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Responses of Neurons in the Primate Taste Cortex to the Glutamate Ion and to Inosine 5′-Monophosphate

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ROLLS, E. T., H. D. CRITCHLEY, E. A. WAKEMAN AND R. MASON. *Responses of neurons in the primate taste cortex to the glutamate ion and to inosine 5′-monophosphate.* PHYSIOL BEHAV **59**(4/5) 991–1000, 1996.—To investigate the neural encoding of glutamate taste in the primate, recordings were made from taste responsive neurons in the cortical taste areas in macaques. Most of the neurons were in the orbitofrontal cortex taste area, with a small number in adjacent taste areas. First, it was shown that single neurons that had their best responses to sodium glutamate also had good responses to glutamic acid. The correlation between the responses to these two tastants was higher than between any other pair of tastants, which included glucose (sweet), sodium chloride (salty), HCl (sour), and quinine HCl (bitter). Accordingly, the responsiveness to glutamic acid clustered with the response to monosodium glutamate in a cluster analysis with this set of stimuli, and glutamic acid was close to sodium glutamate in a space created by multidimensional scaling. Second, it was shown that the responses of these neurons to the nucleotide umami tastant inosine 5′-monophosphate were more correlated with their responses to monosodium glutamate than to any prototypical tastant. Third, concentration response curves showed that concentrations of monosodium glutamate as low as 0.001 *M* were just above threshold for some of these neurons. Fourth, neurons have not yet been found in this cortical region that showed synergism of monosodium glutamate and the nucleotide inosine 5′-monophosphate: it was shown that mixtures of 0.0001 *M* inosine 5′-monophosphate with different concentrations (0.001, 0.01, and 0.1 *M*) of monosodium glutamate did not have a greater effect than the monosodium glutamate alone. Fifth, some neurons in the orbitofrontal region, which responded to monosodium glutamate and other food tastes, decreased their responses after feeding with monosodium glutamate to behavioural satiety. In some cases this reduction was sensory-specific. These findings show that the taste neurons activated by monosodium glutamate can also be activated by other umami tastants, including glutamic acid and the nucleotide inosine 5′-monophosphate. The responses to these umami tastants were more similar to each other than to any of the other prototypical tastants, providing evidence that in this system umami is encoded differently from the other tastants. Moreover, the findings with these tastants provide additional evidence that the responses to monosodium glutamate are not due just to activation of a sodium taste channel.

Taste cortex Orbitofrontal cortex Insular cortex Glutamate Umami Primate Nucleotide

TO understand how appetite and food intake are controlled by the brain, and disorders in appetite and feeding, the neural mechanisms involved are being analysed (26). It has been shown that in the orbitofrontal cortex of primates, there is a region of secondary taste cortex in which neurons are activated by the taste of food (31). This region is implicated in the control of feeding, for it is the first part of the taste system of primates in which neuronal responses to the taste of food occur while hungry, but not after satiation (25,27,30).

An important food taste that appears to be different from that produced by sweet, salt, bitter, or sour is the taste of protein. At least part of this taste is captured by the Japanese word umami, which is a taste common to a diversity of food sources including fish, meats, mushrooms, cheese, and some vegetables. Within

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these food sources, it is the synergistic combination of glutamates and 5'-nucleotides that creates the umami taste (13,41,42). Monosodium L-glutamate (MSG), guanosine 5'-monophosphate (GMP), and inosine 5'-monophosphate (IMP) are examples of umami stimuli.

Umami does not act by enhancing the tastes of sweetness, saltiness, bitterness, or sourness in foods, but instead may be a flavour in its own right, at least in humans. For example, Yamaguchi (41) found that the presence of MSG or IMP did not lower the thresholds for the prototypical tastes (produced by sucrose, NaCl, quinine sulphate, and tartaric acid), suggesting that umami did not improve the detection sensitivity for the four basic taste qualities. Also, the detection thresholds for MSG were not lowered in the presence of the prototypical taste stimuli. This suggests that the receptor sites for umami substances are different from those for other prototypical stimuli (42). (A synergistic effect was found when IMP was added to MSG in that the detection threshold for MSG was dramatically lowered.) However, Schiffman and colleagues (34) demonstrated an interaction between L-glutamate and cations. The addition of sodium, potassium, or calcium salts lowered the taste thresholds to L-glutamate, in young subjects, but this effect was not seen when salts were added to sodium glutamate solutions. Further, Yamaguchi and Kimizuka (42) tested the "singularity" of umami by presenting human subjects with 21 taste stimuli including single and mixture solutions of MSG and sucrose, NaCl, tartaric acid, and quinine sulphate. The subjects sorted the stimuli based on taste quality similarity. These scores were placed into a similarity matrix and analysed using multidimensional scaling procedures. The results revealed that, within a three-dimensional tetrahedron, the four prototypical stimuli were located at the vertices of a tetrahedron. The mixtures containing two, three, or four prototypical stimuli were located on the edges or surfaces of the tetrahedron. However, MSG was located outside of the tetrahedron, implying that the taste of umami is qualitatively different from the four prototypical stimuli used. In spite of this perceptual distinctiveness, traditional taste–quality descriptors are frequently used in describing the quality evoked by monosodium glutamate, particularly saltiness (20,48).

These findings raise the question of whether umami taste operates through channels in the primate taste system that are separable from those for the "prototypical" tastes sweet, salt, bitter, and sour. Although the concept of four prototypical tastes has been used by tradition, there is increasing discussion about the utility of the concept [e.g., (9,35)], and increasing evidence that the taste system is more diverse than this [e.g., 9,33); see also (14)]. To investigate the neural encoding of glutamate in the primate, Baylis and Rolls (4) made recordings from 190 taste-responsive neurons in the primary taste cortex and adjoining orbitofrontal cortex taste area in macaques. Single neurons were found that were tuned to respond best to monosodium glutamate (umami taste), just as other cells were found with best responses to glucose (sweet), sodium chloride (salty), HCl (sour), and quinine HCl (bitter). Across the population of neurons, the responsiveness to glutamate was poorly correlated with the responsiveness to NaCl, so that the representation of glutamate was clearly different from that of NaCl. In addition, the representation of glutamate was shown to be approximately as different from each of the other four tastants as they are from each other, as shown by multidimensional scaling and cluster analysis. Moreover, it was found that glutamate is approximately as well represented in terms of mean evoked neural activity and the number of cells with best responses to it as the other four stimuli glucose, NaCl, HCl, and quinine. It was concluded that in primate taste cortical areas, glutamate, which produces umami taste in humans, is approximately as well represented as are the tastes produced by: glucose (sweet), NaCl (salty), HCl (sour), and quinine HCl (bitter) (4). This is consistent with evidence from behavioural experiments in rodents, human psychophysical studies, and biochemical analysis of competitive receptor mechanisms in tongue preparations (7,16,43). These studies have all indicated that a separate mechanism in the perception of umami operates, but problems still remain. One such problem is the role played by the sodium cation in the MSG molecule, and the degree to which this contributes to umami taste quality has not been clearly elucidated. In addition, the mechanism involved in the perceptual synergism of flavour intensity when glutamate is mixed with umami 5'-nucleotides is poorly understood. Studies of bovine tongue preparations, rat and to some extent chimpanzee chorda tympani recordings, have indicated a peripheral mechanism for this synergism (7,11,47). However, the neural basis of such synergism in higher taste centres has not been addressed.

The aims of the present investigation were to provide further information about how, and where, information about umami taste (and therefore the taste of many foods) is represented and processed in the primate brain. The first aim was to extend the previous investigation in primates in which monosodium glutamate was used as the umami tastant, by using glutamic acid as the tastant. The use of glutamic acid as a tastant potentially allows further evidence to be obtained for activation by umami of a different system to that activated by NaCl, for it allows administration of the glutamate ion without administration of the sodium ion. The second aim was to test the responsiveness of taste neurons to one of the nucleotide umami tastants, inosine 5'-monophosphate, and to investigate in particular whether neurons activated by glutamate were also activated by inosine 5'-monophosphate, or whether inosine 5'-monophosphate activated a separate population of neurons. The third aim was to assess the responsiveness of the system to monosodium glutamate, by measuring dose–firing rate response curves. The fourth aim, for which the third was necessary, was to investigate whether the nucleotide inosine 5'-monophosphate potentiates the effect of monosodium glutamate on neurons that respond to monosodium glutamate. This was performed by performing dose–response curves for different concentrations of glutamate with and without the addition of inosine 5'-monophosphate (IMP).

There have been only a very limited number of previous neurophysiological studies on the effects of umami encoding in the primate central nervous system. One is the study of Baylis and Rolls (4,22); in a study of the responsiveness of neurons in the primary taste cortex of macaques to amino acids they have shown that some neurons respond to MSG. Studies in primates are important because they allow closer comparison to be made to processing in humans. For example, although some neurophysiological investigations on umami have been performed in nonprimates (e.g., mice and rats), in at least some studies no clear evidence for processing of MSG differently from NaCl has been found (44), and in any case there is evidence that some nonprimate species do not respond behaviourally to umami in the same way as humans (38).

Another reason for performing these experiments with primates is that the primate taste system may be organised even anatomically differently to the taste system of nonprimates (6,17,19,25,27). In primates, the three taste nerves terminate in the rostral part of the nucleus of the solitary tract, which projects monosynaptically to the thalamic taste nucleus, the parvicellular division of the ventralposteromedial thalamic nucleus (VPMpc) (19). A remarkable difference from the taste system of rodents is this direct projection from the NTS to the taste thalamus. In rodents, there is an obligatory relay from the NTS to the pontine

parabrachial taste nuclei (the "pontine taste area"), which in turn projects to the thalamus (6,17,19). The pontine taste nuclei also project to the hypothalamus and amygdala in rodents (18), providing direct subcortical access to these subcortical structures important in motivational behaviour (e.g., feeding) and learning (25,27). In contrast, in primates there may well be no such direct pathway from the brain stem taste areas to the hypothalamus and amygdala (6,19). The thalamic taste area, VPMpc, then projects to the cortex, which in primates forms the rostral part of the frontal operculum and adjoining insula, so that this is by definition the primary taste cortex (23). The responses of neurons in these primary cortical in monkeys have been analysed by Scott et al. (36) and by Yaxley, Rolls, and Sienkiewicz (46). A secondary cortical taste area has recently been discovered by Rolls, Yaxley, and Sienkiewicz (31) in the caudolateral orbitofrontal cortex, extending several millimetres in front of the primary taste cortex. This region has been shown to receive projections from the primary taste cortex (5). Taste cells are also found more medial to this in the orbitofrontal cortex (25,32).

In the study described here, the responses of neurons in the secondary taste cortical region of the orbitofrontal cortex, and in more medial regions of the orbitofrontal cortex are described. In addition, a small proportion (9/75 or 12%) of neurons were located in the ventral striatum/olfactory tubercule region. These neurons were not fundamentally different in their responses, compared to orbitofrontal cells.

GENERAL METHOD

The methods were the same as those described in detail elsewhere (4,28,31,36,37), and are described here only briefly or where they differ.

Recordings

Recordings were made from single neurons in the primary taste cortex in the frontal operculum (37) and rostral insula (46) and in the secondary taste cortex and adjoining region in the orbitofrontal cortex (31) of three behaving macaques (two *Macaca fascicularis* and one *Macaca mulatta*) weighing between 2.5 and 3.0 kg during testing. Monkeys were fed upon their return to their home cages, and were given access to water ad lib. Glass insulated tungsten microelectrodes were constructed in the manner of Merrill and Ainsworth (15), but without platinum plating. A computer (Microvax II: Digital Equipment Corporation or IBM PC) collected the spike arrival times and displayed summary statistics or a peristimulus time histogram and rastergram on line.

Localization of Recording Sites

X-radiographs were used to locate the position of the microelectrode after each recording track relative to permanent reference electrodes and to the anterior sphenoidal process. Sphenoid was used as a reference due to its visibility on X-radiographs and because it is a bony landmark that has a relatively invariant position with respect to brain structures (1,2). The mean position of the tip of the sphenoid process is 11 mm dorsal and 20 mm anterior to ear-bar zero in this species. During the final recording tracks in each monkey, microlesions were made through the tip of the recording electrode to mark the location of typical units. These lesions allowed the positions of all cells that were known with respect to bony landmarks to be reconstructed in the 50-μ brain sections (10).

Gustatory Stimuli

The gustatory stimuli used were 1.0 M glucose (G), 0.1 *M* NaCl (N), 0.01 *M* HCl (H), 0.001 *M* QHCl (Q), and 0.1, 0.01, and 0.001 *M* monosodium glutamate (M), 0.05 *M* glutamic acid, and 0.0001 *M* inosine 5′-monophosphate (I). The concentrations of most of the tastants were chosen because of their comparability with our previous studies, and because they are in a sensitive part of the dose–response curve. The concentration of glutamic acid was chosen to be close to that of 0.1 *M* monosodium glutamate, which we have used in previous studies, but within the solubility at room temperature of glutamic acid. The concentration of inosine 5′-monophosphate used was that which in human studies can potentiate the effects of MSG, and which tests on one of the authors showed could be tasted. For additional comparisons, the neuronal responses were also tested to a range of foods including banana, orange, apple juice, milk, and 20% blackcurrant juice. The monkey's mouth was rinsed with distilled water during the intertrial interval (which lasted at least 30 s, or until neuronal activity returned to baseline levels) between taste stimuli. The stimuli within a set were delivered in random sequence. The stimuli were delivered in quantities of 0.2 ml with a hand-held 1-ml syringe. For chronic recording in monkeys, this manual method for stimulus delivery is used because it allows for repeated stimulation of a large receptive surface despite different mouth and tongue positions adopted by the monkeys (36).

Treatment of Results

Analyses of variance were performed on the responses of each cell to the different stimuli, measured in a 3-s period following the onset of stimulus delivery. The one-way ANOVA for each cell was performed over the entire range of taste stimuli and the spontaneous firing rate to determine whether a neuron responded differently to chemosensory stimulation compared to nonchemosensory activity. If a significant difference between the responses to the different stimuli was indicated ($P < 0.05$ was the criterion, although for most cells described here it was $p << 0.001$), then subsequent post hoc Newman–Keuls' analyses were performed to assess and compare the individual efficacies of the different stimuli. The multidimensional scaling described later was performed with the statistical package SPSS, and the cluster analysis with Systat. Because these analyses were derived from correlations based on firing rates, these analyses were performed to reflect the continuous nature of the data available, by using interval levels in the analyses. The analysis and data presentation in this paper utilised the neuronal responses to the different stimuli, that is, the firing rate to a stimulus minus the spontaneous firing rate of the neuron. (The mean spontaneous firing rate of the neurons described here was 5.4 spikes/s. For comparison, the mean firing rate to the most effective taste stimulus of the neurons described here was 22.7 spikes/s.)

EXPERIMENT 1

The aim was to extend the previous investigation in primates in which monosodium glutamate was used as the umami tastant, by using glutamic acid as the tastant. The use of glutamic acid as a tastant potentially allows further evidence to be obtained for activation by umami of a different system to that activated by NaCl, for it allows administration of the glutamate ion without administration of the sodium ion.

Method

The set of tastants 1.0 *M* G, 0.1 *M* N, 0.01 *M* H, 0.001 *M* Q, 0.1 *M* M, and 0.05 *M* glutamic acid (GLA) was tested in

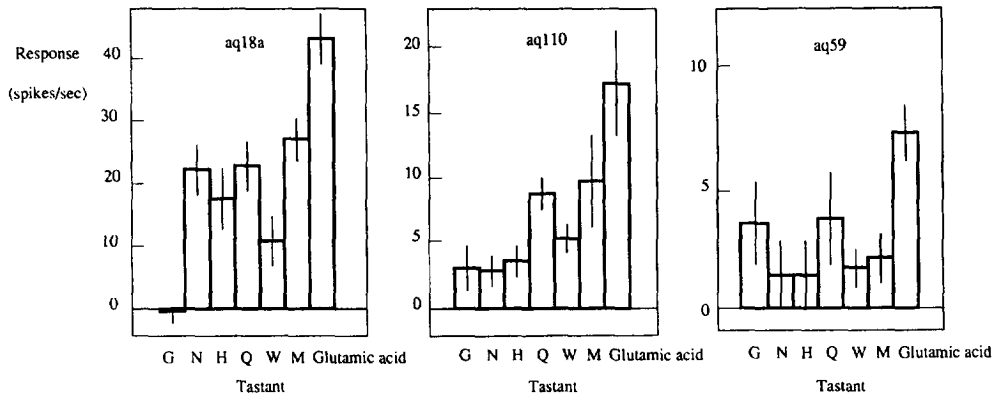

FIG. 1. Examples for three cells of the response profiles of cells in the taste cortex that responded best to glutamic acid. The means and the SEs of the responses calculated over four to six presentations of each tastant in random sequence are shown. The set of tastants was 1.0 *M* glucose (G), 0.1 *M* NaCl (N), 0.01 *M* HCl (H), 0.001 *M* QHCl (Q), distilled water (W), 0.1 *M* monosodium glutamate (M), and 0.05 *M* glutamic acid.

random sequence, as described above. After each tastant had been presented once, the set was repeated in a new random sequence. This was repeated 4–10 times for each cell.

Results

It was possible to complete the testing for 70 taste-responsive cells. The population of neurons analysed for taste responsiveness in this study included more than 1000 cells.) The proportion of taste cells found in the orbitofrontal cortex and these related regions was thus just less than 7%, in line with previous investigations [see (31,32) and discussions therein]. Of the 70 cells analysed in this experiment, the great majority, 63, were in the orbitofrontal cortex, and the remaining cells were in the nearby regions shown in Fig. 11. Examples of the response profiles of the cells to the set of tastants are shown for three cells in Fig. 1. This shows that some of the cells had large responses to 0.05 *M* glutamic acid. It also shows that some of the cells that responded to glutamic acid also responded to monosodium glutamate, and did not necessarily have large responses to 0.01 *M* HCl. (The pH of the glutamic acid was 2.1).

For the cells that responded maximally to glutamic acid ($n = 9$), their mean responsiveness to each of the stimuli in the set is shown in Fig. 2. It is shown that the GLA-best cells tended also to respond well to monosodium glutamate. Across this subset of GLA-best cells, the correlation between the stimuli calculated from the response profiles showed that the correlation of GLA with monosodium glutamate was 0.97, with glucose was −0.04, with NaCl was 0.88, with Q was 0.94, and with HCl was 0.84. Thus, the correlation between the responses of these cells to glutamic acid and monosodium glutamate was high, but there was also a high correlation to quinine in this subset of GLA-best cells.

To test how similarly the whole population of 70 cells respond to the two umami tastants monosodium glutamate and glutamic acid compared to other stimuli, the correlations of the responses of all 70 cells to each pair of stimuli out of M, GLA, G, N, H, and Q were computed. The Pearson correlation coefficients are shown in Table 1. These correlations were based on the response of the 70 neurons to these tastants, that is, they were calculated with the spontaneous firing rate subtracted. It can be seen from Table 1 that the correlation between the responses of this population of neurons to M and GLA was 0.75, and that this similarity was greater than most other correlations between stimuli shown in Table 1, and was higher than the correlation of glutamic acid with any other stimulus. (The other somewhat high correlation of glutamic acid with another tastant was 0.71 with HCl, which is consistent with the fact that both are acidic, and that some neurons in the population reflect the acidity of tastants.)

Another way of indicating the relationships between the responses of this population of 70 neurons to the tastants is by

TABLE 1

CORRELATION COEFFICIENTS BETWEEN THE PROFILES OF RESPONSES GENERATED BY EACH STIMULUS ACROSS THE POPULATION OF 70 CELLS

	G	N	H	Q	W	M	GLA
G	—						
N	0.43	—					
H	0.46	0.70	—				
Q	0.35	0.61	0.66	—			
W	0.47	0.72	0.79	0.66	—		
M	0.60	0.69	0.73	0.57	0.65	—	
GLA	0.34	0.62	0.71	0.61	0.62	0.75	—

G, glucose; N, NaCl; H, HCl; Q, quinine-HCl; W, distilled water; M, monosodium glutamate; GLA, glutamic acid.

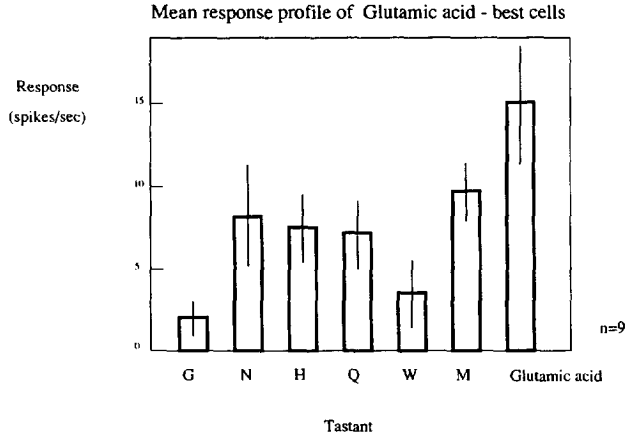

FIG. 2. The mean response profile of the nine cells analyzed in the taste cortex that responded best to glutamic acid. The means and SEs of the means of the responses are shown. The abbreviations are as in Fig. 1.

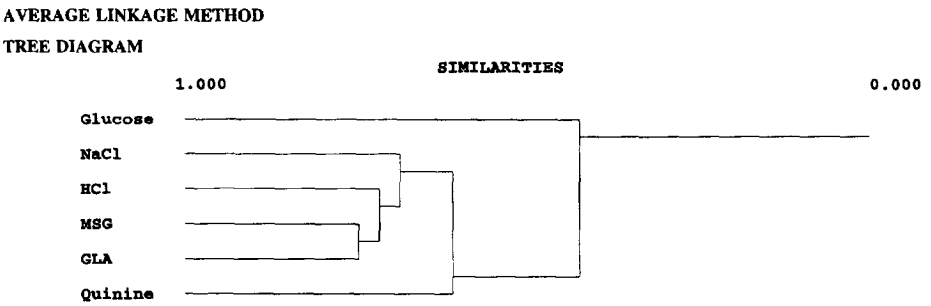

FIG. 3. A dendrogram showing the degree of clustering between the responses to the different stimuli. GLA, glutamic acid. The other abbreviations are as in Fig. 1. A dissimilarity of −1.0 indicates close similarity. This cluster analysis shows that the responses to glutamic acid and monosodium glutamate were relatively similar to each other; and that they were more similar to each other than either was to NaCl.

using cluster analysis. The correlation matrix was used as the basis for a cluster analysis of the responses of the neurons to the set of stimuli, the results of which are shown in Fig. 3. This hierarchical clustering was produced using the average linkage method on the correlation coefficients. It can be seen from this figure that M and GLA cluster together. Moreover they form a separate cluster from N and H (so that the M and GLA cluster does not represent just Na^+ or H^+ sensitivity). Thus, monosodium glutamate and glutamic acid produce responses in the population of neurons that are relatively similar to each other, and relatively different from responses to N and H. The fact that GLA and M do not cluster totally separately from the other tastants may reflect that fact that analyses based on the correlations of the responses of the population of neurons to the set of stimuli reflect how similar the average responsiveness of the population is to the different stimuli, and that GLA and M contain acidic and sodium components, respectively.

To present these data in another readily interpretable form, multidimensional scaling (MDS) was performed on the correlation matrix computed for Table 1. The correlation matrix shows the similarity among stimuli by comparing the profiles of activity that each stimulus evokes across the 70-neuron sample (31). Such a multidimensional space represents the positions of stimuli relative to one another. The results of this are shown in Fig. 4. This figure shows the stimuli spread in general well apart in a three-dimensional space. The major dimension of the figure, dimension 1, emphasises the hedonic qualities of the tastants, with the highly palatable glucose at one extreme and the unpalatable quinine at the other extreme of dimension 1. M and GLA are outside a tetrahedron formed from the responses to G, N, H, and Q. Moreover, M and GLA are placed close together in dimension 2. (Results are shown for a three-dimensional space, which accounted for 98% of the variance. A two-dimensional space accounted for 92% of the variance.)

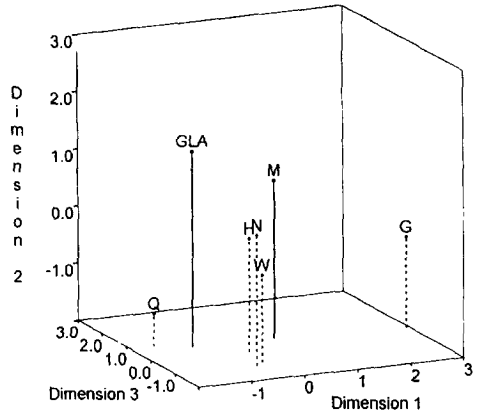

FIG. 4. A three-dimensional scaling of the matrix of correlations between the responses to glutamic acid (GLA), monosodium glutamate (M), the four prototypical stimuli (G, N, H, Q), and water (W).

EXPERIMENT 2

The aim was to test the responsiveness of taste neurons to one of the nucleotide umami tastants, inosine 5′-monophosphate (IMP), and to investigate in particular whether neurons activated by glutamate were also activated by inosine 5′-monophosphate, or whether inosine 5′-monophosphate activated a separate population of neurons, or whether inosine 5′-monophosphate activated neurons that were activated best by one or some of the other tastants.

Method

The set of tastants 1.0 *M* G, 0.1 *M* N, 0.01 *M* H, 0.001 *M* Q, 0.1 *M* M, and 0.0001 *M* inosine 5′-monophosphate (IMP) was tested in random sequence, as described above. This is a low concentration of IMP, but was chosen as it was found in our preliminary studies to be effective in producing neuronal responses in macaques. This concentration is just below the human detection threshold for IMP alone (41), but is a concentration that appears to be able to affect the human taste system, in that in humans this is in the concentration range that has a synergistic effect with monosodium glutamate.

Results

It was possible to complete the testing for 18 cells. Examples of the response profiles of the cells to the set of tastants are shown for three cells in Fig. 5. This shows that some of the cells had responses to 0.0001 *M* IMP. It also shows that some of the cells that responded to IMP also responded to monosodium glutamate.

TABLE 2

CORRELATION COEFFICIENTS BETWEEN THE PROFILES OF ACTIVITY OF 18 CELLS GENERATED BETWEEN INOSINE 5′-MONOPHOSPHATE AND EACH OF THE OTHER STIMULI

	G	N	H	Q	M	W
IMP	0.15	0.55	0.32	0.44	0.80	0.37

IMP, inosine 5′-monophosphate; G, glucose; N, NaCl; H, HCl; Q, quinine-HCl; M, monosodium glutamate; W, water.

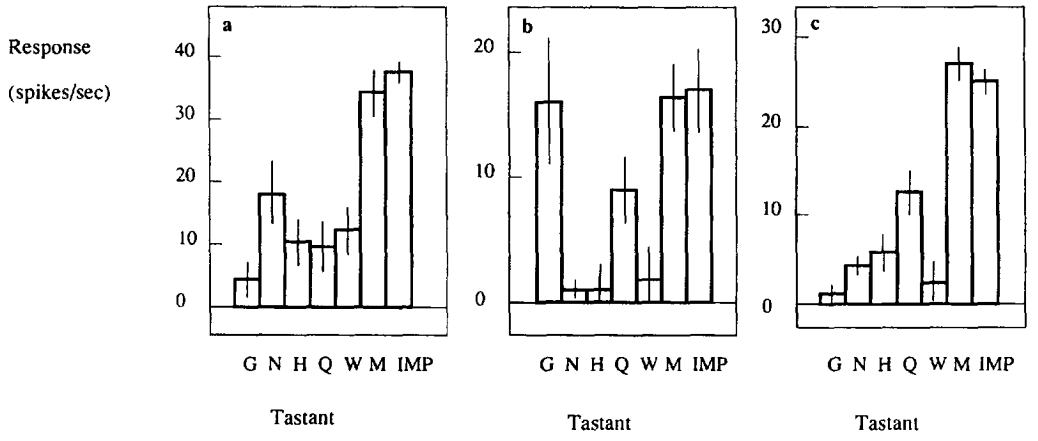

FIG. 5. Examples for three cells of the response profiles of cells in the taste cortex that responded to inosine 5'-monophosphate (IMP, 0.0001 *M*). The mean and SEs of the responses calculated over four to six presentations of each tastant in random sequence are shown. The set of tastants was 1.0 *M* glucose (G), 0.1 *M* NaCl (N), 0.01 *M* HCl (H), 0.001 *M* QHCl (Q), distilled water (W), 0.1 *M* monosodium glutamate (M).

Across this set of 18 cells, the correlations between IMP and the other stimuli calculated from the response profiles are shown in Table 2. This shows that across the population of cells, IMP produced responses that were much more similar to M than to any of the other tastants (Pearson correlation coefficient of 0.80).

EXPERIMENT 3

One aim was to assess the responsiveness of the system to monosodium glutamate, by measuring dose–firing rate response curves. Another aim was to investigate whether the nucleotide inosine 5'-monophosphate synergises with the effect of monosodium glutamate on neurons that respond to monosodium glutamate. We obtained evidence on these issues by performing dose–response curves for different concentrations of glutamate with and without the addition of inosine 5'-monophosphate (IMP), while recording from single neurons.

Method

The set of tastants 0.001, 0.01, 0.1 *M* monosodium glutamate and water, with and without the addition of inosine 5'-monophos-

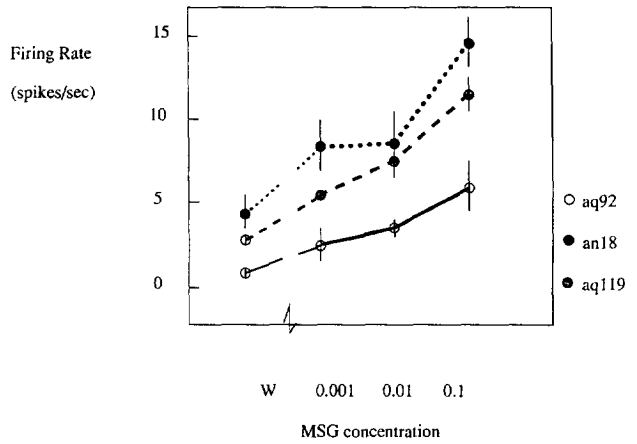

FIG. 6. Concentration–response curves for the effects of monosodium glutamate (MSG) on the firing rate of three cells in the taste cortex. The means and the standard errors of the responses calculated over four to six presentations of each tastant in random sequence are shown. W, water.

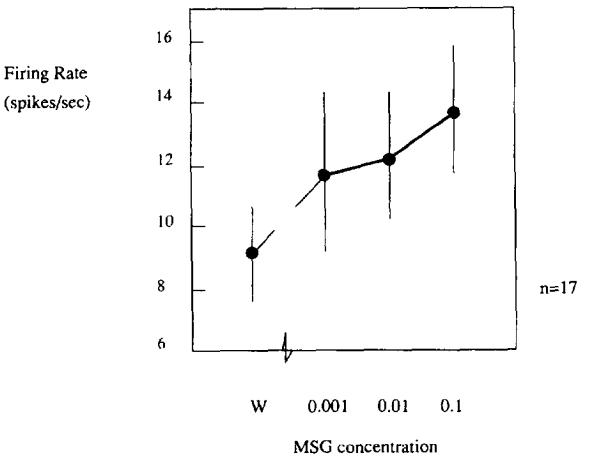

FIG. 7. Average concentration–response curves for the effects of monosodium glutamate (MSG) on the firing rate of 17 cells in the taste cortex. The means and SEs of the responses are shown. W, water.

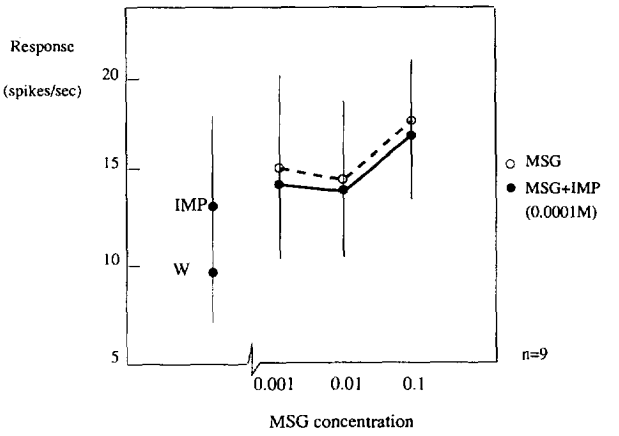

FIG. 8. Concentration–response curves for the effects of monosodium glutamate (MSG) on the firing rate of nine cells in the taste cortex, in the absence or presence of 0.0001 *M* inosine 5'-monophosphate (IMP). For comparison, the response to IMP alone is also shown. The means and SEs of the responses are shown. W, water.

phate to make a concentration of 0.0001 *M*, was tested in random sequence, as described above. The criterion for inclusion in this study was that cells should show a response to 0.1 *M* glutamate that was at least 5 spikes/s greater than the response to water.

Results

Concentration–response curves for the effects of glutamate on the firing rate of three different cells are shown in Fig. 6. Average concentration–response curves for the population of 17 cells tested are shown in Fig. 7. For three cells, the response to the weakest concentration of glutamate was significantly greater than the response to water (*t*-test, $p < 0.05$).

The effects of inosine 5′-monophosphate on the responses to different concentrations of glutamate are shown in Fig. 8, for the nine cells in which responses to the mixtures was measured. It was found that the 0.0001 *M* IMP alone had an effect as great as the 0.01 *M* G. Perhaps partly because even this low concentration of IMP was quite effective as a taste stimulus in producing neuronal responses, synergism was not found in this population of neurons.

EXPERIMENT 4

It has been shown that feeding to satiety decreases the responses of orbitofrontal taste cortex neurons to a food with which a monkey has been fed to satiety (30). Such a modulation of taste responses by hunger has not been found in the primary taste cortex (29,45). Moreover, the reduction in neuronal responsiveness in the secondary taste cortex is at least partly specific to the food with which the monkey has been fed to satiety. This is thus a sensory-specific reduction in responsiveness (30). The aim of this experiment was to investigate whether satiety induced by feeding with monosodium glutamate solution would affect the responses of cells, and if so, whether the response would be sensory-specific. A modulation of responsiveness by hunger would implicate the neurons in a system involved in motivational responses to food. A demonstration of sensory-specific satiety would add further evidence for a separate neural mechanism for the perception of umami taste.

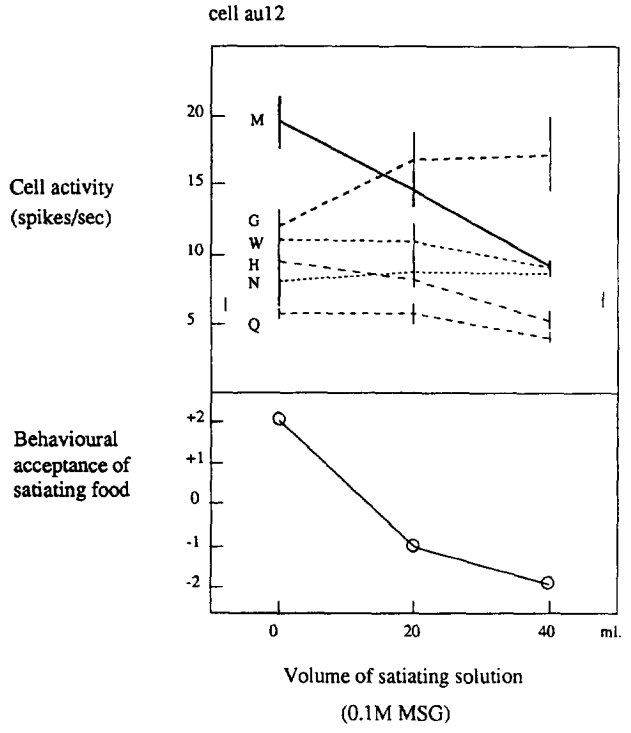

FIG. 9. The effect of satiation induced with 0.1 *M* monosodium glutamate on the response of a cell to the tastes of glucose (G, 1.0 *M*), NaCl (N, 0.1 *M*), HCl (H, 0.01 *M*), quinine-HCl (Q, 0.001 *M*), distilled water (W), and monosodium glutamate (M, 0.1 *M*). The behavioral acceptance of the satiating fluid is illustrated below the neuronal response. The scale runs from keen avid acceptance (+2), through neutral (0), to firm rejection (−2) [see Rolls et al. (30)].

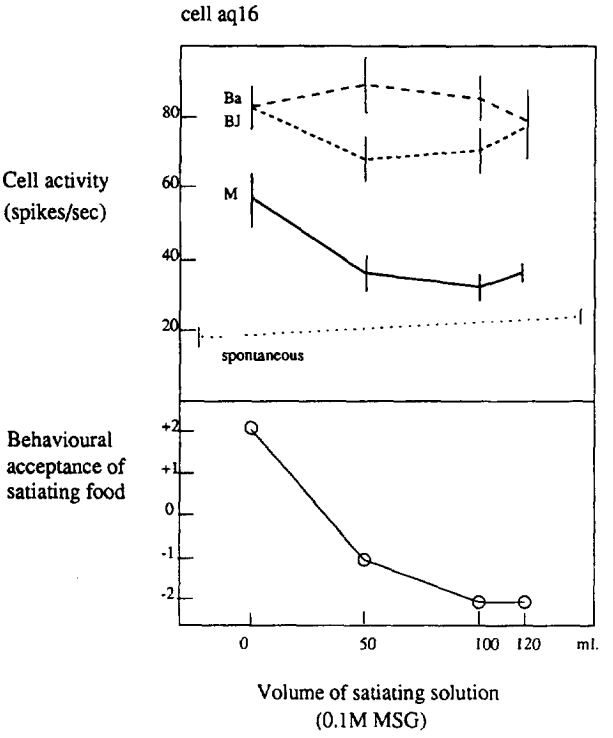

FIG. 10. The effect of satiation induced with 0.1 *M* monosodium glutamate on the responses of an orbitofrontal visual cell responding to the sight of food. The change in neuronal responses to the sight of banana segments (Ba), a syringe containing blackcurrant juice (BJ), and a syringe containing 0.1 *M* monosodium glutamate (M), are illustrated above the data indicating the behavioral stage of satiety.

Method

Cells that responded to the taste of monosodium glutamate, or that responded to the sight of food [see (32,39)], were tested before, during, and after feeding a monkey with 0.1 *M* monosodium glutamate until behavioral satiety was achieved. Taste-responsive cells were tested to measure their responses to the tastants glucose, HCl, NaCl, quinine-HCl, and monosodium glutamate. In cells responsive to the sight of foods, the response to a clearly marked syringe (marked with a large black square) that contained the tastant monosodium glutamate (0.1 *M*) was compared to the responses to the sight of a variety of other foods. Satiety was induced by feeding the monkey 0.1 *M* monosodium glutamate rapidly while recording the behavioural acceptance as a function of volume consumed. The responses of cells were measured at varying stages in the delivery of the satiating solution, and after the monkey was satiated. As the monosodium glutamate was not as preferred as, for instance, glucose, the

volume of monosodium glutamate required to produce satiety was typically 40—150 ml.

Results

It was possible to perform experiments studying the effect of satiety on taste responses to glutamate on five neurons. All these neurons were in the orbitofrontal cortex. The responses of one of these neurons during feeding to satiety are shown in Fig. 9. The response to the taste of glutamate decreased from a value of 19.5 spikes/s when the monkey was hungry to a value of 9.1 spikes/s when the monkey was satiated. A similar reduction was not found for the other tastants, and indeed the response to the taste of glucose increased (see Fig. 9). There was a significant interaction between the responses to the different tastants and feeding to satiety [two-way ANOVA, $p < 0.01$, $F(1, 46) = 9.8$]. In two of the other four taste neurons also tested in this way, there was a generalised decrease in the response to monosodium glutamate and to the other tastants. A larger volume of satiating fluid was used in both cases (125 and 200 ml), and this may account for the non-sensory-specific modulation of responsiveness in these cells. Satiety experiments using monosodium glutamate were performed on three cells responsive to the sight of food. The response to the sight of an MSG-containing syringe was decreased after satiety in two of the three food responsive visual neurons. In one case this was a sensory-specific effect, where the response to the MSG-containing syringe was significantly decreased ($p < 0.02$) (to the level of the spontaneous activity) during behavioural satiation, whereas the cell remained unchanged in its responses to foods such as banana, or a blackcurrant juice-containing syringe (see Fig. 10).

RECORDING SITES

Of 74 cells, 65 were in the orbitofrontal cortex taste region. The remaining nine were located posterior to this and more medially in the olfactory tubercle/ventral striatum region [cf. (40)] shown in Fig. 11. Of the 70 cells in Experiment 1, 63 were in the orbitofrontal taste cortex. The cells with best responses to umami tastants were not clustered together, but were found intermingled with the population of taste neurons analysed, the recording sites of which are shown in Fig. 11.

DISCUSSION

First, it was shown that single neurons that had their best responses to sodium glutamate also had good responses to glutamic acid. The correlation between the responses to these two tastants was higher than between any other pair, which included, in addition, a prototypical set including glucose (sweet), sodium chloride (salty), HCl (sour), and quinine HCl (bitter). Accordingly, the responsiveness to glutamic acid clustered with the response to monosodium glutamate in a cluster analysis with this set of stimuli, and glutamic acid was close to sodium glutamate in a space created by multidimensional scaling. Second, it was shown that the responses of these neurons to the nucleotide umami tastant inosine 5'-monophosphate were more correlated with their responses to monosodium glutamate than to any proto-

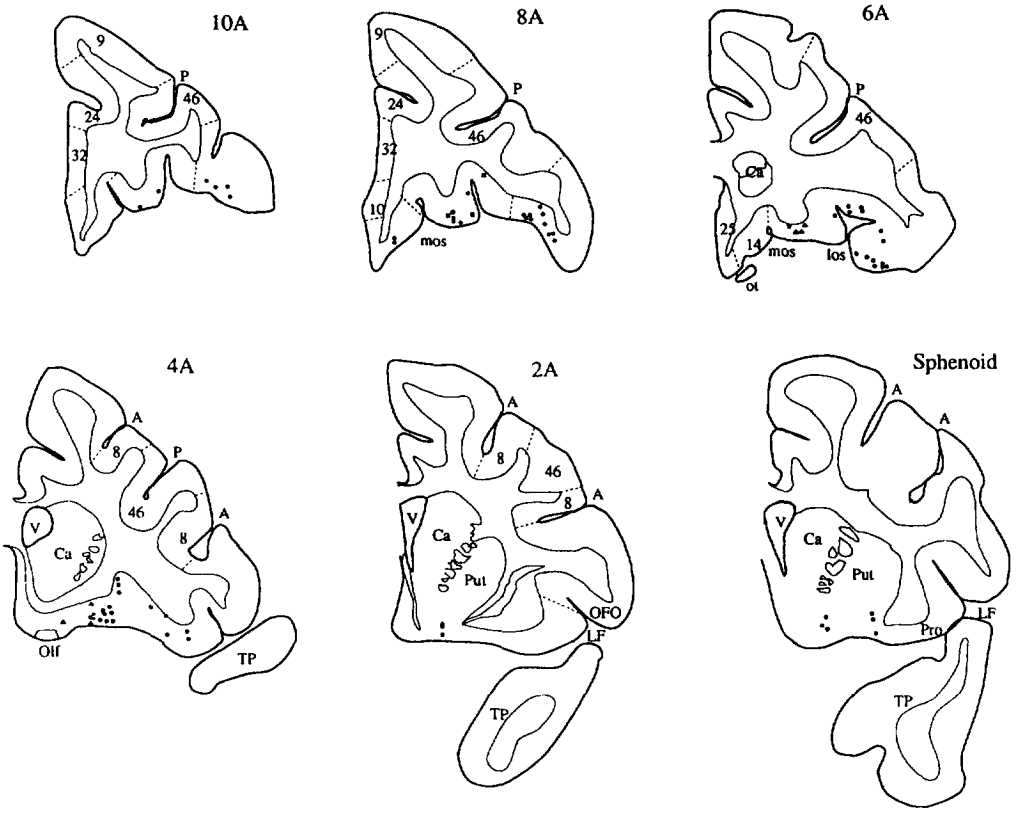

FIG. 11. Examples of the recording sites in the orbitofrontal cortex taste area of neurons recorded in this study. The symbols refer to different monkeys. The coronal sections are at different positions in mm anterior (A) to the sphenoid reference.

typical tastant. Third, concentration–response curves showed that concentrations of monosodium glutamate as low as 0.001 *M* were just above threshold for this population of neurons. These findings show that the taste neurons activated by monosodium glutamate can also be activated by other umami tastants, including glutamic acid and the nucleotide inosine 5′-monophosphate. The responses to these umami tastants were more similar to each other than to any of the other prototypical tastants, providing evidence that in this system umami is encoded differently from the other tastants. Moreover, the findings with these tastants provide additional evidence that the responses to monosodium glutamate are not due just to activation of a sodium taste channel.

Lastly, there is evidence that for at least some taste responsive neurons, their responsiveness was decreased by feeding to satiety. For one of these neurons, the decrease was sensory-specific. It was of interest that feeding an animal to satiety with monosodium glutamate increased the responsiveness of this neuron to a sweet taste. This parallels the finding that in humans, feeding to satiety with a protein meal can increase the pleasantness of the taste of sweet foods (24). Similarly, some evidence was obtained that modulation of hunger, as produced by feeding to satiety with monosodium glutamate, influenced the responses of neurons to the sight of food. These findings are consistent with the hypothesis that the orbitofrontal cortex is involved in behavioral responses to foods (25,27,30).

This study confirms the findings of Baylis and Rolls (4) that the umami taste is encoded independently from the conventional basic taste qualities by orbitofrontal neurons. Gustatory responses of single neurons selective to monosodium glutamate have been reported from the primary taste cortex and lateral hypothalamic areas of macaques (21,22). In the study of primary taste cortical regions in the primate, Plata–Salaman et al. (22) did not demonstrate a clear independence of the umami taste within the primary taste cortex. Given the role of the orbitofrontal cortex in food selection and the control of motivational behaviour to food (3,25,27,30), the independent encoding of motivationally and ethologically significant foods becomes more important than earlier in the taste system. This would enable processes such as sensory-specific satiety to remain specific to individual foods, thereby allowing a finer control of nutrient intake.

A recent study (8) examined the representation of the astringent taste quality conveyed by tannic acid, another taste quality of great biological relevance to primates, including humans. This study demonstrated that the representation of tannic acid in the responses of orbitofrontal gustatory neurons was also independent of the primary taste qualities. Ethologically, the ability to perceive dietary tannins offers a nutritional and hence survival advantage to primates where there is limited availability of dietary protein. The astringent taste sensation of tannins does itself carry a motivational signal. The long-lasting and drying nature of oral astringency is itself an inducement for a leaf-eating monkey to seek out sweet young leaves to eat (12). Although no clear relationship between the representations of tannic acid and monosodium glutamate was demonstrated, the "umami" taste quality is likely to have a reciprocal role to the astringent taste quality of tannins in the regulation of protein intake of both arboreal and more omnivorous primates.

Historically, there have been many attempts to classify tastes, yet the idea that there exist four distinct qualities has been predominant. Sweetness, saltiness, sourness, and bitterness have been considered to be primary taste qualities, and evidence to support this comes from a) independent receptor signal transduction mechanisms; b) the neural code, such that there are distinct channels for each taste quality; c) chemotopic organization over the gustatory receptor surface; d) different concentration response functions; e) a different time course of the perceptual and neural response; f) lack of significant neural or perceptual cross-adaptation between the taste qualities; g) the existence of taste modifiers that are specific to the taste qualities; h) ethological considerations, with each taste quality serving a distinct physiological function (e.g., sweetness—energy requirements, saltiness—electrolyte balance, quinine—avoiding toxins). The sweet, sour, bitter, and salty taste qualities do largely conform to the above criteria, particularly where psychophysical data are used. However, from the evidence from human studies in which semantic components are minimised, and from animal behavioural or neural investigations of taste qualities, the notion of primary taste qualities becomes less clearcut (9,33). Despite this, the concept of taste primaries has been widely accepted as a good general approximation of taste processing [reviewed by Scott and Plata–Salaman (35)].

The present study extends the evidence of Baylis and Rolls (4) that there is a separate neural system involved in representing protein (or umami) taste, by showing that the neurons in this system respond not only to monosodium glutamate, but also to other umami taste stimuli such as glutamic acid and inosine 5′-monophosphate.

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