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AFFERENT CONNECTIONS OF THE CAUDOLATERAL ORBITOFRONTAL CORTEX TASTE AREA OF THE PRIMATE

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Abstract—A cortical taste region has recently been identified in the caudolateral orbitofrontal cortex of the macaque. The afferents to this region were investigated by means of retrograde tracing, using six injections of wheatgerm-conjugated horseradish peroxidase. The area of taste cortex was first identified physiologically in all the monkeys used in this anatomical study. The four injections into the middle and posterior part of this region resulted in large numbers of labelled cell bodies in the insular-opercular primary taste cortex. Following the two more anterior injections, label was found predominantly in the caudal part of the cardolateral orbitofrontal cortex itself. None of the injections resulted in labelled cells in the gustatory thalamic nucleus ventralis posterior medialis, pars parvocellularis, although all injections resulted in label of the mediodorsal nucleus of the thalamus. Afferents were also seen from more anterior parts of the orbitofrontal taste cortex, which may represent backprojections from subsequent taste areas.

These results suggest that the caudolateral orbitofrontal cortex contains a higher-order taste cortex.

The primary gustatory cortex of the primate brain is in an area which folds around the frontal operculum and anterior insula. This region was first implicated as a gustatory region by Bornstein,[12,13] who found that patients with lesions in this area caused by bullet wounds suffered ageusia. Similarly, lesions of this region in monkeys have been shown to lead to elevations in taste thresholds.[5,37]

Benjamin and Burton[11] stimulated the seventh, ninth and tenth cranial nerves of the squirrel monkey and recorded evoked potentials in the insular/opercular cortex. They found that both the chorda tympani and the glossopharyngeal nerves projected predominately ipsilaterally to this region. Benjamin and Burton concluded that this was the primary gustatory cortex since this was the only cortical area in which evoked potentials were seen. Sudakov *et al.*[40] recorded cells which responded to sapid stimulation of the tongue in awake squirrel monkeys in the same cortical region. Scott *et al.*[39] carried out a similar study in the awake cynomolgus macaque. Within the dorsal part of the frontal operculum, 2.2% of neurons responded to gustatory stimulation of the tongue. This figure closely resembles the 3.5% found by Sudakov *et al.*[40] In a second study, Yaxley *et al.*[45] recorded from neurons sensitive to gustatory stimulation in an anterodorsal region of the insula contiguous with the opercular taste region. It was found that cells recorded in the insula had similar selectivity to neurons recorded from the operculum of the same monkeys. It can therefore be concluded that the insular and opercular areas represent a single continuous area of primary gustatory cortex.

Thalamic afferents to the insular-opercular taste cortex were shown to arise from the nucleus ventralis posterior medialis, pars parvocellularis (VPMpc) by Roberts and Akert,[29] using cell degeneration procedures. This finding was later confirmed by Jones and Burton[16] using autoradiographic techniques in New World monkeys. More recently, Pritchard *et al.*[27] injected radioactive isotope into the VPMpc of macaques. The injections were made at locations where cells had previously been found which responded to gustatory stimulation. The terminal fields resulting from these injections were seen in the anterior insula and operculum. This study also included complementary results from retrograde tracing methods. When horseradish peroxidase (HRP) was injected into the insula and operculum retrogradely labelled cell bodies were seen in the VPMpc.

In a new cortical area studied, neurons with gustatory responses were found in the caudolateral part of the orbitofrontal (CLOF) cortex.[36] These neurons were found to be more narrowly tuned to gustatory stimuli than neurons recorded in the frontal

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Abbreviations: Ci, centralis inferior; CLOF, caudolateral orbitofrontal cortex; HRP, horseradish peroxidase; LPOF, lateral posterior orbitofrontal cortex; MD, mediodorsal nucleus; OFC, orbitofrontal cortex; Pcn, posterior central nucleus; Re, nucleus reuniens; VPMpc, nucleus ventralis posterior medialis, pars parvocellularis; WGA-HRP, wheatgerm agglutinin-conjugated with horseradish peroxidase.

opercular-insular cortex. Most of the neurons with gustatory responses responded primarily to sweet stimuli, with a few responding to water or to sodium chloride. None of the neurons in the CLOF were found to respond primarily to quinine or hydrochloric acid. Rolls *et al.*[35] showed that satiety affected the magnitude of the responses of neurons to gustatory stimuli, in that responses to sweet stimuli were seen only if the monkey was hungry. This is in contrast to the finding that neuronal responses in both the insula[45] and operculum[34] are unaffected by the state of hunger of the monkey.

The nature of taste processing in the orbitofrontal cortex was further studied by Baylis and Rolls,[10,33] using a large array of stimuli including complex liquid and solid tastants. These studies showed that neuronal responses in the CLOF taste area did not code stimuli according to their constituent taste primaries. Furthermore, given the fact that neurons in this region may be hunger-modulated, it is possible that processing may reflect hedonic as well as sensory aspects of taste stimuli. Consistent with this suggestion, it has been shown by Baylis and Gaffan[9] that lesions to the orbitofrontal cortex lead to gustatory anhedonia. In this study, monkeys with bilateral lesions showed abnormal choices between apple, lemon, olive and meat. Taken together, these studies of the orbitofrontal cortex taste areas suggest that processing in this region is qualitatively different from the more sensory analysis in primary taste cortex.[30–32]

Given the physiological findings on gustatory processing in the primate, it is important to know the anatomical route by which the CLOF receives gustatory information. Furthermore, the relation between the primary taste cortex and the CLOF taste areas is unknown. In principal, there are three possible patterns of afferents bringing taste information to the CLOF taste area, each indicating a different status for the CLOF taste area, as follows. First, the main taste input into this area may be from the VPMpc, in which case the CLOF would be another primary cortical taste area, or a rostral extension of the established insular-opercular area. Second, the CLOF may receive its main input from the insular-opercular area, with little or no projection from the VPMpc. In this case, the CLOF taste area could be unambiguously described as a secondary taste cortex. The existence of a small input from VPMpc would not diminish the case that the CLOF represented a secondary sensory cortex, since, for example, an input is seen from the lateral geniculate to extrastriate visual cortex.[46] The third possibility is that the CLOF may receive a substantial gustatory input from both insular-opercular cortex and the VPMpc. This result would essentially be ambiguous between the first two possibilities.

It was therefore decided to investigate the afferent inputs into the CLOF taste area using the HRP retrograde fibre tracing method. It was a crucial part of the experimental design that the region of taste cortex first be functionally localized exactly in the monkeys to be used for anatomical tracing. In this way, it was possible to ensure that the HRP injections were made into the gustatory zone of the CLOF.

EXPERIMENTAL PROCEDURES

Recordings were made of the activity of single neurons in the CLOF taste cortex in five alert male cynomolgus monkeys (*Macaca fascicularis*) weighing 3.8–4.0 kg, using methods described previously.[33] The locations of the neurons in each of the monkeys were determined using X-ray localization relative to permanently implanted reference electrodes and relative to bony landmarks such as the anterior sphenoidal process.[2] Six injections of horseradish peroxidase conjugated with wheatgerm lectin (WGA-HRP: Sigma Chemical Co., Poole, U.K.) were then placed into the CLOF gustatory cortical area localized physiologically.

A 5 μl aliquot of 20% WGA-HRP in physiological saline was made the day preceding the injection, and frozen overnight. The following day, the just-thawed WGA-HRP solution was drawn into a Hamilton 7001 10 μl microsyringe with a 150 mm needle (untapered). Each monkey was narcotized with an intramuscular injection of ketamine (Ketalar, 0.1 ml/kg) and anaesthetized with intravenous thiopentone sodium. The monkey was placed in a Kopf stereotaxic instrument. An empty microsyringe was lowered until its tip (measured in X-radiographs) was in the position in which taste cells had been recorded in the CLOF. It was possible to overlay the lateral and coronal X-radiographs of the recording sites and the microsyringe tip until they corresponded exactly. The microsyringe was then removed, and replaced by the syringe containing WGA-HRP. The stereotaxic was then returned to 3 mm above the exact position previously determined for the needle. Coronal and Lateral X-radiographs were taken once again as a final check that the filled syringe needle was in the correct location (i.e. 3 mm above the target location). In every case, this was so, and the needle was then slowly moved the final 3 mm into position by means of the micromanipulator. This procedure successfully avoided the necessity to reposition a syringe needle containing WGA-HRP, since such repositioning would have carried the risk of injection or leakage into incorrect regions.

When in the upper cortical layers of the cortex (i.e. the more ventral half of the thickness of the cortex), a microinjection of 0.1 or 0.15 μl of 20% HRP was made slowly over 10 min. The syringe was left for a further 5 min, and then gradually withdrawn over the next 15 min. Afterwards, the monkey was allowed to recover from the anaesthetic, and a period of 48 h was allowed for axonal transport of HRP to the furthest parts of the brain (a direct projection from the nucleus of the solitary tract, if one existed, would contain axons up to 50 mm long). At the end of this time, the monkey was then given ketamine followed by intravenous pentobarbital sodium until very deeply anaesthetized and perfused with 0.9% saline followed by glutaraldehyde and paraformaldehyde (Karnovsky's fixative).

The brain was cut into 50-μm sections, of which one in five were reacted with tetramethylbenzidine and counterstained with Cresyl Violet according to procedures described in Mesulam.[18] The sections immediately adjacent to these in the series were stained with Cresyl Violet in order to facilitate cytoarchitectural analysis. The location of every cell labelled with HRP found in every section between the far anterior OFC and the nucleus of the solitary trace was marked on an outline made from an adjacent section using an X-Y plotter (Summagraphics BITPAD ONE) electronically coupled to the stage of a Leitz Dialux 22 microscope. If a section is illustrated in a figure, then every labelled cell found in that section is shown in the figure. The centre of

the injection site was determined to be at the level where such intense precipitation of reaction production disabled recognition of cells or axons.

RESULTS

Reconstructions based on HRP injections at three different antero-posterior levels of the CLOF gustatory area are described in Figs 1–7. All injections were placed within Walker's[44] area 12, in the area which has previously been shown to be a cortical taste area.[10,36] The CLOF gustatory area is within the lateral dysgranular field of the posterior OFC. It is bounded posteriorly by the limen of the insula, medially by the lateral orbital sulcus, and laterally by the lateral edge of the OFC. These landmarks are shown in Fig. 1, in a basal view of the OFC of the macaque.

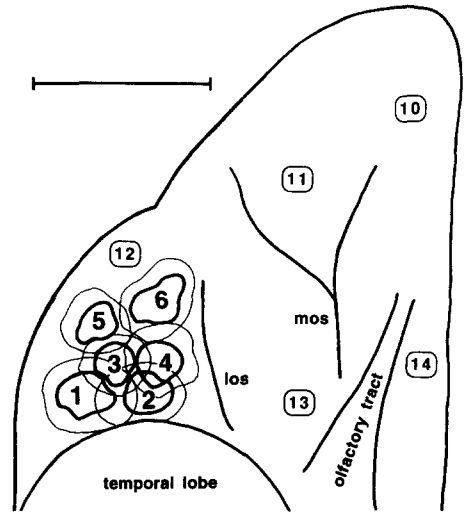

Fig. 1. Basal view of the orbitofrontal cortex showing the location of the six injections of HRP into the CLOF taste area. The extent of the dense centre of each injection site is shown by the bold line around the number of the injection. A larger, concentric fine line shows the limit of the fainter "halo" surrounding each injection. Scale bar = 1 cm.

Injections 1 and 2—Posterior third of caudolateral orbitofrontal cortex taste area

The first injection described was made into the posterior part of the CLOF taste area. The injection site and the cells filled with HRP found between the antero-posterior levels of the OFC and the caudal hypothalamus are shown in Fig. 2. The injection site (Fig. 2b, shown in hatching) was in the caudal third of the CLOF (see also Fig. 1).

Many hundreds of labelled cells were found in the frontal opercular taste cortex. As shown in sections d and e, many cells were labelled in the operculum, and the boundaries of this area of labelling were abrupt. No cells were found in the operculum more than 0.3 mm anterior to the section shown in Fig. 2d, and none more than 0.2 mm posterior to the section shown in Fig. 2e. This area corresponds to the location of taste-responsive cells in the frontal opercular cortex, which are mostly in the caudal part of architectonic region OFO and the anterior part of Prco (both of Roberts and Akert[29]).

As can be seen in the same figure, a well circumscribed region containing many hundreds of labelled cells was found in the insula. The anterior border of this labelled region was near the section shown in Fig. 2c and the posterior boundary was near the section

Abbreviations used in the figures

ABpc[1]	Parvocellular part of the basal nucleus of the amygdala
ABmi[1]	Magnocellular and intermediate parts of the basal nucleus of the amygdala
arc-d	Dorsal limb of the arcuate sulcus
arc-v	Ventral limb of the arcuate sulcus
AL[1]	Lateral nucleus of the amygdala
ATr[1]	Amygdalocortical transition zone
Ca	Caudate nucleus
Ci[2]	Inferior part of the central nucleus of the thalamus
dLGN[2]	Dorsal lateral geniculate nucleus
G.P.	Globus pallidus
Ia-p[3]	Agranular field of the insula
Idg-a[4]	Anterior part of the dysgranular field of the insula
Idg-l[4]	Liminal part of the dysgranular field of the insula
M.B.-l	Lateral mammillary body
M.B.-m	Medial mammillary body
M.D.[2]	Mediodorsal nucleus of the thalamus
OFag[3]	Agranular field of the orbitofrontal cortex
OFdg[3]	Dysgranular field of the orbitofrontal cortex
OFO[5]	Orbitofrontal opercular area
Pcn[2]	Posterior central nucleus of the thalamus
PPC	Prepyriform cortex
Prco[5]	Parietal opercular area
p.s.	Principal sulcus
Pu	Putamen
Re[2]	Nucleus reuniens of the thalamus
Ret[2]	Reticular nucleus of the thalamus
rh.s.	Rhinal sulcus
S.I.	Substantia innominata
SNr	Substantia nigra, pars reticulata
SS I	Primary somaesthetic cortex
s.t.s.	Superior temporal sulcus
VPMpc[2]	Parvocellular part of the ventro-postero-medial nucleus of the thalamus

[1]Amygdaloid nuclei are identified following Ref. 4
[2]Thalamic nuclei are identified following Ref. 24
[3]As defined by Mesulam and Mufson (for review see Ref. 19)
[4]The dysgranular part of the insula was not divided by Mesulam and Mufson,[19] although (as noted by these authors) major differences exist between the anterior, liminal, and posterior parts of the dysgranular insula. Here the areas are considered separately
[5]As defined by Roberts and Akert (for review see Ref. 28)

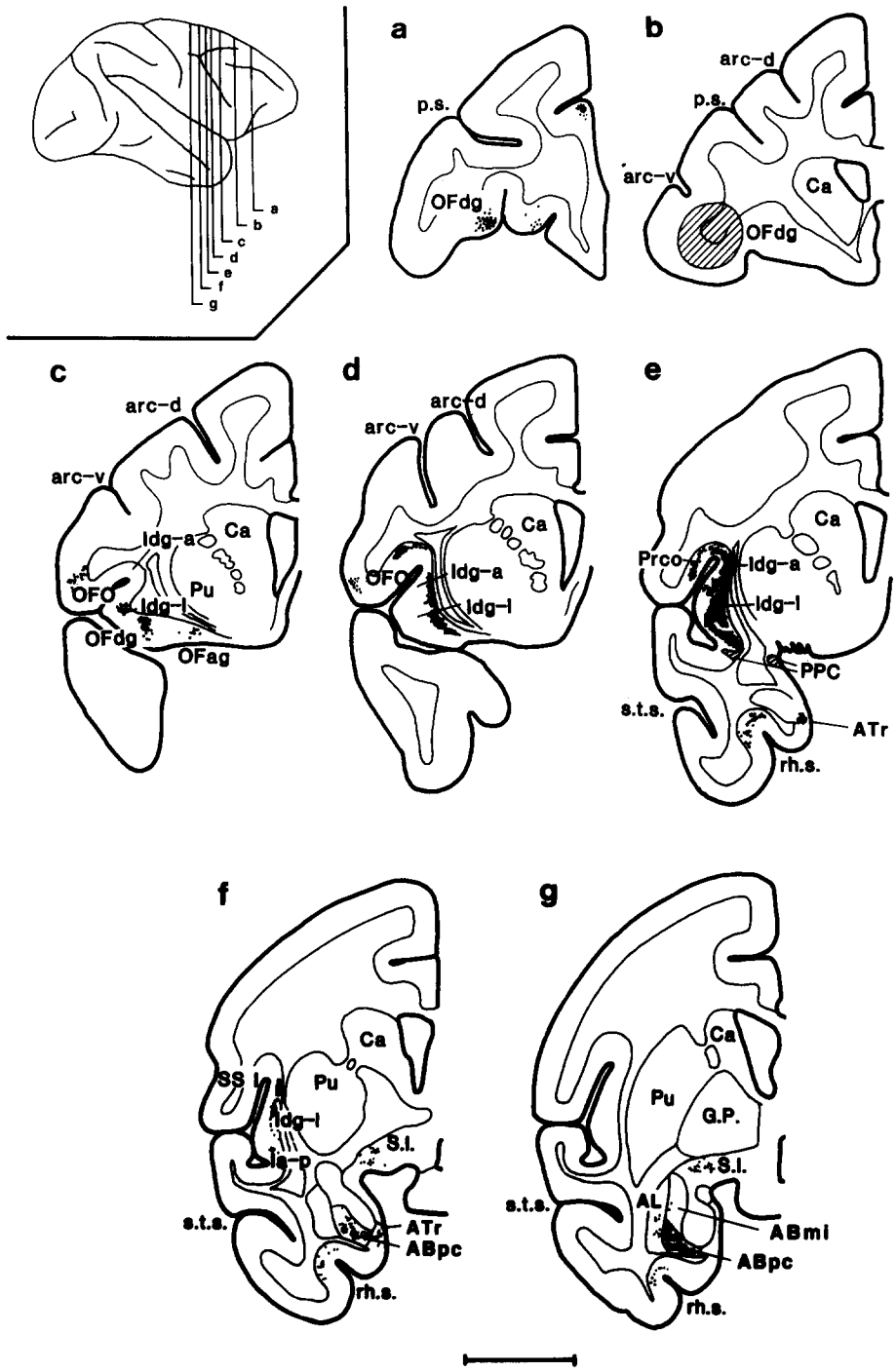

Fig. 2. Injection I into the caudal part of the orbitofrontal cortex taste area. Coronal sections show the position and extent of the injection site and the position of labelled cells. Inset shows the levels at which these sections were taken. Scale bar = 1 cm.

shown in Fig. 2f. Labelled cells were mostly within the liminal part of the dysgranular field of the insula, as described in Mesulam and Mufson.[19,20] This labelled region includes the area of the insula in which taste cells have been recorded,[45] but also extended ventrally and posteriorly beyond this (Fig. 2f).

Labelled cells were found in two areas of OFC anterior to the injection site, as can be seen in Fig. 2a. These correspond to the more medial agranular cortex of the OFC (OFag) and the lateral dysgranular cortex of the OFC (OFdg). The former region has recently been shown to contain many cells responding to olfactory stimuli.[33] Other cortical areas of the frontal lobe in which labelled cells were seen were the cingulate (Fig. 2a), and the far lateral edge of the posterior orbitofrontal cortex (Fig. 2c, d). This latter

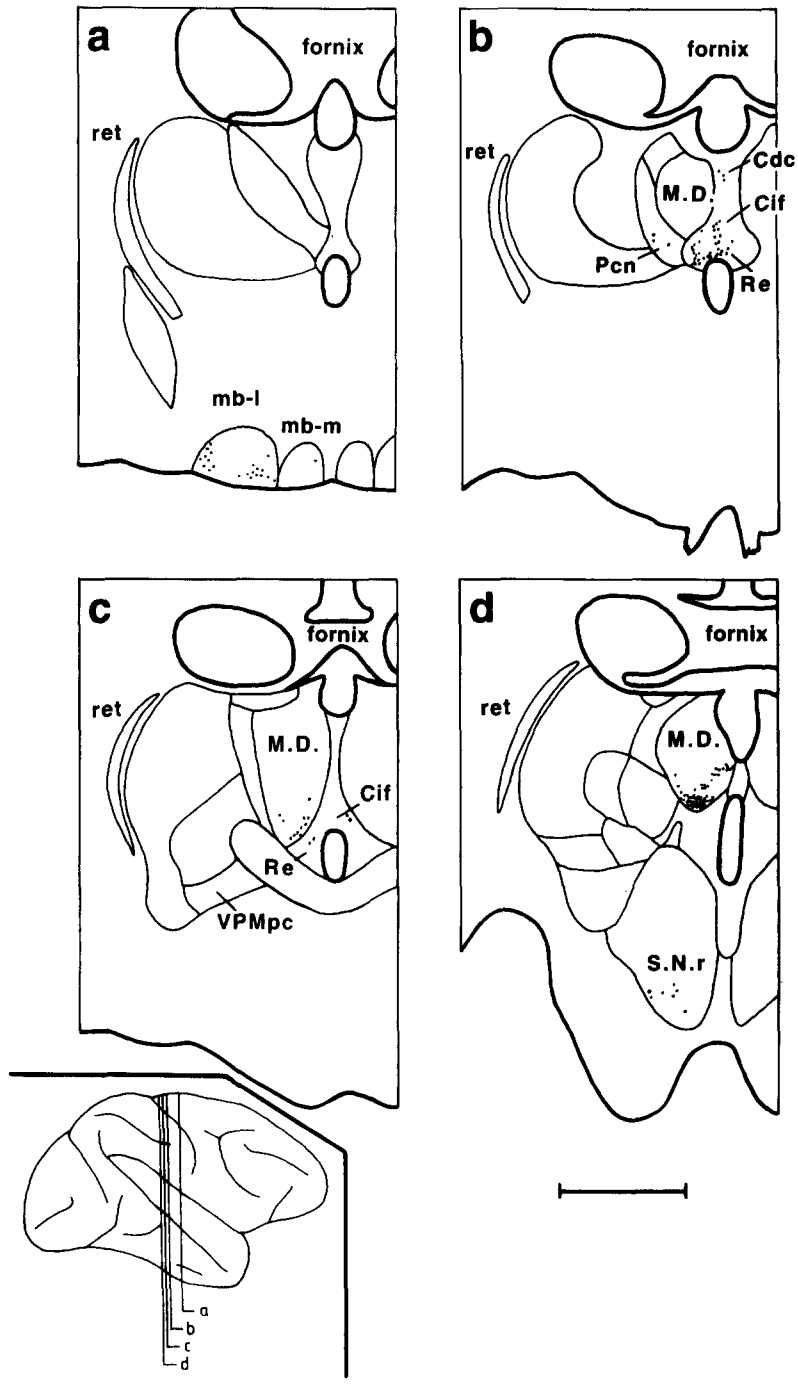

Fig. 3. Injection 1. Coronal sections through the thalamus showing labelled cells in more caudal parts of the brain. Inset shows the levels at which these sections were taken. Scale bar = 5 mm.

region is of particular interest since it may be within the cortical projection area of the VPMpc.[26]

Thalamic labelling from injection 1 is shown in Fig. 3, where it can be seen that no labelled cell bodies were found in the thalamic taste nucleus, VPMpc (Fig. 3c). Rather, the mediodorsal nucleus (MD) and the non-specific midline nuclei, centralis inferior (Ci) and nucleus reuniens (Re), were labelled (Fig. 3b–d). A few labelled cells were also seen in the posterior central nucleus (Pcn) of the thalamus. Heavy labelling was found in the amygdala and adjacent cortex. (Nuclear boundaries and nomenclature follow[4].) Label was most dense in the parvocellular zone of the basal nucleus (ABpc), but labelled cell bodies were also found in the magnocellular and intermediate zones of this nucleus (see ABmi, Fig. 2f, g). Labelled cells were also found in the amygdalocortical transition zone (Fig. 2e) and in the rhinal sulcus (Fig. 2e–g). Other areas of the brain found to project to the site of this injection included the substantia innomi-

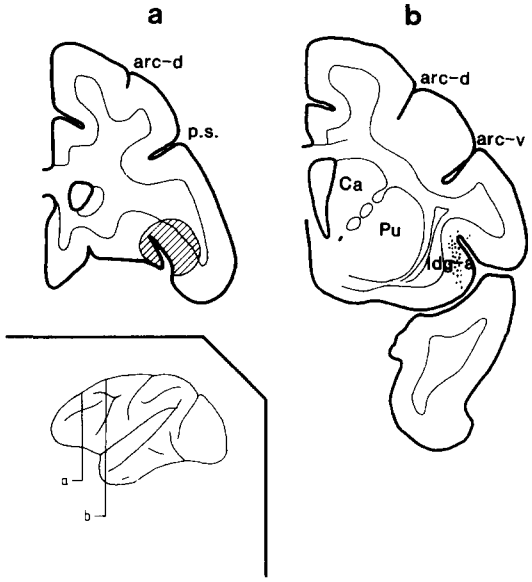

Fig. 4. Injection 2 into the posterior part of the CLOF taste area. Coronal sections show the injection site and some of the areas of labelled cells. Inset shows the levels at which these sections were taken.

nata (Fig. 2f, g), and the lateral mammillary body (Fig. 3a). Cells were apparently labelled in the substantia nigra, pars reticulata, close to the ventral tegmental area (Fig. 2g), but it is likely that this represents spurious reaction product due to the nigral bodies in these cells.

The second HRP injection was medial to the first injection described above (see Figs 1, 4). In Fig. 4 it can be seen that labelling was again found in the frontal opercular and insular taste cortex, and some cells in the far lateral regions of OFC. The distribution of labelled cells in these areas of cortex was slightly further anterior, and slightly less dense compared to injection 1 (cf. Figs 2 and 4). As in the first injection, no labelled cells were found in VPMpc, but many were found in MD, the Ci, and the Re. The other labelling produced by this injection was comparable to that found with the first injection, as shown in Figs 2 and 3, including many cells in the amygdala (especially the ABpc), amygdalocortical transition zone, and in the rhinal sulcus. Again, as in injection 1, labelled cells were seen in the substantia innominata and the lateral mammillary body.

Injections 3 *and* 4*—Middle third of the OLOF taste area*

The site of the third HRP injection is shown in Figs 1 and 5. This and the fourth injection site (see below) lie close to the centre of the CLOF taste area. This injection produced labelling comparable to that found in the first two injections (see Figs 2–4). The insular and opercular taste cortices were heavily labelled (Fig. 5c, d). Cortical label was also found in the OFdg and in the OFag, comparable to injections 1 and 2. Cortical label was also seen in the far lateral edge of the orbital cortex. Thalamic label was mainly confined to MD, with a small number of cells in the Ci and Re. There were no labelled cells seen in VPMpc. In addition, labelled cells were found in the parvocellular regions of the basal nucleus of the amygdala (Fig. 5d).

The fourth injection is illustrated in Figs 1 and 6. The injection site was close to that of the third injection. Heavy labelling was found in the taste regions of the insular and opercular cortices (Fig. 6e, f). The OFC labelling in this monkey was within the same anatomical locations as described above for the third injection, namely the lateral dysgranular area 12,[44] and the medial agranular area 13 (see Fig. 6a–d). Thalamic label was like that in all other injections, and was confined to the MD. This is not illustrated, but corresponds closely to that shown in Fig. 3. Cells in the amygdala and substantia innominata were labelled (Fig. 6f), as were cells in the rhinal sulcus. The subcortical label resulting from this injection closely resembles that resulting from injections 1 and 3.

Injections 5 *and* 6*—Anterior third of the caudolateral orbitofrontal cortex taste area*

The site of the fifth HRP injection was 4.0 mm anterior to the caudal boundary of the OFC, and is shown in Fig. 1. In contrast to the first four injections, no labelled cells were found in the primary taste area of the frontal operculum and insula.[26] A small cluster of labelled cells was found in the far anterior boundary of area OFO (see Fig. 7c). As in the first through fourth injections described above, no labelled cells were found in VPMpc, but numerous cells were found in the MD of the thalamus. The subcortical labelling produced by this injection was similar in location but less dense than that found with injections 1–4 (see Figs 2–6) and is therefore not illustrated. Many labelled cells were seen in the amygdala (within the basal nucleus) and the substantia innominata.

The sixth and final HRP injection was made into the far anterior region of the CLOF taste area, as shown in Fig. 1. With this injection, labelled cell bodies were found primarily in the more posterior parts of the CLOF taste area. Unlike the fifth injection described above, very sparse label was found in the opercular-insular taste cortex. Light labelling was found in the mediodorsal nucleus of the thalamus, but none was found in VPMpc. The pattern of subcortical labelling was generally similar to that seen in the fifth injection, as described previously.

DISCUSSION

This study investigated the afferent projections of a functionally defined region within the orbitofrontal cortex, by means of retrograde HRP tracing. Baylis and Rolls[10,33] have investigated the extent of chemosensory processing within the orbitofrontal

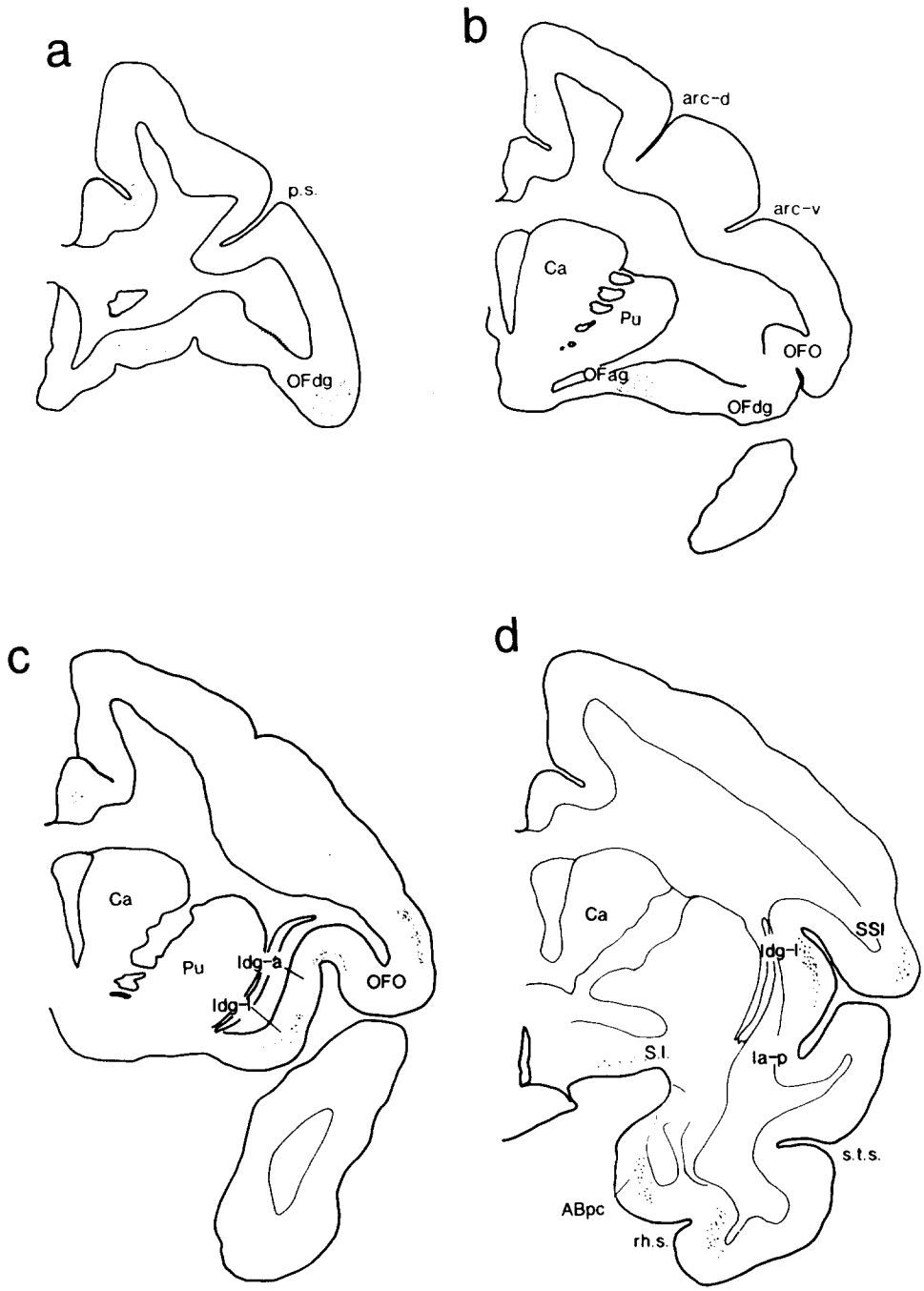

Fig. 5. Injection 3 into the middle third of the CLOF taste area. Coronal sections show some of the areas of labelled cells.

cortex, by means of single-cell recordings. These studies identified a region 16–24 mm lateral to the midline in which many neurons were found to respond only to gustatory stimulation. A small number of neurons responded to the sight of food, with or without associated gustatory responses. Only a very few (Fig. 5) cells with olfactory responsiveness were found within this region. The purpose of the present study was to investigate the routes by which gustatory information reaches this gustatory region of orbitofrontal cortex. All of the injections were made within this gustatory area, and all injections were at sites that were functionally identified in the actual animals used.

The present study demonstrated three main sources of afferents to this region. These are a cortical input, primarily from the insula and operculum, a projection from the thalamus, and a projection from the amygdala. These are each considered in terms of their relation to previous anatomical and physiological studies.

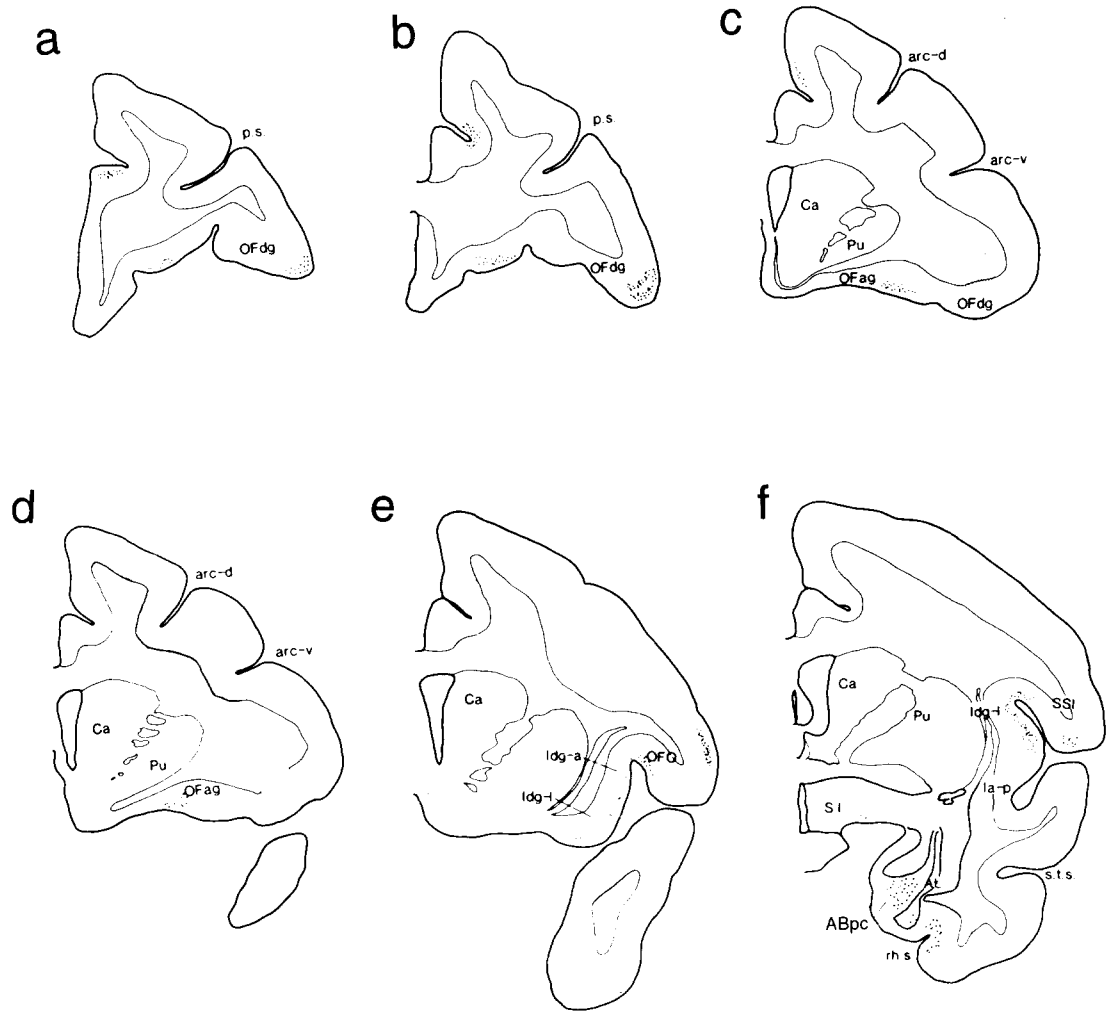

Fig. 6. Injection 4 into the middle third of the CLOF taste area. Coronal sections show some of the areas of labelled cells.

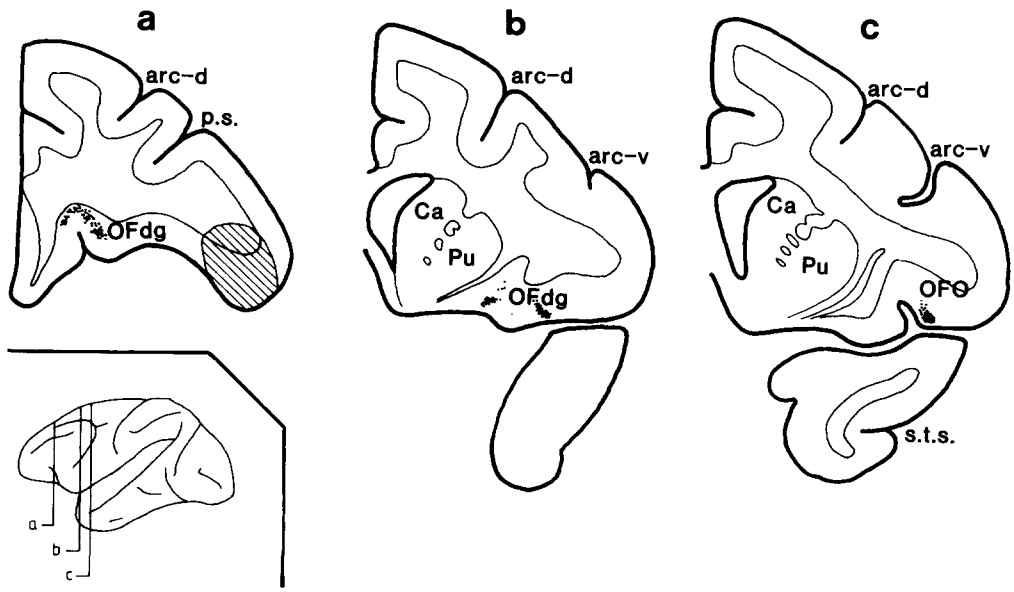

Fig. 7. Injection 5 into the anterior third of the CLOF taste area. Coronal sections show the injection site and some of the areas of labelled cells. Inset shows the levels at which these sections were taken.

Cortical afferents to caudolateral orbitofrontal cortex taste cortex

The main cortical input to the CLOF taste area is a massive projection from all parts of the primary gustatory cortex. This projection is densest for the two most caudal injections (injections 1 and 2), and is absent in the two most anterior injections (5 and 6). This projection comes from the region of the insula and operculum, which has been shown to contain large numbers of neurons responding to gustatory stimuli.[39,44] In addition, a projection is seen from the far lateral edge of the orbital cortex. Although no cellular recordings have been made in this region, Pritchard *et al.*[27] showed a projection from VPMpc to this region, suggesting that it may constitute part of the sensory cortex for taste processing. The projection from primary gustatory cortex is presumably the source of the gustatory input to the CLOF, since the present study did not find an input from the gustatory nucleus of the thalamus (VPMpc—see below). This suggests that the caudal part of the CLOF may contain the secondary gustatory cortex. Note that the possibility (discussed below) of a small projection from VPMpc to CLOF[27] does not undermine the claim that CLOF represents a secondary gustatory cortex. For example, Yuki and Iwai[46] showed that secondary visual cortex receives a direct projection from the visual thalamic nucleus (the dorsal lateral geniculate body). Injections into the most anterior part of the CLOF did not show labelled cells in the primary gustatory cortex, but instead led to a large amount of label in the caudal CLOF. This suggests that the more anterior part of the CLOF taste area might best be characterized as a tertiary gustatory area, although further studies would be needed to confirm such a partitioning.

Other cortical inputs came from the rhinal sulcus, and the cingulate cortex, in broad agreement with the results of Barbas.[6] Unfortunately, none of the injections in study[6] fall entirely within the present gustatory region. Case no. 2 of Barbas is located on the anterolateral boundary of the region currently studied, and shows a very similar pattern of cortical label except that the input from primary gustatory cortex is less dense, and a projection from the superior temporal sulcus is seen. This may be because further anterior in the orbitofrontal cortex there is a lesser representation of taste, and a greater representation of vision.

The area of CLOF into which the present injections were made was shown by Rolls and Baylis[33] to be predominantly gustatory in nature. However, this area overlaps considerably with a part of the orbital cortex previously identified as related to olfactory processing.[41,42] Tanabe *et al.* recorded evoked potentials resulting from electrical stimulation of the olfactory bulb and prepyriform cortex in the lateral posterior orbitofrontal cortex (LPOF). Unfortunately, in the study of Tanabe *et al.* no comparable stimulation of gustatory cortex was performed, nor were physiological recordings made medial to the lateral orbital sulcus. As such, it is impossible to assess whether the LPOF is more directly related to gustatory than to olfactory processing. In a behavioral study, Tanabe *et al.*[43] found some deficit in olfactory discrimination following lesions to the LPOF, but profound deficits were only seen if lesions extended medial to the lateral orbital sulcus.

The present study showed no afferents from prepyriform cortex, but instead showed a projection from anteromedial regions of orbitofrontal cortex within which large numbers of olfactory cells have been recorded.[33] This suggests that there is no direct olfactory input into the CLOF, but that any input of olfactory information into this area must arrive indirectly from the olfactory areas in the anteromedial parts of the orbitofrontal cortex. This is consistent with the findings of Price *et al.*,[26] who found that the direct olfactory projection to the orbitofrontal cortex reaches a small area in and close to an area they name as 13a, medial to the CLOF area described here (see Refs 32, 33 for reviews). The fact that olfactory input into the CLOF must be indirect at best is in contrast to the large monosynaptic projection from primary gustatory cortex. Taken together with the results of Baylis and Rolls,[10,33] these results suggest that olfactory processing in the caudal orbitofrontal cortex takes place primarily in the more medial regions. In contrast, the more lateral region (beyond about 16 mm from the midline) is primarily devoted to gustation. None the less, it is clear from the present study and from the work of Tanabe *et al.*[41-43] that the olfactory and gustatory systems are closely interrelated, as would be expected on the basis of behavioral data.

Thalamic afferents

All injections in the present study resulted in a broadly similar pattern of thalamic input. In common with the entire prefrontal cortex, the CLOF taste area received thalamic input from the mediodorsal nucleus, MD.[29] Goldman-Rakic and Porrino (Ref. 15, case no. 3) made a large injection into the CLOF, including the region studied here, but extending further lateral. The pattern of label seen in MD was similar to that found in the present study. The pattern of label within MD is also in agreement with a recent study of the pattern of MD afferents to the orbitofrontal cortex.[28] In a study by Barbas *et al.*[8] of thalamic inputs into the frontal lobe, no injections included the present region, except perhaps one (case no. 2) in the far anterior part of this region of cortex. This case showed similar patterns of thalamic label, except for a projection from the medial pulvinar which was not seen in the present study. Kievit and Kuypers[17] investigated the thalamic afferents to the orbitofrontal cortex, but the only injection that includes the CLOF taste area (injection A1) also extends far anterior and medial to this region. This

injection resulted in considerable agreement with the present study. A large input from the MD was seen, together with an input from the nucleus centralis and Pcn. In contrast to the present study, Kievit and Kuypers found a substantial projection from the medial pulvinar. However, it is not clear whether this represents afferents to regions anterior and medial to the CLOF taste area.

None of the injections in the present study resulted in any labelled cells in the thalamic taste nucleus, VPMpc. In apparent contrast, one study[27] made injections into the caudal part of the OFC and found some labelled cells in VPMpc. The injections in the present study are mostly located more anterior than those in Ref. 27 with the possible exception of injections 1 and 2. Other thalamic nuclei labelled included the nucleus centralis, principally in the Ci, and a small number of cells in the Re.

The restriction of the thalamic label to the mediodorsal nucleus and the midline thalamic nuclei following CLOF injections is also in broad agreement with Potter and Nauta[25] and Morecroft *et al.*[21] Of particular note is the fact that for case no. 1 of Morecroft *et al.*,[21] and case no. 1 of Potter and Nauta,[25] no labelled cells were found within the taste nucleus VPMpc, following injections in locations similar to the injection sites in the present study. Thus, the preponderance of evidence supports the present contention that there is little or no input from VPMpc to the CLOF, and that instead the major thalamic nucleus which projects to the CLOF is the medial, magnocellular, part of the mediodorsal nucleus. Certainly, in the present study CLOF injections resulted in heavy labelling of cells in MD, but not in VPMpc.

Amygdaloid afferents

There is a very dense projection to the CLOF taste region from the parvocellular part of the basal nucleus of the amygdala, and a large, though less dense, input from the magnocellular and intermediate parts of the basal nucleus. Such a projection has previously been described by Amaral and Price[3] and Potter and Nauta.[25] A similar distribution of label in the amygdala has recently been reported by Barbas and De Olmos (Ref. 7; case no. 3). Neurons in this region of the amgydala have been shown to have gustatory responses[38] in awake monkeys. Neurons with gustatory responses have also been seen in more dorsal parts of the amygdala, including the corticomedial complex.[23] The gustatory input to the amygdala probably arises from the dysgranular region of the insula. Mesulam and Mufson[20] reported a projection from this part of the insular to the amygdala. Yaxley *et al.*[45] showed that the dysgranular region of the insula contains the primary taste cortex. Thus, the primary taste cortex projects to the amygdala, which in turn projects back to the secondary taste cortex.

Other subcortical afferents

A sparse projection from the basal forebrain into the CLOF taste area was also found. The basal forebrain has been previously implicated in taste processing and feeding behaviour. Neurons in this region have been reported to be taste sensitive, in most cases only when the monkey was hungry.[14] The gustatory input to the substantia innominata may come from the CLOF, via a projection described by Nauta.[22] The origin of this projection in the OFC probably corresponds to the CLOF taste area. This suggests that there is a bidirectional connection between the CLOF taste area and the substantia innominata.

CONCLUSION

In conclusion, this study has improved our understanding of the organization of the cortical taste areas in primates. From Ref. 26, it was known that the insular-opercular taste area should be considered the primary cortex, in that it receives a direct projection from the VPMpc nucleus of the thalamus. From the present study it can be concluded that the CLOF taste area contains the second-order cortical representation of taste in that it receives input from the primary taste cortex and does not receive input from VPMpc (for review see Ref. 26). The CLOF taste cortex also receives projections from areas of cortex further anterior and anteromedial in the orbitofrontal cortex. These may represent backprojections from higher-order taste cortices, as well as input from the olfactory area in the OFC.

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REFERENCES

1. Akert K. (1964) Comparative anatomy of frontal cortex and thalamofrontal connections. In *The Frontal Granular Cortex and Behavior* (eds Warren J. M. and Akert K.), pp. 372–396. McGraw-Hill, New York.
2. Aggleton J. P. and Passingham R. E. (1981) Stereotaxic surgery under X-ray guidance in the rhesus monkey, with special reference to the amygdala. *Expl Brain Res.* **44,** 271–276.
3. Amaral D. and Price J. (1984) Amygdalo-cortical projection in the monkey (*Macaca fascicularis*). *J. comp. Neurol.* **230,** 465–496.
4. Amaral D., Price J., Pitkanen A. and Carmichael A. T. (1992) Anatomical organization of the primate amygdaloid complex. In *The Amygdala: Neurobiological Aspects of Emotion, Memory and Mental Dysfunction* (ed. Aggleton J.), pp. 1–66. Wiley-Liss.

5. Bagshaw M. H. and Pribram K. H. (1953) Cortical organization in gustation (*Macaca mulatta*). *J. Neurophysiol.* **16,** 499–508.
6. Barbas H. (1988) Anatomic organization of basoventral and mediodorsal visual recipient prefrontal regions in the rhesus monkey. *J. comp. Neurol.* **276,** 313–342.
7. Barbas H. and De Olmos J. (1990) Projections from the amygdala to basoventral and mediodorsal prefrontal regions in the rhesus monkey. *J. comp. Neurol.* **300,** 549–571.
8. Barbas, H., Henion T. H. H. and Dermon C. R. (1991) Diverse thalamic projections to the prefrontal cortex in the rhesus monkey. *J. comp. Neurol.* **313,** 65–91.
9. Baylis L. L. and Gaffan D. (1991) Amygdalectomy and ventromedial prefrontal ablation produce similar deficits in food choice and in simple object discrimination learning for an unseen reward. *Expl Brain Res.* **86,** 617–622.
10. Baylis L. L. and Rolls E. T. (1995) Responses of neurons in primate gustatory cortex to complex taste stimuli. (submitted).
11. Benjamin R. M. and Burton H. (1968) Projection of taste nerve afferents to anterior opercular-insular cortex in squirrel monkey (*Saimiri sciureus*). *Brain Res.* **7,** 221–231.
12. Bornstein W. S. (1940) Cortical representation of taste in man and monkey. I. Functional and anatomical relations of taste, olfaction and somatic sensibility. *Yale J. Biol. Med.* **12,** 719–736.
13. Bornstein W. S. (1940) Cortical representation of taste in man and monkey. II. The localization of the cortical taste area in man, a method of measuring impairment of taste in man. *Yale J. Biol. Med.* **13,** 133–156.
14. Burton M. J., Rolls E. T. and Mora F. (1976) Effects of hunger on the responses of neurons in the lateral hypothalamus to the sight and taste of food. *Expl Neurol.* **51,** 668–670.
15. Goldman-Rakic P. S. and Porrino L. J. (1985) The primate mediodorsal (MD) nucleus and its projection to the frontal lobe. *J. comp. Neurol.* **242,** 535–560.
16. Jones E. G. and Burton H. (1976) Areal differences in the laminar distribution of thalamic afferents in cortical fields of the insular, parietal and temporal regions of primates. *J. comp. Neurol.* **168,** 197–248.
17. Kievit J. and Kuypers H. G. J. M. (1977) Organization of the thalamo-cortical connections to the frontal lobe in the rhesus monkey. *Expl Brain Res.* **29,** 299–322.
18. Mesulam M.-M. (1982) In *Tracing Neural Connections with Horseradish Peroxidase*, pp. 268. Wiley, Chichester.
19. Mesulam M.-M. and Mufson E. J. (1982) Insula of the Old World monkey. I. Architectonics in the insulo-orbito-temporal component of the paralimbic brain. *J. comp. Neurol.* **212,** 1–22.
20. Mesulam M.-M. and Mufson E. J. (1982) Insula of the Old World monkey. III. Cortical output and a comment on function. *J. comp. Neurol.* **212,** 38–52.
21. Morecroft R. J., Geula C. and Mesulam M.-M. (1992) Cytoarchitecture and neural afferents of orbitofrontal cortex in the brain of the monkey. *J. comp. Neurol.* **323,** 341–358.
22. Nauta W. J. H. (1964) Some efferent connections of the prefrontal cortex in the monkey. In *The Frontal Granular Cortex and Behavior* (eds Warren J. M. and Akert K.), pp. 397–407. McGraw-Hill, New York.
23. Nishijo H., Ono T. and Nishino H. (1988) Topographic distribution of modality-specific amygdalar neurons in alert monkey. *J. Neurosci.* **8,** 3556–3569.
24. Olszewski J. (1952) *The Thalamus of the Macaca mulatta.* Karger, Basel.
25. Potter H. and Nauta W. J. H. (1979) A note on the problem of olfactory associations of the orbitofrontal cortex in the monkey. *Neuroscience* **4,** 361–367.
26. Price J. L., Carmichael S. T., Carnes K. M., Clugnet M.-C., Kuroda M. and Ray J. P. (1991) Olfactory input to the prefrontal cortex. In *Olfaction: A Model System for Computational Neuroscience* (eds Davis J. L. and Eichenbaum H.), pp. 101–120. MIT Press, Cambridge, MA.
27. Pritchard T. C., Hamilton R. B., Morse J. R. and Norgren R. (1986) Projections of thalamic gustatory and lingual areas in the monkey, *Macaca fascicularis. J. comp. Neurol.* **244,** 213–228.
28. Ray J. P. and Price J. L. (1993) The organization of projections from the mediodorsal nucleus of the thalamus to orbital and medial prefrontal cortex in macaque monkeys. *J. comp. Neurol.* **337,** 1–31.
29. Roberts T. S. and Akert K. (1963) Insular and opercular cortex and its thalamic projection in *Macaca mulatta. Schwiz. Arch. Neurol. Neuroch. Psychiat.* **92,** 1–43.
30. Rolls E. T. (1989) Information processing in the taste system of primates. *J. expl Biol.* **146,** 141–164.
31. Rolls E. T. (1993) The neural control of feeding in primates. In *Neurophysiology of Ingestion* (ed. Booth D. A.), Chap. 9, pp. 137–169. Pergamon Press, Oxford.
32. Rolls E. T. (1994) Central taste anatomy and physiology. In *Handbook of Clinical Olfaction and Gustation* (ed. Doty, R. L.), Chap. 24, pp. 549–573. Dekker, New York.
33. Rolls E. T. and Baylis L. L. (1994) Multimodal convergence within the orbitofrontal cortex of the macaque. *J. Neurosci.* (in press).
34. Rolls E. T., Scott T. R., Sienkeiwicz Z. J. and Yaxley S. (1988) The responsiveness of neurones in the frontal opercular gustatory cortex of the macaque monkey is dependent on hunger. *J. Physiol.* **397,** 1–12.
35. Rolls E. T., Sienkiewicz Z. J. and Yaxley S. (1989) Hunger modulates the responses to gustatory stimuli of single neurons in the orbitofrontal cortex. *Eur. J. Neurosci.* **1,** 53–60.
36. Rolls E. T., Yaxley S. and Sienkiewicz Z. J. (1990) Gustatory responses of single neurons in the orbitofrontal cortex of the macaque monkey. *J. Neurophysiol.* **64,** 1055–1066.
37. Ruch T. C. and Patton H. D. (1946) The relation of the deep opercular cortex to taste. *Med. Proc.* **5,** 89–90.
38. Scott T. R., Karadi Z., Oomura Y., Nishino H., Plata-Salaman C., Lenard L., Giza B. K. and Aou S. (1993) Gustatory coding in the amygdala of the alert macaque monkey. *J. Neurophysiol.* **69,** 1810–1820.
39. Scott T. R., Yaxley S., Sienkiewicz Z. J. and Rolls E. T. (1986) Gustatory responses in the frontal opercular cortex of the alert cynomolgus monkey. *J. Neurophysiol.* **56,** 876–890.
40. Sudakov K., MacLean P. D., Reeves A. and Marino R. (1971) Unit study of exteroceptive inputs in claustrocortex in awake, sitting, squirrel monkey. *Brain Res.* **28,** 19–34.
41. Tanabe T., Ooshima Y. and Takagi S. F. (1974) An olfactory area in the prefrontal lobe. *Brain Res.* **80,** 127–130.
42. Tanabe T., Yanita H., Iino M., Ooshima Y. and Takagi S. F. (1975) An olfactory projection area in orbitofrontal cortex of the monkey. *J. Neurophysiol.* **38,** 1269–1283.

43. Tanabe T., Iino M. and Takagi S. F. (1975) Discrimination of odors in the olfactory bulb, pyriform-amygdaloid areas, and orbitofrontal cortex of the monkey. *J. Neurophysiol.* **38,** 1284–1296.
44. Walker A. E. (1940) A cytoarchitectural study of the prefrontal area of the macaque monkey. *J. comp. Neurol.* **98,** 59–86.
45. Yaxley S., Rolls E. T. and Sienkiewicz Z. J. (1990) Gustatory responses of single neurons in the insula of the macaque monkey. *J. Neurophysiol.* **63,** 689–700.
46. Yukie M. and Iwai E. (1981) Direct projection from the dorsal lateral geniculate nucleus to the prestriate cortex in macaque. *J. comp. Neurol.* **201,** 81–97.

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