered to prevent excessive salivation and glycerine applied to the eyes to prevent their drying. The monkey was placed in a Kopf stereotaxic instrument and his position confirmed by X-ray photography. Respiration rate was monitored throughout surgery. A section of skull over the frontal operculum was removed and replaced with a stainless steel ring to which a microdrive could be fitted during recording sessions. This also accommodated an assembly of four steel electrodes, which were implanted stereotactically in the basal forebrain to provide constant referents relative to which the location of the recording electrode could be determined on each recording track by X-ray photography. These reference electrodes also provided the opportunity for antidromically activating recorded neurons so as to give evidence of their outputs, although this was not done in the present study. The implant was fixed in place with dental acrylic. Finally, two stainless steel tubes (8 mm OD, 6 mm ID, 5-cm length) were cemented to the skull cap in front of and behind the ring, through which horizontal support bars could be inserted during data collection. Long- and short-acting antibiotics were administered over the next 2 wk, after which recordings began.

Recording

SESSIONS. Daily recording sessions lasted up to 6 h. Each monkey was transferred from his home cage to the primate chair where his head was supported by slipping bars through the tubes provided as part of the implant. He was otherwise free to move and normally adopted a relaxed sitting position. His comfort was continuously attended to, and he was offered food intermittently throughout the recording session.

ELECTRODES. Electrodes were glass-insulated tungsten, plated with gold and platinum black (22) and had tip sizes of $\sim 2 \times 4$ μm. Such a composition combined the sturdiness required to penetrate through 20 mm of tissue with the fineness needed to isolate small somata from the opercular cortex.

Electrodes were systematically positioned on each track using a Kopf X-Y positioner attached to the implanted ring. The dura was anesthetized with 0.15 ml 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 ($\sim$10 mm dorsal to the operculum) and advanced using a Trent-Wells hydraulic microdrive and chronic adaptor system.

ELECTRICAL SYSTEM. Neural activity passed through a high-input impedance field-effect transistor mounted on the microdrive. It was amplified by conventional band-pass filtered amplifiers and displayed at high-speed time base (0.2 ms/cm) on the main oscilloscope. Action potentials of a single cell were identified by consistency of amplitude and waveform and by the requirement that two spikes never occur within a 2-ms interval. Accepted spikes were converted to transistor-transistor logic pulses for on-line analysis. They were also displayed on a second oscilloscope and were passed to an audio monitor, providing additional visual and auditory cues that permitted corrections as minor changes in recorded voltage occurred with electrode drift. Single-unit data and voice commentary were also stored on magnetic tape for off-line analyses.

Stimuli and stimulus delivery

Thirty-three sapid stimuli were employed. These included eight concentrations, in half-log molar steps, of each of the four prototypical stimuli (10^{-3} – 3.0 M glucose; 10^{-3} – 3.0 M NaCl; 10^{-5} – 3×10^{-2} M HCl; 10^{-6} – 3×10^{-3} M quinine HCl)

plus 20% blackcurrant juice concentrate (Beecham Products, Brentford, UK). Blackcurrant juice was included because it is both highly palatable to the monkey and complex in taste quality, such that most neurons were responsive to it. This combination of attributes made it an effective probe stimulus for identifying gustatory cells.

Stimuli were delivered in quantities of 0.5 ml through a hand-held syringe. Manual delivery was used in the alert monkey because it permitted repeated stimulation of a large and nearly constant receptive field through compensation for the different mouth and tongue positions adopted as the palatability of the solutions varied. The monkey learned to block or partially avoid the delivery of chemicals through fixed tubes placed in the mouth.

Stimulus delivery was followed within 10 s by a 1.0–1.5 ml distilled water rinse. At least 30 s of rest was permitted between stimuli, and if there were indications that either the behavioral (licking, facial expressions) or neural activity had not returned to prestimulus levels, this period was extended.

Fluid consumption

Monkeys were fed and offered water *ad libitum* at the end of each daily recording session so that they began the succeeding day ~18 h food and water deprived. During a typical recording session a subject would consume ~200 ml of fluid and several pieces of fruit over a 5-h period. There was initial concern that increasing satiety might affect taste-evoked responses, but a specific test of this issue showed that such an effect does not occur (42). Over a 5-day wk of data collection, monkeys took nearly half of their food and most of their fluid during recording sessions.

Analysis

A PDP-11 computer counted action potentials for 5 s following stimulus application and performed basic statistics on-line. Spike counts provided material for derived analyses, which included calculations of interneuronal and interstimulus correlation coefficients, multidimensional scaling routines, and cluster analyses as detailed in the results of this paper. These were conducted on a Burroughs 7700 computer.

Localization of recording sites

The position of each recording site was determined in two ways. First, following each track, X-ray photographs were taken from frontal and lateral perspectives. Relative recording positions could then be reconstructed to within 250 μm by reference to the deep electrodes permanently implanted in diencephalic and telencephalic structures during surgery. The positions of the deep electrodes were subsequently determined histologically. Second, in the final several sessions, microlesions were made through the recording electrode (60 μA for 60 s, electrode negative). At the end of these experiments the subjects were tranquilized with ketamine and given a lethal intravenous injection of pentobarbital sodium. They were then perfused with 0.9% saline followed by formal saline. Their brains were stored in sucrose formalin for at least 7 days after which 50-μm serial frozen sections were cut and stained with cresyl violet.

RESULTS

Location and extent

Neurons responsive to chemical stimulation of the oral cavity were encountered in the frontal opercular cortex (FO) beginning at its most rostral edge and extending 4.0 mm posterior (Fig. 1). The dorsoventral extent of the taste area was ~3 mm, and it occupied ~2 mm in the mediolateral coordinate. With posterior progression, taste cells tended to be located more dorsally such that the area assumed a crescent shape. Taste-responsive cells constituted 165 of the 7,839 neurons tested in characterizing and defining the boundaries of this region (2.1%). Their distribution was sufficiently sparse that multiunit taste-evoked activity was never observed and consequently isolation was not difficult. When successive taste cells could be isolated in one track, the distance between them was typically 400–500 μm.

Response characteristics

SPONTANEOUS ACTIVITY. The mean spontaneous rate of taste cells in the operculum was 1.9 ± 0.8 spikes/s (range = 0.0–22.1). To define an evoked response we adopted a dual criterion of spontaneous rate ± 2.33 SD (i.e., $P < 0.01$) with a minimum discharge rate of 1.0 spike/s measured over a 5-s period following stimulus application. The low spontaneous activity precluded inhibition as a response option for 77% of the cells and required imposition of the 1.0 spike/s minimum on 17%.

EVOKED ACTIVITY.

Intensity-response functions. We tested nine neurons for their responses to a 3.5 log molar concentration range of the prototypical taste stimulus to which each was most sensitive (2 cells tested with glucose, 3 with NaCl, 2 with HCl, 2 with QHCl). Although the slopes of intensity-response functions were markedly different for individual neurons, each chemical had a characteristic dynamic range over which

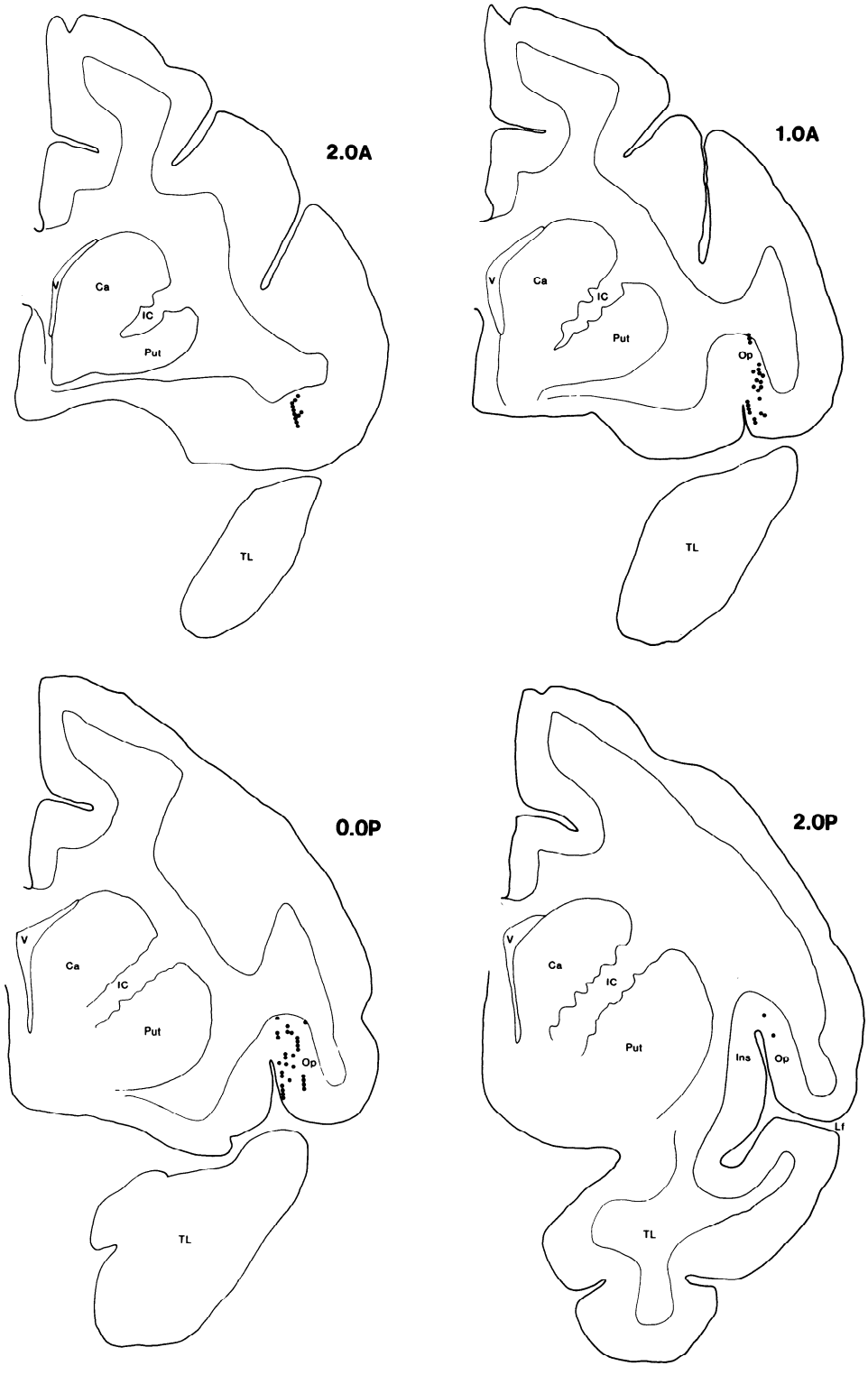

FIG. 1. Coronal sections through the frontal operculum showing the locations of some neurons isolated in this study. A-P coordinates are relative to sphenoid. Ca, caudate; IC, internal capsule; Ins, insula; Lf, lateral fissure; Op, operculum; Put, putamen; TL, temporal lobe; V, lateral ventricle.

neural response rates were most sensitive to concentration (Fig. 2). For glucose, the range was 0.01–0.3 M; for NaCl, from 0.003 to 0.3 M; for HCl, from 10^{-4} to 3×10^{-3} M; for quinine HCl, from 3×10^{-5} M to a concentration beyond that which we were willing to apply to an alert monkey. The initial concentration of each range was at or slightly below the threshold values derived from the monkey nucleus tractus solitarius (43) and the electrophysiological (10) and psychophysical thresholds in humans (33).

Standard concentrations for the remainder of this experiment were chosen on the basis of the intensity-response functions of Fig. 2, the acceptance behavior of the monkeys, our need to effectively stimulate the taste system, and the benefits of using the same stimulus intensities as were employed to study taste in the nucleus tractus solitarius. Standard concentrations were 1.0 M glucose, 1.0 M NaCl, 0.01 M HCl, and 0.001 M quinine HCl. The remainder of this report is based on activity evoked by these four stimuli plus 20% blackcurrant juice and deionized water.

Mean evoked responses and chemotopic organization. The mean evoked responses in spikes per second measured over 5 s for each stimulus were: deionized water = 3.1; 20% blackcurrant juice = 7.2; 1.0 M glucose = 5.7; 1.0 M NaCl = 5.5; 0.01 M HCl = 4.6; 0.001 M quinine HCl = 5.0. With the exception of NaCl, these values are each ~25% greater than the corresponding responses from the solitary nucleus (43), reflecting the higher spontaneous activity of the opercular cells. The mean response to NaCl was similar in medulla and cortex.

In the monkey nucleus tractus solitarius (NTS) some chemotopic arrangement of neural sensitivities was observed (43), which conformed to the topographic organization of sensitivities on the human tongue (9). The anatomical tracing studies of Norgren and his colleagues (2) imply a preservation of this organization from NTS to the gustatory thalamus and cortex (26, 35). Therefore we plotted the responsiveness of each neuron as a function of its location in the operculum to determine whether a chemotopic arrangement was apparent.

The proportion of neurons at each anteroposterior plane, which gave their largest responses to each of the prototypical stimuli, is shown for one monkey in Fig. 3. Glucose sensitivity is greatest in the anterior operculum and declines in orderly fashion with posterior progression. Responsiveness to NaCl appears to peak in the center of the area. Hydrochloric acid is most effective posteriorly, whereas responsiveness to quinine HCl is rather constant along the anteroposterior dimension. These

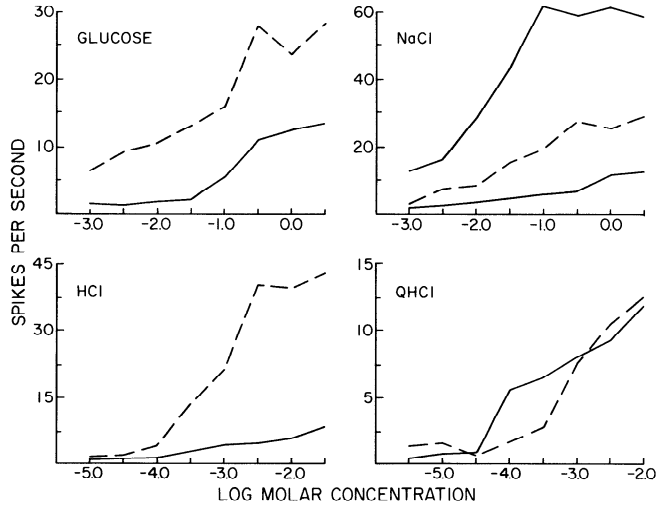

FIG. 2. Intensity-response functions of 9 individual neurons from the frontal operculum of the monkey. Each cell was tested with a 3.5 log molar concentration series of the prototypical stimulus to which it was most sensitive. Hence the rates shown here are higher than the mean response rates from all neurons.

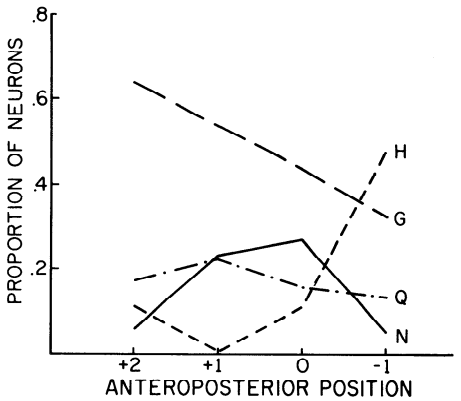

FIG. 3. The proportion of taste cells at each anteroposterior position of 1 monkey that responded best to glucose (G), NaCl (N), HCl (H), or quinine HCl (Q). Anteroposterior position is shown in millimeters relative to sphenoid. The sample of neurons at −2.0 mm was too small to be meaningful.

trends are in general agreement with the chemotopic organization observed in the monkey NTS. There was no apparent relationship between responsiveness and either mediolateral or dorsoventral position within the operculum.

Breadth of sensitivity. Of the 165 neurons tested with all solutions, 37 (22%) exceeded our 2.33 SD response criterion for responding to all four basic stimuli at these moderate-to-high concentrations. Thirty (18%) responded to three stimuli, 42 (25%) to two, 38 (23%) to one, and 18 (11%) did not exceed criterion to any basic taste solution (but, of course, they did in response to blackcurrant juice and/or deionized water). Conversely, the percentages of neurons that responded to each stimulus were 25% to water, 63% to blackcurrant juice, 60% to glucose, 51% to NaCl, 51% to HCl, and 56% to quinine HCl. Thus, ignoring water, 463 (56.1%) of the stimulus-neuron interactions resulted in responses that exceeded criteria: 458 (55.5%) excitatory and five (0.6%) inhibitory. The five inhibitory responses were not associated with a particular stimulus or neuron, and since their frequency approximated the level of error permitted by our criterion (i.e., $P < 0.01$), we conclude that inhibition is not a useful response option in the operculum. In general, the percentage of responses in cortex was lower than the corresponding values from NTS, implying that the cortical taste neurons are more narrowly tuned.

To quantify this range of neuronal sensitivity, the breadth-of-tuning metric developed by Smith and Travers (44) was applied. The proportion of a neuron's total response that is devoted to each of the four basic stimuli can be used to calculate its coefficient of entropy (H). The coefficient ranges from 0.0, representing total specificity to one of the chemicals, to 1.0, which indicates an equal response to all four.[1] The mean coefficient for 165 taste neurons in monkey operculum was 0.67 (range = 0.12–0.99), which is lower than the mean value derived from NTS, where H = 0.87 (t = 8.77; $P < 0.001$; df = 215). Thus opercular taste cells are more specifically tuned across the standard range of taste qualities. There was no evidence that the breadth of tuning varied systematically with neuronal location. The discharges of one neuron to the array of six chemicals are shown in Fig. 4. This cell tended to respond phasically, was activated primarily by sweet stimuli, and had a breadth-of-tuning coefficient of 0.57.

Neuron types. It is not yet resolved whether the taste system is composed of a small number of neuron types, each replicated to generate

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

where H = breadth of responsiveness, k = scaling constant (here k = 1.661 such that H = 1.0 when a neuron responds equally to all four basic stimuli), p_i = proportional response to each of n compounds (in this case n = 4). For example, suppose a neuron responds to the successive application of the four basic taste stimuli with a total of 100 spikes, distributed as follows: 1.0 M glucose = 60; 1.0 M NaCl = 30; 0.01 M HCl = 8; 0.001 M quinine HCl = 2; then

$$H = -1.661 \sum_{1}^{4} p_i \log p_i$$

$$= -1.661\,([0.6 \log 0.6) + [0.3 \log 0.3] + [0.08 \log 0.08] + [0.02 \log 0.02])$$

$$= -1.661\,(0.6[-.22] + 0.3[-0.52] + 0.08[-1.10] + 0.02[-1.70])$$

$$= -1.661\,(-0.13 - 0.16 - 0.09 - 0.03)$$

$$= -1.661\,(-0.41)$$

$$H = 0.68$$

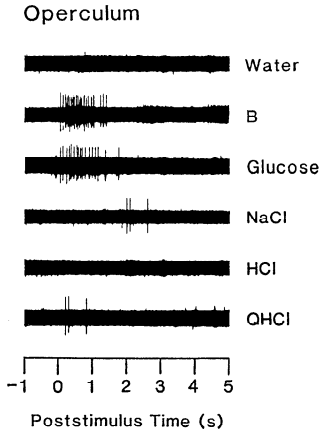

FIG. 4. Responses of a single neuron in the frontal operculum to the stimulus array. B, blackcurrant juice.

all cells of the system, or if each gustatory neuron is individualistic in its characteristics. One approach to the resolution of this issue is to determine whether there is a limited number of response profiles to which all taste neurons conform.

Figure 5 shows 40 representative response profiles of opercular neurons categorized according to the chemicals to which each responded best: deionized water (5*A*), blackcurrant juice (5*B*), and each of the four prototypical taste stimuli (5, *C–F*). Although absolute discharge rates are quite variable, the shapes of the profiles are rather similar within each category, generating the high mean product-moment correlations shown in each frame. The most consistent profiles come from those

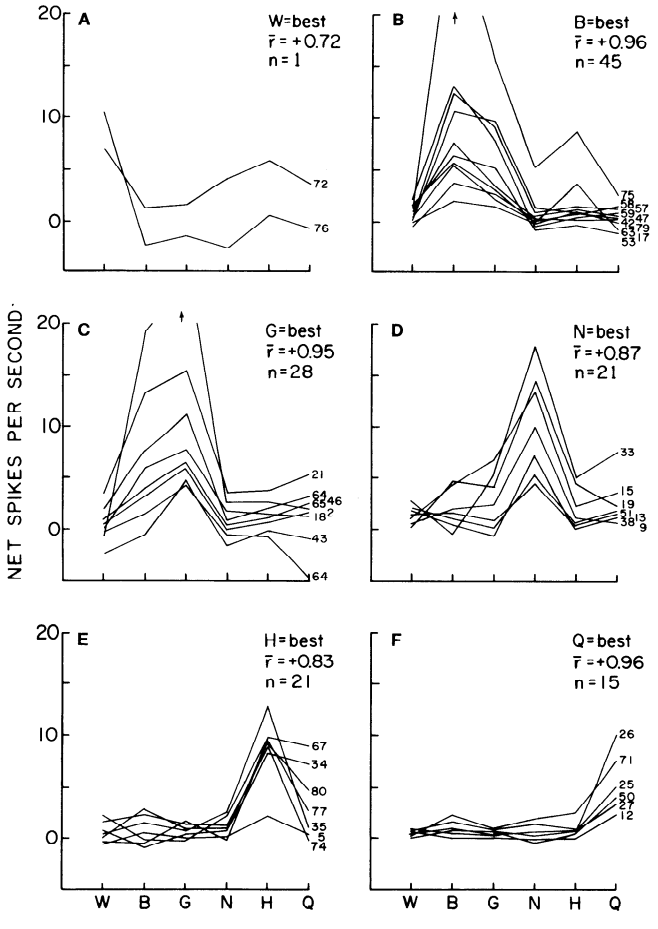

FIG. 5. Response profiles of 40 randomly selected neurons from the frontal operculum. W, deionized water; other abbreviations as in Figs. 3 and 4. In each frame, n = no. of intercorrelations among profiles; r = mean correlation among those profiles.

neurons most responsive to quinine HCl (r = +0.96), blackcurrant juice (r = +0.96), and glucose (r = +0.95). Cells most responsive to HCl (5*E*) are consistent in their insensitivity to all other stimuli except quinine, the variable responses that reduce the mean intercorrelation among these seven profiles to +0.83. Sodium-best cells (5*D*) also show variable responsiveness to quinine and, to a lesser degree, to the three other sapid stimuli, for a mean intercorrelation of +0.87. Neurons most responsive to water are also sensitive to HCl, although there is sufficient variability between the two profiles to reduce their intercorrelation to +0.72. Although the general consistency within each category suggests a limited number of response profiles, there are two other considerations. First, the stimulus array is quite limited such that each profile is defined by the neuron's response to just six chemicals. More accurately defining each profile through use of a larger stimulus set could alter measures of similarity among profiles in either direction (19). Second, neurons were assigned to the categories of Fig. 5 in a manner that could falsely promote the appearance of fiber types. Even along a smooth continuum of sensitivities, neighboring cells would have similar response profiles. By categorizing each neuron according to the stimulus which maximally excites it, we may be creating spurious neighborhoods of cells, sheltered from one another by the axes on which the profiles are plotted. A more comprehensive analysis would include the profiles of a large sample of neurons not prearranged in any manner. Thus we randomly selected and compared 80 profiles (the limit of the analysis program) and organized the resulting 3,160 correlations [$n(n-1)/2$] into a matrix that, in turn, was used to construct a multidimensional space (15) (Fig. 6). The more similar two profiles are, the more closely the cells that generated them will be aligned in the space. Thus neuron types, as defined by recurring sensitivity to the five stimuli plus water employed here, would be indicated by clusters of points, whereas their absence would result in a homogenous distribution throughout. For ease of interpretation, Fig. 6 is presented in two dimensions, which together account for 84% of the data variance. While neurons do not appear to be randomly distributed across the space, neither are there obvious clusters beyond the densely populated region in the right center of the space.

To objectively determine the extent to which neurons may be grouped, a cluster analysis (49) was performed on the same correlation matrix as was used to generate the space. This appears in Fig. 7 as a dendrogram. Neurons are numbered along the abscissa in the order in which they were isolated (their numbers are the same as those assigned in the profiles of Fig. 5 and the space of Fig. 6). Beneath each number is indicated the stimulus to which that cell gave its largest response, followed by any other stimulus that evoked at least 80% as much activity. The correlation between the two most similar pairs is represented by the lowest horizontal connecting line. Other similar pairs are joined until a connection between pairs is made at a height representing the mean correlation among the neurons involved. As more dissimilarity is permitted, larger groups are connected until all neurons are incorporated into the dendrogram. The more tightly a cluster is intercorrelated, the lower will be the horizontal line connecting its constituent cells. The more independent a cluster is from foreign neurons, the longer will be the vertical line leading to it. The four long vertical lines in the top half of the figure lead to neural groups whose predominant sensitivities are to (left to right) sugar, salt, acid, and quinine, respectively. The most massive of these (*neurons 1–49*) includes 37 of the 80 cells. There is a clear subdivision within this group between those most sensitive to blackcurrant (*neurons 1–56*) and those that respond best to glucose (2–49). The fact that these subgroups are so closely related is a testimony to the consistency of sweet-oriented response profiles (Figs. 5, *B* and *C*) and to the similarity of these two taste qualities (see "*Stimulus qualities*" below) such that they activate a common neuronal pool.

On the far right is a second cluster (*neurons 7–62*) of 11 cells among which quinine sensitivity dominates. The strong coherence of this group is a reflection of the highly consistent profiles of quinine-best neurons (Fig. 5*F*).

Each of the middle two groups (salt and acid) subdivides at a lower correlational level (i.e., spatially higher) signifying a lower level of coherence. This is anticipated from the variability in response profiles of NaCl-best

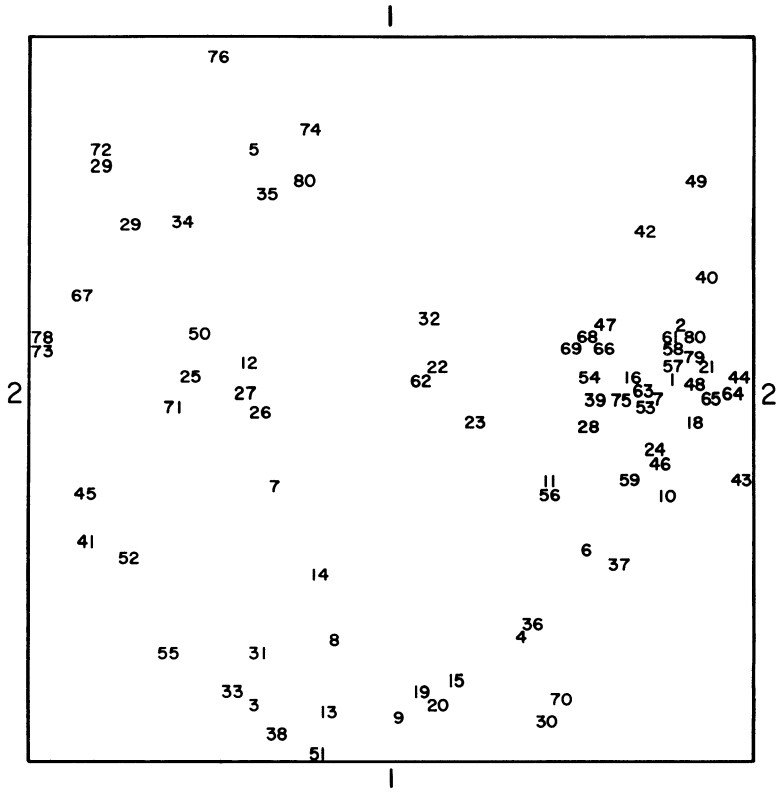

FIG. 6. A two-dimensional spatial representation of relative similarity among neuronal response profiles. The more highly the response profiles of 2 neurons correlate, the closer they are positioned to one another in the space. This solution accounts for 84% of the data variance. *Dimensions 1* and *2* are undefined.

and HCl-best neurons (Fig. 5, *D* and *E*). The larger cluster in the salt group (*neurons 3–70; n* = 16) responds rather specifically to NaCl, whereas the smaller (*neurons 41–55; n* = 4) is nearly equally responsive to NaCl, HCl, and quinine. Correspondingly, the mean breadth-of-tuning coefficient among neurons in the former group is 0.65, whereas that of the latter is 0.81. The larger subgroup in the acid-sensitive cluster (*neurons 5–77; n* = 7) responds rather specifically to acid, with a mean breadth-of-tuning coefficient of 0.57; the smaller subgroup (*neurons 29–76; n* = 5) mixes acid, quinine, and water sensitivities and has a mean breadth of 0.76. As expected from their activation of a common neuron group, these three taste qualities are significantly intercorrelated (see "*Stimulus qualities*").

Whether these various groups are sufficiently coherent to warrant being designated types becomes definitional. It is possible either to affirm or reject the concept of gustatory neuron types, depending on the criteria that one applies. The easier condition to satisfy is one that requires that a candidate cluster fully intercorrelate at or above (in the dendrogram, spatially below) a level that is statistically significant. Critical values of Pearson *r*'s at a $P < 0.05$ confidence level for the four main clusters range from $r = +0.52$ for the smallest (quinine oriented, $n = 11$) to $r = +0.25$ for the largest (sweet oriented, $n = 37$). The sweet, sodium, and quinine-oriented clusters are fully intercorrelated at levels that exceed the critical values, as is the larger subgroup (*neurons 5–77; n* = 7) within the acid-oriented cluster. All interconnections that cross clusters do so at correlational levels that do not exceed the critical *r* values. Thus, if the question is whether each of the four clusters is statistically consistent and separate from one another, it could be answered affirmatively for all but five (*neurons*

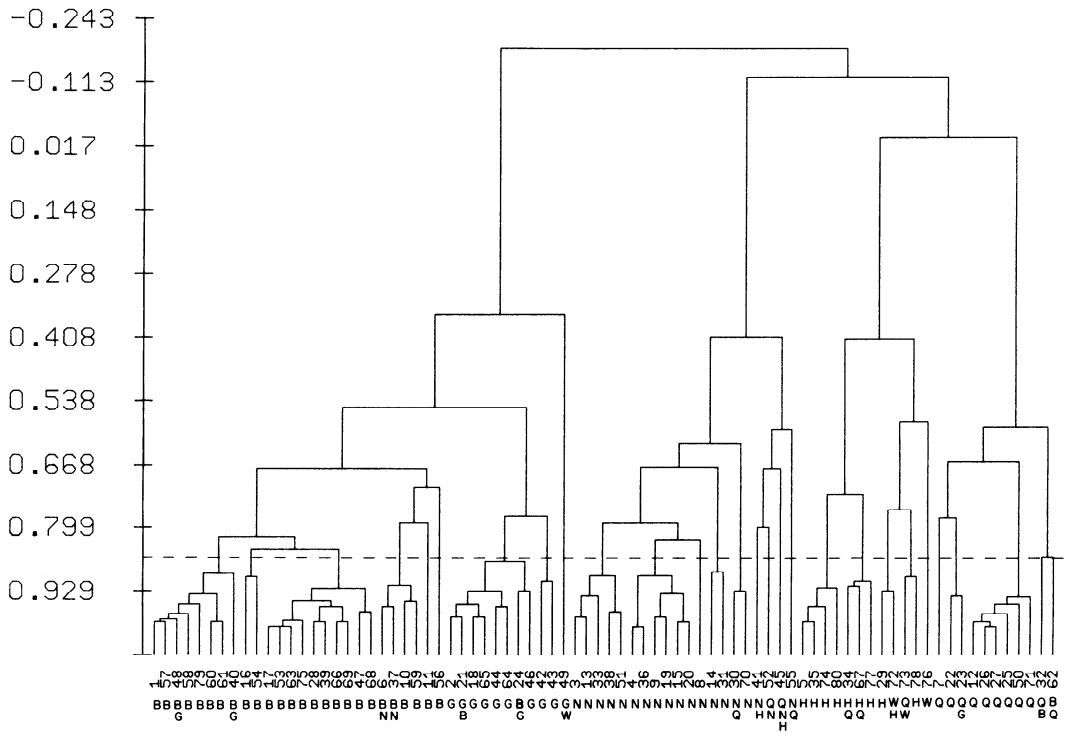

FIG. 7. An analysis for possible clusters of neurons in the spatial representation of Fig. 6. Beneath the number of each cell is an abbreviation indicating the stimulus to which it gave its largest response followed by any other stimulus that evoked >80% of the best response. Any cluster that is not fully intercorrelated spatially below the *dotted horizontal line* can be excluded from being composed of neurons with identical response profiles at a $P < 0.05$ confidence level. Abbreviations are identified in the legends to Figs. 3, 4, and 5.

29–76) of the 80 cells represented here. Thus the visual impression of four groups gained from the dendrogram is largely confirmed by this level of statistical analysis. Moreover, the interconnections among clusters (near the top of the dendrogram) occur in a sensible progression: the quinine and acid-oriented groups are joined first (these are the basic stimuli most commonly confused by human subjects) followed by the addition of the sodium-oriented neurons to complete the nonsweet system, which is only then joined by the sweet-oriented cells.

The more stringent condition to satisfy is one that requires that cells within a candidate cluster be identical to within the limits of experimental error (50). Experimental error is defined by the reapplication of stimuli during recordings from each neuron. With no error, the reapplication would evoke an identical response, and the correlation between the two patterns so generated across neurons would be +1.00. In fact, the reapplication of five stimuli plus water to all 80 cells yields two 480-entry patterns, the correlation between which is +0.88. The 90% confidence interval around this value, as determined by the procedure outlined in Cohen and Cohen (8), extends from +0.86 to +0.90, being rather restricted because of the large number. Any neurons whose profiles do not intercorrelate at a level that meets or exceeds the lower limit of this interval are in the bottom five percent of the distribution and may be excluded from being functionally identical with a confidence of $P < 0.05$. A horizontal line marks this criterion level in Fig. 7. The largest groups fully intercorrelated at a level exceeding (spatially below) the line are composed of *neurons 17–68* ($n = 10$), *neurons 1–40* ($n = 8$), *neurons 2–46* ($n = 8$), *neurons 4–20* ($n = 6$) and *neurons 12–71* ($n = 6$). Thus even the five most com-

TABLE 1. *Correlation coefficients among profiles of activity generated by each stimulus*

	W	B	G	N	H	Q
W						
B	−0.02					
G	0.14	0.75				
N	0.17	0.41	0.47			
H	0.60	0.25	0.26	0.32		
Q	0.43	0.16	0.18	0.31	0.57	

W, water; B, blackcurrant juice concentrate; G, glucose; N, NaCl; H, HCl; Q, quinine HCl.

prehensive clusters can incorporate only a minority (38/80) of the neurons. To include all cells, 27 neuron "types" would be necessary. By the more strict criterion requiring neuronal identity, then, the notion of gustatory neuron types largely fails.

A reasonable conclusion is that taste neurons in the monkey operculum are statistically divisible into a relatively few functional groups, but that within each group there is considerable variability. The variability would not appear sufficient, however, to justify thinking in terms of a smooth continuum of sensitivities. A commitment regarding the existence of gustatory neuron types in the monkey's cortex must await an accepted definition of what constitutes a "type." We employ two statistically based definitions above, by the more liberal of which the notion is acceptable, and by the more stringent of which it is not.

It should be remembered that this analysis is based on the similarity among profiles that were defined only by their responses to five sapid chemicals plus distilled water. The use of a more comprehensive stimulus array, which defines response profiles more precisely, is still needed.

Stimulus quality. Just as the similarity among neuronal response profiles may be indexed by calculating correlation coefficients between each pair, so the similarity among stimuli may be measured by comparing the profiles of activity that each evokes across the full 165 neuron sample. The correlations among these profiles have been shown in the rodent to offer accurate predictions of discriminative capacity (25, 29). The 15 corre-

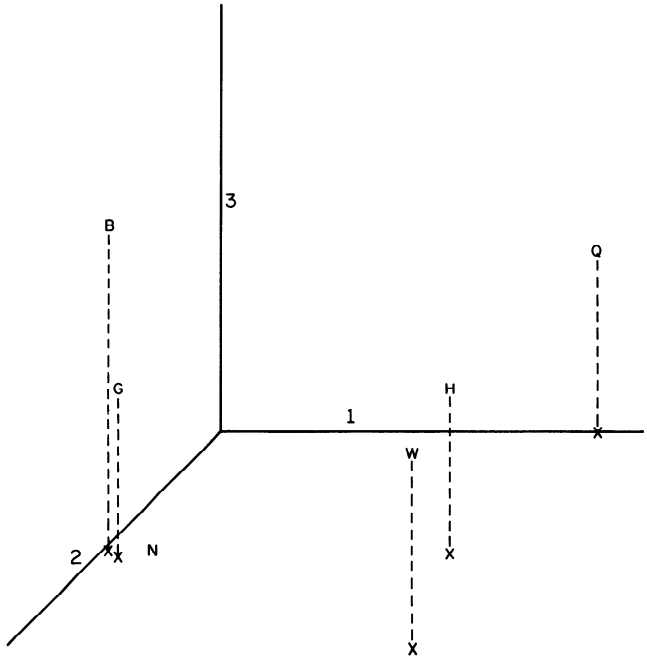

FIG. 8. A three-dimensional spatial representation of similarity among taste qualities. This solution accounts for 99% of the data variance. The 3 dimensions are undefined. Abbreviations as in Figs. 3, 4, and 5.

lation coefficients between all pairs of profiles elicited by these six stimuli are shown in Table 1. The greatest similarity is between blackcurrant juice and glucose ($r = +0.75$), both of which are intensely sweet to humans. The next highest correlations are between HCl and deionized water ($r = +0.60$) and HCl and quinine HCl ($r = +0.57$). Blackcurrant juice and glucose also generate profiles that correlate significantly with NaCl (+0.41 and +0.47, respectively), and water and quinine profiles are related ($r = +0.43$).

As with neurons before, the correlation matrix may be used to generate a multidimensional space, this one containing the positions of stimuli relative to one another (41). A three-dimensional solution accounting for 99% of the data variance is shown in Fig. 8. In broad terms, *dimension 1* separates sweet and salty qualities (normally considered appetitive, though NaCl at 1.0 M is rejected), from those which are described by humans as sour, bitter, or insipid (hence usually aversive). *Dimension 2* gives no further information regarding the appetitive qualities, for all are at nearly the same point on this dimension. It does, however, provide clear discrimination among those that are aversive. *Dimension 3* does the reverse. The aversive qualities are all at approximately the same height, and hence indiscriminable, whereas the appetitive tastes are widely distributed across this dimension.

DISCUSSION

Recordings from the alert macaque

Gustatory data derived from psychophysical studies on humans imply a set of conclusions that have not been fully reconciled with those of electrophysiological experiments on anesthetized rodents. Psychophysical evidence, although not uniform, inclines toward the position that there are independent coding channels, each responsible for mediating one of perhaps four primary tastes (20, 21, 27). The electrophysiological results are also the subject of varied interpretations (11, 12, 13, 50), but do not clearly establish neuron types, channels, or primary tastes. Recent anatomical (30) and physiological (51) findings have made it apparent that a full reconciliation of rodent electrophysiology with primate psychophysics is not to be expected. Despite their obvious similarities, rodent and primate taste systems present structural and functional distinctions; the chemically oriented rodent may actually process taste information differently from the visually oriented primate (14, 51). Thus an understanding of human gustation requires an analysis of the primate taste system and in particular that of the macaque. Following an exhaustive review of the literature on taste electrophysiology, Pfaffmann concluded "Indeed only the macaque chorda tympani seems to behave electrophysiologically as we would expect man's to behave—the chordas of other species do not" (34).[2] The macaque's alert condition further increases the relevance of these results, although at some cost of experimental precision, as detailed elsewhere (43).

A comparison of NTS and FO

The second- and fourth-order neurons of the monkey's gustatory system are in NTS and FO, respectively. Comparisons between their characteristics may be made in the following categories.

SIZE AND CELL DENSITY. FO is larger but less densely populated with taste cells. Neurons sensitive to gustatory stimulation could be isolated over a region of $\sim$24 mm^3 in FO (see Fig. 1). The corresponding volume in NTS is $\sim$3 mm^3 (43). Although we have no quantitative measure of relative cell density, it is clear that neurons are much less compacted in FO.

ACTIVITY LEVELS. FO cells are slightly more active. The mean spontaneous rate of NTS taste neurons is 1.2 spikes/s, whereas that of FO cells is 1.9 spikes/s. Mean evoked rates in spikes per second for cells in NTS vs. FO are deionized water: 2.0 vs. 3.1; 20% blackcurrant juice: 6.1 vs. 7.2; 1.0 M glucose: 4.7 vs. 5.7; 1.0 M NaCl: 5.6 vs. 5.5; 0.01 M HCl: 3.5 vs. 4.6; and 0.001 M quinine HCl: 4.4 vs. 5.0. Thus, for all responses except that evoked by NaCl, there is an increase of $\sim$1 spike/s in FO. Scott et al. (43) note, however, that the difficulty of neuronal isolation in NTS may have resulted in a sample that underrepresents more active cells. Thus the apparent small increase in FO responsiveness must be viewed tentatively.

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[2] Activity from the taste nerves of apes now seems even more directly applicable to humans (16).

TABLE 2. *Three comparisons of breadth of sensitivity between NTS and FO*

	NTS	FO
Percentage of neurons fulfilling the response criterion for each prototypical stimulus		
1.0 M glucose	86	60
1.0 M NaCl	92	51
0.01 M HCl	63	51
0.001 M quinine HCl	86	56
Mean	81.8	54.5
Percentage of neurons responding to 4, 3, 2, 1, or none of the prototypical stimuli		
4/4	42	22
3/4	42	18
2/4	17	25
1/4	0	23
0/4	0	11
Mean	3.25/4	2.17/4
Mean (range) breadth-of-tuning metric		
	0.87 (0.63–0.99)	0.67 (0.12–0.99)

NTS, nucleus tractus solitarius; FO, frontal opercular cortex.

BREADTH OF SENSITIVITY. Taste cells in FO are more narrowly tuned than are those of NTS. Three comparisons are available using the same stimuli and the same response criterion at each level (Table 2). A lower percentage of FO neurons responded to each basic stimulus. Accordingly, a lower percentage responded to 4/4 and 3/4 of the stimuli, and a higher percentage to 2/4, 1/4, and 0/4. Finally, the mean breadth-of-tuning coefficient decreased from 0.87 in NTS to 0.67 in FO. This trend continues at higher-order levels of the monkey's gustatory system: H = 0.56 among taste-responsive neurons in the insula (52) and may be as low as 0.32 among cells in orbitofrontal cortex (37). In this latter area, the extreme specificity centers around responses to sweet stimuli, which are modifiable according to physiological need (37). The apparent decrease in breadth of tuning that accompanies progress through the monkey's taste system stands in distinction to the situation in rodents, where Smith and his colleagues (45, 47) have documented an increase in breadth across successive synaptic levels.

The frontal operculum, in conjunction with the adjacent insular and orbital cortices, could prove a crucial area for processing taste information and relating it to motivation, emotion, and visceral condition. The orbitofrontal cortex supports self-stimulation behavior in the monkey (24), suggesting an involvement with reinforcement and motivation. Electrical stimulation of these paralimbic regions in monkeys (48) and humans (32) causes changes in gastric motility, visceromotor effects, and sensations in the epigastrium, reinforcing MacLean's (18) designation of this area as the "visceral brain." Anatomically, the region provides for communication between association neocortex and core limbic structures (17, 23). The convergence of visceral, gustatory, and motivational influences on this transitional area suggests its involvement in the regulation of food intake.

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