C_S

Responses of Primate Taste Cortex Neurons to the Astringent Tastant Tannic Acid

Hugo D. Critchley and Edmund T. Rolls

Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford OX1 3UD, UK

Correspondence to be sent to: Dr E.T. Rolls, Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford OX1 3UD, UK

Abstract

In order to advance knowledge of the neural control of feeding, we investigated the cortical representation of the taste of tannic acid, which produces the taste of astringency. It is a dietary component of biological importance particularly to arboreal primates. Recordings were made from 74 taste responsive neurons in the orbitofrontal cortex. Single neurons were found that were tuned to respond to 0.001 M tannic acid, and represented a subpopulation of neurons that was distinct from neurons responsive to the tastes of glucose (sweet), NaCl (salty), HCl (sour), quinine (bitter) and monosodium glutamate (umami). In addition, across the population of 74 neurons, tannic acid was as well represented as the tastes of NaCl, HCl quinine or monosodium glutamate. Multidimensional scaling analysis of the neuronal responses to the tastants indicates that tannic acid lies outside the boundaries of the four conventional taste qualities (sweet, sour, bitter and salty). Taken together these data indicate that the astringent taste of tannic acid should be considered as a distinct taste quality, which receives a separate representation from sweet, salt, bitter and sour in the primate cortical taste areas. **Chem. Senses 21: 135–145, 1996.**

Introduction

Tannic acid is a member of the class of compounds known as polyphenols, which are present in a wide spectrum of plant matter, particularly in foliage, the skin and husks of fruit and nuts, and the bark of trees. The tannic acid in leaves is produced as a defence against insects. There is less tannic acid in young leaves than in old leaves. Large monkeys cannot obtain the whole of their protein intake from small animals, insects, etc, and thus obtain some of their protein from leaves. Tannic acid binds protein (hence, its use in tanning) and amino acids, and thus prevents their absorption. Thus, it is adaptive for monkeys to be able to taste tannic acid, so that they can select food sources (typically leaves) without too much tannic acid (Hladik, 1978; C.M. Hladik, personal communication).

In humans, tannic acid elicits a characteristic astringent taste. Oral astringency is perceived as the feeling of long-lasting puckering and drying sensations on the tongue and membranes of the oral cavity. High levels of tannic acid in some potential foods makes them unpalatable without preparative techniques to reduce its presence (e.g. Johns and Duquette, 1991), yet in small quantities it is commonly used to enhance the flavour of food. In this context tannic acid is a constituent of a large range of spices and condiments, such as ginger, chillies and black pepper (Uma-Pradeep *et al.*, 1993). [Tannic acid itself is not present in tea, yet a range of related polyphenol compounds are, particularly in green tea (Graham, 1992)].

In human studies, the mechanical effect of decreased

salivary lubrication has been implicated in the sensation of astringency (Green, 1993; Breslin *et al.*, 1993). The view is held that precipitation of oral mucoproteins produces a rise in friction between oral mucous membranes which results in sensations of dryness and roughness. This has led the authors to label astringency 'a tactile component of flavour'. In a study of the time course of astringent sensations (Lee and Lawless, 1991), the authors found that by subdividing the astringent sensation into six attributes, psychophysical ratings of astringent properties were partly dependent on the taste stimulus used, indicating that there may be multiple subqualities within the perception of astringency. This would be consistent with both gustatory and tactile components to the sensation.

A relationship between tannic acid and sweet taste sensation has been suggested by the following human psychophysical studies. Modulation of the chemical structure of a sweet-tasting protein, thaumatin, by the addition of a pyridoxal group to the molecule, has been found to transform the sweet sensation it elicits to a strongly astringent sensation. The loss of sweetness intensity and the presence of the astringency are likely to be affected by the same mechanisms (Shamil and Benyon, 1990). When tannic acid is repeatedly sampled by sipping, the perceived dryness and bitterness of the astringent sensation both increase with time. The addition of sweeteners attenuates both the dry and bitter aspects of the astringent sensation, and induces an increase in salivary flow (Lyman and Green, 1990).

The following studies in animals provide evidence for an at least partly gustatory, rather than purely tactile, transduction of astringent taste sensation. The astringent taste sensation of tannic acid may be due in part to the action of tannic acid on taste receptor ion channels. Simon *et al.* (1992) demonstrated an inhibitory effect of tannic acid on sodium influx in a canine tongue preparation. There was a pH-independent decrease in measures of amiloride-sensitive sodium channels in the tongue, which are regarded as conveying salty taste, when tannic acid was applied to the lingual surface. Schiffman *et al.* (1992a) showed, in addition, that tannic acid inhibited evoked potentials to glucose in a lingual epithelium. The same group investigated the effect of tannic acid on the chorda tympani responses of rodents (gerbil). A pH-independent inhibition of chorda tympani responses to sweet, sour, bitter and salty stimuli was demonstrated in the presence of tannic acid at a concentration of 0.24 M (Schiffman *et al.*, 1992a). In a second study, a range of astringent compounds, including tannic acid, was shown to elicit robust chorda tympani responses on their own. Tannic acid was effective at concentrations above 0.001 M. There were no responses to astringent compounds in the lingual nerve at concentrations of 0.05 and 0.3 M, implying that the taste system is solely responsible for the responses and that they were not a result of oral mechanical stimulation (Schiffman *et al.*, 1992b). This is consistent with work by Kawamura *et al.* (1969), which showed that glossopharyngeal and chorda tympani nerve fibres, but not lingual nerve fibres, in the rat responded to tannic acid at a concentration of 0.12 M. Kosar and Schwartz (1990) recorded responses from single cells in the rat taste cortex to the oral administration of tannic acid and found responses in acid-sensitive cells to tannic acid at a concentration of 0.05 M (pH = 2.8), so that they did not demonstrate a separate cortical representation of tannic acid (astringent) to that produced by HCl (acid).

In non-human primates there have been few studies of the neurophysiological basis of astringent taste quality. Hellekant *et al.* (1993) mention that 0.0002 M tannic acid was able to elicit a neural response in the chorda tympani of one *Microcebus murinus* monkey.

Tannic acid is a natural antioxidant by virtue of its chemical structure. The dietary intake of tannic acid has a variety of beneficial effects. Studies in which rats were fed high fat diets have demonstrated a cardioprotective effect of tannic acid supplements in lowering plasma lipid and cholesterol levels in both normo- and hyper-cholesterolaemic strains (Yugarani *et al.*, 1992, 1993). The antioxidant properties also show beneficial effects on the development and growth of carcinomas, particularly of the colon in an animal model (Hirose *et al.*, 1991) and a potent inhibitory effect on gastric acid secretion via H + K + ATPase indicates anti-ulcerogenic properties (Murakami *et al.*, 1992).

The aim of this study is to advance knowledge of the control of feeding and food intake through the investigation of the brain mechanisms involved in the identification, selection and processing of sensory information about food substances. The study is conducted in a brain area known to be involved in the linking of taste sensation with motivational factors involved in satiety, and thereby has direct relevance to pathological states, such as eating disorders (Rolls, 1993, 1994, 1995; Rolls *et al.*, 1989), and also to the behavioural changes found in humans after damage to this brain region (Rolls *et al.*, 1994). The majority of the neurons in this study were in the orbitofrontal cortex, which includes the secondary taste cortex (Rolls *et al.*, 1990; Baylis *et al.*, 1994) and also multimodal cortical areas involved in flavour information processing (Rolls and Baylis, 1994), but some

neurons were also recorded in the adjoining primary taste cortex (Yaxley *et al.*, 1990). The particular aim of the current study is to elucidate the neural processing of the astringent tastant tannic acid, which as described above is of biological relevance to the dietary intake of primates including man. Recent investigations have demonstrated the independence of umami (the taste quality ascribed to meat and protein, particularly elicited by monosodium glutamate and 5′-ribonucleotides) from the classical prototypical tastants of sweet, sour, bitter and salt both psychophysically and electrophysiologically (Yamaguchi and Kimizuka, 1979; Baylis and Rolls, 1991; Rolls *et al.*, 1996). It has been hypothesized that astringency is itself a fundamental taste property analogous to sweet, sour, salty and bitter (Boring, 1942). In the light of these findings, the present study was performed to answer similar questions concerning the qualities of the taste of tannic acid, namely:

(i) Are some cortical taste cells specifically tuned to the taste of tannic acid? Particular attention is focused on whether cells respond differently to tannic acid and to HCl (i.e. is the perceived taste derived from the anionic component), and to quinine as an aversive taste.

(ii) Do cells respond on average to tannic acid, as well as to the prototypical taste stimuli or MSG?

(iii) Is tannic acid as different a taste stimulus as the prototypical stimuli are from each other? If tannic acid shares properties similar to the prototypical taste stimuli in these experiments then this is strong evidence that astringency of taste is itself an important prototypical taste in primates.

It is particularly relevant to study the neurophysiology of the astringent taste quality of tannins in rhesus macaques as tannins have been shown to be a major determinant in which foods are selected by rhesus monkeys in their natural environment. Marks *et al.* (1988) studied the influence of hydrolysable tannins on the food choice in a population of free-ranging adult rhesus-macaques in the Himalayan foothills of Pakistan. Records of the feeding behaviour and food selection of a troop of nine monkeys were recorded over a 1-year period. Ninety-one plant samples were collected, representing the range of potential vegetable foods available to the monkeys. These were chemically analysed with respect to the content of condensed and hydrolysable tannins and total astringency. The total astringency was defined as the protein-precipitating capacity of the extractable tannins. It was found that there was a significant negative correlation between the feeding of the rhesus monkeys and the content of phenolic compounds, total astringency and hydrolysable tannins in the food. Other chemicals within the plant matter were not significantly correlated with the food selection of the monkeys. In particular, the total protein and non-structural carbohydrate content of the plant material were not related to food choice. Thus, in the study by Marks *et al.* (1988) it was demonstrated that food selection of this group of rhesus monkeys in their natural habitat was related much more to the astringency of the available food sources rather than the presence of nutritionally more valuable compounds within the plant matter.

Materials and methods

Recordings

Recordings were made from single neurons in the orbitofrontal cortical region which includes the secondary taste cortex of two male behaving rhesus macaques (*Macaca mulatta*) weighing 2.6–3.8 kg. Neurophysiological methods were the same as described previously (Rolls *et al.*, 1976, 1990; Scott *et al.*, 1986; Yaxley *et al.*, 1990). All procedures, including preparative and subsequent ones, were carried out in accordance with the 'Guidelines for the use of animals in neuroscience research' of the Society for Neuroscience, and were licensed under the UK Animals (Scientific Procedures) Act 1986. The monkey was fed on return to its home cage and was allowed access to water *ad lib*. Glass-coated tungsten microelectrodes were constructed in the manner of Merrill and Ainsworth (1972) without the platinum plating. A computer (IBM 486 DX) collected spike arrival times and displayed on-line summary statistics, or a peristimulus time histogram and rastergram. We ensured that recordings were from only a single cell by continuously monitoring the interspike interval to make sure that intervals of less than 2 ms were not seen, and by continuously monitoring the complete waveform of the recorded action potentials using an analog delay line.

Localization of recording sites

X-radiography was used to determine the position of the microelectrode after each recording track relative to permanent reference electrodes and to the anterior sphenoidal process. This is a bony landmark whose position is relatively invariant with respect to brain structures (Aggleton and Passingham, 1981). Microlesions made through the tip of the recording microelectrode during the final few tracks were used to mark the location of typical units. These lesions allowed the position of all cells relative to bony landmarks

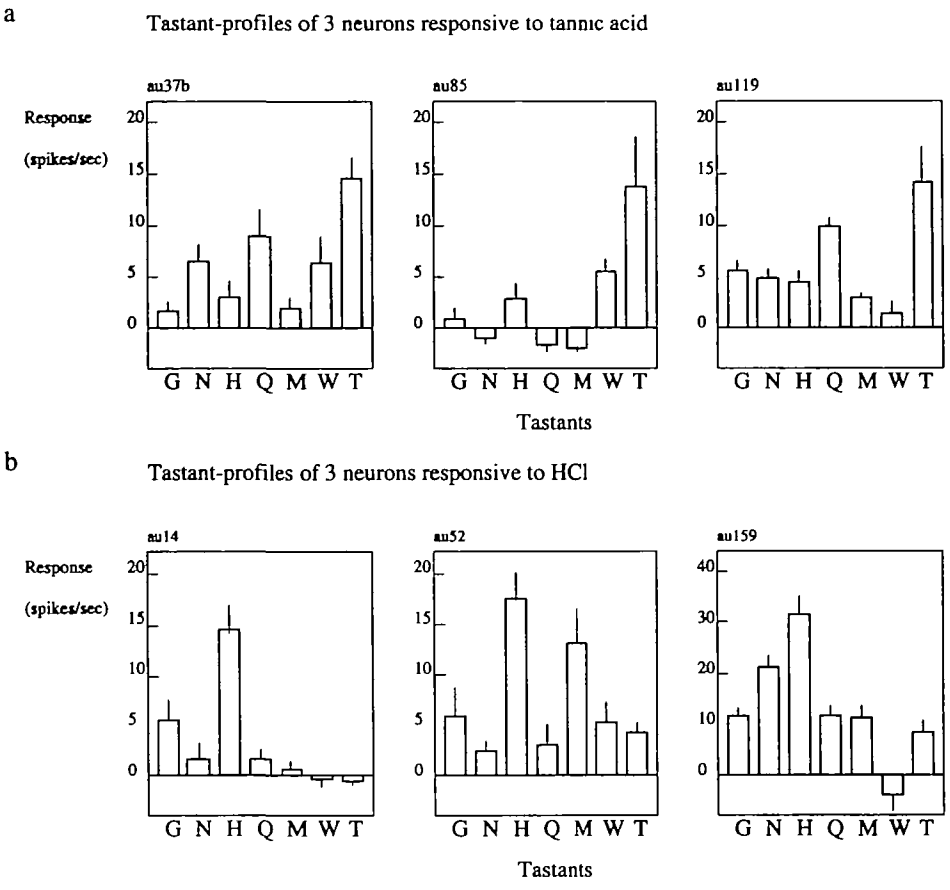

Figure 1 (a) Examples of the response profiles of three single cells in the orbitofrontal cortex which responded best to the taste of tannic acid. The means and standard errors of the responses calculated over four to six presentations of each tastant in random sequence are shown. The set of tastants was 1 0 M glucose (G), 0.1 M NaCl (N), 0.01 M HCl (H), 0.001 M QHCl (Q), deionized water (W), 0 1 M monosodium glutamate (M) and 0 001 M tannic acid (T) (b) Examples of the response profiles of three single cells in the orbitofrontal cortex which responded best to the taste of HCl. The means and standard errors of the responses calculated over the four to six presentations of each tastant in random sequence are shown The abbreviations are as in Figure 1a

to be reconstructed in the 50-μm brain sections with the methods described in Feigenbaum and Rolls (1991).

Stimulus presentation

The gustatory stimuli used were 1.0 M glucose (G), 0.1 M NaCl (N), 0.01 M HCl (H), 0.001 M quinine-HCl (Q), 0.1 M monosodium glutamate (M) and 0.01–0.0001 M tannic acid (T) (generally 0.001 M). The liquid taste stimuli were delivered manually in aliquots of 0.2-ml via a 1-ml syringe. The monkey's mouth was rinsed with distilled water during the intertrial interval, which lasted a minimum of 30 s, or until activity returned to baseline levels. The manual presentation of taste stimuli was chosen to allow the repeated stimulation of a large receptive field despite changing mouth and tongue positions of the monkey (see Scott *et al.*, 1986; Rolls *et al.*, 1990). Neuronal responses were also tested to a selection of complex liquid and solid foods for comparison, e.g. blackcurrant juice, and bite-sized (~1 g) pieces of apple, orange, banana and cereal.

Statistical analyses

Analyses of variance were performed on the response of each cell to the different stimuli. Each firing rate measurement was taken in the 2.5-s time period following stimulus delivery. The response to each stimulus in the set was measured once, then the set was repeated with a new random sequence of tastants 3–7 times, so that there were four to eight measurements of firing rate to each stimulus. A one-way ANOVA was performed to compare the responses to the different stimuli and to the spontaneous firing rate, to determine if there was a significant response to the stimuli. A subsequent one-way ANOVA was performed to determine whether there was a significantly differential response to the stimuli (i.e. the spontaneous firing rate was not included as a condition

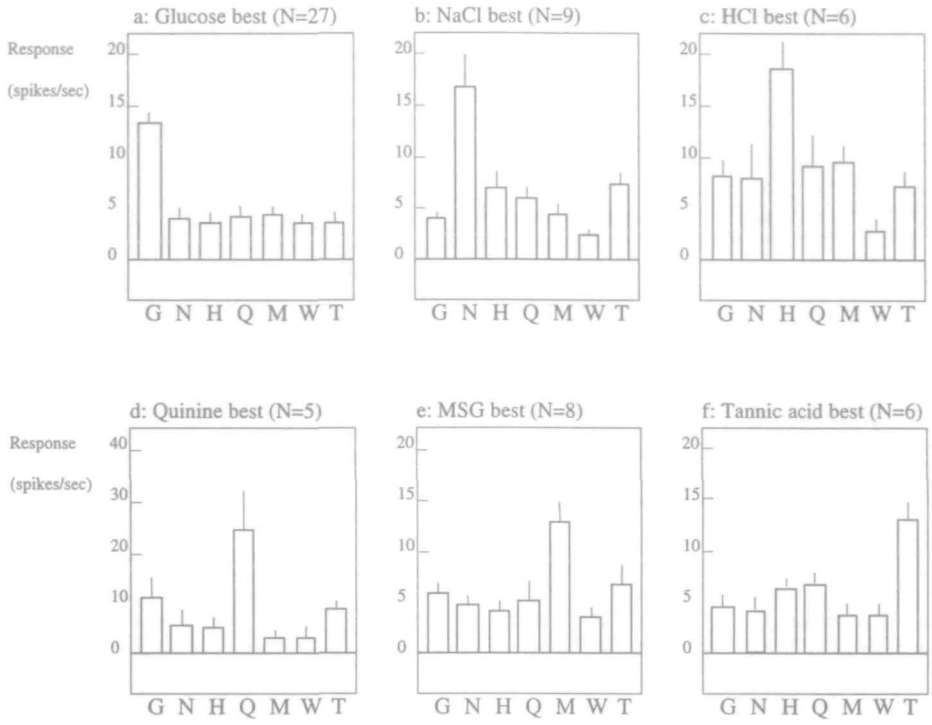

Figure 2 (a–f) The mean response profiles of neurons that respond best to (a) glucose, (b) NaCl, (c) HCl, (d) quinine, (e) monosodium glutamate and (f) tannic acid. The means and standard errors of the responses across the groups of cells are shown The abbreviations are as in Figure 1.

in this ANOVA). If this ANOVA was significant, subsequent *post-hoc* Newman–Keuls' analysis was used to determine the significance of the difference of the responses between particular stimuli. The correlations, cluster analyses and multidimensional scaling described here were performed using the SPSS and Systat statistical packages. In all cases the response of the neuron is given, that is the evoked firing rate of the cell minus the mean spontaneous rate. Overall, the average spontaneous firing rate of all the cells described here was 5.1 (SD = 3.6) spikes/s. (The average peak response of the neurons to this set of tastants was 20.5 (SD 11.7) spikes/s.)

Results

Overview

Seventy-four taste-responsive neurons were tested for responses to the oral administration of tannic acid, glucose, NaCl, HCl, quinine-HCl, monosodium glutamate and distilled water. The cells were located predominantly within the orbitofrontal cortex which includes the region of the secondary taste cortex (Rolls *et al.*, 1990; Baylis *et al.*, 1994).

Cells responding best to tannic acid

Figure 1a illustrates the responses of three neurons with best responses among the tastants to tannic acid. The ANOVAs for each of these cells showed that the cells responded differently to the different tastants, and the *post hoc* tests showed for each of the cells that its responses to tannic acid were greater ($P < 0.05$) than to any of the other tastants. [One-way ANOVAs: cell au37b $F(6,21) = 7.3$, $P < 0.01$; cell au85 $F(6,21) = 8.0$, $P < 0.01$; cell au119 $F(6,21) = 16.8$, $P < 0.01$.)

The data shown in Figure 1a indicate that the cells with best responses to tannic acid do not respond to the tannic acid just because of its acidity, in that the cells had little response to HCl (sour). Further evidence on the separate encoding of tannic acid and HCl by the population of neurons analysed is that the HCl-best neurons did not necessarily respond to the tannic acid. Examples of such cells are shown in Figure 1b. The data shown in Figure 1 indicate that some cortical taste neurons respond to HCl and tannic acid in very different ways.

Responses to the different tastants of the population of neurons

Twenty-seven of the 74 cells analysed (36%) had responses which were strongly preferential to the sweet taste of 1.0 M glucose. Nine of the 74 (12%) responded preferentially to the taste of salt (0.1 M NaCl), 6/74 (8%) to HCl, 5/74 (7%) to quinine-HCl and 8/74 (11%) responded maximally to the

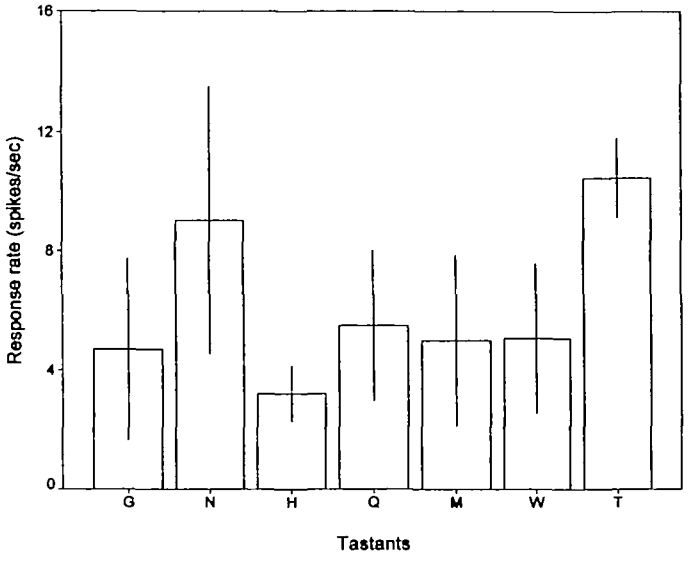

Figure 3 The mean response profile of 11 cells which responded more to tannic acid than to HCl. The difference in the response had to be significant and greater than 5 spikes/s. The means and standard errors of the responses across the cells are shown

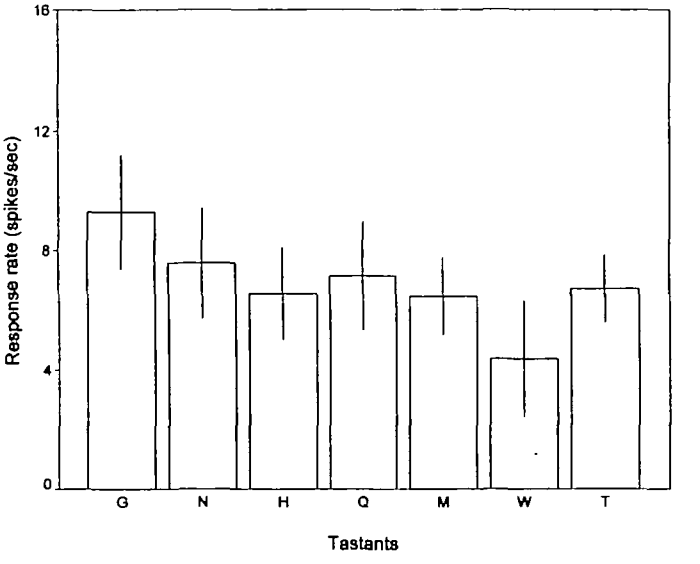

Figure 4 The mean response profile of the 74 taste responsive cells in this study The means and standard errors of the responses across the cells are shown The abbreviations are as in Figure 1.

taste of monosodium glutamate. In addition to these cells, there were six cells (8%) that responded maximally to the taste of tannic acid. The remaining cells (13/74 or 18%) had near-maximal responses to more than one of the prototypical stimuli (including MSG). One cell was preferentially responsive to water.

Further evidence on the tuning of these neurons is shown by the mean response profiles of the neurons to each of the tastants (Figure 2). Separate profiles are shown for the groups of neurons with best responses to each of the tastants. It can be seen from Figure 2f that across the group of cells with best responses to tannic acid, there was no significant difference between the mean responses to the other stimuli. This indicates that information about the taste of tannic acid is unlikely to be carried by the same set of neurons that respond to, for example, the bitter taste of quinine, or the sour taste of HCl. Furthermore, it is also shown in Figure 2a–e in the response profiles of glucose, NaCl, HCl, quinine and MSG-best cells, that tannic acid does not produce large responses on average in these populations of cells. These profiles indicate that the taste of tannic acid is encoded differently from the other tastants and with the same degree of independence as the other tastants.

Further evidence on the different encoding by this population of neurons of tannic acid (astringent) and HCl (sour) is that a total of 11 (15%) neurons responded significantly more to tannic acid than to HCl (a minimal difference of 5 spikes/s was also required as a criterion). The mean response profile of this group of 11 neurons is illustrated in Figure 3. The subpoulation of neurons that responded much more to tannic acid than to HCl included some neurons that responded maximally to NaCl. Taken together these data indicate that cortical taste neurons exist that can differentiate between the tastes of tannic acid and HCl, and thus the neuronal responses reflect the different perceptual qualities (sour and astringent) ascribed to these tastants.

Mean responses of the taste cells

Figure 4 shows the average responses across all the cells to the tastants. The glucose preference of the cells is reflected in the higher mean response to the taste of glucose. Distilled water did not evoke a strong response in these cells. For the remaining tastants, there was no significant difference in the magnitude of the firing across the population of cells produced by the different tastants. Tannic acid, therefore, produces as much neuronal activity in this population of cells as each of the other tastants apart from glucose (and water). This adds to the evidence that the taste quality of tannic acid is strongly represented in this brain region.

Concentration of tannic acid

The concentration of tannic acid used in these experiments (0.001 M) elicited a perceptible and distinct taste in human subjects, and was effective in activating a set of neurons in the orbitofrontal taste cortex as described here. In addition, an extensive concentration-response curve was completed

Table 1 Correlation coefficients beteween the profile of activity across 74 neurons generated by each stimulus

	G	N	H	Q	M	W
N	0.16					
H	0.21	0 62				
Q	0.27	0.60	0.54			
M	0.41	0.49	0.49	0.44		
W	0.42	0.24	0.24	0.42	0.24	
T	0.02	0.62	0.56	0.44	0.40	0.20

G—glucose; N—NaCl; H—HCl; Q—quinine-HCl; W—distilled water, M—monosodium glutamate; T—tannic acid.

for one neuron. A graded increase in the firing rate from 12 spikes/s to 20 spikes/s was found as the concentration increased from 0.0001 to 0.1 M. (The firing rate to water was 9 spikes/s.) Concentrations of tannic acid below 0.001 M did not give neuronal responses that were significantly different from distilled water or from the spontaneous firing rate of the cell.

Similarity measures across the cell population

A 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 74 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 $N(N-1)/2$ ($N = 74$) correlations into a matrix. This procedure was used to provide a measure of how similar the responses of the cells were to tannic acid and to each of the other stimuli. A high Pearson correlation between two stimuli would indicate that the responses to these tastants were related across the cell population. Table 1 shows the correlation matrix for the tastants. The correlation matrix as a whole is significant (Bartlett's Chi-square, $\chi^2 = 184.7$, d.f. $= 21$, $P < 0.001$). The matrix shows that generally tannic acid is as similar to the tastants NaCl, HCl, quinine and MSG as they are to each other. Tannic acid is least well correlated with the taste of glucose, a feature which may underlie the perception of astringency.

Hierarchical cluster analysis of the responses of cells to taste stimuli was used to provide a means of visualizing the relationship between the stimuli. The Pearson correlation matrix was used to provide the distance measures between stimuli. In the hierarchical cluster analysis procedure, the first link is made between the two most highly correlated stimuli, which are joined at the point of their Pearson correlation. The resulting dendrogram has links which indicate the way in which the stimuli are grouped, with the most similar stimuli in the part of the dendrogram where the Pearson correlation is highest. Figure 5 shows the hierarchical cluster analysis for the 74 cells in this experiment. There are two main groups; glucose and water are strongly separated from the remaining stimuli. The dendrogram serves to emphasize the finding that tannic acid is as different from the other prototypical stimuli as they are from each other.

Stimulus space and Henning's tetrahedron

Another effective way of illustrating the relationship between the taste stimuli is by means of multi-dimensional scaling (MDS) (see Schiffman and Erickson, 1971; Rolls *et al.*, 1994). The Euclidean distance between the data points (using an interval measure of response firing rates between the stimuli) was used to calculate a space of one or more dimensions of a hypothetical space that explains as much of the variance between the stimuli as possible. For the data from 74 cells, a one-dimensional solution explained 68% of the variance, a two-dimensional space explained 93% of the variance, and a three-dimensional solution explained 98% of the variance accounted for by the responses to the stimuli. Figure 6 illustrates the three-dimensional scaling of the stimuli used in this study. Dimension 1 reflects to a degree the hedonic value of the stimuli, with glucose and water separated from the other stimuli. Among the other tastants, tannic acid occupies a position away from NaCl, HCl, MSG and quinine.

In 1916, Henning postulated that the four taste primaries (sweet, sour, bitter and salt), should in theory account for all the perceived tastes and, therefore, if these primaries were to be represented as point positions in a three-dimensional space, any combination of the four taste primaries would lie on the faces of, or be contained within, the tetrahedron that results from joining the locations of taste primaries. This has been used primarily to invalidate the concept of primary taste qualities, as taste stimuli that lie outside the tetrahedron formed by three-dimensional scaling of psychophysical or cellular responses to the sweet, sour, bitter and salty stimuli cannot be explained only in terms of these qualities (Yamaguchi, 1979; Erickson, 1982; Rolls and Baylis, 1990). Within the three-dimensional space illustrated in Figure 6, tannic acid and MSG lie outside the boundaries of the tetrahedron formed by joining sweet, sour, bitter and salt. This has been shown already for the umami taste quality

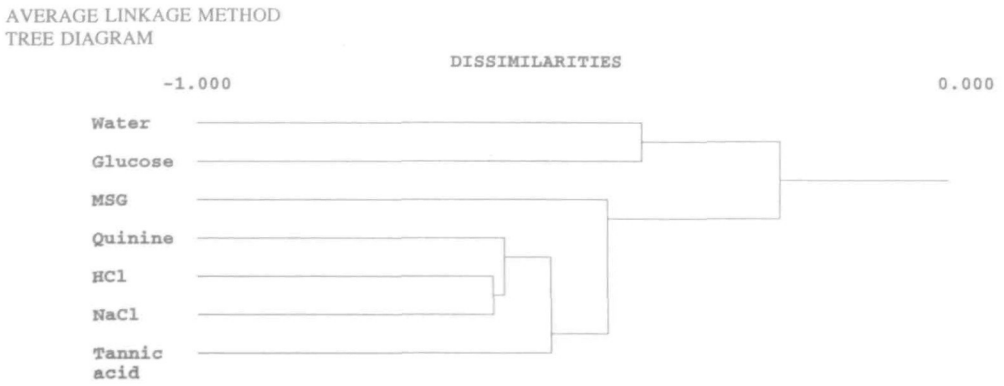

Figure 5 A dendrogram showing the degree of clustering between the responses to the different stimuli. This cluster analysis shows that tannic acid is less similar to HCl than NaCl, and that the tastants quinine, NaCl, HCl and tannic acid are of approximately equal similarity to each other. This figure was obtained using Systat (average linkage method, distances represent Pearson correlations)

of MSG (Baylis and Rolls, 1991; Yamaguchi and Kimisaka, 1979) which has as a result been considered a separate taste in its own right. The finding that tannic acid also lies outside this tetrahedron indicates that there is a separate representation of the taste quality of tannic acid in this population of neurons which is not formed by a linear combination of the other tastes.

Location of cells

Figure 7 shows the location of the 74 taste-responsive cells analysed in this study. All the neurons were located in the orbitofrontal cortex. The caudolateral orbitofrontal cortex is a region of secondary taste cortex, receiving a direct projection from the primary gustatory areas of the insula and frontal operculum (Baylis *et al.*, 1994). Taste-responsive neurons have been reported previously in this region (Rolls *et al.*, 1989, 1990), and in the orbitofrontal regions medial to this (Thorpe *et al.*, 1983; Rolls and Baylis, 1994). In the current study the region containing taste-responsive neurons has been shown to extend (at least) as far anterior as 14 mm in front of the anterior clinoid process of the sphenoid. In this study there was no evidence for chemotopic organization of cortical taste neurons.

Discussion

Evidence is presented above for a distinct representation within gustatory cortical regions of the taste quality elicited by tannic acid. Of particular importance was the relationship between the neuronal responses evoked by tannic acid and by HCl, as evidence from rat gustatory cortical neurons indicated that at high concentrations (0.05 M) tannic acid is

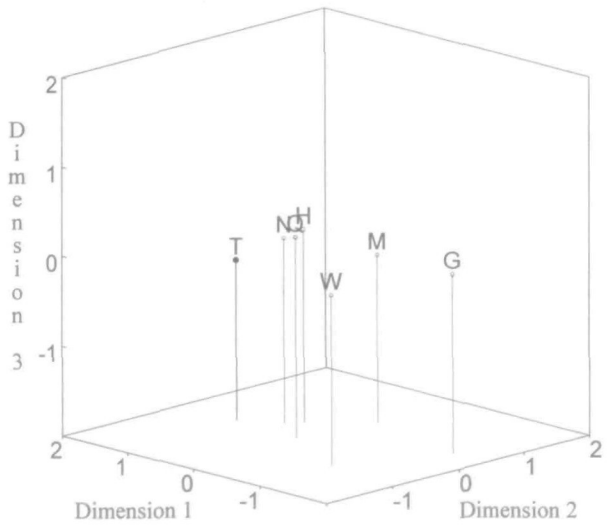

Figure 6 A three-dimensional scaling of the matrix of correlations between the responses to the tastants tannic acid (T), monosodium glutamate (M), the four prototypical tastants (G, N, H, Q) and water (W).

encoded by acid responsive units (Kosar and Schwartz, 1990).

In the present study it was shown that the mean response to tannic acid across the population of 74 cells (Figure 3) was not different from the mean neuronal activity generated by NaCl, HCl, quinine or monosodium glutamate, indicating the tannic acid elicits potent activity in this population of neurons. The taste of glucose had a large effect, and water a smaller effect, than the other tastants on the mean activity of this population of neurons.

The mean response profiles of neurons with maximal responses to glucose, NaCl, HCl, quinine and MSG (Figure 1b) show that these subsets of neurons (and, most importantly, HCl-best neurons) do not encode the taste quality of tannic acid. This is supported by the findings that

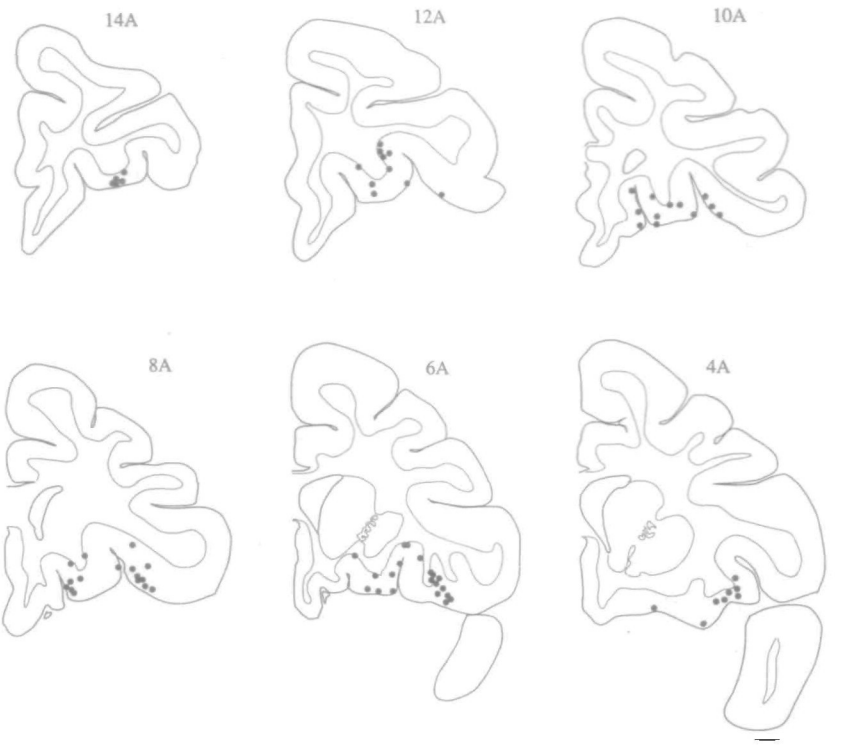

Figure 7 The reconstructed position of the recording sites of neurons in this study The coronal sections are at different positions in mm anterior (A) to the sphenoid reference The majority of the neurons were located in the orbitofrontal cortex.

some cells respond preferentially to tannic acid (Figure 1a) and not to HCl, and that similarly some HCl responsive neurons are unresponsive to the taste of tannic acid (Figure 1b).

Pearson correlations and cluster analysis of the responses of the population of 74 neurons (Table 1 and Figure 5) showed that the tannic acid was approximately equally similar to the tastants NaCl, MSG, HCl and quinine, and that this level of similarity was equivalent to that between these stimuli. Multidimensional scaling analysis of the cellular responses to the tastants also supported the independence of tannic acid from the prototypical tastants (as has been shown for monosodium glutamate - Baylis and Rolls, 1991; Rolls *et al.*, 1996 and further established in this study). Both monosodium glutamate and tannic acid lie outside the space enclosed by the four prototypical tastants and, therefore, can be considered to elicit a distinct taste quality to the conventional sweet, sour, bitter and salty qualities.

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 (i) independent receptor signal transduction mechanisms; (ii) the neural code, such that there are distinct channels for each taste quality; (iii) chemotopic organization over the gustatory receptor surface; (iv) different concentration-response functions; (v) different time courses for different tastants of both the perceptual and neural responses; (vi) lack of significant neural or perceptual cross-adaptation between the taste qualities; (vii) the existence of taste modifiers that are specific to some taste qualities; (viii) 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, in the evidence from human studies in which semantic components are minimized, and from animal behavioural or neural investigations of taste qualities, the notion of primary taste qualities becomes less clearcut. Despite this, the concept of taste primaries is widely accepted as a good general approximation of taste processing (reviewed by Scott and Plata-Salaman, 1991). The present study provides evidence that the neural representation of tannic acid (and by implication, astringency) is distinct from the representations of sweet, sour, bitter and salty taste qualities. Other evidence to support the notion of astringency as a

primary taste quality comes from the ethological significance of tannic acid, its perceptual distinctness and characteristic time course of the astringent response.

Overall, it is shown that tannic acid is represented separately from the prototypical taste stimuli and that this is partly achieved by neurons that specifically respond to the taste of tannic acid. This representation of tannic acid is analogous to that of the prototypical taste stimuli and monosodium glutamate.

The relevance of having a neural representation of the astringent flavour has been described in the Introduction. Ethologically, the ability to perceive dietary tannins offers a nutritional and, hence, survival advantage of 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. The protein taste, 'umami', that is a property of *l*-amino acids such as monosodium glutamate, may have a reciprocal role to the astringent taste of tannins in the regulation of protein intake of more omnivorous primates. However, the data above do not indicate these two systems are strongly connected in the responses of orbitofrontal cortical neurons.

REFERENCES

Aggleton, J.P. and Passingham, R.E. (1981) Stereotactic surgery under X-ray guidance in the rhesus monkey, with special reference to the amygdala. *Exp. Brain Res.*, **57**, 390–399.

Baylis, L.L. and Rolls, E.T. (1991) Responses of neurons in the primate taste cortex to glutamate. *Physiol. Behav.*, **49**, 973–979.

Baylis, L.L., Rolls, E.T. and Baylis, G.C. (1994) Afferent connections of the orbitofrontal cortex taste area of the primate. *Neuroscience*, **64**, 801–812.

Boring, E.G. (1942) *Sensation and Perception in the History of Experimental Psychology*. D. Apple Century Co., New York.

Breslin, P.A.S., Gilmore, M.M., Beauchamp, G.K., Green, B.G. (1993) Psychophysical evidence that oral astringency is a tactile sensation. *Chem. Senses*, **18**, 405–417.

Erickson, R.P. (1982) Studies on the perception of taste: Do primaries exist? *Physiol. Behav.*, **28**, 57–62.

Feigenbaum, J.D. and Rolls, E.T. (1991) Allocentric and egocentric spatial information processing in the hippocampal formation of the behaving primate. *Psychobiology*, **19**, 21–40.

Graham, H.N. (1992) Green tea composition, consumption and polyphenol chemistry. *Prev. Med.*, **21**, 334–350.

Green, B.G. (1993) Oral astringency: a tactile component of flavor. *Acta Psychol. Amst.*, **84**, 119–125.

Hellekant, G., Hladik, C.M., Dennys, V., Simmen, B., Roberts, T.W., Glaser, D., DuBois, G. and Walters, D.E. (1993) On the sense of taste in two Malagasy primates (*Mirocrocebus murinus* and *Eulemur mongoz*). *Chem. Senses*, **18**, 307–320.

Hirose, M., Ozaki, K., Takaba, K., Fukushima, S., Shirai, T. and Ito, N. (1991) Modifying effects of naturally occurring antioxidants gamma-oryzanol, phytic acid, tannic acid and *n*-tritriacotane-16,18-dione in a rat wide spectrum organ carcinogenesis model *Carcinogenesis*, **12**, 1917–1921.

Hladik, C.M. (1978) Adaptive strategies of primates in relation to leaf-eating. In Montgomery, C.G. (ed.), *The Ecology of Arboreal Folivores*. Smithsonian Institute Press, Washington DC, pp. 272–395.

Johns, T. and Duquette, M (1991) Detoxification and mineral supplementation as functions of geophary. *Am. J. Clin Nutr.*, **53**, 448–456.

Kawamura, Y., Funakoshi, M., Kasahara, Y. and Yamamoto, T. (1969) A neurophysiological study on astringent taste. *Jpn. J. Physiol.*, **19**, 851–865.

Kosar, E. and Schwartz, G.H. (1990) Cortical unit responses to chemical stimulation of the oral cavity in the rat. *Brain Res.*, **513**, 212–224.

Lee, C.B. and Lawless, H.T. (1991) Time course of astringent sensations. *Chem. Senses*, **17**, 347–361.

Lyman, B.J. and Green, B.G. (1990) Oral astringency: effects of repeated exposure and interactions with sweeteners. *Chem. Senses*, **15**, 151–164.

Marks, D., Swain, T., Goldstein, S., Richard, A. and Leighton, M. (1988) Chemical correlates of rhesus monkey food choice: the influence of hydrolysable tannins. *J. Chem. Ecol.*, **14**, 213–235.

Merrill, E.G. and Ainsworth, A. (1972) Glass-coated platinum-plated tungsten microelectrodes. *Med. Biol. Eng.*, **10**, 662–672.

Murakami, S., Muramatsu, M. and Otomo, S. (1992) Inhibitory effects of tannic acid on gastric H^+, K^+-ATPase. *J. Nat. Prod.*, **55**, 513–516.

Rolls, E.T. (1993) The neural control of feeding in primates. In Booth, D.A. (ed.), *Neurophysiology of Ingestion*. Pergamon, Oxford, pp. 137–169.

Rolls, E.T. (1994) Neural processing related to feeding in primates. In Legg, C.R. and Booth, D.A. (eds), *Appetite: Neural and Behavioural Bases*. Oxford University Press, Oxford, pp. 11–53.

Rolls, E.T. (1995) Central taste anatomy and physiology. In Doty, R.L. (ed.), *Handbook of Olfaction and Gustation*. Dekker, New York, pp. 549–573.

Rolls, E.T. and Baylis, L.L. (1994) Gustatory, olfactory and visual convergence within the primate orbitofrontal cortex. *J. Neurosci.*, **14**, 5437–5452.

Rolls, E.T., Burton, M.J. and Mora, F. (1976) Hypothalamic neuronal responses associated with the sight of food. *Brain Res*, **111**, 53–66.

Rolls, E.T., Sienkiewicz, Z.J. and Yaxley, S. (1989) Hunger modulates the responses to gustatory stimuli of single neurons in the caudolateral orbitofrontal cortex of the macaque monkey. *Eur. J. Neurosci.*, **1**, 53–60.

Rolls, E.T., Yaxley, S. and Sienkiewicz, Z.J (1990) Gustatory responses of single neurons in the orbitofrontal cortex of the macaque monkey. *J. Neurophysiol.*, **64**, 1055–1066.

Rolls, E.T., Hornak, J., Wade, D. and McGrath, J. (1994) Emotion-related learning in patients with social and emotional changes associated with frontal lobe damage. *J. Neurol. Neurosurg. Psychiat.*, **57**, 1518–1524.

Rolls, E.T., Critchley, H., Wakeman, E.A. and Mason, R. (1996) Responses of neurons in the primate taste cortex to the glutamate ion and to inosine 5'-monophosphate. *Physiol. Behav.* **59**.

Schiffman, S.S. and Erickson, R.P. (1971) A psychophysical model for gustatory quality. *Physiol. Behav.*, **7**, 617–633.

Schiffman, S.S., Suggs, M.S. and Simon, S.A. (1992a) Astringent compounds suppress taste responses in gerbil. *Brain Res.*, **595**, 1–11.

Schiffman, S.S., Suggs, M.S., Sostman, A.L. and Simon, S.A. (1992b) Chorda tympani and lingual nerve responses to astringent compounds in rodents. *Physiol. Behav.*, **51**, 55–63.

Scott, T.R. and Plata-Salaman, C.R. (1991) Coding of taste quality. In Getchell, T.V., Bartoshuk, L.M., Doty, R.L. and Snow, J.B. (eds), *Smell and Taste in Health and Disease*. Raven, New York, pp. 345–368.

Scott, T.R., Yaxley, S., Sienkiewicz, Z.J. and Rolls, E.T. (1986) Gustatory responses in the frontal opercular cortex of the alert monkey. *J. Neurophysiol.*, **56**, 876–890.

Shamil, S. and Benyon, R.J. (1990) A structure-activity study of thaumatin using pyridoxal 5'-monophosphate (PLP) as a probe. *Chem. Senses*, **15**, 457–469.

Simon, S.A., Hall, W.L. and Schiffman, S.S. (1992) Astringent-tasting compounds alter ion transport across isolated canine lingual epithelia. *Pharmacol. Biochem. Behav.*, **43**, 271–283.

Thorpe, S.J., Rolls, E.T. and Maddison, S. (1983) Neuronal activity in the orbitofrontal cortex of the behaving monkey. *Exp. Brain Res.*, **49**, 93–115.

Uma-Pradeep, K., Geervani, P. and Eggum, B.O. (1993) Common Indian spices: nutrient composition, consumption and contribution to dietary value. *Plant Foods Hum. Nutr.*, **44**, 138–148.

Williams, G.V., Rolls, E.T., Leonard, C.M. and Stern, C. (1993) Neuronal responses in the ventral striatum of the behaving macaque. *Behav. Brain Res.*, **55**, 243–252.

Yamaguchi, S. and Kimisuka, A. (1979) Psychometric studies on the taste of monosodium glutamate. In Filer, L.J., Garattini, S., Reynolds, A.R. and Wurtman, R.J. (eds), *Glutamic Acid: Advances in Biochemistry and Physiology*. Raven Press, New York, pp. 35–54.

Yaxley, S., Rolls, E.T. and Sienkiewicz, Z.J. (1990) Gustatory responses of single neurons in the insula of the macaque monkey. *J. Neurophysiol.*, **63**, 689–700.

Yugarani, T., Tan, B.K. and Das, N.P. (1993) The effects of tannic acid on serum cholesterol and liver lipids of RAIF and RICO rats fed on a high fat diet. *Comp. Biochem. Physiol. A.*, **104**, 339–343.

Yugarani, T., Tan, B.K., Teh, H. and Das, N.P. (1992) Effects of polyphenolic natural products on the lipid profiles of rats fed high fat diets. *Lipids*, **27**, 181–186.

Received on February 9, 1995; accepted on 13 November 1995