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Gustatory Responses of Single Neurons in the Insula of the Macaque Monkey

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SUMMARY AND CONCLUSIONS

1. In recordings made from 2,925 single neurons, a region of primary taste cortex was localized to the rostral and dorsal part of the insula of the cynomolgus macaque monkey, *Macaca fascicularis*. The area is part of the dysgranular field of the insula and is bordered laterally by the frontal opercular taste cortex.

2. The responses of 65 single neurons with gustatory responses were analyzed in awake macaques with the use of the taste stimuli glucose, NaCl, HCl, quinine HCl (QHCl), water, and black currant juice.

3. Intensity-response functions showed that the lowest concentration in the dynamic part of the range conformed well to human thresholds for the basic taste stimuli.

4. A breadth-of-tuning coefficient was calculated for each neuron. This is a metric that can range from 0.0 for a neuron that responds specifically to only one of the four basic taste stimuli to 1.0 for one that responds equally to all four stimuli. The mean coefficient for 65 cells in the taste insula was 0.56. This tuning is sharper than that of neurons in the nucleus of the solitary tract of the monkey, and similar to that of neurons in the primary frontal opercular taste cortex.

5. A cluster analysis showed that at least six different groups of neurons were present. For each of the taste stimuli, glucose, NaCl, HCl, QHCl, water, and black currant juice, there was one group of neurons that responded much more to that tastant than to the other tastants. Other subgroups of these neurons responded to two or more of these tastants, such as glucose and black currant juice, or NaCl and QHCl.

6. On the basis of this and other evidence, it is concluded that the primary insular taste cortex, in common with the primary frontal opercular taste cortex, represents a stage of information processing in the taste system of the primate at which the tuning of neurons has become sharper than that of neurons in the nucleus of the solitary tract, and is moving toward the fineness achieved in the secondary taste cortex in the caudolateral orbitofrontal taste cortex, where motivation-dependence first becomes manifest in the taste system.

INTRODUCTION

In primates there is a cortical representation of taste in the frontal operculum and anterior insula. This region of cortex was implicated in gustatory function by Bornstein (1940a,b) who observed ageusias in a dozen patients with bullet wounds in this area. Patton (1960), Ruch and Patton (1946), and Bagshaw and Pribram (1953) performed lesions in the same region in monkeys and noted a reliable, if temporary, elevation of taste thresholds. Benjamin and Emmers (1960) and Benjamin and Burton (1968) 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 frontal operculum and insula. Burton and Benjamin (1971) interpreted this latter region as the pure taste area. Anatomic investigations with the autoradiographic anterograde fiber-tracing technique by Pritchard et al. (1986) have shown that there are projections from the thalamic taste area to the frontal opercular and insular cortices. These frontal opercular and insular regions of cortex have been shown to be cytoarchitectonically distinct by Jones and Burton (1976), Mesulam and Mufson (1982a,b), Roberts and Akert (1963), and Sanides (1968, 1970).

To investigate this region in the primate physiologically, Sudakov et al. (1971) recorded single neuron activity in the frontal operculum and insula of the monkey 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, and milk) employed. In a more extensive investigation, we have analyzed the responses of 165 neurons in the frontal operculum with gustatory responses to stimuli that included NaCl, glucose, HCl, quinine HCl (QHCl), water, and a complex taste stimulus, black currant juice (Scott et al. 1986b). The taste region was found to be located in the dorsal and anterior part of the frontal operculum, the neurons were found to be more specifically tuned to these stimuli than were neurons recorded in the same monkeys in the nucleus of the solitary tract (Scott et al. 1986a), and it was found that satiety did not affect the magnitude of the responses of these opercular neurons to gustatory stimuli (Rolls et al. 1988).

The aims of the present investigation were to determine whether there is a gustatory area in the insula of the primate, which, as noted above, is architectonically distinct from the frontal operculum; and, if so, to determine the limits of this area and to define the ways in which the neuronal responses occur to gustatory stimuli. To allow quantitative comparisons between the different parts of the gustatory system, not only were data on the tuning of the neurons expressed quantitatively, but also the present recordings were made in the same species, cynomolgus monkeys (*Macaca fascicularis*), in which the tuning of neurons in the nucleus of the solitary tract and in the frontal operculum was investigated (Scott et al. 1986a,b). One of the two monkeys in which recordings were made was even the same individual as in the earlier studies. The overall aim of the current series of recordings is to advance understanding of the control of food intake, and of its disorders (Rolls 1986).

METHODS

Subjects

The subjects were two male cynomolgus monkeys (*M. fascicularis*) weighing 3.8–4.0 kg during the course of data collection. They were prepared for recording by implanting a ring over the area from which recordings were to be made, as described previously (Scott et al. 1986a,b). Full sterile precautions were observed throughout surgery. Each monkey was sedated with an intramuscular injection of ketamine (10 mg/kg im) and anesthetized with intravenous thiopentone sodium (50 mg/ml). The depth of anesthesia was monitored by frequently testing for the presence of a leg flexion reflex, and if this was present supplemental anesthetic was administered. Respiration rate was monitored throughout surgery. Atropine (0.1 ml/kg) was administered to prevent excessive salivation, and glycerine was applied to the eyes to prevent their drying. The monkey was placed in a Kopf stereotaxic instrument and his position confirmed by X-radiography. A section of skull over the insula was removed and replaced with a stainless steel ring to which an X-Y positioner and microdrive could be fitted during recording sessions. The implant also held four electrodes that were placed 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. The implant was fixed in place with dental acrylic. 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 two weeks, 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 (Merrill and Ainsworth 1972) and had tip sizes of about 2×4 μm.

The electrodes were systematically positioned from track to track by the use of 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 just through it. The sterile electrode was then lowered to a predetermined depth (about 10 mm dorsal to the insula) and advanced by the use of a Trent-Wells hydraulic microdrive and chronic adaptor system.

ELECTRICAL SYSTEM. Neuronal 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 per 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 TTL pulses for on-line analysis. They were also displayed on a second oscilloscope and audio monitor, providing additional visual and auditory cues that permitted corrections if minor changes in recorded voltage occurred with electrode drift. Single unit data, stimulus onset trigger, where applicable, 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 NaCl, 10^{-3} – 3.0 M glucose, 10^{-5} – 3×10^{-2} M HCl, 10^{-6} – 3×10^{-3} M QHCl), plus 20% black currant juice concentrate (Beecham Products, Brentford, U.K.). Black currant juice was included because it is both highly palatable to the monkey and complex in taste quality such that many 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 handheld 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 evidence that a large and nearly constant receptive field was stimulated was that different neurons with best responses to all the different prototypical stimuli were found and that the responses of these neurons in repeated tests were consistent. If fixed delivery tubes were located in the mouth, the monkey learned to block or partially avoid the delivery of tastants through them.

Stimulus delivery was followed within 10 s by a 1.0- to 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

The 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 in the insula (Yaxley et al. 1988). Over a 5-day week of data collection, monkeys took nearly one-half of their food and most of their fluid during recording sessions.

Analysis

A PDP11 computer measured the firing rate of the neuron in a 5-s period after stimulus application and performed basic statistics on-line. It also calculated and displayed the time course of spontaneous and evoked neural activity (peristimulus time histograms) in 50-ms bins. Spike firing rates in the 5-s period after stimulus application provided material for derived analyses that included calculations of interneuronal and interstimulus correlation coefficients, multidimensional scaling routines, and cluster analyses as detailed in the results of this paper.

Localization 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. The recording positions could then be reconstructed to within 250 μm relative to the deep electrodes permanently implanted in diencephalic and telencephalic structures. 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 sodium pentobarbital. They were then perfused with 0.9% saline followed by formal saline. Their brains

were stored in sucrose formalin for at least seven 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 found in the rostral and dorsal part of the insular cortex, in the sites shown in Fig. 1. It was possible in the experiments described here to find and analyze the responses of 65 such taste neurons in the two monkeys. The area within which taste neurons were found is indicated quite precisely in Fig. 1, in that, altogether, in tracks made to define the limits of the area the responses of ~2,925 neurons round this region were analyzed. The cortex surrounding the area indicated in Fig. 1 was so extensively sampled that each individual neuron could not be indicated in Fig. 1. Many of the neurons in these surrounding areas (e.g., laterally) had somatosensory responses, in that they responded, for example, to touch to the mouth. These somatosensory responses are consistent with the fact that a rostral part of the somatosensory cortex concerned with the mouth is found laterally on the gyrus at the anterior end of the Sylvian fissure, and with the projections from the somatosensory cortex to part of the insula (Mesulam and Mufson 1982a,b; Mufson and Mesulam 1982).

Cytoarchitectonic and myeloarchitectonic analysis using the criteria for distinguishing each of the areas described by Mesulam and Mufson (1982a) showed that the gustatory neurons were in the dysgranular part of the insula (Idg) and were located anteriorly within Idg.

Spontaneous activity

The mean spontaneous firing rate of taste cells in the insular taste cortex of the awake monkey was 2.3 ± 0.4 spikes/s (mean ± SD, range = 0.0–13.3). To define an evoked response we adopted a dual criterion of spontaneous rate ± 2.33 SD (i.e., $P < 0.01$) and a minimum discharge rate of 1.0 spike/s measured over a 5-s period after stimulus application. This is a strict criterion of a response, in that it implies that, with a single measurement (on a single trial) of firing rate to a stimulus, a statistically significant response should be detected by the single neuron. In fact, we made 4–8 measurements of firing rate (in random sequence) to each chemical, and with the resulting small standard error of the mean response and the use of a *t* test, we could detect as significant and reliable much smaller changes than the ±2.33 SD criterion adopted. The criterion corresponds to detecting as significant a taste response based on one tasting of the chemical. The low spontaneous activity precluded inhibition as a response option for 83% of the cells and required imposition of the 1.0 spike/s minimum on 29%.

Intensity-response functions

We tested 10 neurons for their responses to a 3.5-log molar concentration range of a prototypical taste stimulus (3 cells tested with glucose, 3 with NaCl, 2 with HCl, and 2 with QHCl). While the slopes of the intensity-response functions were somewhat different for different neurons, each chemical had a characteristic dynamic range over which neural response rates were most sensitive to concentration (Fig. 2). For glucose, the range was 10^{-2} to 3.0 M; for NaCl, from 10^{-2} to 1.0 M; for HCl, from 10^{-4} to 3×10^{-2} M; for QHCl, from 3×10^{-5} M to a concentration beyond that which we could apply to an alert monkey. The lowest effective concentration of each range was at or slightly below the threshold values derived from the monkey tractus solitarius (Scott et al. 1986a) and the electrophysiological (Diamant et al. 1963) and psychophysical (Pfaffman 1959) thresholds in humans.

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 (Scott et al. 1986a) and frontal opercular taste cortex (Scott et al. 1986b). The standard concentrations were 1.0 M glucose, 1.0 M NaCl, 0.01 M HCl and 0.001 M QHCl. The remainder of this paper is based on activity evoked by these four stimuli plus 20% black currant juice and deionized water. Examples of the responses evoked in one neuron in the insula are shown in Fig. 3.

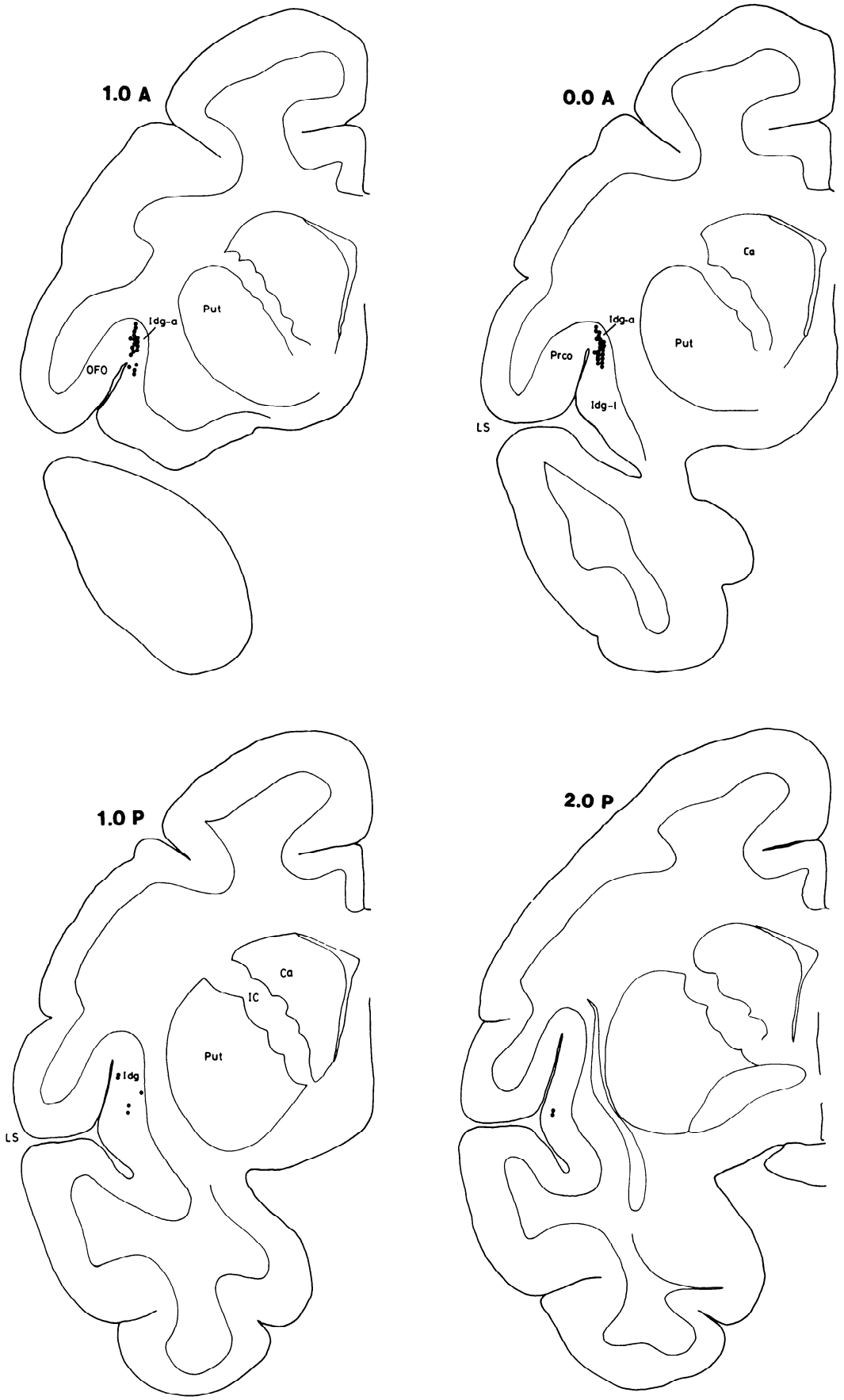
Mean evoked responses and chemotopic organization

The mean evoked responses in spikes/s measured over 5 s for each stimulus were as follows: deionized water = 6.2, 20% black currant juice = 9.3, 1.0 M glucose = 9.2, 1.0 M NaCl = 9.8, 0.01 M HCl = 6.3, and 0.001 M QHCl = 5.4. It is worth noting that it was possible to classify neurons as responding to water if their firing rates altered when water was used as the stimulus but not when other tastants were used as the stimuli. Examples of the responses of three such neurons are shown in Fig. 5*a*. The responses of such neurons cannot be simply the result of tactile stimulation.

In the monkey nucleus tractus solitarius (NTS) some chemotopic arrangement of neural sensitivities was observed which conformed to the topographic organization of sensitivities on the human tongue (Scott et al. 1986a). The anatomic tracing studies of Norgren and his colleagues suggest as a possibility preservation of this organization from NTS to the gustatory thalamus and cortex (Norgren 1984; Pritchard et al. 1986). Therefore we plotted the responsiveness of each neuron as a function of its location in the insula to determine whether a chemotopic arrangement was apparent. No clear topography was apparent from the sample of neurons.

Breadth of sensitivity

Of the 65 neurons tested with all solutions, 10 (15%) fulfilled our response criterion for responding to all four basic stimuli at these moderate-to-high concentrations. Ten (15%) responded to three stimuli, 17 (26%) to two, 22 (34%) to one, and 6 (9%) did not exceed criterion to any basic taste solution (but, of course, they did in response to black currant juice and/or deionized water) (see Table 1*B*). Conversely, the percentages of neurons that responded to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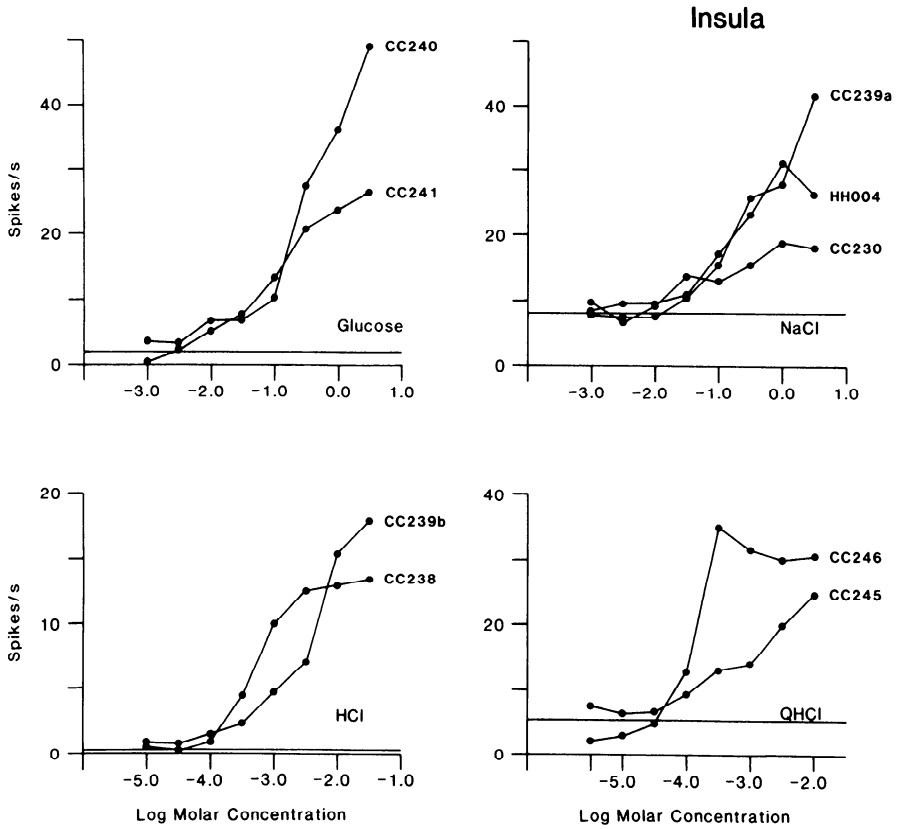

FIG. 2. Intensity-response functions of 9 individual neurons from the insula of the monkey. Each cell was tested with a 3.5 log molar concentration series of the prototypical stimulus to which it was most sensitive.

each stimulus were 34% to water, 62% to black currant juice, 52% to glucose, 52% to NaCl, 48% to HCl, and 42% to QHCl (see Table 1*A*). Thus, ignoring water, 188 (47.7%) of the stimulus-neuron interactions resulted in responses that exceeded criteria: 186 (47.7%) excitatory and two (0.5%) inhibitory. These percentages are lower than the corresponding values from NTS, implying that the mean breadth of tuning is narrower in the insula.

To quantify this range of neuronal sensitivity, the breadth-of-tuning metric, developed by Smith and Travers (1979), 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 measure of entropy is derived from information theory, and is calculated as

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

where H = breadth of responsiveness, k = scaling constant (set so that $H = 1.0$ when the neuron responds equally well to all stimuli in the set of size n), p_i = the response to stimulus i expressed as a proportion of the total response to all the stimuli in the set. The coefficient ranges from 0.0, representing total specificity to one of the stimuli, to 1.0, which indicates an equal response to all of the stimuli. The values calculated from the responses to the four prototypical stimuli for the population of 65 insular neurons analyzed are shown in Fig. 4. The mean coefficient was 0.56 (range = 0.12–0.99). This is lower than the mean value derived from NTS (where $H = 0.87$), and also lower than the mean value of $H = 0.67$ of neurons in the opercular taste cortex (see Table 1*C*). Thus insular taste cells tend to be more specifically tuned across the standard range of taste qualities than do NTS cells. There was no evidence that the breadth of tuning varied systematically with neuronal location.

Neuron types

It is not yet resolved whether the taste system is composed of a small number of neuron types, each replicated to generate all cells of the system, or if each gustatory neuron is individualistic in its characteristics (Woolston and Erickson 1979; Erickson 1985; Scott 1987; Smith et al. 1983). 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.

Shown in Fig. 5 are 18 examples of the response profiles of single insular neurons categorized according to the chemicals to which each responded best: deionized water (5*a*), black currant juice (5*b*) and each of the four prototypical taste stimuli (5, *c–f*). The shapes of the profiles are rather similar within each category, generating high mean product-moment correlations between the responses of the

FIG. 1. Coronal sections through the insular cortex showing sites at which neurons with gustatory responses were recorded. A-P coordinates are in millimeters relative to sphenoid. Ca, caudate nucleus; IC, internal capsule; Idg-a, anterior part of the dysgranular field of the insula; Idg-1, liminal part of the dysgranular field of the insula; LS, lateral sulcus; OFdg, dysgranular field of the orbitofrontal cortex; OFO, orbitofrontal opercular area; Put, Putamen.

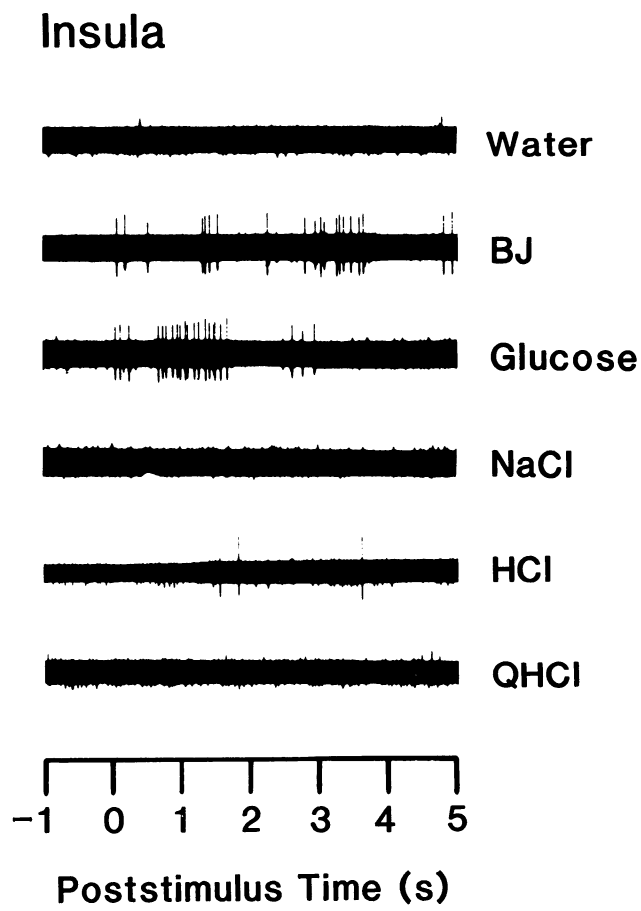

FIG. 3. Examples of the responses recorded from one insular neuron to the six taste stimuli, water, 20% black currant juice (BJ), 1 M glucose, 1 M NaCl, 0.01 M HCl, and 0.001 M quinine HCl (QHCl).

neurons within a category. The cells that responded best to water, glucose, NaCl, and HCl were all quite sharply tuned to these stimuli (see Fig. 5). The neurons shown in Fig. 5 that responded best to black currant juice also responded to glucose. The cells most responsive to HCl were consistent in their insensitivity to all other stimuli except sometimes water or black currant juice.

The general consistency within each category based on the stimulus to which a neuron responds best suggests a limited number of response profiles and so gustatory neuron types. However, it is possible that neurons that respond best to one stimulus may respond differently to other stimuli so that there may be more types of neuron than indicated by the optimal stimulus categorization. Therefore a more comprehensive analysis that took account of the responses of every neuron to every stimulus was performed. To do this, we compared the profiles of the 65 neurons with the use of (Pearson product-moment) correlation of the responses shown by each neuron to that of all the other neurons and organized the resulting 2,080 correlations [$N(N-1)/2$] into a matrix that, in turn, was used to construct a multidimensional space by the use of multidimensional scaling (Bieber and Smith 1986; Gurrman 1968) (Fig. 6). (For any two neurons the correlation between them was measured by calculating the Pearson product-moment correlation based on their responses to

TABLE 1. *Three comparisons of the breadth of tuning of neurons in different areas*

	NTS	OPC	INS
A. Percentage of neurons fulfilling the response criterion for each prototypical stimulus			
1.0 M glucose	85	60	52
1.0 M NaCl	92	51	52
0.01 M HCl	63	51	48
0.001 M QHCl	86	56	42
Mean	82	54	49
B. Percentage of neurons responding to 4, 3, 2, 1, or none of the prototypical taste stimuli			
4/4	42	22	15
3/4	42	18	15
2/4	17	25	26
1/4	0	23	34
0/4	0	11	6
Mean	3.3/4	2.2/4	1.9/4
C. Mean breadth-of-tuning metric			
	0.87	0.67	0.56

NTS, nucleus of the solitary tract; OPC, frontal opercular taste cortex; INS, insular taste cortex.

the four prototypical stimuli, to water, and to black currant juice.) The goal of the multidimensional scaling was to produce a map (i.e., an arrangement of the neurons in space) in which the interneuron distances most closely match the associated proximities. The more similar two profiles are, the more closely the two cells that generated them will be placed in the multidimensional space. Thus neuron types as defined by recurring sensitivity to the five stimuli plus water employed here would be indicated by groups of points, whereas the absence of groups would result in a homogeneous distribution throughout. The three dimensions in the space plotted in Fig. 6 accounted for 92.7% of the variance. The first two dimensions accounted for 87% of the variance. In Fig. 6 there is an interesting separation of neurons into different regions of the taste space. It is clear that the neurons are not scattered homo-

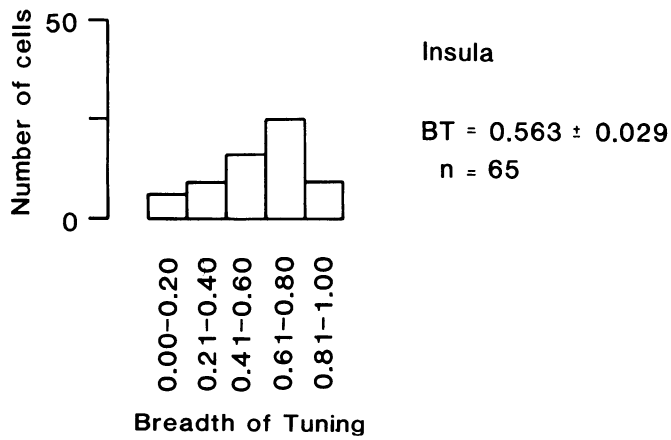

FIG. 4. Breadth of tuning of the 65 insular neurons analyzed.

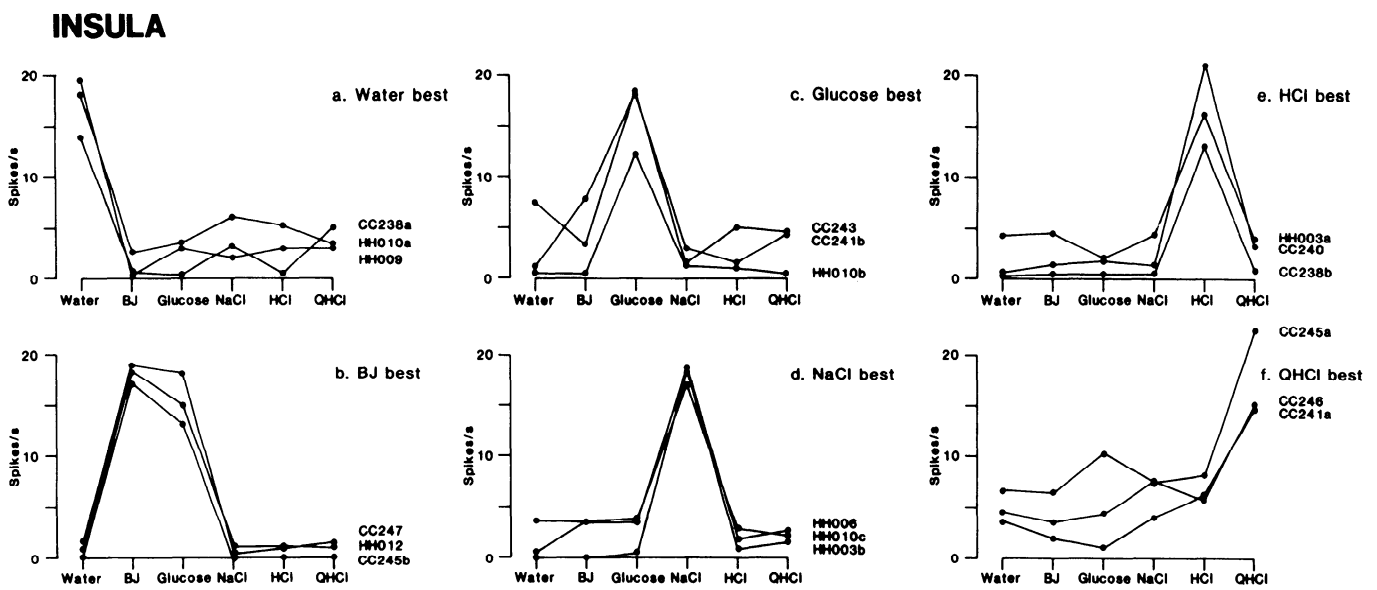

FIG. 5. Response profiles of 18 insular neurons to the different stimuli. Neurons are plotted according to stimulus to which they responded best.

geneously throughout the space, but, instead, there is some grouping of neurons into different regions of the 3-D space. This is an indication that there are different groups of neurons, and that at least three dimensions are needed to represent these groups. The different neurons were grouped as follows. The neurons shown on the right of dimension 1 had their best response to black currant juice (at the back of dimension 2), glucose (in the middle of dimension 2), or water (at the front of dimension 2). Neurons with best responses to sodium occurred at the left end of dimension 1. The neurons with best responses to quinine were towards the left end of dimension 1, but were distinguished from the sodium-best neurons by having, in addition, intermediate values on the third dimension. The neurons with best

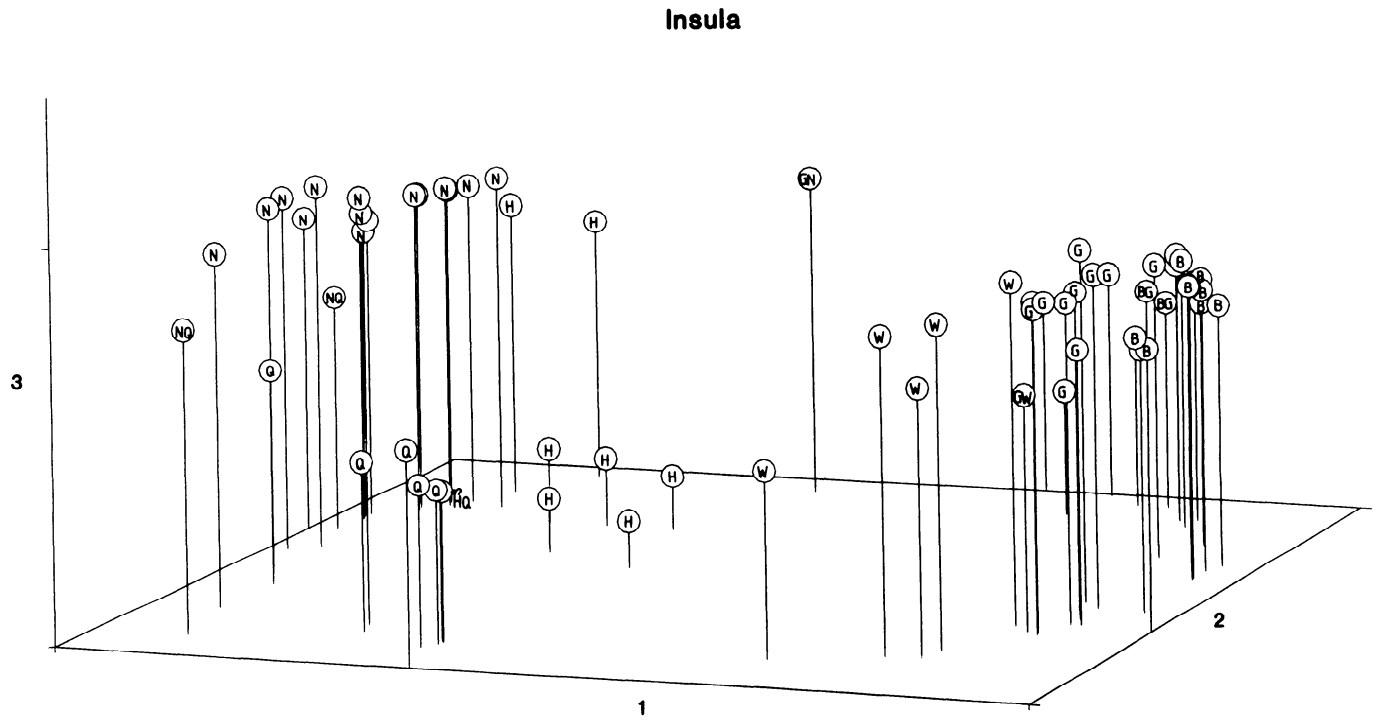

FIG. 6. A three-dimensional representation of relative similarity among neuronal response profiles. The more highly the response profiles of two neurons correlate, the closer they are positioned to one another in the space. This solution accounts for 92.7% of the data variance. Dimensions are numbered in order of the amount of the variance for which they account. Stimulus or stimuli to which each neuron responded best is shown by the following symbols: N, NaCl; W, water; G, glucose; B, black currant juice; H, HCl; Q, quinine HCl.

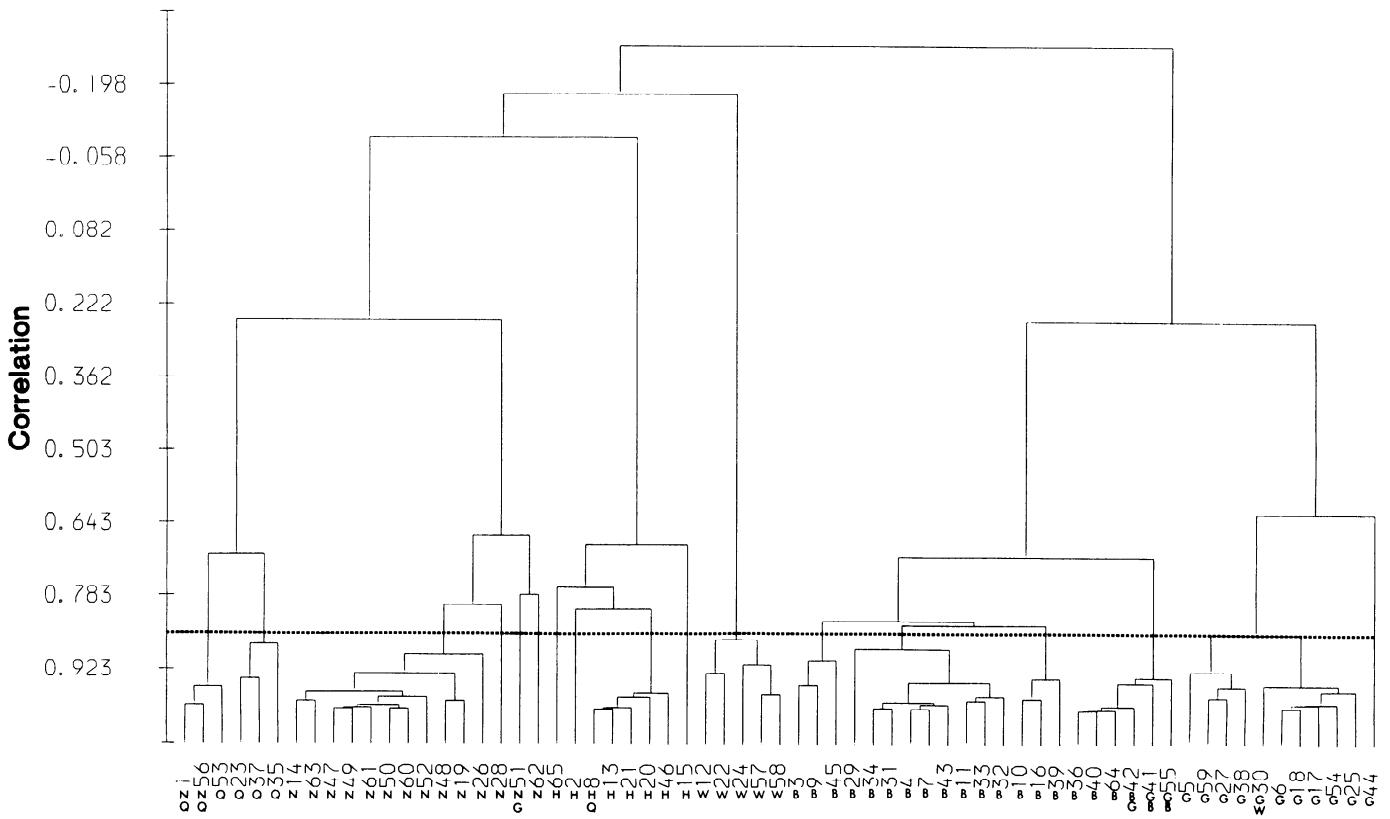

FIG. 7. Results of a cluster analysis performed on response profiles of the 65 insular taste neurons analyzed. Beneath the number of each cell is an abbreviation indicating the stimulus to which it gave its largest response, followed by any other stimulus that evoked >80% of the best response. Any cluster 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. Ordinate indicates correlation level between the profiles of neurons joined by a horizontal line at that level. N, NaCl; W, water; G, glucose; B, black currant juice; H, HCl; Q, quinine HCl.

responses to HCl were distinguished from the other neurons, not only by an intermediate value on the first dimension, but also by very low values on the third dimension.

To provide further evidence on the extent to which the putative groups of neurons apparent in Fig. 6 are internally consistent with clusters of neurons occurring, a cluster analysis (Wishart 1978) was performed on the same correlation matrix as was used to generate the space shown in Fig. 6. This appears in Fig. 7 as a dendrogram. Neurons are numbered along the abscissa in the order in which they were isolated. 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 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 different a cluster is from other neurons, the longer will be the vertical line leading to it. (A hierarchical cluster analysis with the average linking method was used.) It is shown in Fig. 7 that the largest main division is into neurons that respond best to glucose or black currant juice and those that respond to other stimuli best. The next division in the sweet group is into those that respond primarily to glucose (*neurons 5–44*) and those that respond primarily to black currant juice (*neurons 3–55*). The other neurons, after a first division, divide into groups that respond primarily to QHCl (*neurons 1–35*), NaCl (*neurons 14–65*), HCl (*neurons 2–15*), and water (*neurons 12–58*). The cluster analysis thus corroborates the analysis shown in Fig. 6 providing evidence that the response profiles of insular neurons fall into a number of different groups.

To provide an indication of the profiles of responsiveness to the different stimuli of the clusters of neurons detected by the cluster analysis (see Fig. 7), the mean profile of each of the 11 clusters of neurons with high (see below) interneuron correlations was calculated. These mean profiles (shown as a percentage of the maximal response of the neuron to any tastant) are shown in Fig. 8. For each of the taste stimuli glucose, NaCl, HCl, QHCl, water, and black currant juice, there was one group of neurons that responded much more to that tastant than to the other tastants. The other groups of neurons in some cases responded primarily to two of the tastants, such as glucose and black currant juice or NaCl and QHCl. The cluster analysis is thus useful in helping to identify the characteristic response

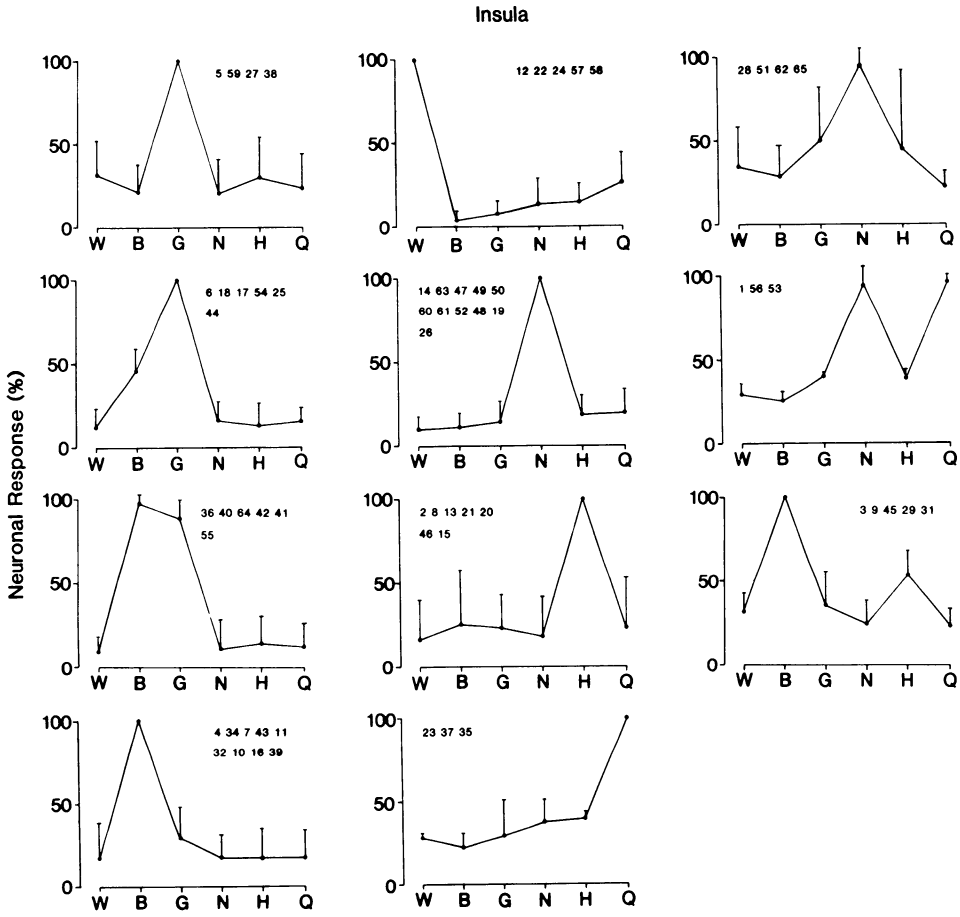

FIG. 8. Mean response profiles (±SD) of each of the main eleven clusters of neurons indicated in Fig. 7. Numbers of the cells included in each cluster are annotated.

profiles of different groups of neurons in the insular taste cortex.

Whether the groups or clusters shown in Fig. 7 are sufficiently coherent and different from other groups to warrant being designated neuron types is of interest (Erickson 1985; Scott 1987; Smith et al. 1983; Woolston and Erickson 1979). One definition of neuron type derives from the suggestion of Woolston and Erickson (1979) that all cells of a putative neuron type should have identical response profiles to within the limit of experimental accuracy. Experimental accuracy was determined by repeating the application of a chemical during a session with one neuron and analyzing the responses by the same means as described above. The correlation between profiles generated by repeated applications should approach 1.00 as experimental error approaches zero. The mean correlation calculated by this method was 0.91. The lower limit of the 95% confidence interval for this distribution, as determined by the procedure described in Cohen and Cohen (1975) (see also Schiffman and Erickson 1971), is indicated by the dotted line in Fig. 7. Any neurons whose profiles do not intercorrelate at a level that meets or exceeds the lower limit of this distribution may be excluded from being functionally identical with a confidence of $P < 0.05$. Thus the groups of neurons apparent above the dotted line in Fig. 7 are more different from each other than would be expected by chance, with a probability of error of 0.05. The mean profiles of each of the 11 main such different groups of neurons are shown in Fig. 8.

Another way of assessing the number of clusters that are meaningful in a cluster analysis is to prepare a scree diagram in which the correlation between clusters is plotted as a function of the number of clusters (Bieber and Smith 1986). Using this method on the data in Fig. 7 shows that there are six main groups of cells, which can be seen, reading from the left of Fig. 7, to have best responses to quinine, NaCl, HCl, water, black currant juice, and glucose. Within these six main cell types, the analysis of the previous paragraph suggests that there may be further subtypes.

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

TABLE 2. *Correlation coefficients between the profiles of activity generated by each stimulus*

	W	B	G	N	H	Q
W						
B	−0.01					
G	0.21	0.37				
N	0.28	−0.10	0.25			
H	0.24	0.06	−0.03	0.12		
Q	0.31	0.01	0.12	0.41	0.32	

W, water; B, black currant juice; G, glucose; N, NaCl; H, HCl; Q, quinine HCl.

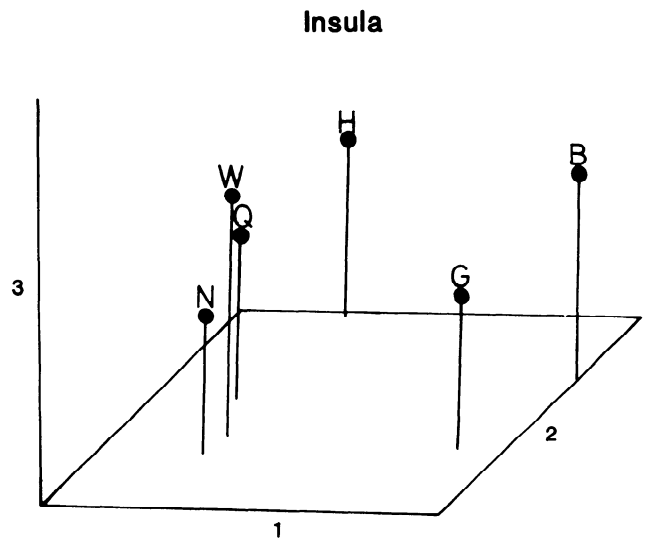

FIG. 9. A three-dimensional spatial representation of similarity among taste qualities as indicated by the responses of insular neurons. The 3 dimensions are undefined. Dimensions are numbered in order of the amount of the variance for which they account. Symbols are as in the previous figures.

measured by comparing the profiles of activity that each evokes across the 65 neuron sample. The correlations among these profiles have been shown in the rodent to offer accurate predictions of discriminative capacity. The 15 correlation coefficients between all pairs of profiles elicited by these six stimuli are shown in Table 2. In general, the correlations between the different stimuli are relatively low, indicating that these neurons provide a representation of the stimuli in which the stimuli are well separated from each other. The greatest similarity is between black currant juice and glucose (r = 0.37), both of which are intensely sweet to humans; but it is of interest that even this correlation is relatively low compared to the correlation between these stimuli for neurons in the frontal opercular taste cortex (r = 0.75). It is also of interest that the correlation between responsiveness to HCl and QHCl is relatively low (0.32) compared, for example, to 0.60 for neurons in the nucleus of the solitary tract (Scott et al. 1986a).

As with the neurons, the correlation matrix may be used to generate a multidimensional space; this one containing the positions of stimuli relative to one another (Schiffman and Erickson 1971). A three-dimensional solution is shown in Fig. 9. In broad terms, dimension 1 separates sweet from other qualities, dimension 2 separates stimuli according to their acidity (black currant juice is more acidic than glucose), and water is highest on dimension 3.

DISCUSSION

In this study, the function of a gustatory area in the rostral insula of the macaque monkey has been investigated. Very few neurons have been recorded in this area previously (Sudakov et al. 1971).

The insular gustatory cortex is in the rostral and dorsal part of the Idg, as shown in Fig. 1. The area is probably well delimited by the sites of the gustatory neurons shown in Fig. 1, in that altogether ~2,925 neurons were recorded in this study in the area immediately surrounding the area of gustatory neurons shown in Fig. 1. The majority of these other neurons had somatosensory or movement-related responses, typically responding to touch to or movement of the mouth. It was of interest that the gustatory neurons in the insula described here appeared to be unimodal in that they were not affected by touch to or within the mouth, by olfactory stimuli, or by visual stimuli even when these signified food (compare Thorpe et al. 1983; Rolls 1986). It will be of interest in future investigations to determine where in the taste system there is convergence with inputs from other modalities. The insular taste cortex is continuous laterally with the frontal opercular taste cortex (Scott et al. 1986b).

Taste cells in the insular taste cortex are more sharply tuned (mean breadth-of-tuning index, H = 0.56) to the stimuli used than are cells in the NTS (H = 0.87, χ^2 = 58, df = 2, $P < 0.001$) and a little more sharply tuned than are cells in the frontal opercular taste cortex (H = 0.67) (see Table 1*C*). Consistent with this, a relatively high proportion of insular cortex gustatory neurons responded to only one of the prototypical taste stimuli, and a relatively low proportion to four or three of the prototypical taste stimuli (see Table 1*B*). The same stimuli and the same response criteria were used for these comparisons.

The cluster analysis helped to identify groups of neurons that had high similarity of the within-group response profiles to the different stimuli, and differences from the response profiles of the other groups. It is of interest that taste processing in the insular cortex is different from that in the NTS in that there are high within-group correlations of the response profiles for a number of distinguishable groups of neurons in the insular taste cortex, compared with much more heterogeneity and less specificity of neurons in the NTS (compare Fig. 7 with Fig. 6 of Sanides 1970). The statistical analysis described above on which the dotted line shown in Fig. 7 was based provided an indication that when the experimental error is estimated, 11 main groups of neurons can be distinguished (see Fig. 7). It is possible that with a larger stimulus array than the six used, and perhaps if these stimuli were used in combination, that even more groups of neurons would appear. The profiles of the 11 main groups of neurons found are shown in Fig. 8. It is clear that some of these groups of neurons respond primarily to only one of the taste stimuli used, so that within the insular taste cortex there is a representation of the different tastants in which different neurons respond mainly to one tastant. This is in contrast to the much more distributed representation found in the NTS (Scott et al. 1986a). Some of the reasons for the finer tuning of neurons in the insular cortex than in the NTS and the even finer tuning in a secondary gustatory cortical area in the caudolateral orbitofrontal cortex (Rolls et al., unpublished observations) are discussed elsewhere (Rolls 1987, 1989, and unpublished observations). The fine tuning in the insular taste cortex may be in preparation for the computation of sensory-specific satiety, which is reflected in the responses of neurons in the caudolateral orbitofrontal cortex (Rolls et al. 1989) but not in the insular taste cortex (Yaxley et al. 1988).

The taste space analysis (Fig. 9 and Table 2) and neuronal space analysis (Fig. 6) show that neurons in the insular taste space provide clearly separate representations of the different tastants used.

The insular taste cortex is anatomically adjacent to the opercular frontal taste cortex (see Scott et al. 1986b) from which it is also differentiated by cytoarchitecture and myeloarchitecture (Mesulam and Mufson 1982a; Wiggins et al. 1987), but, functionally, the insular and the frontal opercular taste cortices are relatively similar. Both are parts of the primary taste cortex in that they both receive afferents from the taste thalamus, VPMpc (Pritchard et al. 1986). Both areas project to a secondary area of taste cortex in the caudolateral orbitofrontal cortex (Wiggins et al. 1987). There are projections from the insula into the amygdala (Mesulam and Mufson 1982b), and it is likely that these include projections from the insular taste area. It is not known whether there are similar projections from the frontal opercular taste cortex. The taste neurons are a little more finely tuned in the insular taste area than in the frontal opercular taste area, and the neurons cluster more tightly into separate groups in the insula than in the frontal opercular taste cortex (compare Fig. 7 of this paper with Fig. 6 of Scott et al. 1986b), but these differences are small. In both areas of the primary taste cortex the taste responses are independent of hunger (Rolls et al. 1988; Yaxley et al. 1988), and it is likely that these two cortical areas provide an analysis of the quality of taste input and its intensity but do not reflect the pleasantness of the taste or provide an interface to motivation, both of which occur later in taste processing (Rolls 1986; Rolls et al. 1989).

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REFERENCES

BAGSHAW, M. H. AND PRIBRAM, K. H. Cortical organization in gustation (*Macaca mulatta*). *J. Neurophysiol.* 16: 499–508, 1953.

BENJAMIN, R. M. AND EMMERS, R. Localization of separate cortical areas for taste and tactile tongue afferents in squirrel monkey (*Saimiri sciureus*). *Federation Proc.* 19: 291, 1960.

BENJAMIN, R. M. AND BURTON, H. Projection of taste nerve afferents to anterior opercular-insular cortex in squirrel monkey (*Saimiri sciureus*). *Brain Res.* 7: 221–231, 1968.

BIEBER, S. L. AND SMITH, D. V. Multivariate analysis of sensory data: a comparison of methods. *Chem. Sens.* 11: 19–47, 1986.

BORNSTEIN, W. S. Cortical representation of taste in man and monkey. I. Functional and anatomical relations of taste, olfaction and somatic sensibility. *Yale J. Biol. Med.* 12: 719–736, 1940a.

BORNSTEIN, W. S. Cortical representation of taste in man and monkey. II. The localization of the cortical taste area in man, a method of measuring impairment of taste in man. *Yale J. Biol. Med.* 13: 133–156, 1940b.

BURTON, H. AND BENJAMIN, R. M. Central projections of the gustatory system. In: *Handbook of Sensory Physiology,* edited by L. M. Beidler. Berlin: Springer-Verlag, 1971, Vol. IV, Part 2, p. 148–164.

COHEN, J. AND COHEN, P. *Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences.* New York: Wiley, 1975.

DIAMANT, H., FUNAKOSHI, M., STROM, L., AND ZOTTERMAN, Y. Electrophysiological studies on human taste nerves. In: *Olfaction and Taste,* edited by Y. Zotterman. Oxford, UK: Pergamon, 1963, p. 193–203.

ERICKSON, R. P. Grouping in the chemical senses. *Chem. Sens.* 10: 333–340, 1985.

GURRMAN, L. A general nonmetric technique for finding the smallest coordinate space for a configuration of points. *Psychometrika* 33: 469–507, 1968.

JONES, E. G. AND BURTON, H. Areal differences in the laminar distribution of thalamic afferents in cortical fields of the insular, parietal and temporal regions of primates. *J. Comp. Neurol.* 168: 197–248, 1976.

MERRILL, E. G. AND AINSWORTH, A. Glass-coated platinum-plated tungsten microelectrodes. *Med. Biol. Eng.* 10: 662–672, 1972.

MESULAM, M.-M. AND MUFSON, E. J. Insula of the old world monkey. I: Architectonics in the insulo-orbito-temporal component of the paralimbic brain. *J. Comp. Neurol.* 212: 1–22, 1982a.

MESULAM, M.-M. AND MUFSON, E. J. Insula of the old world monkey. III: Efferent cortical output and comments on function. *J. Comp. Neurol.* 212: 38–52, 1982b.

MUFSON, E. J. AND MESULAM, M.-M. Insula of the old world monkey. II: Afferent cortical input and comments on the claustrum. *J. Comp. Neurol.* 212: 23–37, 1982.

NORGREN, R. Central neural mechanisms of taste. In: *Handbook of Physiology. The Nervous System.* Bethesda, MD: Am. Physiol. Soc., 1984, vol. III, part 2, chapt. 24, p. 1087–1128.

PATTON, H. D. Taste, olfaction and visceral sensation. In: *Medical Physiology and Biophysics* (18th ed.), edited by T. C. Ruch. Philadelphia, PA: Saunders, 1960, p. 369–385.

PFAFFMANN, C. The sense of taste. In: *Handbook of Physiology. Neurophysiology.* Washington, DC: Am. Physiol. Soc., 1959, sect. I, p. 507–533.

PRITCHARD, T. C., HAMILTON, R. B., MORSE, J. R., AND NORGREN, R. Projections of thalamic gustatory and lingual areas in the monkey, *Macaca fascicularis. J. Comp. Neurol.* 244: 213–228, 1986.

ROBERTS, T. S. AND AKERT, K. Insular and opercular cortex and its thalamic projection in *Macaca mulatta. Schweiz. Arch. Neurol. Neurochir. Psychiatr.* 92: 1–43, 1963.

ROLLS, E. T. Neuronal activity related to the control of feeding. In: *Feeding Behavior: Neural and Humoral Controls,* edited by R. C. Ritter, S. Ritter, and C. D. Barnes. New York: Academic, 1986, chapt. 6, p. 163–190.

ROLLS, E. T. Information representation, processing and storage in the brain: analysis at the single neuron level. In: *The Neural and Molecular Bases of Learning,* edited by J.-P. Changeux and M. Konishi. Chichester, UK: Wiley, 1987, p. 503–540.

ROLLS, E. T. The representation and storage of information in neuronal networks in the primate cerebral cortex and hippocampus. In: *The Computing Neuron,* edited by R. Durbin, C. Miall, and G. Mitchison. Wokingham, UK: Addison-Wesley, 1989a, chapt. 8, p. 125–159.

ROLLS, E. T. Information processing in the taste system of primates. *J. Exp. Biol.* 146: 141–164, 1989b.

ROLLS, E. T., SANGHERA, M. K., AND ROPER-HALL, A. The latency of activation of neurons in the lateral hypothalamus and substantia innominata during feeding in the monkey. *Brain Res.* 164: 121–135, 1979.

ROLLS, E. T., SCOTT, T. R., SIENKIEWICZ, Z. J., AND YAXLEY, S. The responsiveness of neurones in the frontal opercular gustatory cortex of the macaque monkey is independent of hunger. *J. Physiol. Lond.* 397: 1–12, 1988.

ROLLS, E. T., SIENKIEWICZ, Z. J., AND YAXLEY, S. Hunger modulates the responses to gustatory stimuli of single neurons in the orbitofrontal cortex. *Eur. J. Neurosci.* 1: 53–60, 1989.

RUCH, T. C. AND PATTON, H. D. The relation of the deep opercular cortex to taste. *Med. Proc.* 5: 89–90, 1946.

SANIDES, F. The architecture of the cortical taste nerve areas in squirrel monkey (*Saimiri sciureus*) and their relationships to insular, sensorimotor and prefrontal regions. *Brain Res.* 8: 97–124, 1968.

SANIDES, F. Functional architecture of motor and sensory cortices in primates in the light of a new concept of neocortex evolution. In: *Advances in Primatology. The Primate Brain,* edited by C. Noback and W. Montagna. New York: Appleton, 1970, Vol. I, p. 137–208.

SCHIFFMAN, S. S. AND ERICKSON, R. P. A psychophysical model for gustatory quality. *Physiol. Behav.* 7: 617–633, 1971.

SCOTT, T. R. Coding in the gustatory system. *Neurobiology of Taste and Smell,* edited by T. E. Finger and W. L. Silver. New York: Wiley, 1987, p. 355–378.

SCOTT, T. R., YAXLEY, S., SIENKIEWICZ, Z. J., AND ROLLS, E. T. Taste responses in the nucleus tractus solitarius of the behaving monkey. *J. Neurophysiol.* 55: 182–200, 1986a.

SCOTT, T. R., YAXLEY, S., SIENKIEWICZ, Z. J., AND ROLLS, E. T. Gustatory responses in the frontal opercular cortex of the alert cynomolgus monkey. *J. Neurophysiol.* 56: 876–890, 1986b.

SMITH, D. V. AND TRAVERS, J. B. A metric for the breadth of tuning of gustatory neurons. *Chem. Sens.* 4: 215–219, 1979.

SMITH, D. V., VAN BUSKIRK, R. L., TRAVERS, J. B., AND BIEBER, S. L. Gustatory neuron types in hamster brain stem. *J. Neurophysiol.* 50: 522–540, 1983.

SUDAKOV, K., MACLEAN, P. D., REEVES, A., AND MARINO, R. Unit study of exteroceptive inputs in claustrocortex in awake, sitting, squirrel monkey. *Brain Res.* 28: 19–34, 1971.

THORPE, S. J., ROLLS, E. T., AND MADDISON, S. Neuronal activity in the orbitofrontal cortex of the behaving monkey. *Exp. Brain Res.* 49: 93–115, 1983.

WIGGINS, L. L., ROLLS, E. T., AND BAYLIS, G. C. Afferent connections of the caudolateral orbitofrontal cortex taste area of the primate. *Soc. Neurosci. Abstr.* 13: 1406, 1987.

WISHART, D. Clustan. Program Library Unit, Edinburgh University, 1978.

WOOLSTON, D. E. AND ERICKSON, R. P. Concept of neuron types in gustation in the rat. *J. Neurophysiol.* 42: 1390–1409, 1979.

YAXLEY, S., ROLLS, E. T., SIENKIEWICZ, Z. J., AND SCOTT, T. R. Satiety does not affect gustatory activity in the nucleus of the solitary tract of the alert monkey. *Brain Res.* 347: 85–93, 1985.

YAXLEY, S., ROLLS, E. T., AND SIENKIEWICZ, Z. J. The responsiveness of neurones in the insular gustatory cortex of the macaque monkey is independent of hunger. *Physiol. Behav.* 42: 223–229, 1988.