THE PRIMATE AMYGDALA: NEURONAL REPRESENTATIONS OF THE VISCOSITY, FAT TEXTURE, TEMPERATURE, GRITTINESS AND TASTE OF FOODS

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Abstract—The primate amygdala is implicated in the control of behavioral responses to foods and in stimulus-reinforcement learning, but only its taste representation of oral stimuli has been investigated previously. Of 1416 macaque amygdala neurons recorded, 44 (3.1%) responded to oral stimuli. Of the 44 orally responsive neurons, 17 (39%) represent the viscosity of oral stimuli, tested using carboxymethyl-cellulose in the range 1–10,000 cP. Two neurons (5%) responded to fat in the mouth by encoding its texture (shown by the responses of these neurons to a range of fats, and also to non-fat oils such as silicone oil $((Si(CH_3)_2O)_n)$ and mineral oil (pure hydrocarbon), but no or small responses to the cellulose viscosity series or to the fatty acids linoleic acid and lauric acid). Of the 44 neurons, three (7%) responded to gritty texture (produced by microspheres suspended in cellulose). Eighteen neurons (41%) responded to the temperature of liquid in the mouth. Some amygdala neurons responded to capsaicin, and some to fatty acids (but not to fats in the mouth). Some amygdala neurons respond to taste, texture and temperature unimodally, but others combine these inputs. These results provide fundamental evidence about the information channels used to represent the texture and flavor of food in a part of the brain important in appetitive responses to food and in learning associations to reinforcing oral stimuli, and are relevant to understanding the physiological and pathophysiological processes related to food intake, food selection, and the effects of variety of food texture in combination with taste and other inputs on food intake. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: appetitive learning, Pavlovian conditioning, obesity, food intake, reward.

The amygdala is implicated in emotion and motivation by lesion, single neuron recording, and neuroimaging investigations (Sanghera et al., 1979; Nishijo et al., 1988a; Davis, 1994; Francis et al., 1999; Rolls, 1999; Schoenbaum et al., 1999; LeDoux, 2000; Rolls, 2000). Part of the function of the amygdala in motivation and emotion appears to be in associating previously neutral (e.g. auditory or visual) stimuli to primary (unlearned) reinforcers such as taste and somatosensory including painful stimuli (Davis, 1994; Rolls, 1999, 2000; LeDoux, 2000), and in primates, for example, reward devaluation learning depends on the amygdala (Murray et al.,

1996). This associative function (Rolls and Treves, 1998) implies a representation of primary reinforcers such as the taste of food, and indeed taste neurons have been described in the amygdala (Sanghera et al., 1979; Nishijo et al., 1988a,b; Scott et al., 1993; Yan and Scott, 1996) and taste responsiveness in the human amygdala (O'Doherty et al., 2001). Almost nothing is known, however, about whether aspects of food other than taste and smell are represented in the amygdala. The texture of food is important in its palatability and acceptability (Bourne, 2002, consider e.g. damp cereal or potato chips), and temperature may also be important (Zellner et al., 1988). We describe here for the first time the responses of primate amygdala neurons to oral texture and temperature stimuli, and show that they are combined in some neurons with responsiveness to the taste of food. The investigation was performed in macaques in order to make it as relevant to understanding the operation of this system in humans as possible, and in the context that the taste system is differently connected in rodents and primates, with taste pathways in primates to the amygdala only after cortical processing (Norgren, 1984; Rolls et al., 2003). Understanding the factors that determine the palatability of food is currently of great interest, given the role of palatability in the control of food intake, and the rapidly increasing incidence of obesity which is accompanied by serious health risks (Berthoud, 2003; Steinberger and Daniels, 2003). The factors investigated included the texture of food as reflected by viscosity (tested parametrically with a viscosity series made with cellulose); oral fat; oral fatty acids which might signal the presence of fat in the mouth if salivary lipase was present (Gilbertson, 1998); oral texture as manipulated by inert microspheres; oral temperature; taste; and the effects of capsaicin, an oral irritant present in a number of foods.

EXPERIMENTAL PROCEDURES

Subjects

The recordings were made in three hemispheres of two rhesus macaques (*Macaca mulatta*; one female weighing 2.6–3.3 kg and one male weighing 6.1–6.7 kg). 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 pop corn) and fluid (1 h/day *ad libitum* water) deprivation, in that both were provided after the daily recording session. The monkeys showed steady increases in bodyweight. All procedures, including preparative and subsequent ones, were carried out in accordance with the National Institutes for Health Guide for the Care and Use of Laboratory Animals, and were licensed under the UK Animals (Scientific Procedures) Act, 1986, and were designed to minimize

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Abbreviations: BJ, blackcurrant juice; CMC, carboxymethylcellulose; CO, coconut oil; GR, gritty texture stimulus; LaA, lauric acid; LiA, linoleic acid; MO, mineral oil; SaO, safflower oil; SC, single cream; SiO, silicone oil; VO, vegetable oil.

the number of animals used and maximize their welfare by adopting, for example, group housing, and environmental enrichments.

Recordings

Recordings were made with epoxylite-coated high impedance (2–10 MΩ at 1 kHz; Frederic Haer & Co., St. Bowdoinham, ME, USA) tungsten microelectrodes from single neurons in the amygdala, which included areas in which taste responses have previously been described (Sanghera et al., 1979; Nishijo et al., 1988a,b; Scott et al., 1993), using neurophysiological methods as described previously (Scott et al., 1986a,b; Rolls et al., 1990, 1999, 2003; Verhagen et al., 2003). The signal-to-noise ratio was typically 3:1 or higher as illustrated in Fig. 4. The data were collected using a Datawave Discovery Inc. (Tucson, AZ, USA) system which digitized the signal (12 bit, 16 kHz) for 8 s after stimulus onset. The spikes were sorted off-line using the cluster cutting method provided with the Datawave system, and this procedure was straightforward as the data were collected with single neuron microelectrodes which typically recorded from only one neuron at a time. To prevent visual associative input from evoking neural activity, we prevented the monkeys from seeing the stimuli and experimenter by a view-obstructing screen.

Localization of recordings

X-radiography was used to determine the position of the micro-electrode after each recording track relative to permanent reference electrodes and to the anterior sphenoidal process, followed by microlesions on selected tracks and reconstruction from histological sections of the brain using the methods described by Feigenbaum and Rolls (1991).

Stimuli

The neurons of the amygdala 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. (2003). 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 SmithKline Beecham), because with its complex taste and olfactory components and high palatability it is an effective stimulus when searching for and analyzing the responses of cortical neurons (Rolls et al., 1990). The viscosity series was made with carboxymethylcellulose (CMC; Sigma; high viscosity, Mw 700,000, dialysed, code C5013), a virtually odor- and tasteless thickening agent used widely in the food industry (see Rolls et al., 2003). 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 analyze 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 (VO; viscosity 55 cP at 23 °C), safflower oil (SaO), and coconut oil (CO). Single cream (SC; 18% fat, viscosity: 12 cP; Coop 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. Four of the orally responsive neurons were tested with mineral oil (MO), a hydrocarbon mixture with a viscosity of 25 cP. All the neurons with fat-related responses described in this and our earlier study (Rolls et al., 1999) responded well to SC. As Gilbertson (1998) had reported differential effects in isolated taste cells to linoleic and lauric acid (LaA) *in vitro*, suggesting that the

gustatory modality might be involved in orally sensing fat, we included (Verhagen et al., 2003) in the stimulus set free linoleic (LiA; 100 μM) and LaA (100 μM) sodium salts (Sigma), as well as oils rich in conjugated LiA (SaO, 68–83%, 50 cP; Aldrich), and LaA (CO, 45–50%, 40 cP; Sigma; Weiss, 1983; Wills et al., 1998). All fatty oils were kept in the dark under N_2 at 4 °C to avoid oxidation.

To investigate whether the neurons responsive to fat-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/MO (pure hydrocarbon, viscosity 25 cP at 23 °C, Sigma), and silicone oil (SiO; $Si(CH_3)_2O)_n$, 10, 100 or 280, and 1000 cP (Brookfield viscometer calibration fluid except 280 cP; Aldrich).

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).

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

The monkeys' preference for the stimuli was measured objectively by an acceptability rating, where +2 indicates that the macaque reached for the stimulus and placed it in the mouth, +1 indicates that the macaque actively opened the mouth to receive and swallow the stimulus, 0 indicates neutrality in which the stimulus was accepted only passively by mouth opening, −1 indicates that the macaque closed the mouth to try to reject the stimulus; and −2 indicates that the macaque used the hand to push away the stimulus from the mouth (Rolls et al., 1977, 1989). This rating scale has been extensively validated by comparison with neuronal activity in the lateral hypothalamus and orbitofrontal cortex in studies of sensory-specific satiety, in the sense that there was a close relation found between the acceptability rating and the neuronal response in these regions, as shown in previously published data (Rolls et al., 1986, 1989; Critchley and Rolls, 1996). The ratings were repeated a total of four (or for one macaque 5) times by two independent investigators, with a high correlation between the ratings given by the two investigators ($r=0.85$, $n=25$, $P<10^{-7}$).

Stimulus delivery

The general method for stimulus delivery and accurate stimulus onset marking (Rolls et al., 1990) was modified by introducing repeater pipettes (Verhagen et al., 2003). 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 which are consistent from trial to trial, and yet which 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. 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, while allowing the subjects to swallow after each rinse. For V1000 and V10,000, 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.

Table 1. Stimuli

Stimulus	Abbrev	Concentration	MW	Temp (°C)	Visco (cP)	Chemical group	Acceptability bk mean±S.E.M.	Acceptability bo mean±S.E.M.
Glucose	G	1 M	180	23	1	Monosaccharide aldohexose	1.8±0.1*	1.3±0.3
Black currant juice	BJ	20%		23	1	Mixture	1.8±0.1*	1.0±0.0
Monosodium glutamate	M	0.1 M	187	23	1	Amino acid salt	−0.7±0.7	−0.6±0.3
NaCl	N	0.1 M	58	23	1	Inorganic salt	−1.6±0.1*	−0.8±0.1
HCl	H	0.01 M	36	23	1	Inorganic acid	0.40±0.48	−0.13±0.83
Quinine HCl	Q	0.001 M	387	23	1	Alkaloid	0.10±0.33	0.63±0.43
Water	T10			10	1		0.60±0.37	1.25±0.29
Water	T23/V1			23	1		0.06±0.13*	1.00±0.00
Water	T37			37	1		1.50±0.20	1.00±0.00
Water	T42			42	1		1.46±0.23	1.25±0.29
CMC	V10	0.2 g+1IV1	700,000	23	10	Polysaccharide	0.05±0.52	1.00±0.00
CMC	V100	4.0 g+1IV1	700,000	23	100	Polysaccharide	−0.76±0.28*	1.00±0.00
CMC	V1000	11.0 g+1IV1	700,000	23	1000	Polysaccharide	−0.83±0.33*	1.00±0.00
CMC	V10000	24.0 g+1IV1	700,000	23	10000	Polysaccharide	−1.56±0.26*	1.00±0.00
Gritty	Gr	100 g Fillite+ 9.4 g CMC+1IV1	700,000	23	1000	SiO_2 + polysaccharide	−1.34±0.18*	1.25±0.29
Silicone oil	SiO10	100%		23	10	Silicon-oxygen polymer	—	−1.13±0.34
Silicone oil	SiO100 or SiO 280	100%		23	100 Or 280	Silicon-oxygen polymer	0.032±0.63	−1.13±0.34
Silicone oil	SiO 1000	100%		23	1000	Silicon-oxygen polymer	—	1.00±0.00
Vegetable oil	VO	100%		23	55	Fat	−0.93±0.17	−1.38±0.42
Coconut oil	CO	100%		23	40	Fat	−1.00±0.31	−0.13±0.83
Safflower oil	SaO	100%		23	50	Fat	−1.14±0.11*	0.63±0.43
Single cream	SC	100%		23	12	Emulsion	1.02±0.65*	−1.69±0.36
Lauric acid	LaA	100 μM	222	23	1	ffa	−0.13±0.68	−0.50±1.00
Linoleic acid	LiA	100 μM	280	23	1	ffa	0.00±0.17	0.25±0.87
Capsaicin	Cap	10 μM	377	23	1	Vanillyl amide	−1.10±0.11	0.25±0.87

* $P<0.05$ acceptability rating bk vs bo; $n=4$–5. NA, not applicable.

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. 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, as shown in Fig. 2 of Rolls et al. (2003) and in Fig. 4. 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–V10,000, the set of fat stimuli (MO, SiO10, 100 or 280, 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 analyzed, and graphical presentation of stimulus similarity using multidimensional scaling (loss function: Kruskal; regression: mono).

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–V10,000. 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 significant larger 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 α level was set at $P<0.05$. Further, the tests for capsaicin, LaA, and LiA sensitivity were a two-tailed t-test comparing the responses of the neuron to capsaicin, LaA, and LiA 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 (Gr; which has a viscosity of 1000 cP) and to the 1000 cP stimulus from the viscosity series made with CMC.

A Fisher (1932) probability combination (or generalized significance or exact probability) test was performed to check that the statistically significant results in the orally responsive cells could not reflect just chance statistical results. (By chance, if for example one statistical test was performed on 100 cells, then five of the tests might be expected to be significant at $P<0.05$.) The Fisher combination test calculates the exact probability of obtaining a set of significance values by chance in independent tests. The procedure calculates $-2\sum \ln p_i$, which has a χ^2 distribution with $2n$ degrees of freedom, and the sum is over the n probability values p_i obtained in separate tests. This measure is well established and asymptotically Bahadur optimal (Littell and Folks, 1971; Zaykin et al., 2002).

To quantify the tuning of the neurons to the stimuli, the breadth of tuning metric H of Smith and Travers (1979) was calculated as
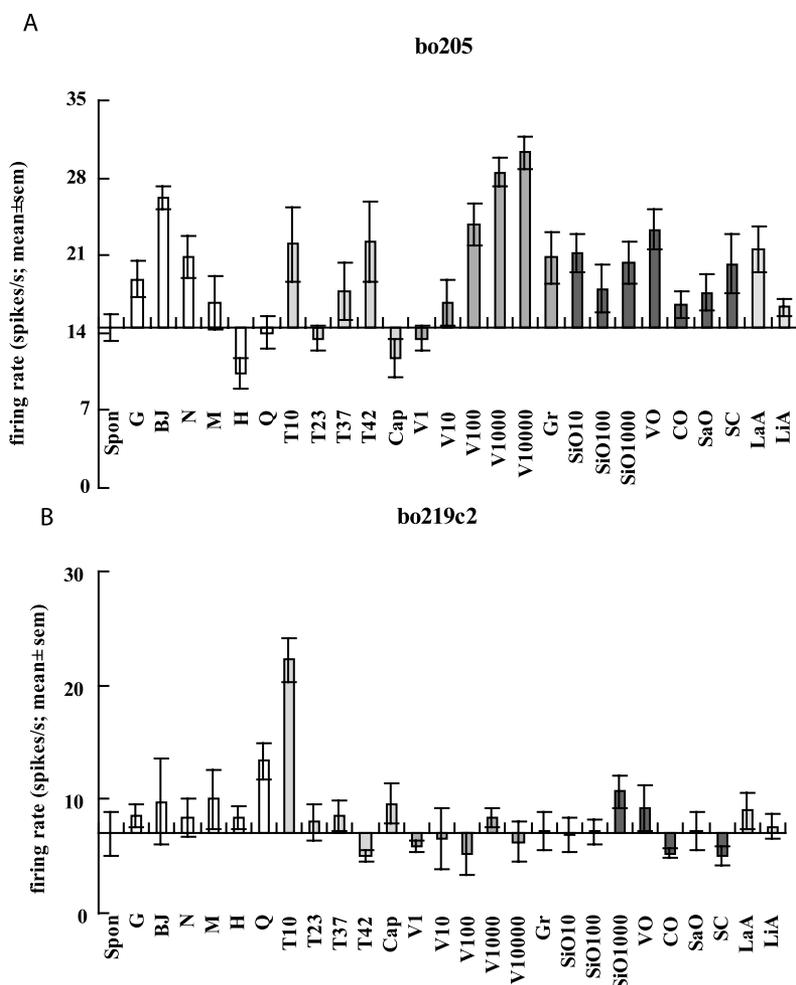
$$H = -k \sum_i p_i \log p_i$$

Fig. 1. (A) Responses of an amygdala neuron (bo205) with differential responses to viscosity, temperature and taste. The mean (±S.E.M.) firing rate responses to each stimulus calculated in a 1 s period over four to six trials are shown here and elsewhere unless otherwise indicated. The spontaneous (Spon) firing rate is shown. 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 V10,000 where the numeral indicates the viscosity in cP at 23 °C); fat texture stimuli were SiO10, SiO100, SiO1000 (SiO with the viscosity indicated), VO, CO and SaO. BJ is fruit juice; Cap is 10 μM capsaicin; LaA is 0.1 mM LaA; LiA is 0.1 mM LiA; see Table 1. (B) The responses of an amygdala neuron (bo219c2) responding to temperature. Conventions as in Fig. 1A.

where k=scaling constant (set so that H=1.0 when the neuron responds equally well to all stimuli in the set of size n), p_i=the response to stimulus i expressed as a proportion of the total response to all the 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 was also measure quantitatively 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 (Rolls and Tovee, 1995; Rolls and Treves, 1998; Rolls and Deco, 2002). 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).

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 also tested for visual responsiveness (to the sight of food, a saline-associated square plaque, the approach of a taste stimulus toward the mouth, objects, faces, head movement, and lip-smacking) and auditory responsiveness (a 500 Hz tone, coo-calls, grunts and vocalization) as stimuli of these types do activate some amygdala neurons (Sanghera et al., 1979). When neurons were insensitive to these stimuli, we classified them as nonresponsive. Only cells responding consistently to at least one stimulus of the array were recorded, all stimuli being applied four to six times in permuted sequences.

RESULTS

The data described in this paper were obtained in three hemispheres of two monkeys. Out of 1416 screened neurons in the amygdala, 44 neurons (3.1%) responded in
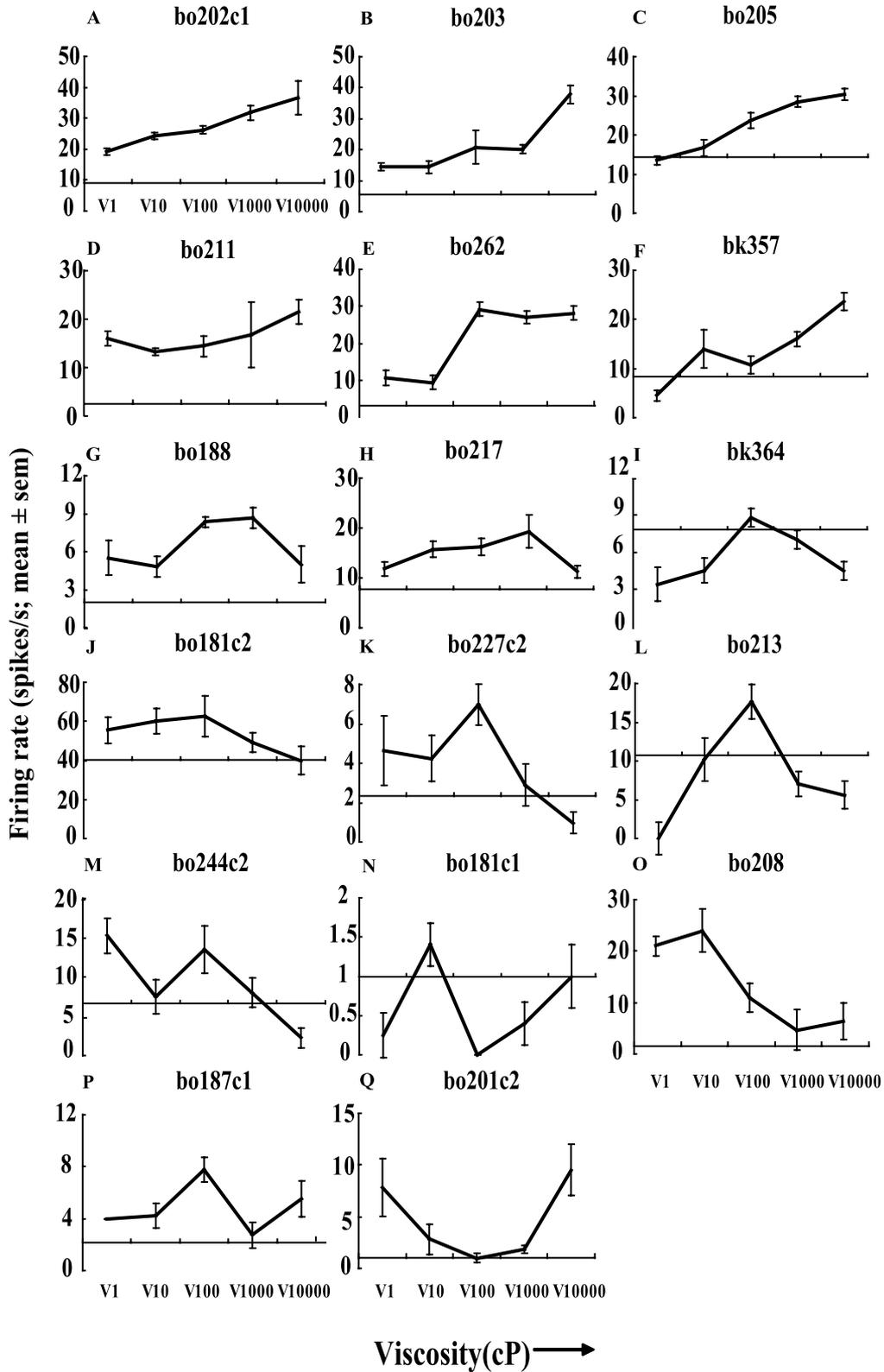
Fig. 2. The response functions of all the viscosity-sensitive neurons to 1, 10, 100, 1000 and 10,000 cP CMC. The mean and the S.E.M. are shown. The spontaneous firing rate for each neuron is shown by the horizontal line.

Table 2. Numbers of amygdala neurons with different types of input[a]

Unimodal		Bimodal		Multimodal		Others
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

[a] Note: the percentage indicated is of the 44 orally responsive neurons.

relation to oral viscosity, gritty texture, fat, taste, temperature, capsaicin, LaA, and/or LiA included in the 25 oral stimulus sets. The remainder of the neurons was unresponsive to the oral stimuli used, except for four neurons that increased their firing rates above the spontaneous level non-specifically to all the stimuli applied. (In contrast to the 40 differential orally responsive neurons described in detail here, they had no significant differential activity in a one-way ANOVA that tested for differences between the whole set of oral stimuli.) Of the 40 neurons with selective responses to the stimulus array, the responses were typically very significant, as shown by the one-way ANOVA across the 25 stimuli (in 35/40 cases at $P<0.001$, in 28/40 cases at $P<0.0001$, and in 25/40 cases at $P<10^{-6}$, and in 12/40 cases at $P<10^{-10}$; see Experimental Procedures). To confirm that the significant responses of this population of 40 neurons could not have arisen by chance, we performed a Fisher (1932; see Experimental Procedures) probability combination test across the population of 1416 neurons, and found a χ^2 value of 3664 ($df=2832$), which corresponds to a z value of 10.36, $P\ll10^{-16}$. Thus, the responses of the 40 neurons to taste, viscosity, etc (across all 25 stimuli) were very unlikely to be due to chance. Visual, auditory, and/or olfactory responses were clear in 7.4% of the total sample. Neuronal responses related to mouth movements comprised 0.2% of the total sample. These neurons responded phasically whenever the monkey moved the mouth, and could be made to fire when a control syringe containing no liquid touched the mouth and produced mouth movements. These neurons could have been somatosensory or motor. The remainder of the neurons (89%) was unresponsive to the stimuli used.

Amygdala neurons with responses related to the viscosity of oral stimuli

Fig. 1A shows a neuron (bo205) which is tuned to viscosity, with graded responses to the 1–10,000 cP stimuli from the CMC viscosity series (ANOVA within the viscosity stimuli, ($F(4,16)=7.95$, $P=0.0012$). The neuron also responded when the viscosity was produced by stimuli in the SiO series. (Post hoc tests showed that the responses were for a given viscosity not significantly different to the CMC and the silicone, though the correspondence of firing rates is not as close as in some other brain areas, as considered in the Discussion.) This viscosity-sensitive neuron also had taste ($F(5,22)=6.98$, $P<0.0007$) inputs, and the difference of

firing rates to different temperatures was not significant ($F(3,12)=2.83$, $P=0.08$). This neuron was unusual in responding to LaA.

The way in which each of the 17 viscosity-sensitive neurons responded to the different members of the viscosity series is shown in Fig. 2, in which the abscissa is the viscosity of the stimulus on a log scale for the five viscosity stimuli in the range 1–10,000 cP. For the purpose of ordering the neurons in Fig. 2, the neurons were grouped into six sets based on cluster analysis using the neuronal responses to V1–V10,000. The first set of neurons in the diagram (A–F) tended to have increasing firing rates as a function of viscosity. The other sets of neurons (G–I, J–L, M and N, O and P) were tuned within the viscosity series, with increasing and decreasing parts of their response functions.

While three of the viscosity-sensitive (i.e. tuned) neurons responded to the SiO and the other oils in ways that would be predicted if they were responding to the viscosity of the oils, the majority (10) of the viscosity-sensitive neurons responded to the CMC viscosity series more than they responded to the equivalent viscosity when provided by an oil, and some (3) did not respond to the oil at all (see Table 2). An example of the latter is shown in Fig. 3 (bo217). The neuron was broadly tuned to viscosity (V10–V1000), but its firing when any oil (silicone, VO, CO, SaO; and SC which is an oil in water emulsion) was in the mouth was at the spontaneous firing rate level. (The firing to most of the aqueous stimuli was a small amount above the spontaneous firing rate.) Thus the neuron can discriminate between fat texture and viscosity (t-test, $P<10^{-6}$ using all oils, and CMC V10–V1000), and this is made clear in Fig. 3B. This neuron also had responsiveness to taste ($F(5,22)=3.72$, $P=0.014$) and temperature ($F(3,13)=10.47$, $P=0.001$), as shown in Fig. 3A.

The typical time course of the responses of a viscosity-sensitive neuron is shown in the peristimulus rastergrams and time histograms in Fig. 4. The neuron had a larger response to 10,000 than to 1 cP CMC.

Fat-responsive neurons

Fig. 4A shows a neuron (bk361) which responded only to the set of oils (with these responses being significantly different from both spontaneous and from all members of the viscosity series V10–V1000; $P=0.00005$). There was no significant response to any of the members of the CMC viscosity series, nor to any of the taste stimuli, nor to any of the temperature stimuli. (The neuron did not respond to SC, which although it contains fat, also contains sweet and other tastants which may have prevented the neuron responding to the fat in the cream.) Interestingly, the neuron did not respond to the fatty acids LaA and LiA, indicating that the responses to fat were based on its texture, and not on any fatty acids that might possibly be produced if fat is lipolyzed at all in the mouth by any salivary lipase that might be present. Further evidence that the neuronal response was not based on fatty acids is that the neuron responded to the SiOs (which contain no fat or fatty acids, but have a similar texture to the fatty oils such as VO, CO and SaO). Fig. 4B illustrates the neuronal responses in
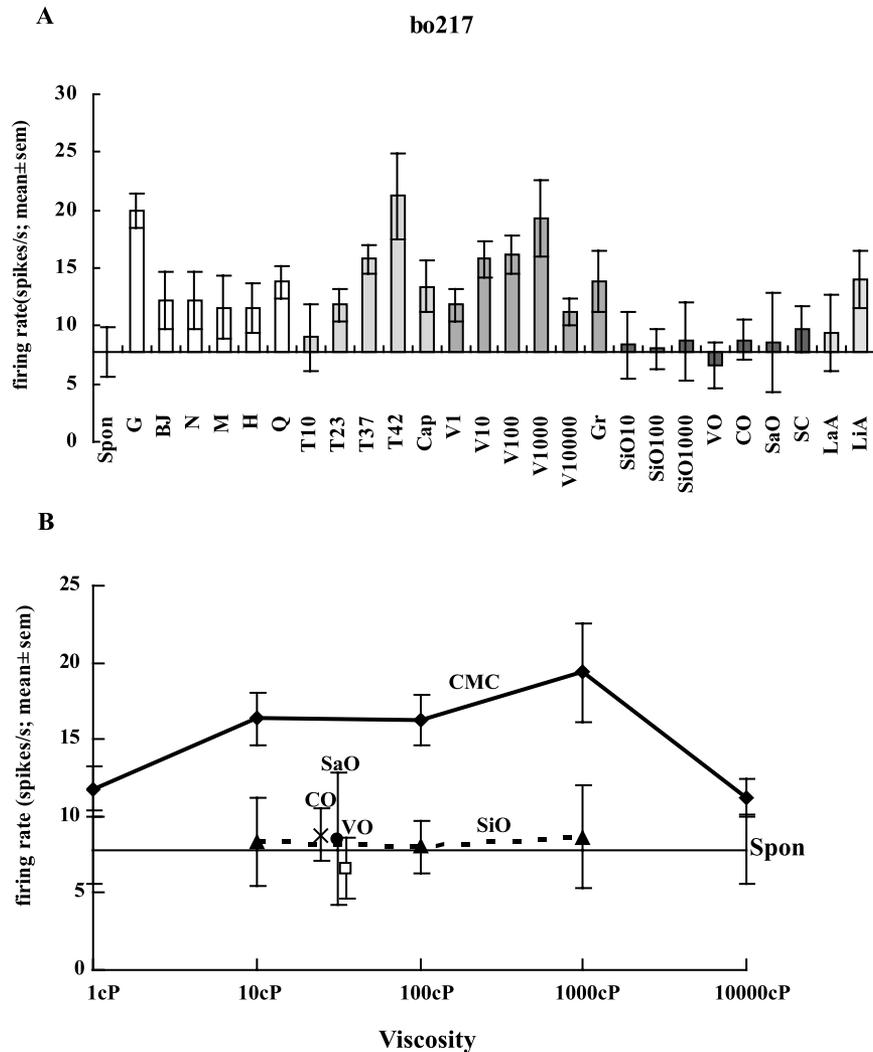
Fig. 3. (A) The responses of an amygdala neuron (bo217) with differential responses to taste, temperature and viscosity. The neuron did not respond to fat texture. Conventions as in Fig. 1. G, N, M, H and Q are the taste stimuli. T10–T42 are the temperature stimuli. V1–V10,000 are the CMC viscosity series with the viscosity in cP. The fat texture stimuli were SiO10, SiO100, SiO1000 (SiO with the viscosity indicated), VO, CO and SaO. BJ is fruit juice; Cap is 10 μM capsaicin; LaA is 0.1 mM LaA; LiA is 0.1 mM LiA; see Table 1. (B) The firing rate (±S.E.M.) to different viscosities of CMC shown as a graph. The horizontal line shows the spontaneous activity (±S.E.M.). There was no response to the fatty oils. Abbreviations as in Fig. 1.

more detail, by showing peristimulus time firing rate histograms and rastergrams for some of the stimuli, together with an inset to show the spike being recorded, which was, as was usual, very well isolated.

As shown in Table 2, two of the neurons (bk361 and bk364) were fat sensitive, in that their responses were large to the oils and occurred in a way that would not be predicted from any smaller response they might have to the CMC viscosity series (see example in Fig. 4A). Both of these neurons also responded to the SiO, and neither of them responded to fatty acids. In addition, none of nine other neurons with a response to one or both of the fatty acids was classified as fat sensitive.

Temperature-responsive neurons

Fig. 1B shows a neuron (bo219c2) with differential responses to different temperatures ($F_{(3,16)}=9.179$, $P=0.001$). The

neuron responded primarily to T10 from the temperature series (and had no significantly different responses within the taste series, within the viscosity series, or within the fats or oils).

The profiles of the responsiveness to the different temperature stimuli of the thermosensitive neurons are shown in Fig. 5. The order of the neurons in Fig. 5 just for the purposes of illustration is based on six clusters identified by cluster analysis. In the first set (A–G), the neuronal responses show a generally upward trend with increasing temperature (T10–42); in the second set (H–L), the neurons had the best responses to T10; in the third set (M–P), the neurons showed more activity to the low than to the high temperatures. The other neurons (Q and R in Fig. 5) were separate clusters.

Of the 18 temperature-sensitive neurons, six responded only to temperature (see Table 2). Thus the temperature of
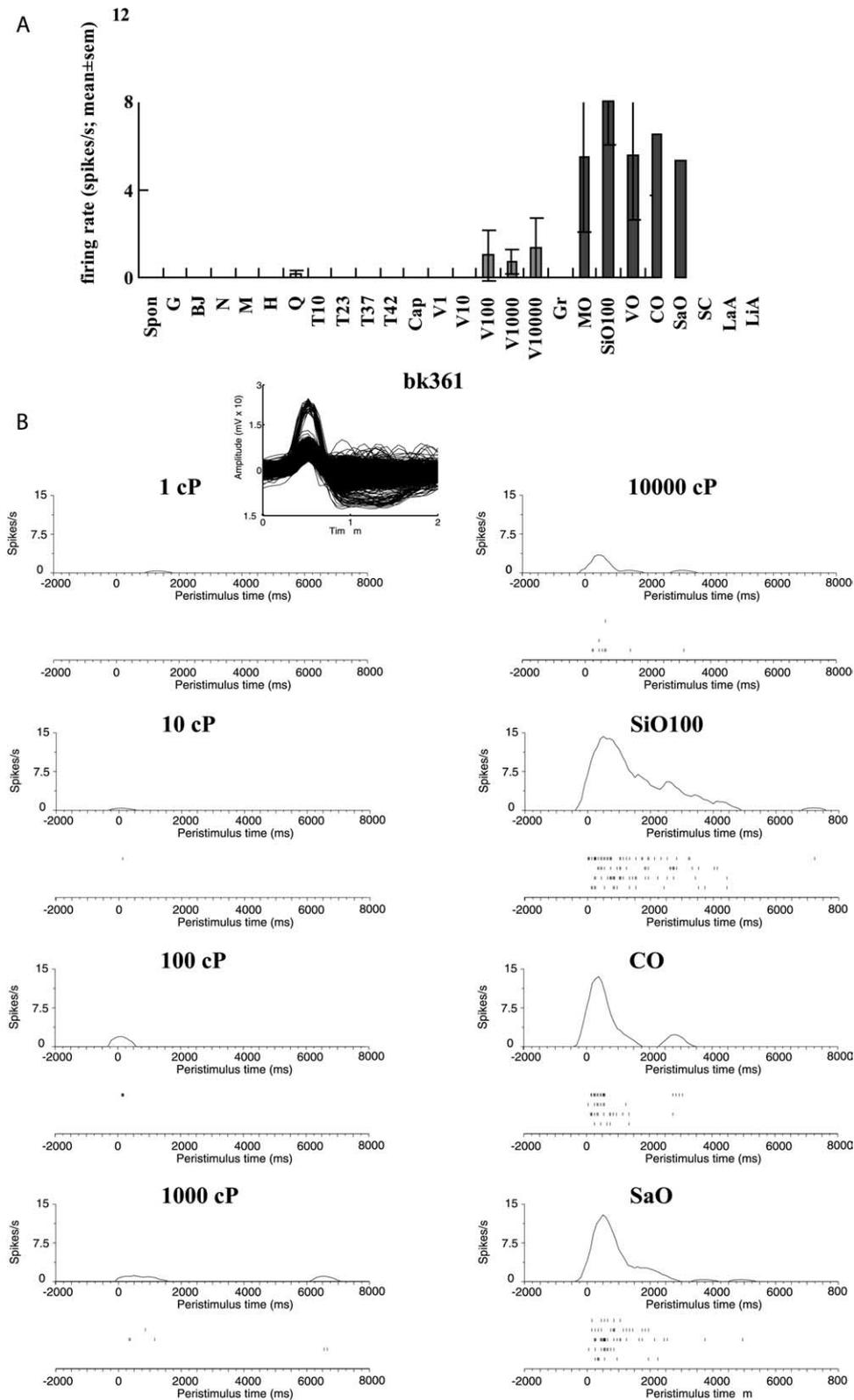
Fig. 4. (A) Responses of an amygdala neuron (bk361) responding to fat. Conventions as in Fig. 1. G, N, M, H and Q are the taste stimuli. T10–T42 are the temperature stimuli. V1–V10,000 are the CMC viscosity series with the viscosity in cP. The fat texture stimuli were SiO10, SiO100, SiO1000 (SiO with the viscosity indicated), VO, CO and SaO. BJ is fruit juice; Cap is 10 μM capsaicin; LaA is 0.1 mM LaA; LiA is 0.1 mM LiA; see Table 1. (B) Post-stimulus-time histogram and rastergram of the same neuron (bk361) to show the nature of the neuronal responses on typical trials to each stimulus. Spont, spontaneous firing rate. The recording on each trial started at time 0, when the stimulus was delivered. The poststimulus time histograms were Gaussian smoothed with a S.D. of one time bin, each 100 ms wide. The inset shows spikes from the neuron being recorded to show the typically very good isolation of the spikes, and there is no overlap with the noise.
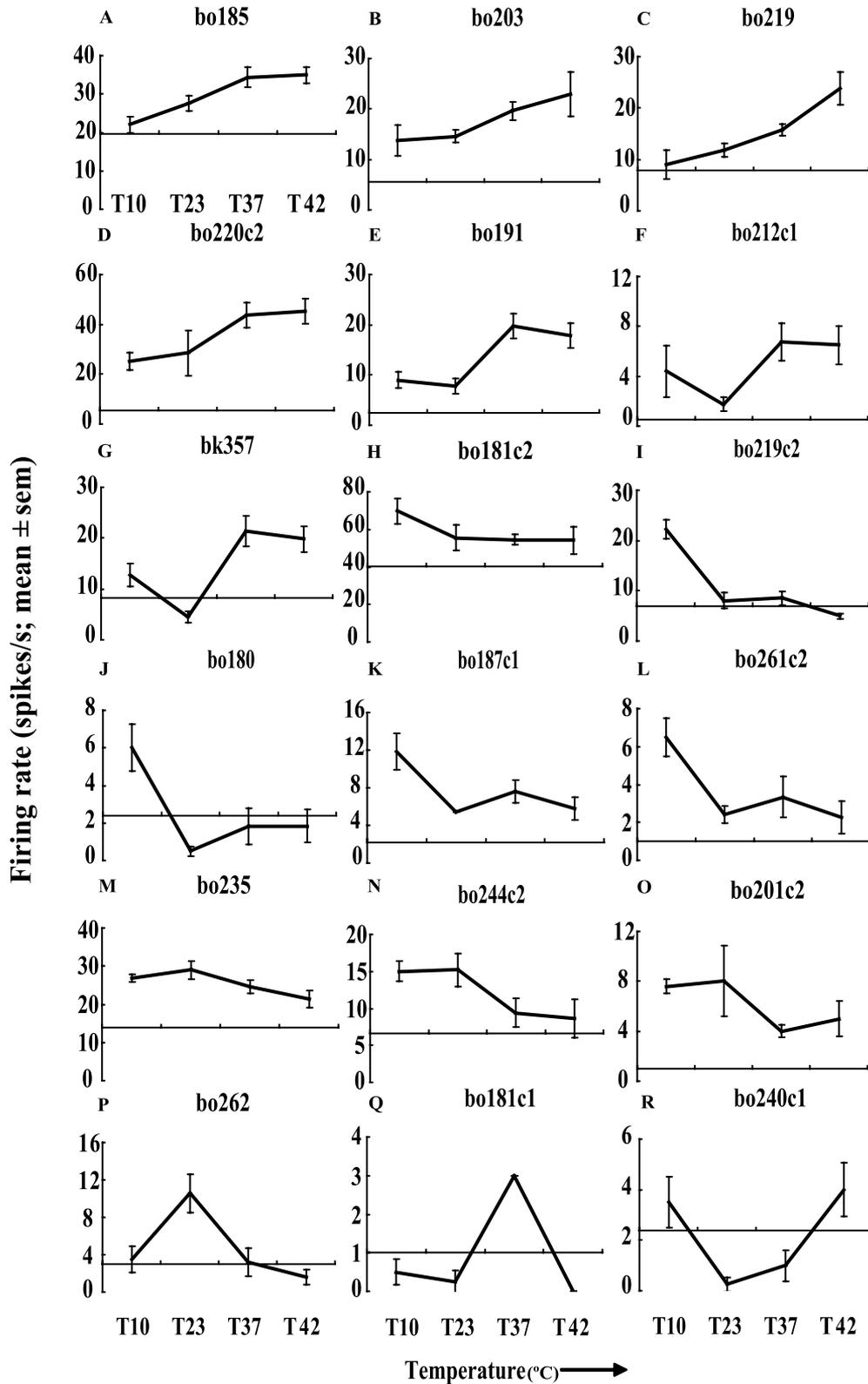
Fig. 5. The response functions of all the thermosensitive neurons to different temperature in °C (T10, T23, T37 and T42). The mean and the S.E.M. are shown. The spontaneous firing rate for each neuron is shown by the horizontal line.

what is in the mouth is represented independently of taste, viscosity, and fatty texture in the primate amygdala. In addi-

tion, 10 neurons responded to both taste and temperature, showing that the primate amygdala represents combinations
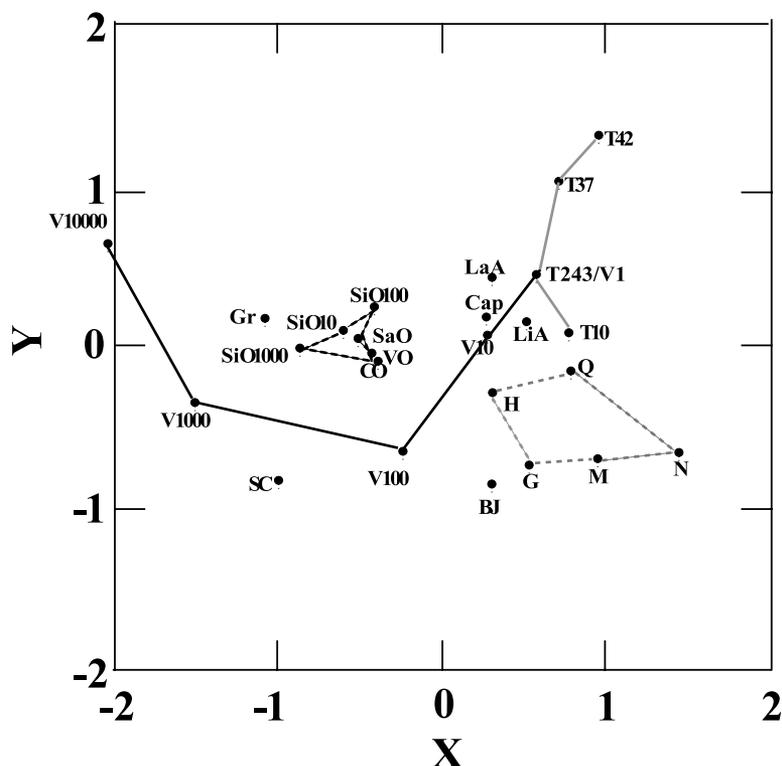
Amygdala (n=44)

Fig. 6. A stimulus space (multidimensional scaling) of the stimulus similarity based on the across-neuron response profiles of the 44 orally responsive amygdala neurons. 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 V10,000 where the numeral indicates the viscosity in cP); fat texture stimuli were SiO10, SiO100, SiO1000 (SiO with the viscosity indicated), VO, CO and SaO. BJ is fruit juice; Cap is 10 μM capsaicin; LaA is 0.1 mM LaA; LiA is 0.1 mM LiA. 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 accounted for 91% of the variance (*n*=44).

of these two modalities, potentially providing the basis for different behavioral responses to particular combinations of the taste and temperature of the food or fluid in the mouth. As shown in Table 2, some neurons in the primate amygdala respond to oral temperature and to viscosity, and in addition other neurons respond to temperature combined with several other types of oral sensory stimulus including taste and viscosity.

Population analyses

The representation of the similarity of the stimuli by the population of neurons was approached with multidimensional scaling analysis, based on the first 1 s of post-stimulus activity, and was performed on the responses of the same 44 neurons (Fig. 6). The segregations between modalities are clearly shown in the multidimensional space. The different modalities have been joined by lines to help clarify the representation in this multidimensional space. First, the viscosity series is very well separated in the space (primarily along the *x* axis). The five taste stimuli are well separated from each other, but contained in their own part of the space separate

from all the other stimuli. The temperature series are again clearly laid out in the space (primarily along the *y* axis). The oils are located closely together and clearly separate from the viscosity series parametric representation. It is of considerable interest that the oil stimuli are not separated out in the space according to their viscosity, as this provides further evidence that the viscosity of stimuli is encoded parametrically in the amygdala; and that fatty texture is coded as a fatty texture independently of its viscosity. In a check that the multidimensional space represented the distances between the stimuli reasonably and in a way that could be interpreted, we found that removing different randomly permuted sets of five neurons from the calculations used for the multidimensional space did not influence what is shown in Fig. 6.

Of the 44 neurons in the sample, 23 (52%) neurons were unimodal (13 unimodal taste, six unimodal temperature, three unimodal viscosity and one unimodal fat neurons), 10 (23%) neurons were bimodal, and seven (16%) neurons were multimodal with responses to taste, temperature and viscosity (see Table 2; the four neurons shown as 'other' had significantly different responses to the set of

oral stimuli based on the ANOVA performed across the whole set of oral stimuli, but were not further classified on the basis of further ANOVAs as temperature sensitive etc). The findings provide clear evidence for convergence of taste and somatosensory (thermosensitive, texture-sensitive and/or fat sensitive) inputs onto some neurons in the amygdala (see Figs. 1A and 3), and also that each type of input is represented independently of the others (see Figs. 4 and 1B). Further, some neurons had responsiveness to LaA, LiA and Cap when compared with their solvent water (T23/V1): four neurons to LaA, seven neurons to LiA (with two of these neurons responding to both LaA and LiA); and four neurons to Cap. None of the fatty acid sensitive neurons were classified as fat responsive. In addition, three neurons responded to the Gr (when compared with the equally viscous but not gritty V1000).

The breadth-of-tuning metric (Smith and Travers, 1979) calculated across the taste stimuli H, Q, N and G was 0.85 ± 0.03 (mean$\pm$S.E.M.) for the (13) neurons with only taste inputs (i.e. without somatosensory-thermosensitive, texture-sensitive and/or fat sensitive-input), and for (14) neurons with both taste and somatosensory inputs was 0.93 ± 0.02 ($P=0.07$). The corresponding sparsenesses were 0.77 ± 0.04 and 0.87 ± 0.04 ($P=0.092$). In addition, 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 44 amygdala neurons was 0.79 ± 0.18 (mean$\pm$S.D.). This compares to the mean sparseness of 52 orbitofrontal cortex neurons to the same set of stimuli (Verhagen et al., 2003) of 0.67 ± 0.23 (mean$\pm$S.D.; $P=0.006$), which indicates the amygdala neurons were tuned more broadly to the set of stimuli.

Localization of recordings

The reconstructed positions of the neurons analyzed in this study are shown in Fig. 7. The top row shows the locations of the neurons responding to oral stimuli. Most of these neurons were located in the basal/basal accessory amygdaloid nucleus, central amygdaloid nucleus and lateral amygdaloid nucleus. Although it was not an aim of this study to investigate amygdala neurons with other responses, we did find a number of neurons with visual responses (93; categorized as such by the criteria of Sanghera et al., 1979; Leonard et al., 1985), with auditory responses (11), with olfactory responses (2), or with activity associated with mouth movements (4), as shown in the middle row of Fig. 7, primarily for comparison with the locations of the orally responsive neurons. Some of these neurons with other than oral responses responded to stimuli in several modalities, as shown in Fig. 7. Eight of the 44 neurons with oral responses analyzed here and shown in Table 2 had visual (6) or auditory (2) responses. The bottom row shows the sites of all the recorded neurons, to indicate the parts of the amygdala that were sampled.

DISCUSSION

The results in this paper describe the discoveries of neurons in the primate amygdala with activity related to the viscosity, the fat texture, and the temperature of food in the mouth. The results also describe the discovery of amygdala neurons tuned to capsaicin, oral grittiness, and to fatty acids. These results also show that the primate amygdala contains separate representations of the properties of oral stimuli, but also has other neurons that combine them with each other, and also with taste. These findings provide the first detailed evidence on the representation in the primate amygdala of non-taste oral stimuli, and are relevant to understanding what is represented in the human amygdala. Such stimuli, sometimes combined with taste, provide important (mainly primary or unlearned) reinforcers (rewards and punishments) for food intake, and in this way are involved in the control of appetite and the control of food intake. These stimuli also provide the primary reinforcer for much stimulus-reinforcer association learning with appetitive stimuli, in which the amygdala is implicated (Everitt and Robbins, 1992; LeDoux, 1995, 2000; Gallagher and Chiba, 1996; Robbins and Everitt, 1996; Rolls, 1999, 2000; Schoenbaum et al., 1999; Baxter and Murray, 2000; Davis and Whalen, 2001; Everitt et al., 2003; Petrovich and Gallagher, 2003). This stimulus-reinforcer associative learning allows previously neutral visual, olfactory, and auditory stimuli to become associated with the sensory properties of foods in the mouth. By this associative learning, such previously neutral stimuli can gain control of behavior. For example, in the rat the central nuclei of the amygdala encode or express Pavlovian stimulus-response (CS-UR) associations (including conditioned suppression, conditioned orienting, conditioned autonomic and endocrine responses, and Pavlovian-instrumental transfer); and modulate, perhaps by arousal, the associability of representations stored elsewhere in the brain (Gallagher and Holland, 1994; Holland and Gallagher, 1999). In contrast, the basolateral amygdala encodes or retrieves the affective value of the predicted US, and can use this to influence action–outcome learning via pathways to brain regions such as the nucleus accumbens and prefrontal cortex including the orbitofrontal cortex (Cardinal et al., 2002). For both types of learning in the amygdala, the nature of the primary reinforcer is important, for this defines what the learning is about, and in the case of appetitive oral reinforcers, the types of neuron described in this paper are fundamental.

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 in that different neurons have different response functions, as shown in Fig. 2. Further evidence for this is provided by the multidimensional space shown in Fig. 6, 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 three neurons (when compared with equally viscous cellulose).

Fat in the mouth was represented in two ways by the amygdala neurons described here. One way was by the amygdala neurons that respond to fat and not to the cellulose
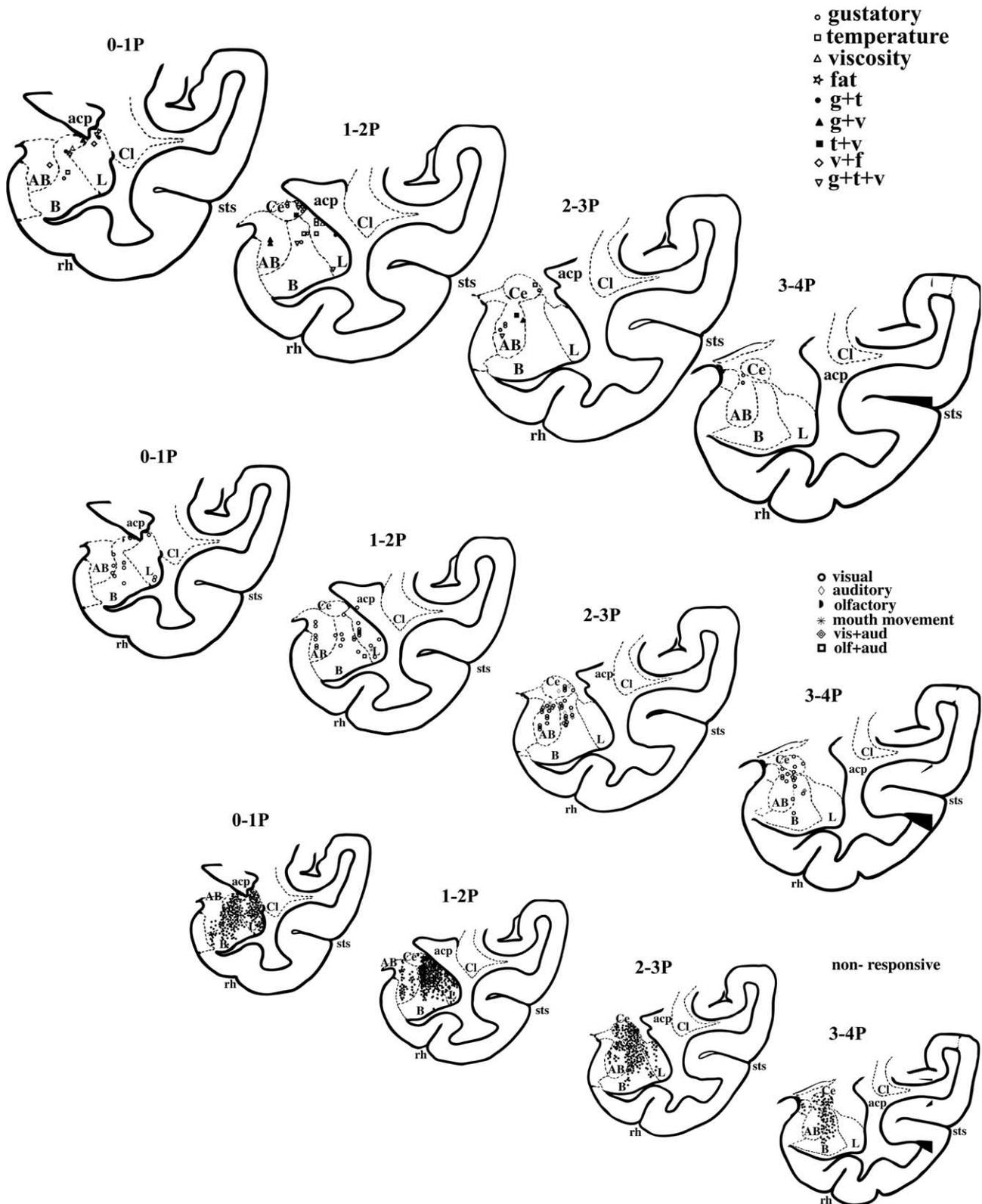
Fig. 7. The reconstructed positions in the brain of the neurons in this study. Top: the symbol with which the location of each neuron is indicated shows whether the neuron was tuned to g=taste, v=viscosity, t=temperature, f=fat or to combinations of these. Middle: the symbol with which the location of each neuron is indicated shows whether the neuron was tuned to vis=visual, aud=auditory, olf=olfactory or to combinations of these, or to mouth movement. Bottom: the sites of the neurons that were not differentially responsive to the stimuli used in this investigation, to show the area of amygdala
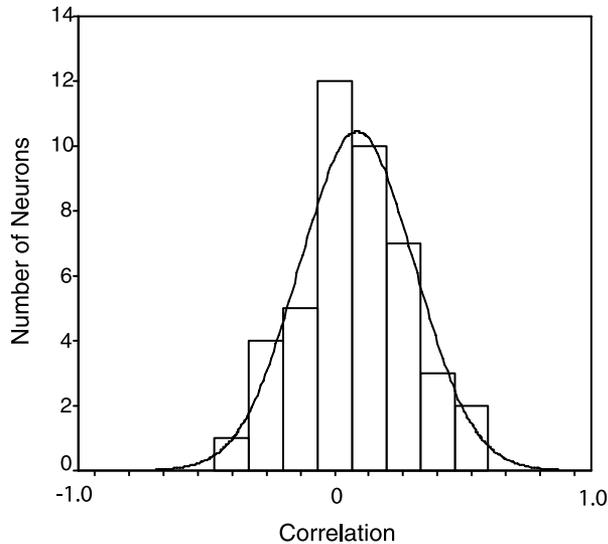
Fig. 8. A frequency histogram showing the correlations between the firing of each of the 44 orally responsive neurons and the acceptability ratings. A normal distribution has been fitted. The mean correlation was 0.09 and the S.D. was 0.22.

viscosity series (Fig. 4). These neurons encode fat by its texture (and not by any odor or free fatty acid cue), in that the same neurons respond to SiO, to MO, and not to fatty acids (Gilbertson, 1998; Verhagen et al., 2003). The second way in which fat is distinguished from non-fat textures in the amygdala is by the neurons that respond to viscosity and not to the oils (see example shown in Fig. 3). Indeed, it was of interest that most of the neurons tuned to the cellulose viscosity series (13/17) tended to have smaller responses to the same viscosity when produced by fat, providing a further way in which the population of amygdala 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. We note that the responses of these neurons to the oils (Si series) or the texture (carboxymethyl cellulose) stimuli cannot be accounted for by taste responses, as shown by the facts that 7/18 amygdala neurons that responded to these texture stimuli had no responses to the taste stimuli G, N, H, Q and M; that neurons that did respond to taste and texture stimuli had different responsiveness to each other to the taste and texture stimuli (see examples in Figs. 1 and 3); and that human psychophysical rating on a 100 mm visual analog rating scale of the intensity of the taste of the texture stimuli was 12.5±3.6 (S.D.), and of the water was 11.6±3.5, whereas that for the taste stimuli was 61.0±10.0. (Consistent with this, carboxymethyl cellulose is used by industry as a tasteless and odorless thickening agent.) Further, the responses of these amygdala texture neurons cannot be accounted for by responsiveness to odor, in that the human psychophysical rating on the 100 mm visual

analog rating scale of the intensity of the odor of the CMC texture and oil (SiO series) stimuli was 8.6±2.7 (mean± S.D.), and of the water was 7.8±2.6.

The representation of temperature provided by these primate amygdala neurons was graded, as shown by the responses of the neurons illustrated in Fig. 1B and Fig. 5, and by the multidimensional space shown in Fig. 6 in which the warm temperatures T42, T37 are parametrically separated from the other stimuli including T23/T10. Four of the 44 amygdala orally responsive neurons tested in this study had responses to capsaicin that were different from water. The neurons did not 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, which responds to temperatures above 43 °C (Caterina et al., 1999).

The responses of the viscosity, fat texture, and temperature responsive neurons were not related to any mouth movements as shown by the following. First, Fig. 4 shows rastergrams and peristimulus response histograms for a neuron that responded to all the oils, and no phasic responsiveness was found to these stimuli that might have been related to any mouth movement. Moreover, the neuron did not respond to any of the CMC series, to which mouth movements were also made. Further, these amygdala neurons in their responsiveness to textured stimuli were very similar to the responses of neurons in the orbitofrontal cortex and insular taste cortex that respond to viscosity and fat texture, which also, as illustrated in the following papers, do not have phasic responsiveness related to mouth movements (Rolls et al., 2003; Verhagen et al., 2003, 2004). Moreover, the tuning functions of different amygdala neurons to the set of viscosity, oil, and temperature stimuli were very different to each other, so that no single factor such as mouth movements could easily account for the responses. Further, neuronal responses related to mouth movements were found and comprised 0.2% of the total sample. These neurons responded phasically whenever the monkey moved the mouth, and could be made to fire when a control syringe containing no liquid touched the mouth and produced mouth movements. These neurons in their phasic, mouth-movement related, responses were very different from those of the taste, texture, oil and temperature sensitive neurons.

Some of the amygdala neurons described here provide separate representations of viscosity, fat texture, temperature, taste, capsaicin, grittiness, and fatty acids, and other neurons combined inputs from different subsets of these properties of sensory stimuli (see Table 2). The combination-responding neurons provide one possible basis for different behavioral and/or endocrine responses to particular combinations of the sensory properties of stimuli such

in which neurons were sampled. 1–2 P shows that the coronal section was taken 1–2 mm posterior to the sphenoid process used as a landmark, which is at approximately the A-P level of the optic chiasm. The architectonic boundaries as described by Amaral et al. (1992) are indicated. AB, accessory basal amygdaloid nucleus; acp, anterior commissure posterior part; B, basal amygdaloid nucleus; Ce, central amygdaloid nucleus; Cl, claustrum; L, lateral amygdaloid nucleus; rh, rhinal fissure; sts, superior temporal sulcus.

as food in the mouth. Other brain areas that also provide possible bases for particular behavioral responses to different combinations of oral stimuli include the orbitofrontal cortex (Rolls et al., 2003). These behaviors include sensory-specific satiety, in which the pleasantness of a food eaten in a meal decreases relative to other foods not eaten in the meal (Rolls et al., 1981; Rolls, 1997, 1999, 2004; Kringelbach et al., 2003). With respect to the pleasantness of the sensory properties of food, there is now very strong evidence that orbitofrontal cortex neurons are related to sensory-specific satiety (Rolls et al., 1989; Critchley and Rolls, 1996; Kringelbach et al., 2003), whereas there is less evidence for strong modulation by hunger or hedonics of taste responsiveness in the amygdala (Nishijo et al., 1988a,b; Yan and Scott, 1996; Rolls and Scott, 2003). The fact that some amygdala neurons respond to both taste and temperature shows that the temperature of what is in the mouth is not encoded only separately from the other sensory properties of the food, but also in combination with other sensory properties of food. Thus this temperature representation may not only allow hot or cold substances to be rejected (or accepted), but also enable foods that have particular combinations of temperature, taste and texture to be reacted to differently.

We note that although human fMRI results are consistent with those described here in showing for example that the orbitofrontal cortex, which has major reciprocal connections with the amygdala (Amaral et al., 1992), is strongly activated by textured whole food stimuli such as tomato juice and chocolate (Kringelbach et al., 2003), and has a representation of oral viscosity (de Araujo and Rolls, 2004). However, the details of the representation as described here, with both unimodal neurons, and bimodal 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. In addition, fMRI studies provide little evidence on the proportion of neurons in a structure that respond in different ways. One rather interesting result of what was found in the primate amygdala is that provided that care is taken, as we did, to exclude movement-related neurons from the sample, then the number of neurons specifically tuned to oral sensory stimuli in the amygdala appears quite modest, 3.1%. Even though the proportion is not large, the population of neurons described here did provide very detailed representations of what is in the mouth, and indeed, if scaled up according to the number of neurons in the extensive part of the amygdala sampled (see Fig. 7), would amount to thousands of neurons, which is sufficient to provide a great deal of information about which stimulus is present in the mouth (Rolls and Treves, 1998; Rolls and Deco, 2002). Indeed, information of this type, the proportion of neurons, and how each one responds, is of course essential to understanding how the brain operates, and the importance of these neurons to overall behavior is attested to by the evidence that the amygdala is involved in associative learning for which one important type of unconditioned stimulus is food in the mouth (Aggleton, 2000). The fact that the proportion of amygdala neurons devoted to oral stimuli is not high is consistent with the fact that other inputs, including visual (Sanghera et al., 1979; Leonard et al., 1985; Rolls, 2000), are represented in the primate amygdala, and if the overall representation is to be maintained sparse, which has many advantages for neural computation (Olshausen and Field, 1996; Rolls and Deco, 2002), then the proportion devoted to any one modality cannot be high. Moreover, the precise identification described here of the proportions of neurons activated by oral stimuli in the amygdala is of interest in relation to functional magnetic resonance neuroimaging, in which activations to the taste of glucose or salt can be demonstrated in the human amygdala. This is an indication that even quite low proportions of neurons that are activated may be sufficient to produce a very significant fMRI signal, at least in a 3T scanner (O'Doherty et al., 2001).

In this paper the reward value of the textured stimuli has not been manipulated, by for example feeding the monkey to satiety to decrease the reward value of the stimulus, and then re-measuring the neuronal response. It has previously been shown that satiety produces a rather modest (on average 58%) reduction in the responses of macaque amygdala neurons to taste (Yan and Scott, 1996), in comparison to the essentially complete reduction of responsiveness found in orbitofrontal cortex taste neurons (Rolls et al., 1989). Further, the representation in the amygdala of these oral stimuli does not appear to be on any simple hedonic basis, in that no direction in the multidimensional space in Fig. 6 reflects the measured preference of the monkeys for the stimuli. Moreover, for each monkey, there was no correlation between the acceptability ratings of each stimulus by the monkeys and the average neuronal responses to each stimulus (BK: $F(1,21)=0.33$, $P=0.570$; BO: $F(1,23)=1.85$, $P=0.187$). We also calculated a correlation between the acceptability ratings of the monkey and the responses of each neuron that had a significant response to the oral stimuli. The values of the correlations are shown in Fig. 8, together with a fitted normal distribution. For 39 of the 44 neurons there was no significant correlation between the neuronal response and the preference rating across the 25 stimuli. For five neurons, there was a significant correlation at $P<0.05$, and for these neurons the correlation values were 0.40 ($P<0.05$), 0.42 ($P<0.05$), 0.46 ($P<0.05$), 0.57 ($P=0.003$) and 0.58 ($P=0.002$). Across all 44 neurons, the mean correlation was 0.08 with a standard deviation of 0.22 (as shown in Fig. 8). (This analysis takes into account that the acceptability measure for each stimulus was different for some of the stimuli, as shown in Table 1.) A further analysis showed that the largest differences were that the carboxymethyl cellulose (V10,000) stimulus was less acceptable in bk, the SC was less acceptable in bo; and the gritty stimulus was lower in bk. Nevertheless, the firing rates of amygdala neurons to these three particular stimuli showed no difference between the monkeys, $P=0.43$, 0.55 and 0.80, respectively between bo and bk across all their amygdala neurons; t-test. Moreover, even when the firing rates to these three stimuli were scaled in each monkey relative to the firing rates to the acceptable glucose and unpleasant saline, the scaled values for the three stimuli were not significantly dif-

ferent for the two monkeys ($P>0.33$ for all three comparisons) and were not related to the monkeys' preferences ($P>0.12$) for these three stimuli. Thus we conclude by these distinct types of analysis, that the differences in the firing rates of these neurons to the set of stimuli in most cases did not reflect just the acceptability to the monkey of the stimuli, but reflect attributes of the stimuli such as their texture, taste, and temperature. Only two neurons had reasonably significant correlations of their firing rates with the acceptability ratings (given that 44 correlations were tested, and a more stringent criterion than $P<0.05$ should be applied), and even for these two neurons the correlations were only moderate, 0.57 and 0.58.

In comparison to the orbitofrontal cortex neurons that respond to the same set of stimuli (Rolls et al., 2003; Verhagen et al., 2003; Kadohisa et al., 2004), the amygdala neurons had less fine tuning across the set of 16 stimuli used in both areas, as indicated by the sparseness measures presented here. Interestingly, the amygdala neurons with oral responsiveness had very clearly separated responses to the viscosity series and the temperature series in the multidimensional space, with a relatively compact part of the space devoted to taste (see Fig. 6). In contrast, the multidimensional space for 53 orbitofrontal cortex neurons tested with the same set of stimuli (apart from minor differences in the oily stimulus subset) in the same two monkeys showed a relatively greater area devoted to taste, and relatively more compressed representations of viscosity and temperature (Rolls et al., 2003; Verhagen et al., 2003; Kadohisa et al., 2004). This may be consistent with the major gustatory inputs to the orbitofrontal cortex from the primary taste cortex in the insular/opercular regions (Baylis et al., 1994), and with the major somatosensory inputs to the amygdala from somatosensory parts (Friedman et al., 1986) and also from taste parts (Verhagen et al., 2004) of the insula. A further interesting difference is that in terms of best responses to different tastes, 57% of the orbitofrontal cortex taste neurons had their best responses to glucose, whereas 21% of the amygdala neurons had their best response to glucose ($\chi^2=12.5$, $df=5$, $P<0.03$). (More amygdala neurons had their best responses to H (18%) and M (14%).)

In conclusion, we show for the first time that amygdala neurons can be specifically tuned to five types of oral somatosensory stimulus: viscosity, grittiness, fat texture, capsaicin, and temperature. In addition to separate representations provided by some neurons, other neurons respond to combinations of viscosity and/or taste and/or gritty and/or capsaicin and/or fat inputs, thereby providing a rich representation of the sensory properties of food in the mouth, in which particular combinations of the above properties can be represented separately from the components. This allows for subjective and behavioral responses that can be based on particular combinations of these inputs, providing for great sensory capability in choosing and learning about particular complex foods, and in extending the flavor space.

REFERENCES

Aggleton JP, ed (2000) The amygdala. Oxford: Oxford University Press.

Amaral DG, Price JL, Pitkanen A, Carmichael ST (1992) Anatomical organization of the primate amygdaloid complex. In: The amygdala (Aggleton JP, ed), pp 1–66. New York: Wiley-Liss.

Baxter MG, Murray EA (2000) Reinterpreting the behavioural effects of amygdala lesions in non-human primates. In: The amygdala: a functional analysis (Aggleton JP, ed), pp 545–568. Oxford: Oxford University Press.

Baylis LL, Rolls ET, Baylis GC (1994) Afferent connections of the orbitofrontal cortex taste area of the primate. Neuroscience 64:801–812.

Berthoud HR (2003) Neural system controlling food intake and energy balance in the modern world. Curr Opin Clin Nutr Metab Care 6:615–620.

Bourne MC (2002) Food texture and viscosity: concept and measurement. London: Academic Press.

Cardinal N, Parkinson JA, Hall J, Everitt BJ (2002) Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev 26:321–352.

Caterina MJ, Rosen TA, Tominaga M, Brake AJ, Julius D (1999) A capsaicin-receptor homologue with a high threshold for noxious heat. Nature 398:436–441.

Critchley HD, Rolls ET (1996) Hunger and satiety modify the responses of olfactory and visual neurons in the primate orbitofrontal cortex. J Neurophysiol 75:1673–1686.

Davis M (1994) The role of the amygdala in emotional learning. Int Rev Neurobiol 36:225–266.

Davis M, Whalen PJ (2001) The amygdala: vigilance and emotion. Mol Psychiatry 6:13–34.

de Araujo IET, Rolls ET (2004) The representation in the human brain of food texture and oral fat. J Neurosci 24:3086–3093.

Everitt B, Cardinal RN, Parkinson JA, Robbins TW (2003) Impact of amygdala-dependent mechanisms of emotional learning. Ann NY Acad Sci 985:233–250.

Everitt B, Robbins TW (1992) Amygdala-ventral striatal interactions and reward-related processes. In: The amygdala (Aggleton JP, ed), pp 401–430. Chichester: Wiley.

Feigenbaum JD, Rolls ET (1991) Allocentric and egocentric spatial information processing in the hippocampal formation of the behaving primate. Psychobiology 19:21–40.

Fisher RA (1932) Statistical methods for research workers. London: Oliver and Boyd.

Francis S, Rolls ET, Bowtell R, McGlone F, O'Doherty J, Browning A, Clare S, Smith E (1999) The representation of the pleasant touch in the brain and its relationship with taste and olfactory areas. Neuroreport 10:453–459.

Friedman DP, Murray EA, O'Neill JB, Mishkin M (1986) Cortical connections of the somatosensory fields of the lateral sulcus of macaques: evidence for a corticolimbic pathway for touch. J Comp Neurol 252:323–347.

Gallagher M, Chiba AA (1996) The amygdala and emotion. Curr Opin Neurobiol 6:221–227.

Gallagher M, Holland PC (1994) The amygdala complex: multiple roles in associative learning and attention. Proc Natl Acad Sci USA 91:11771–11776.

Gilbertson TA (1998) Gustatory mechanisms for the detection of fat. Curr Opin Neurobiol 8:447–452.

Holland PC, Gallagher M (1999) Amygdala circuitry in attentional and representational processes. Trends Cogn Sci 3:65–73.

Kadohisa M, Rolls ET, Verhagen JV (2004) Orbitofrontal cortex neuronal representation of temperature and capsaicin in the mouth. Neuroscience 127:207–221.

Kringelbach ML, O'Doherty J, Rolls ET, Andrews C (2003) Activation of the human orbitofrontal cortex to a liquid food stimulus is correlated with its subjective pleasantness. Cereb Cortex 13:1064–1071.

LeDoux JE (1995) Emotion: clues from the brain. Annu Rev Psychol 46:209–235.

LeDoux JE (2000) Emotion circuits in the brain. Annu Rev Neurosci 23:155–184.

Leonard CM, Rolls ET, Wilson FAW, Baylis GC (1985) Neurons in the amygdala of the monkey with responses selective for faces. Behav Brain Res 15:159–176.

Littell RC, Folks JL (1971) Asymptotic optimality of Fisher's method of combining independent tests. J Am Stat Assoc 66:802–806.

Murray EA, Gaffan EA, Flint RW (1996) Anterior rhinal cortex and amygdala: dissociation of their contributions to memory and food preference in rhesus monkeys. Behav Neurosci 110:30–42.

Nishijo H, Ono T, Nishino H (1988a) Single neuron responses in amygdala of alert monkey during complex sensory stimulation with affective significance. J Neurosci 8:3570–3583.

Nishijo H, Ono T, Nishino H (1988b) Topographic distribution of modality-specific amygdalar neurons in alert monkey. J Neurosci 8:3556–3569.

Norgren R (1984) Central neural mechanisms of taste. In: Handbook of physiology: the nervous system: III. Sensory processes 1 (Darien-Smith I, ed), pp 1087–1128. Washington, D.C.: American Physiological Society.

O'Doherty J, Rolls ET, Francis S, Bowtell R, McGlone F (2001) The representation of pleasant and aversive taste in the human brain. J Neurophysiol 85:1315–1321.

Olshausen BA, Field DJ (1996) Emergence of simple-cell receptive field properties by learning a sparse code for natural images. Nature 381:607–609.

Petrovich GD, Gallagher M (2003) Amygdala subsystems and control of feeding behaviour by learned cues. Ann NY Acad Sci 985:251–262.

Robbins TW, Everitt B (1996) Neurobehavioural mechanisms of reward and motivation. Curr Opin Neurobiol 6:228–236.

Rolls ET (1997) Taste and olfactory processing in the brain and its relation to the control of eating. Crit Rev Neurobiol 11:263–287.

Rolls ET (1999) The brain and emotion. Oxford: Oxford University Press.

Rolls ET (2000) Neurophysiology and functions of the primate amygdala, and the neural basis of emotion. In: The amygdala: a functional analysis (Aggleton JP, ed), pp 447–478. Oxford: Oxford University Press.

Rolls ET (2004) The functions of the orbitofrontal cortex. Brain Cogn 55:11–29.

Rolls ET, Critchley HD, Browning AS, Hernadi A, Lenard L (1999) Responses to the sensory properties of fat of neurons in the primate orbitofrontal cortex. J Neurosci 19:1532–1540.

Rolls ET, Deco G (2002) Computational neuroscience of vision. Oxford: Oxford University Press.

Rolls ET, Judge SJ, Sanghera M (1977) Activity of neurones in the inferotemporal cortex of the alert monkey. Brain Res 130:229–238.

Rolls ET, Murzi E, Yaxley S, Thorpe SJ, Simpson SJ (1986) Sensory-specific satiety: food-specific reduction in responsiveness of ventral forebrain neurons after feeding in the monkey. Brain Res 368:79–86.

Rolls BJ, Rolls ET, Rowe EA, Sweeney K (1981) Sensory specific satiety in man. Physiol Behav 27:137–142.

Rolls ET, Scott TR (2003) Central taste anatomy and neurophysiology. In: Handbook of olfaction and gustation (Doty RL, ed), pp 679–705. New York: Dekker.

Rolls ET, Sienkiewicz ZJ, 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 ET, Tovee MJ (1995) Sparseness of the neuronal representation of stimuli in the primate temporal visual cortex. J Neurophysiol 73:713–726.

Rolls ET, Treves A (1998) Neural networks and brain function. Oxford: Oxford University Press.

Rolls ET, Verhagen JV, Kadohisa M (2003) Representations of the texture of food in the primate orbitofrontal cortex: neurons responding to viscosity, grittiness and capsaicin. J Neurophysiol 90:3711–3724.

Rolls ET, Yaxley S, Sienkiewicz ZJ (1990) Gustatory responses of single neurons in the caudolateral orbitofrontal cortex of the macaque monkey. J Neurophysiol 64:1055–1066.

Sanghera MK, Rolls ET, Roper-Hall A (1979) Visual responses of neurons in the dorsolateral amygdala of the alert monkey. Exp Neurol 63:610–626.

Schoenbaum G, Chiba AA, Gallagher M (1999) Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. J Neurosci 19:1976–1884.

Scott TR, Karadi Z, Oomura Y, Nishino H, Plata-Salaman CR, Lenard L, Giza BK, Ao S (1993) Gustatory neural coding in the amygdala of the alert monkey. J Neurophysiol 69:1810–1820.

Scott TR, Yaxley S, Sienkiewicz ZJ, Rolls ET (1986a) Gustatory responses in the frontal opercular cortex of the alert cynomolgus monkey. J Neurophysiol 56:876–890.

Scott TR, Yaxley S, Sienkiewicz ZJ, Rolls ET (1986b) Taste responses in the nucleus tractus solitarius of the behaving monkey. J Neurophysiol 55:182–200.

Smith DV, Travers JB (1979) A metric for the breadth of tuning of gustatory neurons. Chem Senses 4:215–219.

Steinberger J, Daniels SR (2003) Obesity, insulin resistance, diabetes, and cardiovascular risk in children. Circulation 107:1448–1453.

Szolcsanyi J (1990) Capsaicin, irritation, and desensitisation: neurophysiological basis and future perspective. Chem Senses 2:141–168.

Verhagen JV, Kadohisa M, Rolls ET (2004) The primate insular/opercular taste cortex: neuronal representations of the viscosity, fat texture, grittiness and taste of foods in the mouth. J Neurophysiol 92:1685–1699.

Verhagen JV, Rolls ET, Kadohisa M (2003) Neurons in the primate orbitofrontal cortex respond to fat texture independently of viscosity. J Neurophysiol 90:1514–1525.

Weiss TJ (1983) Food oils and their uses. Chichester: Horwood Ltd.

Wills WM, Lencki RW, Marangoni AG (1998) Lipid modification strategies in the production of nutritionally functional fats and oils. Crit Rev Food Sci Nutr 38:639–674.

Yan J, Scott TR (1996) The effect of satiety on responses of gustatory neurons in the amygdala of alert cynomolgus macaques. Brain Res 740:193–200.

Zaykin DV, Zhivotovsky LA, Westfall PH, Weir BS (2002) Truncated product method for combining P-values. Genet Epidemiol 22:170–185.

Zellner DA, Stewart WF, Rozin P, Brown JM (1988) Effect of temperature and expectations on liking for beverages. Physiol Behav 44:61–68.