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Cortical Activation Induced by Intraoral Stimulation with Water in Humans

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Abstract

Studies of gustatory processing frequently utilize water as a control stimulus. However, the neural representations of intraoral stimulation with water have received little attention. We report a series of positron emission tomography studies involving intraoral stimulation with deionized distilled water. Attempting to taste water produced large, bilateral activations in insular, opercular, Rolandic and cerebellar cortices relative to resting with eyes closed or 'smelling' odorless air. The magnitude and volume of activation was substantially reduced when tasting water was contrasted with voluntary swallowing. This indicates that much of the activity induced by water reflects intraoral somatosensory or motor processing. Nevertheless, portions of the insula, operculum, post-central gyrus and cerebellum remained significantly activated in the contrast between 'tasting' water and swallowing. This activity appears to represent a specific neural correlate of fluid stimulation, and may reflect aspects of trigeminal, gustatory or thermal coding. These findings emphasize the large volume of cortex dedicated to intraoral processing, and highlight the importance of controlling for nongustatory factors in studies of gustation.

Introduction

The perception of intraoral foodstuffs involves a complex interaction of gustatory, olfactory, tactile and thermal information. A full understanding of the cerebral processing of intraoral perception requires a determination of how each of these perceptual characteristics are represented within the human brain. Modern cognitive neuroimaging techniques can elucidate the neural correlates of different aspects of sensory perception. Although such techniques provide increasingly detailed information on different aspects of visual, auditory and somatosensory processing, the functional neuroanatomy of intraoral perception remains largely unexplored.

Electrophyshiological and neuroanatomical studies of the gustatory system in nonhuman primates identify the anterior/dorsal insula and buried frontal operculum as primary gustatory cortex (Benjamin and Burton, 1968; Sudakov *et al.*, 1971; Pritchard *et al.*, 1986; Scott *et al.*, 1986; Ogawa, 1994). Positron emission topography (PET) studies in humans similarly demonstrate increased activity in the insular–opercular region during gustatory stimulation (see Figure 1C) (Kinomura *et al.*, 1994; Small *et al.*, 1997a, 1999; Zald *et al.*, 1998). Recent functional magnetic resonance imaging (fMRI) and magneto-encephalography studies similarly support the involvement of this region in gustatory processing (Kobayakawa *et al.*, 1996, 1999; Faurion *et al.*, 1999; Francis *et al.*, 1999). However, neuroimaging studies also indicate that this area frequently shows only modest or nonsignificant activation in contrasts between gustatory stimulation and stimulation of the tongue with water. For instance, Small and colleagues (Small *et al.*, 1997b) did not observe regional cerebral blood flow (rCBF) increases in the insula or frontal operculum in a PET study in which tasting citric acid was contrasted with water. In another PET study, Zald *et al.* observed a significant increase in insular activity in subjects tasting aversive saline relative to water, but only subjects who experienced the saline as extremely aversive demonstrated this increase (Zald *et al.*, 1998). The inconsistency in the activation of presumed primary gustatory cortex is surprising when considered in the light of neuroimaging studies of other sensory modalities, which consistently demonstrate robust increases in primary sensory cortices.

The lack of insular–opercular activation in some studies might be explainable if water itself induces activation of this region. Electrophysiological studies in rodents and nonhuman primates indicate that stimulation of the tongue with deionized water selectively activates cells within many stages of the gustatory pathway, including the insular–opercular taste cortex (Scott *et al.*, 1986; Yamamato *et al.*, 1989; Smith-Swintowsky *et al.*, 1991). Water responsive cells in the insula-operculum are less numerous and exhibit weaker electrophysiological responses than those responding to basic tastants. Nevertheless, their presence suggests that

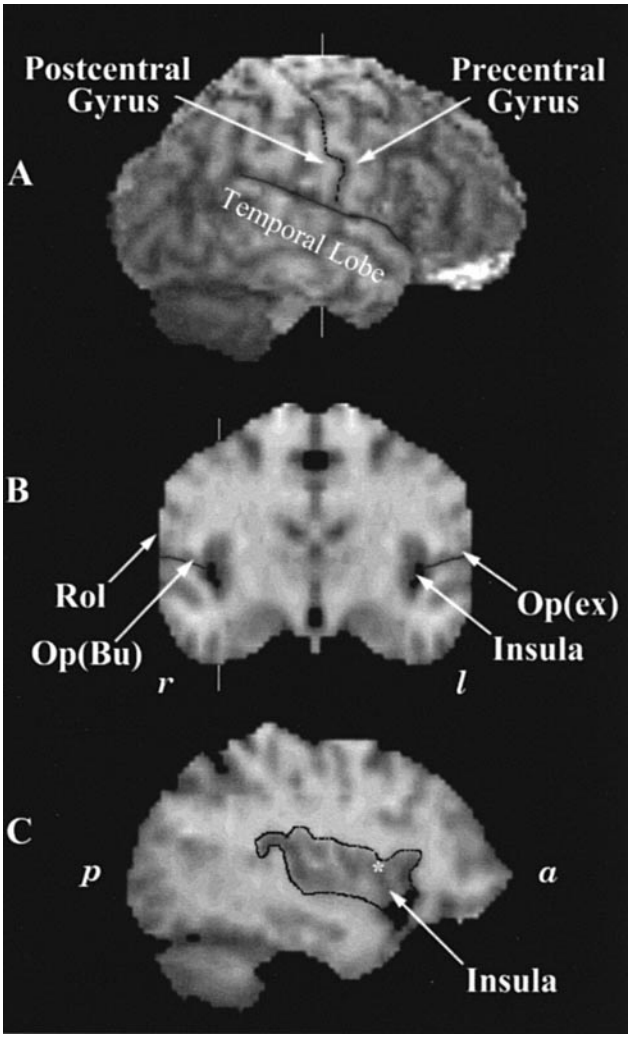

Figure 1 Location of primary cortical areas involved in lingual and gustatory processing. **(A)** Lateral surface rendering of the brain. The dashed vertical line follows the central sulcus. The cortex falling within and on either side of the central sulcus represents Rolandic cortex and is subdivided into the pre- and post-central gyri. The arrow heads point to the general location of the primary motor (pre-central) and somatosensory (post-central) representations of the mouth. The solid horizontal line follows the Sylvian fissure. The exposed area immediately above the Sylvian fissure is referred to as exposed operculum. The white vertical line above and below the image indicates the location of the coronal slice displayed in **(B)**. (B) Coronal view 10 mm posterior to the anterior commissure showing the location of the insula, buried operculum [Op(bu)], exposed operculum [Op(ex)] and inferior Rolandic (Rol) cortex. The solid horizontal line follows the Sylvian fissure, which divides the operculum from the underlying temporal cortex. The white vertical line above and below image indicates the location of the sagittal slice displayed in **(C)**. (C) Sagittal view 35 mm lateral to the midline. The insula is shown surrounded by the black dashed line. The superior portion of this dashed line follows the superior limiting sulcus which forms the boundary between the insula and the neighboring operculum. The white asterisk marks the dorsal anterior area that appears to represent the homologue of primary gustatory cortex as identified in nonhuman primates. Abbreviations: *a* = anterior, *P* = posterior, *l* = left, *r* = right.

water itself may directly activate insular taste cortex in humans. Less specific activations might also occur due to water's ability to activate nonspecific somatosensory, thermal or motor processing in the insular–opercular region. To examine the neural correlates of attempting to taste water, we conducted a series of PET studies in which subjects attempted to taste water relative to different control conditions.

Materials and methods

Human subjects

Twenty-three healthy subjects participated in study 1 (nine right-handed females, nine right-handed males, four left-handed males and one ambidextrous male; mean age 26 years, range 18–38 years). The data for many of these subjects were collected in the course of a series of gustatory studies in which water was utilized as a control condition. Eight subjects from study 1 participated in study 2 (three right-handed females, five right-handed males). Finally, a separate group of 11 healthy right-handed female subjects (mean age 37 years, range 25–62 years) participated in study 3. All subjects completed informed consent following procedures approved by the Veterans Affairs Medical Center's Institutional Human Subjects and Radioactive Drug Research Committees.

Stimulation conditions

Study 1

The first study examined the level of activation induced by water relative to a resting condition. Activity induced by such a comparison includes representations of multiple types of intraoral sensory processing, as well motor processing related to tongue movements and swallowing. Thus, this contrast provides an overall estimate of the extent to which intraoral sensory stimulation with a liquid engages cortical processing. During separate PET scans, subjects attempted to taste pure deionized, distilled water or rested with eyes closed (ECR). Each subject contributed one scan pair to the analysis. In both conditions subjects lay in a quiet, darkened room. During the water condition, subjects held a small plastic cannula between their teeth. Prior to the water condition, subjects received the following instructions: 'You are about to receive a liquid in your mouth. Close your eyes, and see if you can taste anything. When you feel the liquid in your mouth, swish it around a couple of times and then allow your tongue to rest. Try not to move your tongue during the course of the scan. If there is too much fluid in your mouth, go ahead and swallow, and then allow your tongue to rest again.' Approximately 3 s (s) before the start of scan acquisition, 3 cc of water at room temperature was injected through the cannula into the subject's mouth. An additional 3–7 cc of water was then injected slowly into the mouth during the course of the 90 s scan. Subjects were instructed to briefly raise their left hand if there was too much fluid in their mouth, and the flow

of fluid was stopped and only restarted if the subject swallowed. On average, subjects swallowed once during the course of the scan, but this usually did not occur until the second half of the scan, when the sensitivity to changes in rCBF is reduced. Two subjects reported detecting a faint taste. In both cases the subjects were scanned again with spring water, whose taste they did not detect, and only the scans with spring water were included in the data analysis.

A potential confound of the contrast between the water and ECR conditions arises because subjects had to hold a cannula between their teeth in the water condition, but this was not the case during the ECR condition. To ensure that the simple act of holding the cannula between one's teeth did not dramatically change the pattern of results, the final eight subjects from study 1 (three right-handed females, four right-handed males, one left-handed male) completed an additional control condition in which they rested with eyes closed while holding the cannula between their upper and lower incisors.

Study 2

Although study 1 provides an overall picture of the areas activated by stimulation of the mouth with a liquid, it provides little information on the extent to which these activations are specific to stimulation with water. Nonspecific somatosensory features may substantially contribute to these responses. Activations may also relate to motor movement. Subjects reported minimal tongue movement during the water condition. However, in the absence of direct monitoring, it may be assumed that subjects performed at least slightly more tongue movements during the water condition than during the ECR condition. Although the effect of a few small tongue movements on rCBF is unclear, frequent tongue movements produce substantial increases in insular activity (Zald and Pardo, 1999). Subjects were more likely to swallow during the water condition than when resting. Indeed, most subjects swallowed once during the course of the scan. This usually occurred in the later stages of the scan, when the sensitivity of the scanning procedure is low. Although the PET procedures used in this study should be insensitive to a single swallow occurring late in the scan, it remains an uncontrolled factor in study 1. This issue takes on added importance given recent evidence that portions of the insula show increased rCBF during voluntary swallowing (Hamdy *et al.*, 1999; Zald and Pardo, 1999). To limit the effects of tongue movement and swallowing, we asked subjects to swallow once every 5 s as a control condition. Subjects were instructed to produce their own saliva if they needed to have something to swallow. The water versus swallowing comparison represents a conservative contrast in that the swallowing condition involves far greater tongue movement and swallowing than the minimal motor movement involved in the water condition. This condition also cancels out activity related to attending to intraoral sensations, since voluntary swallowing inherently requires substantial attention to intraoral somatomotor sensations. Thus, activations arising in the contrast of the water and swallowing conditions represent a relatively specific correlate of water stimulation.

Study 3

A potential confound of study 1 involves the fact that the water and the ECR conditions differ in their attentional demands. In the water conditions, subjects attended to intraoral stimulation and attempted to detect a taste, whereas no such attentional demands were required in the ECR condition. We therefore implemented an intermodal attention paradigm in which attempting to taste water was contrasted with attempting to smell odorless air. This paradigm enables an examination of modality-specific processing by controlling for nonspecific attentional factors and arousal level. The water condition was identical to the other experiments, but the total amount of fluid was limited to 6 cc, to reduce the need to swallow. During the odor control task, subjects received the instructions: 'Close your eyes, breathe through your nose, and see if you can smell anything.' No odors were presented during the course of the scan, and no subjects reported perceiving any odorants. As in the other conditions, subjects contributed one scan pair to each analysis.

PET imaging and analysis

rCBF was estimated from tissue radioactivity using a Siemens ECAT 953B camera (Knoxville, TN) with septae retracted, a slow-bolus injection of $H_2{}^{15}O$ [0.25 mCi/kg infused at a constant rate over 30 s (Silbersweig *et al.*, 1993)], a 90 s scan acquisition beginning upon radiotracer arrival into the brain, and a 10 min interscan interval. Images were reconstructed using a 3-D reconstruction algorithm with a Hanning filter (0.5 cycles/pixel) (Kinahan and Rogers, 1989) and were corrected for attenuation with a measured 2-D transmission scan. Measured coincidences were corrected for randoms and electronic dead time; no corrections were made for decay or scatter. Normalization for global activity (1000 counts), coregistration within each study session, placement of the intercommissural line from image fiducials, nonlinear warping of each subject's scans to a reference stereotactic atlas (Talairach and Tournoux, 1988) and statistical analyses were accomplished with software developed and provided by Minoshima and co-workers (Minoshima *et al.*, 1992, 1993, 1994). Images were blurred with a 4.5 mm 3-D gaussian filter producing a final image resolution of 7–8 mm full-width at half-maximum (FWHM), which is only slightly lower resolution than the inherent resolution of the ECAT 953B camera. This limited level of smoothing provides greater spatial resolution than is typically reported in PET studies (which frequently use a resolution of 12–20 mm FWHM) and indeed is higher than that reported in many fMRI studies. We adopted this minimal blurring because initial results indicated that water

produces large magnitude and large volume responses, and we wanted to maximize our ability to distinguish adjacent, but distinct areas of activation. This approach has a shortcoming in that it substantially lowers the effect size of large volume foci relative to what would have arisen if greater blurring were applied (this occurs because the global variance of the image increases at high spatial resolutions). For instance, a *Z*-score of 9 using this minimal blurring approaches a *Z*-score of 14 for spatial blurring of 12 mm FWHM. At the same time, this high-resolution technique can increase the risk of observing small volume foci by chance. To compensate for this, we applied a higher than normal criterion for statistical significance ($P < 0.0001$). This represents a more conservative threshold than the $P < 0.001$ threshold typically reported in PET studies. Statistics reflect the maximum magnitude of rCBF increase in a given region relative to the global variance of all pixels within the brain (Worsley *et al.*, 1993). Identification of cerebellar foci utilized the atlas of Schmahmann *et al.* (Schmahmann *et al.*, 1999).

Results

Study 1

Table 1 displays the results from the contrast of the water and ECR conditions. Given the nature of this contrast, these activations cannot be attributed to the processing of a single sensory or motor component, but reflect the multiple types of processing induced by the introduction of water into the mouth. The largest rCBF increases localized to the Rolandic cortex (i.e. pre- and post-central gyrus) and operculum bilaterally (see Figure 2). This activation covered a large volume of cortex (almost 300 cc of this region exceeded the $P < 0.0001$ threshold in each hemisphere) and encompassed much of the exposed operculum (BA 43), the buried operculum, the primary tongue and intraoral somatosensory areas of the post-central gyrus, and parts of the tongue and motor mouth region of the pre-central gyrus. Although this entire region showed activation during the water condition, the relatively high spatial resolution of the present PET technique allowed detection of separate subpeaks in each of these areas. For instance, the peak of the activation within the buried operculum localized near the superior limiting sulcus, although the region of increased rCBF ran continuously from the superior limiting sulcus to the exposed operculum (see Figure 2A).

The water condition produced large rCBF increases in the insula bilaterally. The volume of activation was particularly striking in the right insula (see Figure 2B), where ~50 cc of

Table 1 Areas of increased rCBF during stimulation of the tongue with water relative to eyes closed rest ($n = 23$)

Area	*x*	*y*	*z*	*Z*-score
Right Rolandic cortex (BA 4/3/43)	56	–7	27	10.7
Left postcentral gyrus (BA 2)	–55	–20	25	9.9
Right postcentral gyrus (BA 2/43)	60	–17	20	8.7
Right cerebellum	16	–60	–21	8.0
Left cerebellum	–16	–60	–19	7.9
Left operculum	–39	–8	16	6.2
Right insula	35	–2	–2	6.2
Right insula	37	–6	7	6.0
Left cerebellum	–33	–51	–22	5.4
Left Insula	–35	–1	2	4.9
Left cerebellum	–15	–64	–32	4.7
Right posterior internal capsule	17	–19	–2	4.6
Right frontal operculum	46	5	9	4.5
Brainstem	–3	–33	–27	4.5
Left globus pallidus	–21	–10	–2	4.4
Right orbitofrontal (BA 11)	28	41	–16	4.1
Medial thalamus	–1	–17	6	4.1
Right temporal pyriform cortex	24	3	–2	4.1
Right inferior temporal gyrus	35	–6	–22	3.9
Ventral posterior thalamus	–8	–17	0	3.9
Brainstem	1	–28	–11	3.8
Cerebellar vermis	1	–51	–14	3.7

Stereotactic coordinates in mm of peak rCBF increases. *x* refers to the medial–lateral position relative to midline (positive = right). *y* refers to the anterior–posterior position relative to the anterior commissure (positive = anterior). *z* refers to the inferior–superior position relative to the commissural line (positive = superior).

Figure 2 Cortical activations in the contrast between the water and ECR conditions. In all images, a threshold for rCBF magnitude was applied to display regions with greater than $P < 0.001$ increases in blood flow. Areas displayed in white possess *Z*-scores of >5.0 and rCBF increases of >9 % (see color scale to the right of Figure 3). The thresholded data were then overlayed on the corresponding slice or surface rendering of a standardized MRI. **(A)** Rolandic activation superimposed on a lateral surface rendering of the right hemisphere. Note the large volume of cortex with *Z*-scores exceeding 5.0. A similar pattern of activation emerged in the left hemisphere. **(B)** Coronal section ($y = –8$) demonstrating activation of buried and exposed operculum, inferior Rolandic cortex, and the right insula. In the left hemisphere, weak activity can be seen along the dorsal insula, but the left insular focus localizes several mm anterior to this. **(C)** Sagittal section ($x = 33$) demonstrating the large volume of activated cortex in the right insula.

Figure 3 Surface rendering demonstrating Rolandic activation in the contrast between the water and swallowing conditions. Note the smaller volume and lower magnitude of inferior Rolandic activation relative to Figure 1A.

Figure 4 Cortical and subcortical activation in the contrast between the water and swallowing conditions. **(A)** Coronal section ($y = –12$) demonstrating activation of the Rolandic cortex bilaterally and a portion of left parietal operculum. The posterior extreme of the left insular focus can also be seen in this image. **(B)** Sagittal section ($x = 37$) demonstrating the right insular focus. Note the small volume of these foci relative to Figure 2B,C. **(C)** Sagittal section ($x = –1$) displaying activation in the posterior cerebellar vermis and brainstem. The peak of the brainstem activation falls on the other side of the midline at similar *y* and *z* coordinates.

cortex exceeded the $P < 0.0001$ threshold. The left insula also contained a sizable (albeit smaller) area of activation (30 cc). In both hemispheres, the insular activation included the area that appears to represent primary gustatory cortex in humans (Small *et al.*, 1999). Additional foci localized to the cerebellum, brainstem, medial thalamus, anterior cingulate, globus pallidus and right orbitofrontal cortex (due to its position at the edge of the brain, this final focus may reflect an artifact and must be viewed with caution).

The activations in the Rolandic, insular, opercular and cerebellar cortices could not be explained by the act of holding the cannula between the teeth. Holding the cannula (relative to resting without the cannula) did not induce significant increases in these areas. Moreover, comparison of the water condition to the condition of resting while holding the cannula between the teeth produced results very similar to those arising in the contrast between the water and ECR conditions. (The results of this contrast may be examined on-line at http://james.psych.umn.edu/water_appendix.html.)

Figure 2. Water - Eyes Closed Rest

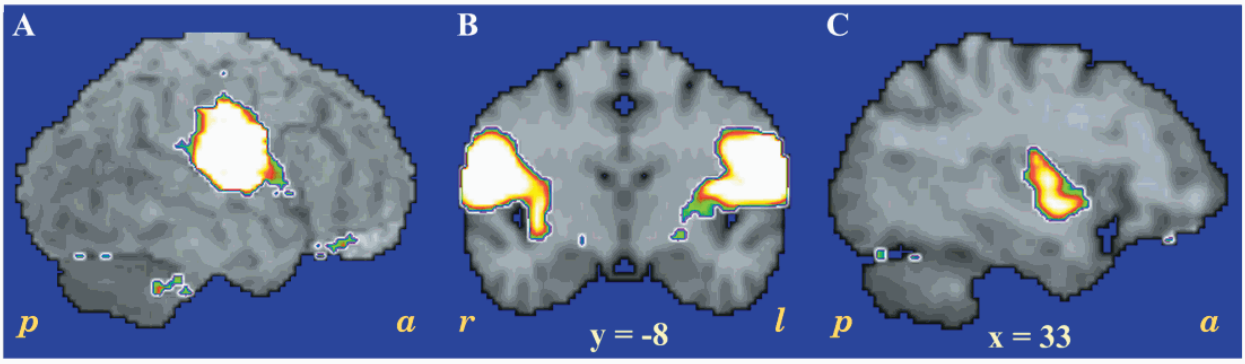

Figure 3. Water - Swallowing

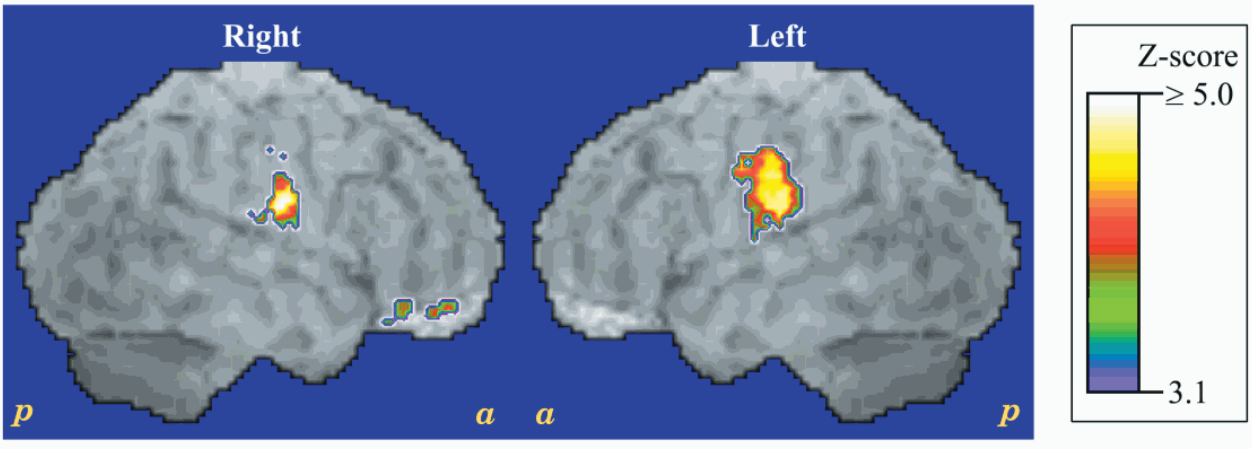

Figure 4. Water - Swallowing

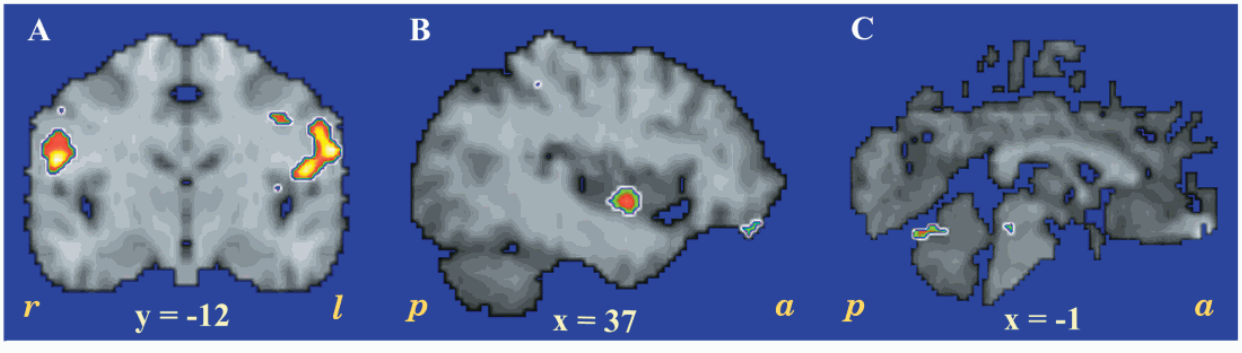

Table 2 Areas of increased rCBF during stimulation of the tongue with water relative to swallowing ($n = 7$)

Area	*x*	*y*	*z*	*Z*-score
Left Rolandic cortex (BA 43/3/4)	–53	–10	22	4.5
Right Rolandic cortex (BA 4/3)	55	–14	34	4.4
Right postcentral gyrus (BA 2/43)	53	–17	22	4.4
Left Rolandic cortex (BA 3/4)	–59	–13	27	4.3
Left operculum	–48	–13	18	4.2
Cerebellar vermis	–1	–67	–16	4.1
Left cerebellum	–24	–71	–18	4.1
Left lingual gyrus (BA 19)	–15	–67	–7	4.1
Left precentral gyrus (BA 4/6)	–51	–8	34	4.0
Left cerebellum	–28	–69	–16	4.0
Brainstem	3	–24	–2	3.9
Left cerebellum	–17	–49	–18	3.9
Right insula	39	–1	–4	3.9
Brainstem	–1	–28	–14	3.8
Left insula	–37	–10	11	3.7
Right rolandic cortex (BA 4/3)	51	–13	32	3.6*
Left precentral gyrus (BA 4/6)	–39	–13	38	3.5*
Cerebellum	17	–60	–20	3.4*
Right postcentral gyrus (BA 43)	57	–10	18	3.4*
Left anterior insula	–33	5	7	3.2*
Cerebellar vermis	–8	–69	–18	3.1*

Asterisks denotes *Z*-scores below the $P < 0.0001$ level but exceeding $P < 0.001$, and overlapping with foci in other comparisons.

Table 3 Areas of increased rCBF during stimulation of the tongue with water relative to odor detection ($n = 11$)

Area	*x*	*y*	*z*	*Z*-score
Right Rolandic cortex (BA 43/3/4)	57	–10	22	9.9
Left Rolandic cortex (BA 43/3/4)	–57	–6	22	8.8
Left cerebellum	–19	–60	–20	6.1
Right cerebellum	21	–60	–18	6.1
Right insula	37	–6	9	5.2
Left insula	–37	–4	4	5.1
Left rolandic cortex (BA 4/3)	–51	–15	38	5.1
Brainstem	3	–31	–29	4.6
Left thalamus	–6	–24	4	4.2
Right Thalamus	8	–15	2	4.2
Right exposed frontal operculum	57	1	7	4.1
Medial dorsal brainstem	3	–31	–18	4.1
Medial dorsal brainstem	–1	–33	–20	4.1
Anterior cingulate (BA 24)	3	3	45	3.9
Left postcentral gyrus (BA 2)	–35	–26	38	3.9
Left cerebellar vermis	–8	–71	–18	3.7
Left cerebellar vermis	–3	–67	–22	3.7
Left cerebellum	–33	–64	–22	3.7
Left superior cerebellar vermis	–8	–64	–11	3.7
Left cerebellum	–26	–46	–32	3.7

Most subjects described the water condition as hedonically neutral or mildly pleasant. A few subjects reported experiencing the water condition as unpleasant, due to the volume of water involved. However, removal of these subjects did not substantially change the pattern, magnitude or volume of the activations.

Study 2

To control for intraoral tongue movement and swallowing, a subgroup of subjects performed a supplemental control condition of repetitive swallowing. The contrast between the water and repetitive swallowing conditions represents a biased contrast in that there was far more swallowing and tongue movement in the swallowing condition than in the condition of tasting water. This means that significant activations arising in this contrast occur despite the greater motor and somatosensory features of the control condition relative to the water stimulation condition. Table 2 displays the results of this contrast. Despite the biased nature of the contrast, significant activations again emerged in the Rolandic, opercular and insular cortices. However, the volume and magnitude of these foci were dramatically reduced. For instance, only 15 cc of Rolandic/opercular cortex activated above the $P < 0.0001$ level in each hemisphere (see Figures 3 and 4A). Insular foci showed similar reductions in volume, with no foci involving >0.5 cc of cortex above the $P < 0.0001$ threshold (see Figure 4A,B). The dramatic reduction in the volume and magnitude of foci in this comparison indicates that much of the Rolandic, opercular and insular activity in study 1 reflects somatosensory or motor processing, rather than a specific coding of water. Nevertheless, smaller portions of these regions show activity that appears relatively specific to intraoral stimulation with a fluid. Interestingly, the insular regions that remained significant in the contrast with swallowing do not overlap with the area that appears to represent the functional and cytoarchitectural homologue of primary gustatory cortex in the monkey. For instance, the focus in the right hemisphere appears inferior to the presumed location of primary gustatory cortex (compare Figure 4A with Figure 1C).

Several foci also localized to the cerebellum in this contrast. These localized to the cerebellar vermis (see Figure 4C), and lobules VI and V of the cerebellar hemispheres. Discrete foci also again emerged in the brainstem (see Figure 4C).

Study 3

Comparison of the water condition to a resting state fails to control for differences in the attentional demands of the two conditions (because the water condition involves focused attention on the gustatory modality, whereas the ECR condition does not). To control for these attentional demands, we utilized an intermodal attention paradigm in which subjects either attempted to taste water or attempted to smell odorless air. Table 3 shows the peak areas of activation during the contrast of the conditions of tasting water

and smelling odor-free air. The results of this contrast were highly consistent with study 1. As in study 1, stimulation with water produced large magnitude and large volume activations of the Rolandic, opercular, insular and cerebellar cortices, bilaterally. More discrete activations emerged again in the medial–dorsal brainstem, thalamus and cingulate. These data both replicate the findings of study 1 in a separate sample of subjects and indicate that attentional factors do not substantially alter the pattern of results.

Discussion

The current series of studies demonstrate that intraoral stimulation with pure water at room temperature produces rCBF increases within the perisylvian (insula, operculum, ventral pre- and post-central gyrus), Rolandic and cerebellar cortices. The activity in these areas increased dramatically when stimulation with water was compared with resting with eyes closed, with statically holding a cannula between the teeth or with attempting to smell odorless air. A number of component processes likely contribute to these responses, including somatosensory, thermal and gustatory perception, as well as motor processing of the tongue and pharynx. Indeed, somatosensory and motor processing contribute substantially to responses in all of these areas, as indicated by the significant reduction in the magnitude and volume of activated cortex when tasting water was contrasted with repetitive swallowing. However, even in this comparison, significant activations localized to all of the perisylvian areas.

The present series of studies demonstrate that pure water activates the insula and buried frontal operculum, despite being perceived as tasteless. When water was contrasted with resting or with attention to olfaction, the activated region extended throughout large areas of the insula, including an area that probably contains oral secondary somatosensory cortex (Kaas and Pons, 1988) as well as primary gustatory cortex (Small *et al.*, 1999). However, it should not be concluded from this finding that the activity must represent gustatory coding. Only a small proportion of cells in the primary gustatory region of primates code gustatory information, with many more cells coding for somatosensory and motor features (Scott *et al.*, 1986; Smith-Swintowsky *et al.*, 1991). Nevertheless, the present study indicates that parts of the insula and operculum show responses to water that cannot be easily explained by motor or general somatosensory factors (since they remained significant even compared with swallowing). Exactly what factors contribute to these responses remains unclear. It could reflect thermal coding, since the water was experienced as cool, and studies in rodents indicate that some cells in this region respond to thermal information (Yamamato *et al.*, 1989). Alternatively, it might represent a specific trigeminally mediated correlate of fluid stimulation. These areas might also relate to gustatory attention or gustatory imagery, since subjects were not merely passive recipients of the water stimulation, but were actively told to see if they could taste anything. Finally, it might represent a neural correlate of the chemical transduction of water (i.e. a coding of the shift in pH and osmolarity caused by the introduction of water into the mouth). Interestingly, the insular responses to water were consistently of a larger volume in the right hemisphere. This appears consistent with the more frequent right hemisphere involvement of the insula in neuroimaging studies of gustation (Small *et al.*, 1999). However, it is important to note that the area of the insula that remained significantly activated by water relative to swallowing was somewhat ventral to the region that appears homologous to primary gustatory cortex in monkeys (Small *et al.*, 1999). Thus, the area that appears most consistently responsive to water, regardless of the control condition, may represent a proximal area, rather than the primary gustatory cortex.

The highest magnitude rCBF increases localized to the Rolandic cortices, primarily involving intraoral somatosensory regions. The peak focus in this region frequently fell slightly below the maximal location of the somatosensory representation of the tongue, although the area of activation extended throughout much of the tongue, mouth and intraoral representations of the post-central gyrus (Penfield and Boldrey, 1937; McCarthy *et al.*, 1993; Urasaki *et al.*, 1994; Sakai *et al.*, 1995; Pardo *et al.*, 1997). The focus also extended in to the ventral pre-central gyrus, which may reflect motor movement (especially in the studies involving comparisons with rest), or the somatosensory representations of the pre-central gyrus (Urasaki *et al.*, 1994). The large magnitude of these Rolandic responses likely arise as a result of the dense sensory innervation of the mouth and tongue. The shear volume of cortex activated by water is striking. This confirms the disproportionately broad representation of the tongue relative to other body areas, as identified previously in electrophysiological studies (Penfield and Boldrey, 1937; McCarthy *et al.*, 1993; Urasaki *et al.*, 1994). This large volume could not be attributed to spatial blurring since the imaging resolution was <8 mm FWHM. The high resolution of the current methods is further demonstrated by the ability to observe discrete bilateral foci in the thalamus and dorsomedial brainstem. Given this high resolution, these data indicate that a vast area of the Rolandic cortex becomes active during intraoral stimulation with water.

As expected, much of the Rolandic activity disappeared when tasting water was contrasted with swallowing. However, multiple discrete foci within this region remained significantly activated in the contrast between tasting water and swallowing. While this activity may simply reflect additional somatosensory or thermal coding in the water condition relative to the swallowing condition, it may have implications for gustatory studies. In nonhuman primates, the frontal extension of area 3B (which lies in the ventral pre-central gyrus, adjacent to the subcentral sulcus) receives

projections from the thalamic gustatory relay (ventroposteriomedial nucleus, pars parvocellularis) (Pritchard *et al.*, 1986). The ventral central sulcus shows electrophysiological responses to lingual nerve, chorda tympani and glossopharyngeal nerve stimulation, suggesting that both taste and somatosensory information reach this area (Ogawa *et al.*, 1985). This area has been proposed as an interface between gustatory and somatosensory information (Benjamin and Burton, 1968). The Rolandic area that continues to show activation even in the contrast between the water and the swallowing conditions appears too superior to represent this secondary taste area. Nevertheless, the comparisons with resting conditions makes it evident that stimulation with water, and the somatosensory and motor processing that accompanies such stimulation, activates a massive area that includes the presumed location of the ventral Rolandic secondary taste cortex. Most neuroimaging studies have failed to observe greater activation in this area during gustatory conditions when they are contrasted with water [see (Faurion *et al.*, 1998) for the only exception to date]. The present data suggest that the difficulty observing gustatory responses in this area likely reflects the robust ability of water, and the somatosensory and motor processing that accompany it, to induce substantial activations in this area. Any additional rCBF increases caused by gustatory stimulation must occur on top of this already high level of activation. Thus, to the extent that gustatory stimuli increase rCBF in this area, the increases may be obscured by the large magnitude of activations induced by control conditions.

The current study also demonstrates that water activates portions of the cerebellum in humans. Studies in frogs indicate that stimulation of the tongue with both salt and water produces alterations in cerebellar electrophysiological activity (Hanamori and Ishiko, 1987). An orofacial somatosensory representation has been identified in portions of Crus II and the paramedian cerebellar lobule in rodents and cats (Shambes *et al.*, 1978; Kassel *et al.*, 1984). However, the locations of the responses in the present study appear more consistent with the vermis, and lobules V and VI of the cerebellar hemispheres. The activity in the cerebellum cannot be dismissed as simply relating to motor movement, since they emerged even when tasting water was contrasted with swallowing.

Converging data demonstrate the ability of modern functional neuroimaging techniques to study gustation in humans. However, unlike other sensory systems that are easily activated in isolation, it is extremely difficult to produce gustatory sensation without simultaneously activating other intraoral processes. The present studies with water, as well as other recent studies of intraoral somatomotor functioning (Hamdy *et al.*, 1999; Zald and Pardo, 1999), make it evident that the processing of information other than taste can induce strong activations within or near gustatory areas. Indeed, the magnitude of the signal produced by somatosensory or motor factors appears substantially larger than that produced by gustatory factors, and may potentially mask the smaller activations induced by gustatory stimuli.

In summary, stimulation of the tongue with water increases rCBF within the perisylvian, Rolandic and cerebellar cortices, as well as the brainstem, thalamus and cingulate. These findings highlight the complex and distributed processing of different aspects of intraoral perception, and emphasize the importance of controlling for the effects of water and other nongustatory factors when investigating the functional neuroanatomy of taste.

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