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Neuronal Representations of Stimuli in the Mouth: The Primate Insular Taste Cortex, Orbitofrontal Cortex and Amygdala

Mikiko Kadohisa, Edmund T. Rolls and Justus V. Verhagen

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

Correspondence to be sent to: Professor E.T. Rolls, University of Oxford, Department of Experimental Psychology, South Parks Road, Oxford OX1 3UD, UK. e-mail: edmund.rolls@psy.ox.ac.uk

Abstract

The responses of 3687 neurons in the macaque primary taste cortex in the insula/frontal operculum, orbitofrontal cortex (OFC) and amygdala to oral sensory stimuli reveals principles of representation in these areas. Information about the taste, texture of what is in the mouth (viscosity, fat texture and grittiness, which reflect somatosensory inputs), temperature and capsaicin is represented in all three areas. In the primary taste cortex, taste and viscosity are more likely to activate different neurons, with more convergence onto single neurons particularly in the OFC and amygdala. The different responses of different OFC neurons to different combinations of these oral sensory stimuli potentially provides a basis for different behavioral responses. Consistently, the mean correlations between the representations of the different stimuli provided by the population of OFC neurons were lower (0.71) than for the insula (0.81) and amygdala (0.89). Further, the encoding was more sparse in the OFC (0.67) than in the insula (0.74) and amygdala (0.79). The insular neurons did not respond to olfactory and visual stimuli, with convergence occurring in the OFC and amygdala. Human psychophysics showed that the sensory spaces revealed by multidimensional scaling were similar to those provided by the neurons.

Key words: fat, flavour, gritty texture, insular taste cortex, macaque, primary taste cortex, taste, texture, viscosity

Introduction

Until recently almost nothing was known about the representation of the sensory properties apart from taste of what is in the mouth. Understanding how the sensory properties of food are represented in the brain provides fundamental information about the separate sensory information channels that can contribute independently to the palatability of food. Understanding the factors that determine the palatability of food is currently of great importance, given the role of palatability in the control of food intake, and the increasing incidence of obesity which is accompanied by serious health risks (Berthoud, 2003; Steinberger and Daniels, 2003). Recently, however, a series of neurophysiological investigations has been performed to show how these processes operate in humans by analysing the responses of single neurons in the macaque primary taste cortex (Verhagen *et al.*, 2004) and two regions to which it projects, the orbitofrontal cortex (OFC) (Rolls *et al.*, 2003; Verhagen *et al.*, 2003b; Kadohisa *et al.*, 2004) and the amygdala (Kadohisa *et al.*, 2005). The purpose of this paper is to compare the processing of oral sensory stimuli in these three areas, in order to provide a basis for understanding the hierarchical processing of oral sensory stimuli in the brain, and what types of information processing are being performed. These comparisons are powerful, in that the same stimuli, the same testing and even some of the same macaques were used in the different investigations. Moreover, the whole set of neurons studied was very large, with 3687 neurons included in the studies compared in this paper. In addition to this comparison, we also in this paper present new psychophysical data in humans with the same set of stimuli so that human subjective responses to the stimuli can be compared with the neuronal representations.

The macaque primary taste cortex is in the anterior insula and adjoining frontal operculum, as shown by the anatomical inputs to these regions from the thalamic taste nucleus, VPMpc (the parvicellular division of the ventroposteromedial thalamic nucleus) (Pritchard *et al.*, 1986). In this paper, we use the term primary taste cortex and insular taste cortex to refer to the insular/frontal opercular region which receives inputs from the thalamic taste nucleus, and which projects to the secondary taste cortex (Baylis *et al.*, 1994), and in which

we analyzed neuronal activity with the set of oral stimuli described here (Verhagen *et al*., 2004). Examples of the recording sites are shown in Figure 4.

A remarkable difference from the taste system of rodents is that in primates there is a direct projection from the first central relay, the nucleus of the solitary tract (NTS), to the gustatory thalamus (Beckstead *et al.*, 1980; Norgren, 1984; Pritchard *et al.*, 1989). In rodents, there is an obligatory relay from the NTS to the pontine parabrachial taste nuclei (PBN), which in turn project to the thalamus (Norgren and Leonard, 1973; Norgren, 1984). The pontine taste nuclei also project to the hypothalamus and amygdala in rodents (Norgren, 1976), providing direct access in rodents to these subcortical structures important in motivational behavior (e.g. feeding) and learning (Rolls, 1999). In contrast, in primates there appears to be no such direct pathway from the brainstem taste areas to the hypothalamus and amygdala (Norgren, 1984), and instead taste information reaches structures such as the amygdala and orbitofrontal cortex from the primary taste cortex (Turner *et al.*, 1980; Baylis *et al.*, 1994). This fundamental difference in the anatomy of the rodent and primate taste pathways shows that even in a phylogenetically old system such as taste, the way in which the system functions and processes information may be different across mammalian orders. It is because of its potentially greater relevance to understanding the taste system in humans, that we analysed the oral sensory responses of neurons in the macaque primary taste cortex (Verhagen *et al*., 2004), in which the gustatory responses of single neurons have been analysed previously (Scott *et al.*, 1986a, 1991; Rolls *et al.*, 1988; Yaxley *et al.*, 1988, 1990; Scott and Plata-Salaman, 1999), and also in the secondary taste cortex (Baylis *et al*., 1994) in the orbitofrontal cortex (Rolls *et al.*, 2003; Verhagen *et al.*, 2003b; Kadohisa *et al.*, 2004), and in the amygdala (Kadohisa *et al*., 2005).

The aims of the investigations compared here were to examine whether the primary taste cortex, and the OFC and amygdala, also receive and represent other information about the properties of oral stimuli, including their viscosity, fat texture and temperature; and if so, whether this information is represented independently of taste information (i.e. by separate neurons), and whether some neurons combine information about taste and these other oral properties, as such neurons would potentially provide a neural basis for behavioral responses that could be selective for particular combinations of taste and these other oral properties. Another aim was to determine whether fatty acids are represented in these areas, and if so, if the representation is separate from that of fat texture and of acid. A further aim was to determine whether gritty oral texture is represented separately from these other properties of oral stimuli. Another part of the interest of the investigations is that given that some neurons in the orbitofrontal cortex and amygdala do show convergence from some of the different sensory properties of oral stimuli (such as taste, texture and temperature), it is of interest to investigate whether this convergence happens for the first time in these secondary taste areas in primates, or whether the convergence is present in some neurons in the primary taste cortex. Another aim was to compare the nature of the representations in the three areas, in order to advance understanding of what processing is taking place as one moves up from the primary taste cortex in these hierarchies. A further aim was to determine whether olfactory and orally related visual stimuli (such as the sight of food) are represented in the primary taste cortex, or whether this type of convergence is left to the secondary taste cortex, in the OFC (Rolls *et al.*, 2003; Kadohisa *et al.*, 2004, 2005), where we know that single neurons reflect these types of convergence (Thorpe *et al.*, 1983; Rolls and Baylis, 1994; Critchley and Rolls, 1996; Rolls *et al.*, 1996). Finally, an aim was to compare the neuronal representations of these stimuli with the psychophysical similarity of the different stimuli in new psychophysical investigations described here.

Materials and methods

Subjects

The recordings in these investigations were made in three rhesus macaques (*Macaca mulatta*): OFC (Rolls *et al.*, 2003; Verhagen *et al.*, 2003b; Kadohisa *et al.*, 2004); amygdala (Kadohisa *et al*., 2005); insula (Verhagen *et al*., 2004). All the recordings were from very well-isolated single neurons. To ensure that the macaques were willing to ingest the test foods and fluids during the recording sessions, they were on mild food (150 g of nutritionally balanced mash plus fruits, boiled chicken eggs, nuts, seeds and popcorn) and fluid (1 h/day *ad libidem* water) deprivation, in that both were provided after the daily recording session.

Stimuli

The neurons were tested for their responsiveness to the set of taste, viscosity, gritty, oily stimuli and capsaicin, at room temperature (23°C), and also the set of temperature stimuli as shown in Table 1. Details of the rationale for the choice of the stimuli are given by Rolls *et al*. (2003) and Verhagen *et al*. (2003b). The gustatory stimuli used included 1.0 M glucose (G), 0.1 M NaCl (N), 0.01 M HCl (H), 0.001 M Quinine–HCl (Q) and 0.1 M monosodium glutamate (M). 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 (Scott *et al.*, 1986b, 1991; Rolls *et al.*, 1989). Distilled water at 23°C was one member of the temperature series (T23), and with its viscosity of 1 cP was also one member (V1) of the viscosity series. For an additional comparison, the neuronal responses were tested to 20% blackcurrant juice (BJ, Ribena), because with its complex taste and olfactory components and high palatability it is an effective stimulus when searching for

Table 1 Stimuli

Stimulus	Abbreviation	Concentration	Mol. wt	Temperature (°C)	Viscosity (cP)	Chemical group
Glucose	G	1 M	180	23	1	monosaccharide aldohexose
Blackcurrant J	BJ	20%		23	1	Mixture
Monosodium glutamate	M	0.1 M	187	23	1	amino acid salt
NaCl	N	0.1 M	58	23	1	inorganic salt
HCl	H	0.01 M	36	23	1	inorganic acid
Quinine–HCl	Q	0.001 M	387	23	1	Alkaloid
Water	T10			10	1	
Water	T23 / V1			23	1	
Water	T37			37	1	
Water	T42			42	1	
CMC	V10	0.2 g + 1 l V1	700 000	23	10	Polysaccharide
CMC	V100	4.0 g + 1 l V1	700 000	23	100	Polysaccharide
CMC	V1000	11.0 g + 1 l V1	700 000	23	1000	Polysaccharide
CMC	V10000	24.0 g + 1 l V1	700 000	23	10000	Polysaccharide
Gritty	Gr	100 g Fillite + 9.4 g CMC + 1 l V1	700 000	23	1000	SiO_2 + polysaccharide
Mineral oil	MO	100%		23	25	Hydrocarbon mixture
Silicone oil	SiO10	100%		23	10	silicon-oxygen polymer
Silicone oil	SiO100	100%		23	100	silicon-oxygen polymer
Silicone oil	SiO 1000	100%		23	1000	silicon-oxygen polymer
Vegetable oil	VO	100%		23	55	Fat
Coconut oil	CO	100%		23	40	Fat
Safflower oil	SaO	100%		23	50	Fat
Single cream	SC	100%		23	12	Emulsion
Lauric acid	LaA	100 μM		23	1	Ffa
Linoleic acid	LiA	100 μM		23	1	Ffa
Capsaicin	Cap	10 μM		23	1	Vanillyl amide

and analysing the responses of cortical neurons (Rolls *et al.*, 1990).

A viscosity series was made with carboxymethyl-cellulose (CMC, Sigma, high viscosity, mol. wt 700 000, dialysed, Code C5013), a virtually odour- and tasteless thickening agent used widely in the food industry. To confirm this, we performed preliminary psychophysical investigations (Rolls *et al.*, 2003) on four expert subjects and found that for no CMC stimulus was the mean intensity, on a 100 mm visual analogue rating scale, greater than 3/100 for sweet, salt, bitter and sour taste, compared to intensity values for the taste stimuli used that were in the range 35–65/100; and that for odour, no CMC stimulus was higher than 3/100. In contrast, the rated thickness of the CMC series increased approximately logarithmically from 3/100 (10 cP) to 76/100 (10 000 cP). We extend in this paper the human psychophysical comparison with neuronal data by describing new psychophysical investigations on 12 human participants of the taste, thickness, oiliness and pleasantness of the range of taste and texture stimuli used in the neurophysiological investigations. Viscosity (or apparent viscosity given that CMC is non-Newtonian and shows some shear-thinning) was assessed using a calibrated Brookfield rotary viscometer (type LVT, Brookfield Engineering Laboratories Inc., Middleboro, MA) at 60 r.p.m. (shear rate $\sim$12 s^{-1}, spindles 1–4) at 23°C. Concentrations (in g CMC added to 500 ml water) yielding 1, 10, 100, 1000 and 10 000 cP (V1, V10, V100, V1000 and V10000; reliability ±10%) solutions were: 0.0, 0.1, 2.0, 5.5 and 12.0 g CMC respectively (Theunissen and Kroeze, 1995). The solutions were mixed until they

were optically clear. Viscosity was assessed at room temperature after air bubbles had disappeared. (Note that 1 cP = 1 mPa s.)

The gritty stimulus consisted of hard (Mohs scale 5) hollow microspheres (Fillite grade PG, with 87% having a diameter with the range 100–300 μm, Trelleborg Fillite, Runcorn, UK) made up in methylcellulose to have a measured viscosity of 1000 cP (100 g of Fillite PG was added to 4.7 g of CMC in 500 ml of water).

To test for and analyse the effects of oral fat on neuronal activity, a set of oils and fat-related stimuli was included. The triglyceride-based oils consisted of vegetable oil, safflower oil and coconut oil. These were used in order to examine whether fat is represented by the responses of insular cortex neurons. Single cream (SC, 18% fat, viscosity 12 cP, Co-op brand, pasteurized) was used as an exemplar of a natural high-fat-content food of the type for which we wished to examine the neural representation and sensing mechanisms. All the neurons with fat-related responses described in this and our earlier study (Rolls *et al.*, 1999) responded well to single cream. The monkeys had been raised on their mother's milk, which is a good source of dietary fat. Vegetable oil (VO, viscosity 55 cP at 23°C), coconut oil (CO, viscosity 40 cP at 23°C) and safflower oil (SaO, viscosity 50 cP at 23°C, Aldrich) were used as natural high-fat stimuli. As Gilbertson and colleagues (Gilbertson, 1998) had reported differential effects in isolated taste cells to linoleic and lauric acid *in vitro*, suggesting that the gustatory modality might be involved in orally sensing fat, we included (Verhagen *et al.*, 2003b) in the stimulus set free linoleic (LiA, 100 μM) and lauric acid (LaA, 100 μM, sodium salt) (Sigma), as well as oils rich in conjugated linoleic acid (68–83% in the safflower oil), and lauric acid (coconut oil, CO, 45–50%, 40 cP, Sigma) (Weiss, 1983; Wills *et al.*, 1998).

To investigate whether the neurons responsive to fatty-acid-based oils were in some way responding to the somatosensory sensations elicited by the fat, stimuli with a similar mouth feel but non-fat chemical composition were used. These stimuli included paraffin/mineral oil (pure hydrocarbon, viscosity 25 cP at 23°C, Sigma) and silicone oil $(Si(CH_3)_2O)_n$, SiO, 10, 100 and 1000 cP (Brookfield viscometer calibration fluid).

The temperature series was provided by water at 10°C (chosen as the cold stimulus — commercial cold drinks are served at 6°C), at 42°C (warm/hot but not noxious), 37°C (body temperature) and 23°C (room temperature). These temperature stimuli were produced by keeping the 10 ml applicator pipettes (described under stimulus delivery) in a 100 ml bottle containing the same water as that inside the applicator pipette, with the bottle itself maintained in a separate waterbath controlled at 10°C, 37°C and 42°C (T10, T37, T42). As the temperature stimulus was delivered directly from the applicator to the mouth, there was no effect of the heat capacity of the applicator on the temperature of the water delivered to the mouth.

The capsaicin was made up as a 10 μM solution (containing 0.3% ethanol). This is ~15 times the human recognition threshold of 0.66 μM (Szolcsanyi, 1990).

Stimulus delivery

The stimuli were delivered intra-orally in the awake, behaving macaque using repeater pipettes (Verhagen *et al.*, 2003b). For chronic recording in monkeys, a 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 (Scott *et al.*, 1986a,b). The stimulus application volume was 200 ± 10 μl, because this is sufficient to produce large gustatory neuronal responses that are consistent from trial to trial, and yet do not result in large volumes of fluid being ingested which might, by producing satiety, influence the neuronal responses (Rolls *et al.*, 1989, 1990). The monkey's mouth was rinsed with 200 μl T23/V1 (water) during the inter-trial interval (which lasted at least 30 s, or until neuronal activity returned to baseline levels) between taste stimuli. The complete stimulus array was delivered in random sequence. Due to the tenacious nature of the oral coating resulting from the delivery of cream or of oil, and also for gritty and capsaicin, four 200 μl rinses with T23/V1 were given, and the subjects were allowed to swallow after each rinse. For V1000 and V10000, we used two such rinses. All the stimuli shown in Table 1 were delivered in permuted sequences, with the computer specifying the next stimulus to be used by the experimenter. The spontaneous firing rate of the neuron was measured from trials in which no stimulus delivery occurred.

Screening cells

While searching for neurons, we continuously applied samples from our stimulus set: G, N, Q, BJ, SC, VO, SO, V100, V1/T23, T10, T42. We tested for olfactory responses using the odours vanilla, eugenol, naphthalene or amyl acetate held close to the nostril on a perfumer strip (with a blank perfumer strip as a control), as this is an effective way of locating neurons with olfactory responses, in for example the OFC (Rolls and Baylis, 1994; Critchley and Rolls, 1996; Rolls *et al.*, 1996). Only cells responding consistently to at least one oral stimulus of the array were used in the experiments described here, all stimuli being then applied 4–6 times in permuted sequences. What we defined as consistent responses are illustrated in Figure 1, in which it is seen that on the different trials for any one stimulus, run originally in permuted sequences, the neuron's response is very similar. Further evidence for the consistency of the responses to a given stimulus is that with the 4–6 trials of data for each stimulus, very highly significant differences in the mean firing rate to particular stimuli were found, as described in more detail for neurons in the OFC (Rolls *et al.*, 2003; Verhagen *et al.*, 2003b; Kadohisa *et al.*, 2004), amygdala (Kadohisa *et al.*, 2005) and insula (Verhagen *et al.*, 2004).

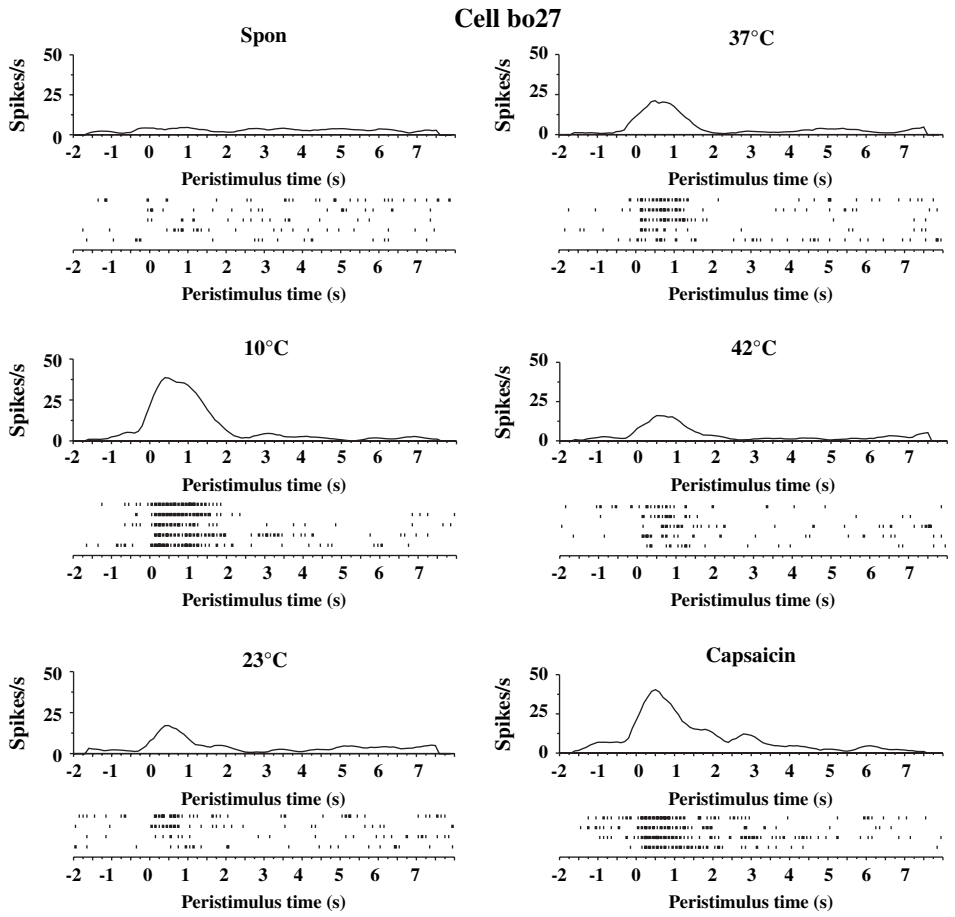

Figure 1 Peri-stimulus–time histograms and rastergrams of a temperature-responsive neuron (bo27) recorded in the macaque orbitofrontal cortex for the different oral temperature stimuli tested. The stimulus was delivered at time 0. The neuron also responded to capsaicin. Spon shows the spontaneous activity on trials on which a stimulus was not delivered.

Data analysis

After cluster cutting of the spikes with Datawave software, the numbers of spikes of the single neuron in 80 time bins, each 100 ms long, starting at the onset of the stimulus were obtained using SPSS. These time series were useful for calculating peristimulus time histograms. Statistical analysis was performed on the numbers of spikes in the first 1 s period after stimulus onset, which was sufficiently long to include firing to even viscous liquids, and sufficiently short so that low-viscosity taste stimuli were still activating the neurons. The appropriateness of this period is shown by the responses of the neuron illustrated here in Figure 1, and also in Figure 2 of Rolls *et al.* (2003). We repeated the analyses for other periods, and confirmed, for example, that selecting a longer period of 3 s would not in fact have altered the way in which any of the cells described here was classified. An ANOVA was performed (with SPSS) to determine whether the neuron had significantly different responses to the set of stimuli. If the main ANOVA was significant, four further ANOVAs were performed to test for differences in neuronal responses between the set of taste stimuli (G, N, H, Q, M and T23/V1), between the members of the viscosity series V1–V10000, the set of fat stimuli (MO, SiO 10, 100 and 1000, VO, CO, SaO), and the set of temperature stimuli (T10–T42). Systat 10 was used for the generation of Pearson product–moment correlation coefficients calculated between the stimuli using the responses of all the neurons analysed, and graphical presentation of stimulus similarity using multidimensional scaling (MDS) (loss function: Kruskal; regression: mono) and cluster analysis (linkage: average, distance: Pearson).

A taste cell was defined by a significant effect in the ANOVA performed across the stimulus subset (V1, G, N, M, H, Q) on the number of spikes during the first second after stimulus onset. Similarly, the viscosity cell criterion was based on a significant effect in the ANOVA between the set of stimuli V1–V10000. Fat cells were defined by a significantly larger average firing rate to the oils (viscosity 25–100 cP) than to the average rates to V10 and V100; and by in addition a significantly higher average firing rate to the oils than the spontaneous firing rate. The criterion for being sensitive to temperature was based on a significant effect in the ANOVA between the set of stimuli T10–T42. The critical

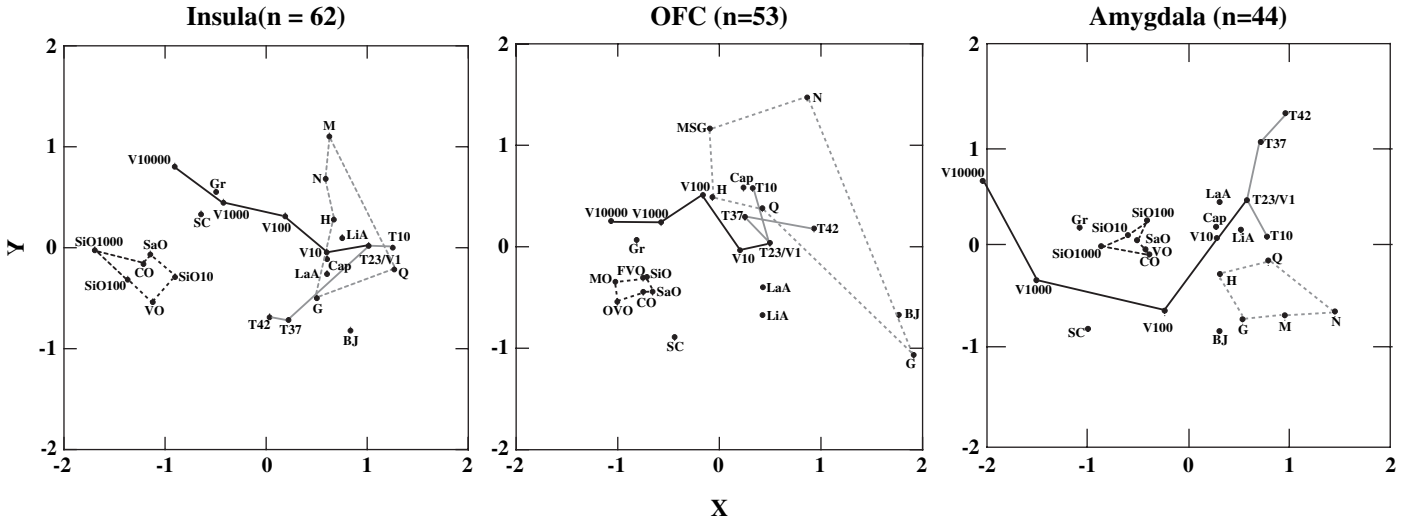

Figure 2 Stimulus space (multidimensional scaling) of the stimulus similarity based on the across-neuron response profiles of the insular taste cortex, orbitofrontal cortex and amygdala neurons. Each space utilizes the interstimulus correlations calculated across the set of neurons analysed in each area. The taste stimuli were 1 M glucose (G), 0.1 M NaCl (N), 0.1 M MSG (M), 0.01 M HCl (H) and 0.001 M Quinine–HCl (Q); the temperature stimuli were T10, T23, T37 and T42 where the number indicates the temperature in °C; the viscosity stimuli were V1, V10, V100, V1000 and V10000 where the numeral indicates the viscosity in cP; fat texture stimuli were SiO10, SiO100, SiO1000 (silicone oil with the viscosity indicated), vegetable oil (VO), coconut oil (CO) and safflower oil (SaO). BJ is fruit juice; Cap is 10 μM capsaicin; LaA is 0.1 mM lauric acid; LiA is 0.1 mM linoleic acid; Gr is the gritty stimulus. The solid line joins the members of the viscosity series. Different line styles join the members of the taste, temperature and oil stimuli. The two-dimensional solution for the insula accounted for 95% of the variance, for the OFC for 89% of the variance, and for the amygdala for 90% of the variance. The numbers of neurons in each area with differential responses to oral stimuli tested on the same set of oral stimuli are indicated.

alpha level was set at $P < 0.05$. Further, the tests for capsaicin, lauric acid and linoleic acid sensitivity were a two-tailed *t*-test comparing the responses of the neuron to capsaicin, lauric acid and linoleic acid, and to water. The test for gritty texture sensitivity was a two-tailed *t*-test comparing the responses of the neuron to the gritty texture stimulus (which has a viscosity of 1000 cP) and to the 1000 cP stimulus from the viscosity series made with CMC.

The breadth of tuning metric of Smith and Travers (1979) was calculated as follows. 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

$$H = -k \sum_i p_i \log p_i$$

where H is the breadth of responsiveness, k a 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 is the response to stimulus expressed as a proportion of the total response to all the n 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 sparseness of the representation a can be measured (Rolls and Tovee, 1995; Rolls and Treves, 1998; Rolls and Deco, 2002) by extending the binary notion of the proportion of neurons that are firing, as

$$a = \left(\sum_{i=1,N} r_i / N \right)^2 \Big/ \sum_{i=1,N} (r_i^2 / N)$$

where r_i is the firing rate of the ith neuron in the set of N neurons. The sparseness is within the range 0–1, and assumes the value 0.5 for a fully distributed representation with binary encoding; and $1/N$ for a local or grandmother cell representation with binary encoding. These measures of the fineness of the tuning of neurons are important in understanding the neuronal encoding of information (Rolls and Treves, 1998; Rolls and Deco, 2002).

Comparison of representations in different brain areas using multidimensional scaling

As described above, MDS (Schiffman *et al.*, 1981), calculated by Systat from the inter-stimulus correlation matrix calculated across the neurons within a brain area (loss function: Kruskal; regression: mono), was used to provide a graphical presentation of stimulus similarity within each brain area. In an MDS graph, the dissimilarity between stimuli is represented by their distance in the space. Examples are shown in Figure 2. Although MDS is primarily used to provide a visual representation of stimulus similarity, we did develop the following new approach to check that the differences in the MDS spaces in different brain areas apparent in Figure 2 were meaningful, and did not arise just by chance random sampling.

The method we used to evaluate similarity among different two-dimensional MDS solutions is as follows. We define our dissimilarity measure as the sum of the Pythagorean distances (based on Cartesian MDS coordinates) between the corresponding stimuli in two MDS graphs with the same set of stimuli. In an MDS space, rotation, scaling, flipping and translation yield equivalent solutions, as the solution consists only of the relative distances among all stimuli. Thus we aligned the two spaces in terms of these transforms before measuring the summed distances. This was performed by using Microsoft Excel's solver add-in to rotate, scale and translate one MDS (and its flipped version), and at the same time to find the minimum of the summed distances between the points for the corresponding stimuli in the two MDS spaces. This allowed equivalent MDS solutions to yield a sum of zero.

We performed comparisons with this approach for 23 identical oral stimuli that had been employed to record neural responses in the three brain areas described in this paper corresponding to the MDS spaces shown in Figure 2. First, we found that the summed distances between the MDSs of the insula and OFC was 14.1, between the amygdala and OFC was 15.7, and between the insula and the amygdala was 15.9. This shows that all three areas have the same degree of dissimilarity to each other. Second, we assessed whether these dissimilarities could have arisen by chance selection of neurons, by randomly removing 20% of the neurons from an area in repeated resampling. The resulting summed distance between the original MDS solution and the resampled one was 4.4 ± 1.0 for the insula (n = 5), being only 29% of the mean distance among the three areas. For the OFC this was 7.4 and for the amygdala 7.8. The resampled MDSs were significantly more similar to each other than to the other areas ($P < 0.002$, n = 3). Third, we used a 50–50 validation procedure whereby two separate MDS spaces were calculated from half the neurons available for an area, and compared. It was found that the reliability of the MDS spaces was high within an area. The dissimilarity between the MDS spaces calculated from each half of the insula dataset were, for example, smaller than those between these split datasets and those of the other areas. Fourth, we used the method to compare the extent of the spaces devoted to each sensory modality in each area. Starting with the stimulus lying on one extreme of the longest axis among the stimuli of a modality, we connected it to its closest neighbour and this second one to its next closest neighbour, etc., until all stimuli of a modality were connected. The mean (± SD) distance of these lines between the tastants was 0.64 ± 0.23 for the insula, 0.97 ± 0.79 for the OFC and 0.46 ± 0.04 for the amygdala. The mean distance between the members of the viscosity series was 0.57 ± 0.10 for the insula, 0.48 ± 0.14 for the OFC and 0.94 ± 0.37 for the amygdala. The mean distance between the members of the fat/oil stimuli was 0.25 ± 0.13 for the insula, 0.16 ± 0.10 for the OFC and 0.13 ± 0.07 for the amygdala. The mean distance between the temperature stimuli was 0.54 ± 0.53 for the insula, 0.49 ± 0.20 for the OFC and 0.13 ± 0.07 for the amygdala. The mean distances between the oil stimuli were lowest of all these 12, which was significant ($P < 0.003$). For the amygdala the distances between the viscosity series (n = 4) were higher than the others (n = 10; $P < 0.001$). For the OFC the distances between the taste series (n = 4) were higher than the others (n = 10; $P < 0.03$). Thus these quantitative analyses of the MDS spaces provided evidence that the OFC separated the taste stimuli from each other more than the insula and the amygdala; that the amygdala separated the viscosity series more than the insula and OFC; and that the representations of the fat/oil stimuli were all very similar to each other, and that this did not differ between areas. Fifth, we used this method to show the average distance between, for example, the stimuli of each modality in the spaces of different brain areas optimally transformed to minimize their summed distances as before. We found that the mean distances apart in the spaces of different areas were rather similar for tastants (0.71 for insula–amygdala, 0.60 for insula–OFC and 0.72 for amygdala–OFC). The distances between the thermal stimuli in the different MDS spaces were less consistent (0.54 for insula–amygdala, 0.89 for insula–OFC and 0.94 for amygdala–OFC). The distances between the viscosity stimuli in the different MDS spaces were 0.38 for insula–amygdala, 0.40 for insula–OFC and 1.0 for amygdala–OFC, showing that for viscosity the largest difference was between the amygdala and OFC. The distances between the fat/oil stimuli in the different MDS spaces were 1.38 for insula–amygdala, 0.77 for insula–OFC and 0.15 for amygdala–OFC.

Overall, this approach to interpreting the MDS spaces thus shows that the spaces for the different brain areas are different in that each space is robust with respect to recalculating it by taking different subsamples of the neurons tested in a given brain area; and helps to express more quantitatively some of the points that are evident when inspecting the MDS spaces for the different brain regions, and that are presented in the Results section.

Psychophysical investigations

Twelve untrained subjects (age 34.4 ± 9.4 years, mean ± SD; range 24–55; 9 males) provided informed consent to participate in the study. The subjects rated their sensations produced by 15 stimuli from the set used in the neurophysiological experiments. The stimuli were (from those shown in Table 1): the tastants G, N, H and Q; the CMC viscosity series V1–V10000; the silicone oil viscosity series SiO10–SiO1000; safflower oil and linoleic acid. A positive taste control consisted of V100 with 0.1 M NaCl. The subjects rated the intensities of each stimulus on a separate 100 mm visual analogue scale, labelled (and anchored) by 'extremely high level' (at the top) and 'extremely low level' (at the bottom). The subjects also rated each stimulus on a separate but similar scale labelled 'taste: salty', 'taste: sour',

'taste: sweet', 'taste: bitter', 'overall taste'; 'odour'; 'texture: thickness', 'texture: slimy', 'texture: oily'; 'any other: specify'. To the right of these scales was a separate line with markers from −2 (bottom) to +2 (top; in 1 point graduations) and anchored with 'Extremely unpleasant' (−2), 'Neutral' ('0') and 'Extremely pleasant' (+2). Subjects were asked to put a horizontal line at the level that best corresponded to the elicited sensation. The stimuli were rated using a different random order for each subject. The subjects rinsed with distilled water between stimuli. The subjects presented 1 ml of each stimulus to themselves from 1 ml syringes, and were asked to sample it freely (while moving their tongues and making chewing mouth movements), in order to provide accurate ratings of the taste, odour and texture components. The subjects were asked to make all of the 11 ratings while sampling and within 30 s of taking in the substance. They were instructed not to swallow any sample, but to expectorate and thoroughly rinse. There was a 30 s delay before the next stimulus was sampled.

Results

An example of the type of neurophysiological data collected is illustrated in Figure 1, which shows the responses of a neuron recorded in the macaque OFC to a set of different temperature stimuli and to capsaicin. The rastergrams show each spike as a vertical line, and one trial is a single row. The peristimulus time histogram above shows the average firing rate across the set of trials with each stimulus. The stimuli were delivered at time 0 in a permuted sequence, except for Spon when no stimulus was delivered in order to measure the spontaneous firing rate of the neuron.

The data sets include a population of 62 neurons (out of 1122 recorded) with differential oral responses in the insular cortex (Verhagen *et al.*, 2004), 53 neurons (out of 1149 recorded) in the orbitofrontal cortex (Rolls *et al.*, 2003; Verhagen *et al.*, 2003b; Kadohisa *et al.*, 2004) and 44 neurons (out of 1416 recorded) in the amygdala (Kadohisa *et al.*, 2005). In all cases, the neuronal populations were statistically highly significant, with individual neurons often having significant effects at $P < 10^{-5}$, and the probability of the populations having such P values of, for example, $\ll 10^{-16}$ (Kadohisa *et al.*, 2004). In each area, a small number of neurons had non-differential responses (as assessed by ANOVA) to the set of oral stimuli, but the activity was different from spontaneous firing, and as these neurons did not convey significant information about which oral stimulus was present, they are not considered further here.

Multidimensional spaces and cluster analysis

The representations of the similarity of the oral stimuli by the populations of neurons in these three areas was approached with MDS analysis, based on the first 1 s of post-stimulus activity, and are compared in Figure 2. Distances in this space represent how dissimilar the representations are of the different stimuli provided by the populations of neurons, based on the inter-stimulus correlation values calculated across the population of neurons with differential oral responses in each area. The area (relative to other stimuli) occupied by the taste stimuli (G, N, H, Q and M) was moderate in the primary taste cortex, small in the amygdala and large in the orbitofrontal cortex. This reflected the average correlations between the taste stimuli across the whole populations of orally responsive neurons, which were 0.84 ± 0.06 in the insula, 0.93 ± 0.02 in the amygdala and 0.71 ± 0.16 (mean ± SD) in the OFC. This property is reflected also in the dendrograms shown in Figure 3. Indeed, the correlations between the taste stimuli are lower for the OFC than the insula ($P < 0.03$) and the amygdala ($P < 0.0004$); and lower for the insula than for the amygdala ($P < 0.0002$). The proportions of neurons with taste responses in the different areas were similar (35/1122 for the insula, 36/1149 for the OFC and 27/1416 for the amygdala, $\chi^2 = 5.01$, df = 2, $P = 0.08$). In the amygdala, the average correlation between the five taste stimuli was lower for the neurons with taste-only responses (0.61 ± 0.15, mean ± SD, $n = 13$) than for the neurons with taste and other oral responses (0.95 ± 0.02, $n = 14$, $P < 10^{-5}$). In the OFC, the reverse was found, in that the average correlation between the five taste stimuli was higher for the neurons with taste-only responses (0.81 ± 0.12, mean ± SD, $n = 12$) than for the neurons with taste and other oral responses (0.43 ± 0.29, $n = 24$, $P < 0.002$). No differences were found in the insula, in which both correlations were 0.84.

The actual values of the correlations obtained between stimuli do depend on whether the spontaneous firing rate is subtracted, with a lower value being obtained if the spontaneous firing rate is subtracted. This must be borne in mind when comparing the correlations with other studies. In the comparisons being performed here, the same methods were used for the calculations of responsiveness in different areas, and the recordings were made with the same stimuli, and even in some of the same monkeys. The actual values of the correlations found in studies such as those of Scott *et al.* (1993) for the amygdala were similar, with the value for the average correlation between four taste stimuli G, N, H and Q provided by amygdala neurons (with the spontaneous rate subtracted) being 0.70. This comparison leads to confidence in the values reported here, and also indicates that the rather higher average correlations between stimuli found when the correlations were measured across all 20 stimuli are reliable. (The set of 20 stimuli was the whole set shown in Table 1 except that only one oil, vegetable oil, VO, was included, as the responses to the different oils were in general very similar, as shown below.)

The difference in the taste representation in the OFC from the other areas (with a larger part of the space occupied by the taste stimuli, as shown in Fig. 2) was accompanied by a large proportion of the taste neurons in the OFC having their best taste response to glucose. In the OFC, 22/36

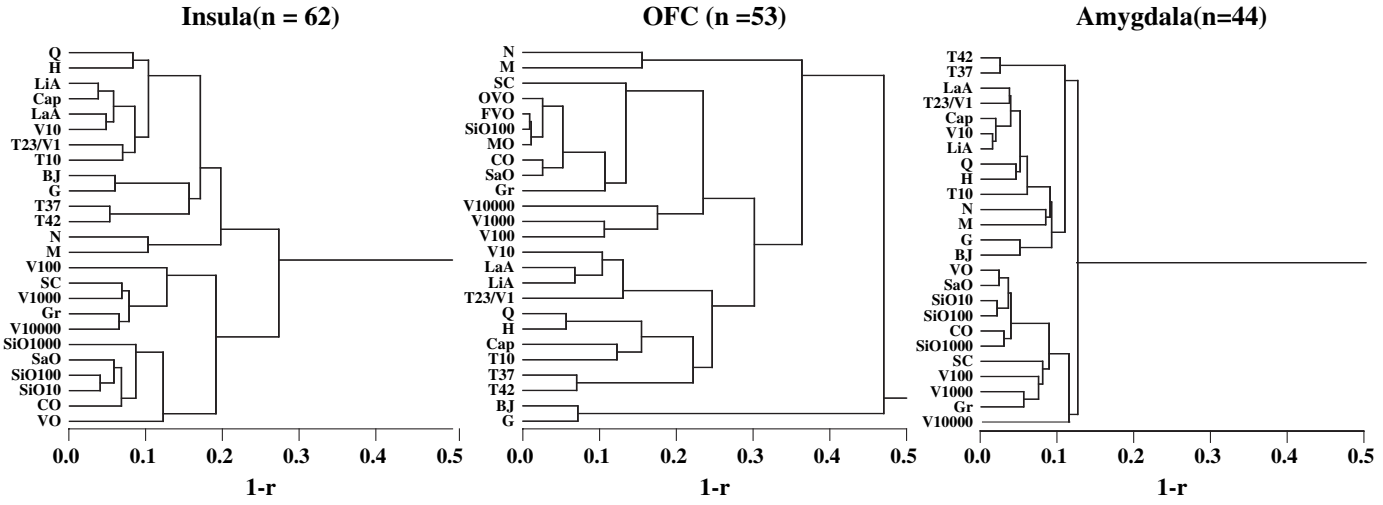

Figure 3 Stimulus dendrograms based on the neurons with differential responses to the set of oral stimuli for the insular taste cortex, orbitofrontal cortex and amygdala. Each dendrogram utilizes the interstimulus correlation coefficients *r* calculated across the set of neurons analysed in each area. (1– *r*) is the measure of dissimilarity of the clusters. The numbers of neurons in each area with differential responses to oral stimuli tested on the same set of oral stimuli and used in the analysis are indicated. Abbreviations as for Figure 2.

(61%) of the neurons with taste responses had their best taste response to glucose, in the amygdala 7/27 (26%) and in the insula 10/35 (29%) (χ^2 = 20.0, df = 10, $P < 0.03$).

A second difference between the three areas is also revealed in the multidimensional scaling analyses shown in Figure 2. The region across which the viscosity is represented is particularly extensive in the insula and amygdala, and less extensive for the OFC. In the dendrogram (Figure 3), this corresponds to the relatively low part of the tree in the hierarchical clustering at which the different stimuli are joined. The correlations between the viscosity stimuli were 0.80 ± 0.07 (mean ± SD) in the OFC 0, 0.89 ± 0.06 in the amygdala and 0.83 ± 0.09 in the insula. This did not reflect different proportions of neurons that were responsive to the viscosity series, which were 42% for the OFC and 39% for the amygdala. For all areas, it was very interesting that the viscosity stimuli were set out in series through the spaces, reflecting a parametric representation of viscosity (i.e. a representation in which the greater the difference between viscosities, the greater was the difference between their representation in the space). A feature of the multidimensional spaces for all three areas (Figure 3) is that the CMC viscosity series is set out almost linearly, indicating a parametric representation. A plane at right angles to the line formed by the series of viscosity stimuli divides all three spaces into regions that contain all stimuli ≤10 cP, and all stimuli >10 cP. This same division is reflected in the dendrograms in Figure 3, where for the insula and amygdala the first main division separates these two classes of stimuli, and for the OFC these stimulus classes are separated by the third main division.

The temperature of oral stimuli was also represented in all three areas, with clearly parametric representations in the insula and amygdala, but a less extensive and less parametric representation in the OFC. This was reflected also in the dendrograms shown in Figure 3, in that in the OFC the different temperature stimuli were joined at a relatively low level in the dendrogram, whereas in the amygdala the different temperatures were separated by the clustering at a relatively higher part of the tree. The correlations between the neuronal representations of the temperature stimuli were 0.80 ± 0.08 (mean ± SD) in the OFC, in the 0.92 ± 0.03 amygdala and 0.84 ± 0.08 in the insula.

In all three areas, the fatty and non-fatty oils were closely grouped together, indicating that there was a similar basis for the representation of the fatty and non-fatty oils. A similarity they share is the slick texture, the nature of the oral coating and immiscibility with saliva. In addition, in all three areas, the oils were separated in the spaces from the CMC viscosity stimuli, indicating that the basis for the detection of fat in the mouth is not viscosity.

Overall, these comparisons show that oral texture, already present in the insular/opercular cortex, may reach the amygdala and OFC through the insular/opercular primary taste cortex. However, the more extensive representation of texture in the amygdala than in the OFC must then be related either to differential input of texture versus taste from the insula to the amygdala, or to further oral somatosensory inputs to the amygdala from other somatosensory areas (Friedman *et al.*, 1986). The relative distances between the stimulus members of each 'modality' of stimulus (taste, viscosity, fat and temperature) in the multidimensional spaces reflect the relative similarity of the stimuli within each modality compared to those in other modalities. Thus Figure 2 shows, for example, that, relative to the other stimulus modalities, taste is well represented in the OFC; whereas relative to the other stimulus modalities, viscosity and temperature

are well represented in the amygdala. What is meant by 'well represented' in this context is that the members of a modality are represented as being very different from each other, i.e. as being highly discriminable. A similar point can be made about the dendrograms shown in Figure 3. In the OFC, the first major division in the hierarchical clustering separates some of the taste stimuli (N and M) from others (G and BJ). Thus there are large differences in the neuronal representation on the OFC of different tastes. Similarly, for the amygdala dendrogram, the first major division in the hierarchical clustering separates some of the viscosity stimuli (V100, V1000 and V10000) from others (V10 and V1). The fact that it is the relative correlation within a brain area of each modality that is important in the representations provided by the multidimensional spaces (Figure 2) and the dendrograms (Figure 3) is made evident in Table 3.

The other major feature of the dendrograms shown in Figure 3 is that the amygdala dendrogram has all the joinings in the hierarchical cluster analysis at relatively high levels of the correlation r, whereas the OFC dendrogram has many of the divisions at relatively low levels of r. This aspect of the dendrograms emphasizes the hypotheses that the neuronal representation in the insula represents a reasonable separation of the different oral stimuli [with a mean correlation (± SD) between the 20 stimuli of 0.81 ± 0.08]; and that the neuronal representation in the orbitofrontal cortex provides a better separation of the different oral stimuli (with a mean correlation between the 20 stimuli of 0.71 ± 0.12). In comparison, that the neuronal representation in the amygdala provides a poorer separation of the different oral stimuli (with a mean correlation between the 20 stimuli of 0.89 ± 0.05). [The OFC mean correlations were significantly lower than the insula ($P < 10^{-22}$) and amygdala ($P < 10^{-58}$) mean correlations. The insula mean correlations were lower than the amygdala mean correlations ($P < 10^{-26}$).]

Unimodal versus multimodal

Table 2 shows for each brain region the numbers of neurons with unimodal, bimodal and multimodal inputs, and the types of those inputs. For the purpose of this analysis, the different modalities were taste (G), temperature (T), viscosity (V) and fat (F). One difference between the areas is that more orally responsive neurons were classified as unimodal in the insula (50%) and amygdala (52%) than in the OFC (30%) (χ^2 =7.59, df = 2, P = 0.02). Thus the OFC appears to provide a site of further convergence for these different oral sensory inputs. Of the unimodal neurons, taste-only neurons were found in all three areas, but it was noticeable that the insular taste cortex had relatively more unimodal differential viscosity neurons (12/62 orally responsive) than the amygdala (3/44) and the orbitofrontal cortex (2/53) (χ^2 = 7.72, df = 2, P = 0.02). Thus the insular cortex has clearly separate representations of taste and viscosity, and these two information channels are more likely to be combined with each or with other oral sensory signals in the amygdala and orbitofrontal cortex. This is consistent with a hierarchical architecture in which convergence occurs upwards in the hierarchy, with the amygdala and OFC being placed above the insula with respect to the convergence of taste and viscosity information. With respect to bimodal and multimodal neurons (three or more oral input types), the insular cortex contains, in addition to many unimodal neurons (50%), relatively many bimodal neurons (31%), and relatively few multimodal neurons (13%). The amygdala, in addition to its unimodal neurons (52%), has many bimodal neurons (23%), and many multimodal neurons (16%). The OFC, with relatively few unimodal neurons (30%), has a number of bimodal neurons (30%), and relatively many multimodal neurons (28%). Thus the main trend appears to be that the OFC

Table 2

Unimodal		Bimodal		Multimodal		Others
Insula (n = 62/1122)						
Taste (G)	15	G + T	6	G + T + V	6	
Temperature (T)	2	G + V	6	G + T + F	0	
Viscosity (V)	12	G + F	0	G + V + F	0	
Fat (F)	2	T + V	6	T + V+ F	0	
		T + F	0	G + T+ V + F	2	
		V + F	1			
Total	31		19		8	4
%	50		31		13	6
OFC (n = 53/1149)						
Taste (G)	12	G + T	5	G + T + V	8	
Temperature (T)	1	G + V	1	G + T + F	2	
Viscosity (V)	2	G + F	4	G + V + F	4	
Fat (F)	1	T + V	5	T+ V + F	1	
		T + F	0	G + T+ V + F	0	
		V + F	1			
Total	16		16		15	6
%	30		30		28	11
Amygdala (n = 44/1416)						
Taste (G)	13	G + T	3	G + T + V	7	
Temperature (T)	6	G + V	4	G + T + F	0	
Viscosity (V)	3	G + F	0	G + V + F	0	
Fat (F)	1	T + V	2	G + T+ V + F	0	
		T + F	0			
		V + F	1			
Total	23		10		7	4
%	52		23		16	9

has relatively more multimodal neurons (relative to unimodal and bimodal) than the insula and amygdala ($\chi^2 = 7.3$, df = 2, $P < 0.03$).

Sparseness and breadth of tuning

The sparsenesses of the neuronal representations in the three different areas are shown in Table 3. The mean sparseness of the representation of 16 stimuli (G, BJ, N, M, H, Q, T23/V1, T10, T37, T42, V10, V100, V1000, SC and VO) of the 62 insula neurons was 0.74 ± 0.21 (mean ± SD). This compares to the mean sparseness of 52 OFC neurons to the same set of stimuli of 0.67 ± 0.23 (mean ± sd) ($P = 0.12$), which indicates the insular neurons were non-significantly tuned more broadly to the set of stimuli. The mean sparseness for the same set of stimuli of the 44 amygdala neurons was 0.79 ± 0.08, which is significantly higher than the value for the OFC ($P = 0.006$), but not significantly different from the insula. Thus the OFC has a relatively sparse representation of this set of stimuli, and the amygdala a rather distributed representation.

A similar pattern of results occurs for the sparseness calculated just across the four taste stimuli G, N, H and Q for the taste-only neurons (see Table 4), though the differences are not sufficiently large, and the numbers of neurons are relatively small (12–15), so that this trend was not significant. A similar pattern of results occurs for the sparseness calculated just across the four taste stimuli G, N, H and Q for the neurons with responses to taste and other stimuli (see Table 4), with the OFC neurons being more sparsely tuned than both the insula ($P < 10^{-4}$) and the amygdala ($P = 10^{-4}$) neurons. [The numbers of neurons involved in these comparisons were 18 (insula), 24 (OFC) and 14 (amygdala).]

These comparisons together provide evidence that the representation of both the whole set of stimuli, and of taste, is more sparse in the OFC than the insula, and less sparse in the amygdala than the insula.

The breadth-of-tuning metric (Smith and Travers, 1979) calculated across the taste stimuli H, Q, N and G revealed similar conclusions. (A low breadth of tuning indicates a sparse representation, in which the sparseness measure is low.) In particular, for the neurons with taste and other responses, the breadth of tuning was significantly lower in the OFC than the insula ($P = 0.001$) and the amygdala ($P = 0.001$). The same trend was present for the taste breadth of tuning for the taste-only neurons, but the trends were not significant in this case.

Table 3 Mean correlation across neurons between the responses of each population of neurons to the stimuli within each modality (taste, viscosity, etc.)

	Insula	OFC	Amygdala
Taste	0.84	0.71	0.93
Viscosity	0.83	0.81	0.89
Temperature	0.84	0.80	0.92
Fat	0.91	0.96	0.96

Gritty texture, capsaicin and fatty acids

In all three brain regions, neurons were found that responded to capsaicin, to one or both of the fatty acids linoleic acid (polyunsaturated) and lauric acid, and to gritty texture, as shown in Table 5. There were no clear differences between the areas, consistent with the hypothesis that the amygdala and OFC receive information about these stimuli from the primary taste cortex. In all areas, the capsaicin-responsive neurons were not particularly likely to be activated by the warmest temperature in our series, 42°C, and this may be related to the fact that the sensation of capsaicin is mediated by the vanilloid receptor subtype 1 (VR1), which responds to temperatures above 43°C (Caterina *et al.*, 1999).

In all three areas, there was almost no overlap between the neurons activated by the fatty acids and by fat, so that the sensory effects produced by fat in the mouth are unlikely in

Table 4 Sparseness and breadth of tuning (entropy) of neurons in different brain areas to taste stimuli, and across a wide range of oral stimuli (sparseness 16)

	Insula	OFC	Amygdala
Sparseness 16	0.74 ± 0.21	0.67 ± 0.23	0.79 ± 0.18
Sparseness 4 taste only	0.74 ± 0.20	0.70 ± 0.25	0.77 ± 0.15
Sparseness 4 taste + other	0.85 ± 0.16	0.60 ± 0.20	0.87 ± 0.16
Breadth of tuning 4 taste only	0.82 ± 0.22	0.75 ± 0.36	0.86 ± 0.12
Breadth of tuning 4 taste + other	0.91 ± 0.12	0.72 ± 0.23	0.93 ± 0.09

Sparseness 16: across 16 stimuli.Sparseness 4 taste only: across taste stimuli (G, N, H, Q) for neurons responding to taste only.
Sparseness 4 taste + other: across taste stimuli (G, N, H, Q) for neurons responding to taste and other modalities.
Breadth of tuning 4: across taste stimuli (G, N, H, Q) for neurons responding to taste only.
Breadth of tuning 4 + other: across taste stimuli (G, N, H, Q) for neurons responding to taste and other modalities.

Table 5 Numbers of neurons with responses to the stimuli indicated in different brain regions

	Insula	OFC	Amygdala
Capsaicin	8/62	8/51	4/44
Lauric acid	9/62	4/34	4/44
Linoleic acid	4/62	2/34	7/44
Gritty	4/62	7/49	3/44

The table shows the number of neurons with responses to each stimulus/number of neurons tested with each stimulus.

primates to be related to free fatty acids released from fats by salivary lipase, which has been suggested as a possibility in rodents (Gilbertson, 1998). Further evidence against the free fatty acid hypothesis of fat sensing in primates is that in all three brain regions considered here, the neurons activated by fat (vegetable oil, safflower oil, coconut oil and cream) in the mouth were also activated by non-fat oils including mineral oil (pure hydrocarbon) and silicone oil $(Si(CH_3)_2O)_n$. Even for the neurons that responded to fatty acids, there is of course the possibility that their responses were related to the acid rather than the fatty acid component, and indeed in the population of cells in the insula, 10 of the 12 fatty acid sensitive cells responded to HCl, and two did not (Verhagen *et al.*, 2004). However, the fatty acid concentration was 0.1 mM (Verhagen *et al.*, 2004), and the measured pH was $\sim$7, so it is unlikely that the fatty acids produced a pH sufficient to activate the acid taste system. In all three brain regions some neurons also responded to another type of oral texture, a gritty texture produced by microspheres suspended in cellulose, and the responses of these neurons were not ascribable to viscosity.

Olfactory and visual response

Neurons with olfactory responses, and with visual responses to, for example, the sight of food, are found in the OFC and amygdala, and are in many cases found in neurons that respond to oral sensory stimuli such as taste (Verhagen *et al.*, 2004). To investigate whether these visual and olfactory inputs are already present in the primary taste cortex in the insula and frontal operculum, Verhagen *et al.* (2004) investigated whether olfactory and taste stimuli activate neurons in this region. Of 62 orally responsive insular/opercular neurons, it was possible to test 25 for responses to olfactory or visual stimuli, and none had significant responses. However, some (19) other neurons recorded in this insular region did have some responses to visual stimuli, such as the sight of food approaching the mouth. As these neurons were not tested in a visual discrimination so that the latency of their neuronal response could be measured, it is possible that the activity of these neurons was related to anticipatory mouth movements made as the object approached the mouth. The activity of such neurons could have been related to somatosensory inputs occurring during small mouth movements, and indeed some other neurons (15) did respond to touch to the perioral region (e.g. the lips), or in two cases clearly in relation to mouth movements. No neurons in the insular/opercular taste cortical region responded to olfactory stimuli.

Localization of recordings

The reconstructed positions of the neurons analysed are shown in Figure 4, which provides representative sections only. Complete details of the histology are provided in the original papers (Rolls *et al.*, 2003; Verhagen *et al.*, 2003b; Kadohisa *et al.*, 2004, 2005). The primary taste cortex neurons are within the region defined as primary taste cortex as shown by the cortical area receiving afferents from the thalamic taste nucleus VPMpc (Pritchard *et al.*, 1986). The OFC neurons are within the area shown to be secondary taste cortex in that it receives afferents from the primary taste cortex (Baylis *et al.*, 1994).

Psychophysics

The results of MDS performed on the taste and texture stimuli are illustrated in Figure 5 to show the dissimilarity of the different stimuli. The MDS space shows the distances between the stimuli based on the correlations between the observers' mean ratings for each stimulus as follows: sweet, salt, bitter, sour, taste intensity, odour intensity, oily, slimy, thickness and pleasantness. The different taste stimuli are represented in one part of the space, with glucose somewhat separated from the other tastes. The viscosity series is represented parametrically in the space. The oils are grouped together in another part of the space. The psychophysical stimulus space has been rotated to be approximately aligned with the stimulus spaces based on the neuronal recordings shown in Figure 2, with which there is an interesting similarity. These points are supported by the results of the cluster analysis shown in Figure 6, which also show that the taste stimuli tend to be separated from the texture stimuli, and from the oily stimuli.

The results of MDS performed on the taste and texture stimuli are shown in Figure 7 to show the dissimilarity of the different ratings across the set of stimuli. The different taste ratings are represented in one part of the space, with sweet and pleasant together but quite separate from the other taste ratings, and all the taste ratings are well separated from the thickness, oily and slimy ratings. The odour rating is not separated from the taste ratings, consistent with the fact described further below that none of these stimuli had significant odour components. This is a useful property of this set of stimuli, which was carefully chosen to have minimal olfactory components, to help ensure that even in the brain regions with olfactory neurons (OFC and amygdala), the neuronal responses to the texture stimuli would be based on their texture and not on any strong olfactory component. The MDS rating space (Figure 7) shows that the human observers did not separate oiliness and sliminess very well from viscosity, but on the other hand the stimulus space based on the ratings made (Figure 5) does show that the oily stimuli and CMC viscosity stimuli can be separated from each other to at least some extent based on the ratings made. The observers were relatively untrained, and it would be expected that with training the cellulose viscosity stimuli could be psychophysically distinguished perhaps even better from the oils. The stimulus space (Figure 5) and dendrogram (Figure 6) also show that the CMC series do not have taste components, in that it is only when Na is added to 100 cP CMC (V100N) that this viscosity stimulus moves close to salty (Na) taste in the dendrogram. Further evidence that the CMC viscosity series and the silicone oil is

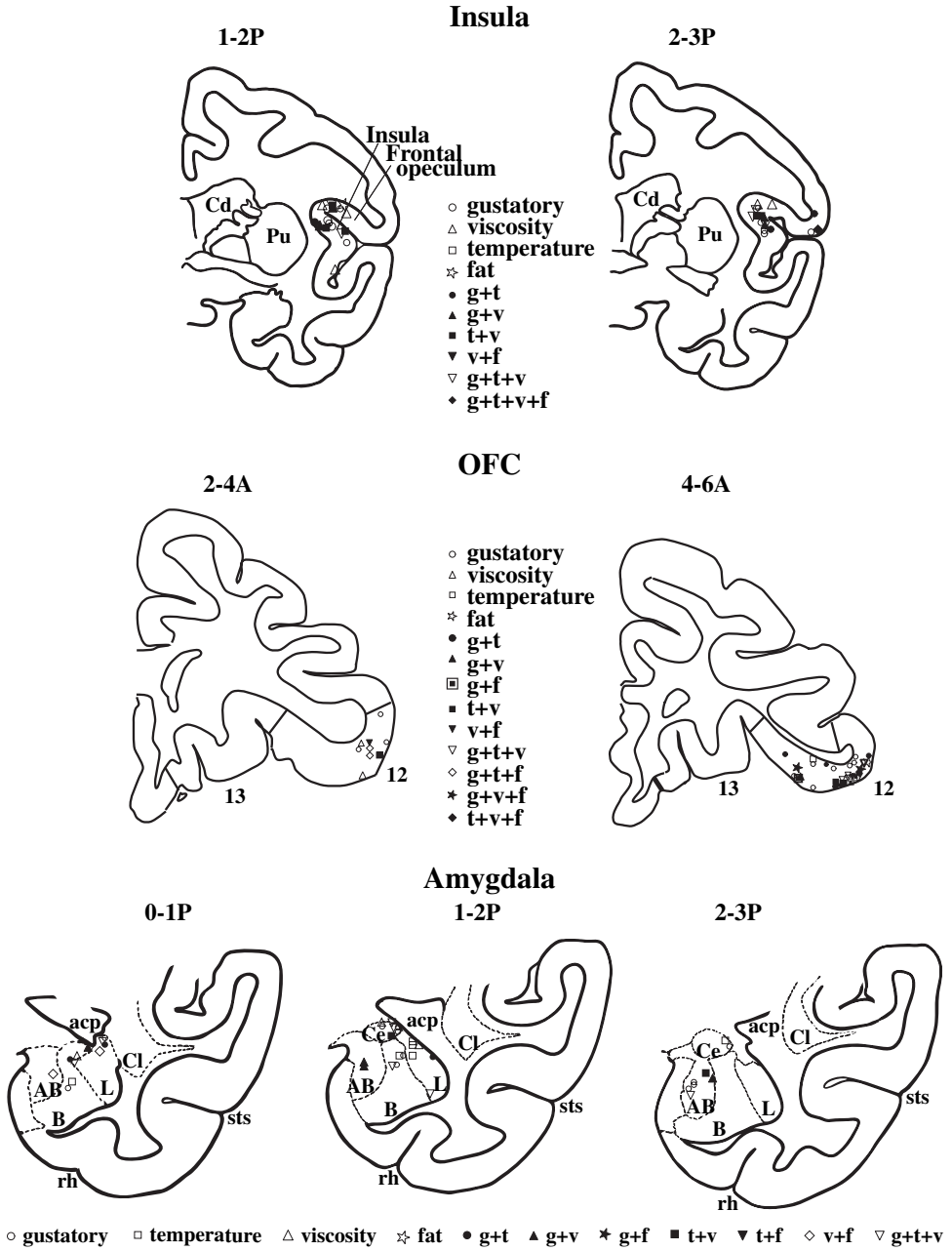

Figure 4 Summary showing the recording sites of some of the orally responsive neurons in the insula, orbitofrontal cortex and amygdala. Representative coronal sections are shown for each brain region. The coronal sections are at different distances in mm posterior (P) to the sphenoid reference (which is at approximately the anterior–posterior level of the optic chiasm). The symbol with which the location of each neuron is indicated shows whether the neuron was tuned to g = taste, v = viscosity, t = temperature, f = fat, or to combinations of these. AB, accessory basal amygdaloid nucleus; acp, anterior commissure posterior part; B, basal amygdaloid nucleus; Cd, Caudate nucleus; Ce, central amygdaloid nucleus; Cl, claustrum; L, lateral amygdaloid nucleus; Pu, Putamen ; rh, rhinal fissure; sts, superior temporal sulcus.

tasteless and odourless is provided by the ratings shown in Figure 8, with only the safflower oil and the linoleic acid having small taste and olfactory ratings.

The human psychophysics did show that the human ratings of the thickness of what was in the mouth were very closely related to the viscosity of the CMC viscosity series, and indeed there is a linear relation between the rated thickness of the CMC and the log of its viscosity [see Figures 8 and 9; r = 0.99 for 10–10 000 cP, omitting 1 cP because it is below the viscosity of saliva; cf. Theunissen and Kroeze (1995)]. This close relation indicates that, although the CMC is non-Newtonian and shows shear thinning at high shear rates and high viscosities, the apparent viscosity measured at 60 r.p.m. (shear rate $\sim 12\ s^{-1}$) in the Brookfield rotary viscometer does have physiological relevance, in that it relates very closely to psychophysics, and to neuronal responses in the

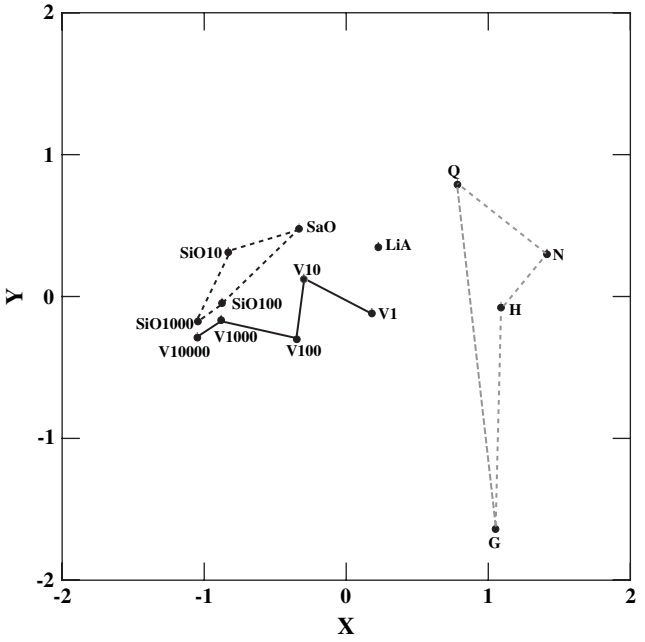

Figure 5 Multidimensional scaling showing the stimulus space based on the human psychophysical ratings of the thickness, taste intensity, taste quality, etc., of many of the taste and texture stimuli used in the neurophysiological experiments. The taste stimuli were 1 M glucose (G), 0.1 M NaCl (N), 0.01 M HCl (H) and 0.001 M Quinine–HCl (Q). The viscosity stimuli were V1, V10, V100, V1000 and V10000 where the numeral indicates the viscosity in cP at 23°C. V100Na is a solution of 0.1 M NaCl made up in 100 cp CMC. The oil texture stimuli were SiO10, SiO100, SiO1000 (silicone oil with the viscosity indicated), and safflower oil (SaO). LiA is 0.1 mM linoleic acid. This two-dimensional space accounted for 97% of the variance. (See Table 1 for details.)

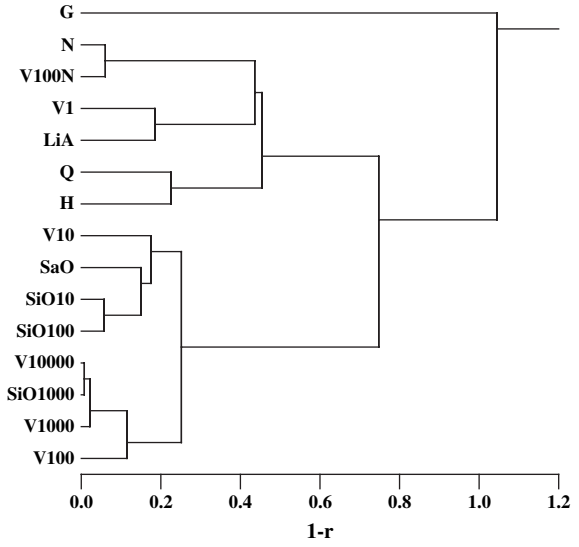

Figure 6 Dendrogram resulting from a cluster analysis showing the clustering of different stimuli based on the human psychophysical ratings as indicated in Figure 7 of each of the stimuli shown in the legend to Figure 5.

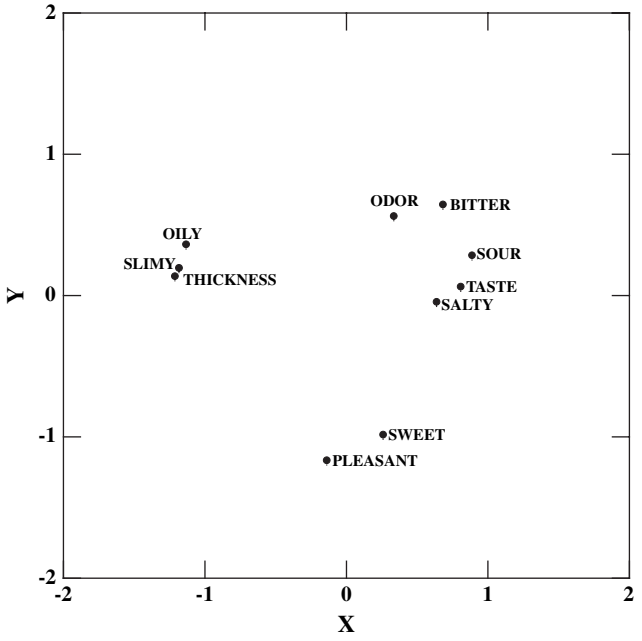

Figure 7 Multidimensional scaling showing the rating space based on the human psychophysical ratings of the thickness, taste intensity, taste quality etc. of many of the taste and texture stimuli used in the neurophysiological experiments. The properties rated are as shown in the multidimensional space. The stimuli were as shown in the legend to Figure 5 and in Table 1.

insula, OFC and amygdala that in some cases also show a linear change in firing rate as a function of the log of the apparent viscosity. Interestingly, the rated thickness for the Newtonian silicone oil series is not a simple log-linear function of viscosity (see Figure 8), indicating that when humans make subjective ratings of the thickness of what is in the mouth, then an oily texture interferes with this thickness rating. In addition, some effect of increasing viscosity of the CMC series on the rated oiliness and sliminess was apparent (see Figure 8). However, at 10 cP, the humans rated the CMC as being not thick or oily, whereas the silicone oil was rated as being slimy and oily, indicating that at low viscosities, oils can be clearly distinguished from non-oily stimuli. Further, across the range of viscosities of the silicone oils (10–1000 cP), the rated oiliness and sliminess remained relatively constant, showing that the humans found that the fat texture of the oils was almost independent of the viscosity of the oils.

Discussion

The MDS analyses and the dendrograms shown in Figures 2 and 3 indicate that relative to the insula, the OFC contains a representation of oral stimuli that is more distinct. The OFC representation is more distinct in that, for example, the average correlations between the 20 different stimuli (including taste, viscosity, temperature and one oil stimulus) are lower for the OFC than for the insula (see Table 3). The representations are also more distinct in the OFC in that the representation is more sparse across the set of 16 stimuli in the OFC (0.67) than it is in the insula (0.74). This principle,

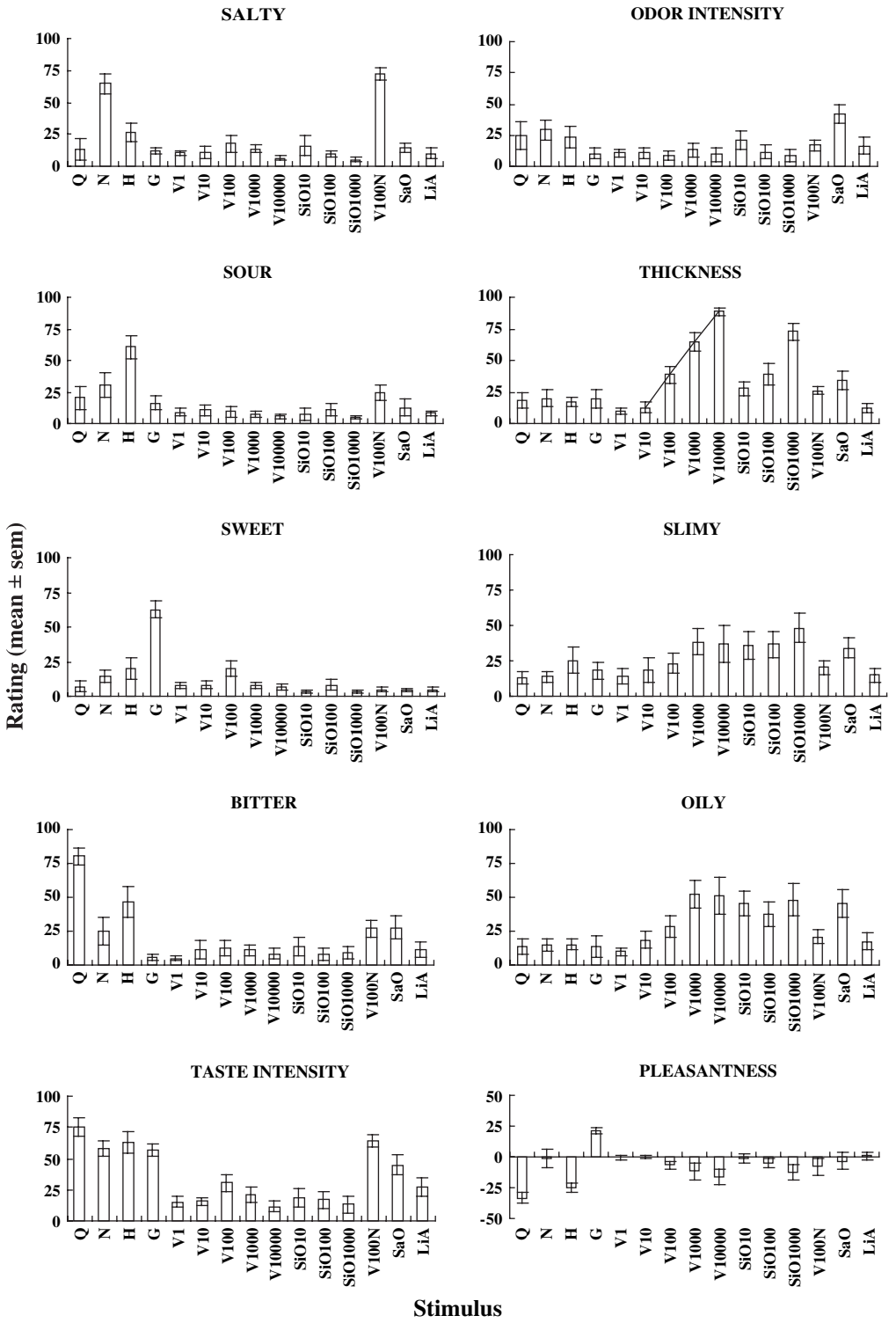

Figure 8 Human psychophysical ratings (mean ± SEM) of the saltiness, sourness, sweetness, bitterness, taste intensity, odor intensity, thickness, sliminess, oiliness and pleasantness of the set of stimuli indicated in the legend to Fig. 5.

of more distinct representations of different stimuli as one proceeds in the hierarchy from the primary taste cortex to the OFC, was suggested originally by a comparison of the average breadths of tuning between the representations of different tastes provided by neurons in the OFC in comparison with the insular/opercular taste areas, and the nucleus of the solitary tract (see Rolls *et al.*, 1990). The new analysis provided in this paper not only reinforces that earlier view,

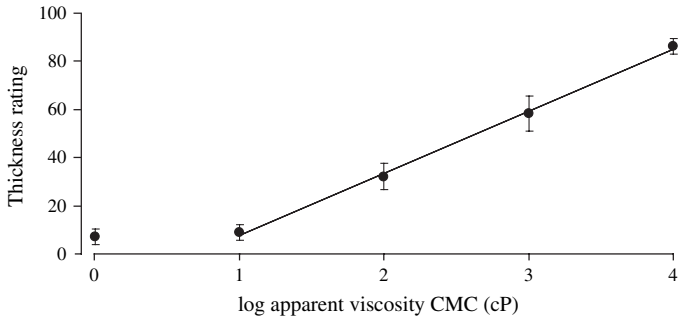

Figure 9 Human psychophysical ratings (mean ± SEM) of the thickness of the CMC viscosity series 1–10 000 cP. The regression line is calculated across all points apart from 1 cP, as this viscosity is below that of human saliva, and a clear relation no longer holds.

but also extends it far beyond taste representations, to include now the representations of a very wide range of oral stimuli, including in addition viscosity and temperature.

In the primary taste cortex, taste and viscosity are more likely to activate different neurons, with more convergence onto single neurons in the OFC and amygdala (Table 2). Most convergence is found in the OFC, with multimodal neurons—responding to three or more of viscosity, fat, temperature and taste—found in the OFC more than in the amygdala and insula (Table 2). This convergence in the OFC potentially provides a basis for different behavioral responses to particular combinations of these oral sensory stimuli. Consistent with this, the mean correlations between the representations of the (20) different oral stimuli provided by the population of OFC neurons were lower (0.71) than for the insula (0.81) and amygdala (0.89). The sparseness of the encoding was consistent with this, in that the encoding was more sparse in the OFC (0.67) than in the insula (0.74) and amygdala (0.79). The interstimulus correlations and MDS showed that taste is relatively more represented in the OFC (with many neurons responding to sweet taste), whereas oral somatosensory stimuli are relatively emphasized in the amygdala (Table 3). The oral sensory neurons in the insula did not respond to olfactory and visual stimuli such as the sight of food (Verhagen *et al.*, 2004), with convergence of this information occurring in the hierarchically higher OFC and amygdala (Rolls and Baylis, 1994). Thus overall, this gives the OFC a special functional role, for it sharpens the tuning of neurons to this broad range of oral stimuli, providing more separate representations of each oral stimulus. This more separate representation in the OFC than the insula or amygdala fits the OFC particularly well for functions such as sensory-specific satiety, which is computed in the OFC (Rolls *et al.*, 1989) and not in the insular/frontal opercular primary taste cortex (Rolls *et al.*, 1988; Yaxley *et al.*, 1988). Sensory-specific satiety could be implemented by synaptic or neuronal adaptation (Deco and Rolls, 2005) occurring over 10–15 min of stimulation by a food, and the effect can only be relatively specific if the tuning of the individual neurons is relatively specific. An important mechanism by which sparse and decorrelated representations are formed in the brain is by competitive learning (Rolls and Deco, 2002).

The separate (relatively uncorrelated) representations of different stimuli in the OFC may also be appropriate for a stage at which learning of associations between visual or olfactory stimuli and oral stimuli occurs (Thorpe *et al.*, 1983; Critchley and Rolls, 1996; Rolls *et al.*, 1996), for then the learned association can reflect particular qualities of individual foods and other oral stimuli much more effectively. Indeed, one of the important findings of these investigations, which is consistent with this hypothesis, is that olfactory stimuli, and visual stimuli such as the sight of food, did not activate orally responsive neurons in the primate insular/frontal opercular taste cortex. This provides further evidence that this type of convergence (Baylis *et al.*, 1994), which is implemented by associative learning (Thorpe *et al.*, 1983; Critchley and Rolls, 1996; Rolls *et al.*, 1996), is an important function of the primate OFC. This overall design of the taste system in primates may result from the great development of the cerebral cortex in primates, and the advantage of using extensive cortical processing from each sensory modality before the representations are integrated in multimodal regions (Rolls, 1999, 2005).

In all three brain regions in primates, the sensing of oral fat was not related to free fatty acids that might be released by any salivary lipase that might be present, as described above. In addition, the sensing of fat (vegetable oil, safflower oil, coconut oil and cream) in the mouth does appear to be related to a texture signal, in that the neurons activated by these fats were also activated by non-fat oils including mineral oil (pure hydrocarbon) and silicone oil $(Si(CH_3)_2O)_n$ (e.g. Verhagen *et al.*, 2003a). The exact physical basis of this texture signal is not yet known, but gives rise to a subjective sensation of slickness. The neuronal populations in all three brain regions clearly separated the fat texture signal from that produced by viscosity, as shown, for example, by the fact that in all three MDS spaces (Figure 2), the oils were grouped closely together, and were in addition separated from the representation of viscosity provided by the cellulose viscosity series.

Fat in the mouth was represented in two ways by the neurons described here. One way was by the neurons that respond to fat and much less to the cellulose viscosity series (e.g. Fig. 3 of Verhagen *et al.*, 2004). These neurons encode fat by its texture (and not by any odour or free fatty acid cue), in that the same neurons respond to silicone oil, to mineral oil, and not to fatty acids (Gilbertson, 1998; Verhagen *et al.*, 2003b). The second way in which fat is distinguished from non-fat textures in the primate insular/frontal opercular taste cortex and in the OFC and amygdala is by the neurons that respond to viscosity and not to the oils. Indeed, it was of interest that in, for example, the insular cortex (Verhagen *et al.*, 2004), most of the neurons differentially responsive to the cellulose viscosity series (11/33) tended to have smaller responses to the same viscosity when produced by fat, providing a further way in which the population of insular/opercular

neurons described here separates the representations of oral viscosity and fat. In addition, the few neurons that responded to fatty acids did not respond to the oil stimuli.

The representation of viscosity described here encodes the degree of viscosity of what is in the mouth, in that each neuron has graded firing to the different viscosities used (CMC in the range 1–10 000 cP), and different neurons have different response functions (as shown, for example, in Figure 2 of Verhagen *et al*., 2004). Further evidence for this is provided by the multidimensional space shown in Figure 2, in which the different viscosity stimuli are parametrically represented and well separated from each other in the stimulus space. The hard, round microspheres we employed (100–300 μm) evoke an oral gritty texture, and this was an effective stimulus when suspended in cellulose for some neurons in these different areas (when compared to equally viscous cellulose).

The representation of temperature provided by these primate neurons was graded, as shown by the responses of the neurons illustrated for the primary taste cortex in Figure 5 and in Figure 4 of Verhagen *et al*. (2004), and by the multidimensional spaces for all three areas shown in Figure 2 in which the temperature stimuli are parametrically organized in space. Some orally responsive neurons in all three regions had responses to capsaicin that were different from those to water. These neurons did not have any strong tendency to respond to 42°C water, and this may be related to the fact that the sensation of capsaicin is mediated by the vanilloid receptor subtype 1 (VR1), which responds to temperatures above 43°C (Caterina *et al*., 1999).

The interesting finding that some primary taste cortex neurons respond to both taste and intra-oral somatosensory stimuli such as viscosity and temperature (Verhagen *et al*., 2004) could reflect convergence in the insular cortex, or the convergence could be present already at earlier stages of taste processing. It is known that some neurons in the taste thalamus (nucleus VPMpc) have thermal responsiveness in monkeys (Pritchard *et al*., 1989) and rats (Verhagen *et al*., 2003a). In the periphery, it is known that chorda tympani fibers in the monkey (Sato *et al*., 1975) and hamster (Ogawa *et al*., 1968) show significant correlations between the responses to HCl and those to cooling (20°C), and between the responses to sucrose and warming (to 40°C). Some lingual nerve fibres in monkeys were activated by cooling to 15°C but not by taste (Danilova and Hellekant, 2002). We know of no studies in the periphery of the effects of food-relevant oral stimuli such as viscosity and fat texture.

Although the effects of intra-oral stimuli other than taste on primate primary taste cortex or amygdala or OFC neurons have not been investigated prior to these studies as far as we know, there are reports that some neurons in the macaque insular cortex respond to tactile stimulation of the mouth region (Scott and Plata-Salaman, 1999), though in the study of Schneider *et al*. (1993) none of these responded to taste. In the rat, there is some evidence that perioral mechanical and/or temperature (Yamamoto *et al*., 1981, 1988; Kosar and Schwartz, 1990a,b) stimuli can activate some taste cortex neurons in rats, but food-related oral stimuli such as texture were not investigated in those studies.

It was noticeable from the dendrograms (Figure 3) that the inter-stimulus correlations across the populations of neurons were relatively high. Further, the mean correlations between the representations of the different stimuli provided by the population of OFC neurons were 0.71, for the insula were 0.81 and for the amygdala were 0.89. [We note that when calculating the correlations between the pairs of 20 stimuli, we do not subtract the spontaneous firing rate, as the spontaneous firing rate would of course not be subtracted before the firing is transmitted to other neurons in the brain.] We note for comparison with other studies (Scott *et al*., 1986a, 1991; Rolls *et al*., 1988; Yaxley *et al*., 1988, 1990; Scott and Plata-Salaman, 1999) that the mean value of the correlations between the six taste stimuli G, N, H, Q, T23/V1 and BJ for the taste responsive neurons in the primary taste cortex with the spontaneous subtracted was 0.75 ± 0.09 (mean ± SD) (Verhagen *et al*., 2004).

Psychophysics performed with the same set of stimuli in humans showed that the cellulose viscosity stimuli used, and most of the oils, had no significant taste or olfactory components (Figure 8). This is further evidence that the neuronal representations of these stimuli described in this paper were not due to olfactory or taste properties of the cellulose and oil stimuli. Further evidence for this is that in the primary taste cortex, in which odour is not represented, the viscosity and oil stimuli had representations (shown in Figure 2 and 3) that had all the properties described in this paper.

The stimulus sensory spaces revealed by MDS from the psychophysics (Figure 5) (based on the correlations between the mean ratings of sweet, salt, bitter, sour, taste intensity, odour intensity, oily, slick, thickness and pleasantness) showed that the taste, viscosity and oil stimuli were represented in different parts of the space. This shows that humans can report independently on these different aspects of oral sensory stimuli; and that the ratings made (of thickness, etc) were sufficient to reveal at least some of the differences between these oral stimuli. These conclusions are supported by the dendrogram shown in Figure 6. In addition, the psychophysically based stimulus spaces bear a striking resemblance to the neuronal spaces shown in Figure 2. The stimulus space determined psychophysically appears to be closer to the neuronal spaces in the insula and OFC, in that viscosity is not emphasized in the psychophysical space. In addition, it is of considerable interest that the human psychophysical space represents the viscosity series parametrically (systematically organized approximately linearly in the space), showing that human observers reflect the neuronal representations of how different the members of the viscosity series are from each other. The space showing the similarity of the different psychophysical ratings (Figure 7) showed that sweet and pleasant are grouped closely, and that the other tastes are organized together. A small surprise was that the thickness ratings were not well separated from the oil ratings in

this space, consistent with the fact shown in Figure 8 that at some viscosities (10 and 100 cP) the oils were rated as having a thickness higher than would be expected if thickness ratings reflected only viscosity. As noted in the Results section, it would probably be possible to train observers to separate these stimuli much better, but the psychophysical investigations were performed with observers who were given some practice in making the ratings, but no training in discriminating between different aspects of oral sensory stimuli.

Although a number of functional neuroimaging studies have shown activation of an insular/frontal opercular cortical region by taste in humans (Zald *et al.*, 1998; Small *et al.*, 1999; O'Doherty *et al.*, 2001; de Araujo *et al.*, 2003b), a recent study has shown that the same insular/opercular region has blood oxygen level dependent (BOLD) activation in a funtional magnetic resonance imaging (fMRI) study which is correlated with the viscosity of carboxymethylcellulose, providing evidence that this region in humans, putatively the primary taste cortex, also receives an oral texture input (de Araujo and Rolls, 2004). In the same fMRI study, oral texture stimuli also activated parts of the OFC, consistent with the neurophysiology described here. Of course, the details of the representation as described here, with both unimodal neurons and multimodal neurons showing convergence, together with the details of the individual neuronal tuning to viscosity and temperature stimuli, and the separateness of the representation from gritty and capsaicin, could not be shown by fMRI studies. Another fMRI study does, though, also indicate that the results described at the neuronal level in primates are relevant to understanding the human insular cortical system in that although the OFC and the most anterior, agranular, insula in humans is activated by both taste and olfactory stimuli, there is a part of the human insular/frontal opercular cortex that is activated only by taste, and not by olfactory, stimuli (de Araujo *et al.*, 2003a).

These results provide fundamental evidence about the information channels used to represent the taste, texture and temperature of food in the first cortical area involved in taste in the primate brain, and in the OFC and amygdala. The current investigation thus greatly extends previous investigations in which taste representations in the primary taste cortex have been analysed (Scott *et al.*, 1986a, 1991; Rolls *et al.*, 1988; Yaxley *et al.*, 1988, 1990; Scott and Plata-Salaman, 1999); have been shown to represent the quality of taste and not its hedonic or reward value in that they are independent of satiety (Rolls *et al.*, 1988; Yaxley *et al.*, 1988); have been shown to correlate with human psychophysical reports of the similarity of different taste stimuli (see Scott and Plata-Salaman, 1999); and damage to which in humans impairs gustatory sensation (Pritchard *et al.*, 1999). The results are relevant to understanding the physiological and pathophysiological processes related to how the properties of oral stimuli are represented in the brain, and thus to the control of food intake and food selection.

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