

# Human Cortical Responses to Water in the Mouth, and the Effects of Thirst

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**de Araujo, Ivan E.T., Morten L. Kringelbach, Edmund T. Rolls, and Francis McGlone.** Human cortical responses to water in the mouth, and the effects of thirst. *J Neurophysiol* 90: 1865–1876, 2003. First published May 28, 2003; 10.1152/jn.00297.2003. In an event-related functional magnetic resonance imaging (fMRI) study in humans it was shown, first, that water produces activations in cortical taste areas (in particular the frontal operculum/anterior insula which is the primate primary taste cortex, and the caudal orbitofrontal/secondary taste cortex) comparable to those produced by the prototypical tastants salt and glucose. Second, the activations in the frontal operculum/anterior insula produced by water when thirsty were still as large after the subjects had consumed water to satiety. Third, in contrast, the responses to water in the caudal orbitofrontal cortex were modulated by the physiological state of the body, in that responses to the oral delivery of water in this region were not found after the subjects had drunk water to satiety. Fourth, further evidence that the reward value or pleasantness of water is represented in the orbitofrontal cortex was that a positive correlation with the subjective ratings of the pleasantness of the water was found with activations in the caudal and anterior orbitofrontal cortex, and also in the anterior cingulate cortex. Fifth, it was found that a region of the middle part of the insula was also activated by water in the mouth, and further, that this activation only occurred when thirsty. Sixth, analyses comparing pre- and postsatiety periods (i.e., when thirsty and when not thirsty) independently of stimulus delivery revealed higher activity levels in the rostral anterior cingulate cortex. The activity of the rostral anterior cingulate cortex thus appears to reflect the thirst level or motivational state of the subjects.

## INTRODUCTION

Thirst is a subjective sensation aroused by the lack of water. Thirst is sensed by an increase in the tonicity of the body fluids by receptors in the preoptic area, and by a decrease in the body fluid volume (Fitzsimons 1979, 1998; Rolls and Rolls 1982). Water in the mouth tastes pleasant when thirsty but not when satiated (Rolls et al. 1980), and this alteration in the reward or affective value of the water is thought to be part of the control mechanism by which water is consumed when thirsty, but not after water has been drunk to satiety (Rolls 1999).

It has been shown in macaques that water in the mouth activates neurons in the primary taste cortex in the anterior insula and adjoining frontal operculum (Scott et al. 1986; Yaxley et al. 1990), and in the secondary taste cortex in the caudal orbitofrontal region (Rolls et al. 1990). In the primary taste cortex the responses to taste stimuli are not decreased

after satiety (Rolls et al. 1988; Yaxley et al. 1988), but in the secondary taste cortex the neuronal responses to water in the mouth are large when thirst is present and decrease to zero during drinking to satiety (Rolls et al. 1989). Thus the reward value of water in the mouth is correlated with the responses of single neurons in the orbitofrontal cortex, but the responses of neurons in the macaque primary taste cortex reflect the identity and concentration of the tastant, but not its affective value.

In humans, much less is known of the neural mechanisms underlying thirst and the reward value of water intake. In a positron emission tomography (PET) study, Denton and colleagues investigated the brain representation of the genesis and satiation of thirst (Denton et al. 1999). The conditions investigated included the initial state, a state after thirst was induced by infusion of intravenous hypertonic saline, and a state after the subjects had drunk to satiety. A correlation between the thirst scores and regional cerebral blood flow was reported in the anterior cingulate cortex. This study did not investigate neural responses to the delivery of water in the mouth, or with any modulation of that response by the level of thirst. In another PET study, water has been shown to activate taste-related cortical areas in humans (Zald and Pardo 2000), but there was no condition to control for placing a fluid in the mouth that was similar to saliva. The aims of the investigation described here were to investigate cortical areas of the human brain activated by the delivery of small quantities of water to the mouth with an adequate control for nonspecific effects of placing a fluid in the mouth provided by a tasteless solution with the main ionic components of saliva; to compare these areas with known taste cortical areas identified by prototypical taste stimuli such as sweet (glucose) and salt (NaCl) incorporated into the experimental design; to investigate which cortical areas were activated by water in the mouth independently of thirst and which cortical areas only respond to water in the mouth when thirst was present; to investigate the brain areas in which the activation is correlated with the subjective pleasantness and refreshment value of water in the mouth; and to investigate brain areas in which the level of activity even in the absence of any oral stimulation was different during thirst produced by mild water deprivation as compared with the satiated state produced after water had been drunk to satiety. How water in the mouth is sensed, and whether it should be classified as a taste, is considered in the DISCUSSION. A wider

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aim of this study was to provide further evidence on the brain areas involved in emotion, for emotions can be defined as states elicited by rewarding (generally subjectively pleasant) and punishing (generally subjectively unpleasant) stimuli, and the investigation of where the pleasantness of water is represented in the brain thus provides one part of a systematic study of the brain regions involved in affect (Rolls 1999).

## METHODS

### Subjects

Eleven healthy right-handed subjects (of which 5 were males) participated in the study. Written informed consent from all subjects and ethical approval from the Central Oxford Research Ethics Committee were obtained before the experiment. Subjects were asked to arrive at the scanning session (which was generally between 5 and 8 P.M.) after 6–8 h of fluid deprivation. They were allowed during this time to take a light meal (e.g., a light sandwich) and to drink one cup of coffee or tea.

### Stimuli

The following three different stimuli were used: mineral water (Evian, France), 0.5 M glucose (Sigma), and 0.05 M salt (NaCl, Sigma). The glucose was chosen in preference to sucrose for direct comparison with the neurophysiological studies described in the INTRODUCTION in which an aim was sometimes to produce rapid satiety during investigations of hunger. The concentrations of the two prototypical tastants were chosen to be approximately equally intense based on psychophysical tests performed on a panel of subjects. All stimuli were diluted and delivered in the mineral water and were delivered at room temperature. A tasteless solution (containing the main ionic components of saliva, 25 mM KCl + 2.5 mM NaCO<sub>3</sub>) was used as a control stimulus. All stimuli were delivered at room temperature, 23°C, and because of the subtraction contrasts using the tasteless control used in this study, any effects of temperature, known to be represented in the insula (Craig et al. 2000), could not have contributed to any of the effects described in our investigation.

### Experimental design

The experimental protocol consisted of an event-related interleaved design using four stimulus conditions (the 3 stimuli described above, and the tasteless control solution). Stimuli were delivered to the subject's mouth through four polythene tubes that were held between the lips. Each tube of approximately 1 m in length was connected to

a separate reservoir via a syringe and a one-way syringe valve (supplied by Fisher Scientific, UK).

Initially, subjects were scanned when mildly fluid deprived as described above. At the beginning of each trial (the timing of which is shown in Fig. 1), one of the four stimuli chosen by random permutation was delivered in a 0.75-ml aliquot to the subject's mouth. The subject was instructed to make one tongue movement to distribute the fluid in the oral cavity, then 11 s after the delivery of the stimulus, swallowing was cued by a visual stimulus (following initial instruction and training). The swallowing period was 3 s long, followed by three rating periods over a period of 21 s. In the first rating period the pleasantness of the stimulus was rated; in the second intensity, and in the third, the refreshment value was also rated. Each rating was cued by a question on the screen for 2 s and was made during 5 s using a button box on a visual analog rating scale shown on the screen anchored at +2 for very pleasant/intense/refreshing, and at -2 for very unpleasant/weak/nonrefreshing. After the three ratings, the tasteless control solution was administered in exactly the same way as the main stimulus with the same single tongue movement being made, and the subject was cued to swallow again after 11 s. The next trial started after a 3-s interval for swallowing, and a 1-s inter-trial gap.

The tasteless solution was used as the comparison condition for the stimulus solution delivered first in each trial, and allowed effects such as somatosensory effects produced by liquid in the mouth, and the single tongue movement made to distribute the liquid throughout the mouth, to be controlled for. The tasteless solution also provided a rinse before the oral stimulus provided on the next trial. A taste trial was performed permutatively for each of the four stimuli, and the whole cycle was repeated nine times.

After the first scanning session, the subjects were taken out of the scanner and allowed to drink mineral water to satiety. Subjects drank on average 1.6 l of water (SD = 0.46), although care was taken to ensure that they could not count how many cups they had consumed by providing the water in a large (0.5 l) jug. A second and identical scanning session was then run.

### fMRI data acquisition

Images were acquired with a 3.0-T VARIAN/SIEMENS whole-body scanner at the Centre for Functional Magnetic Resonance Imaging at Oxford (FMRIB), where 14 T2\* weighted EPI slices were acquired every 2 s (TR = 2). We used the techniques that we have developed over a number of years to carefully select the imaging parameters to minimize susceptibility and distortion artifact in the orbitofrontal cortex as described in detail by Wilson et al. (2002). The relevant factors include imaging in the coronal plane, minimizing voxel size in the plane of the imaging, as high a gradient switching

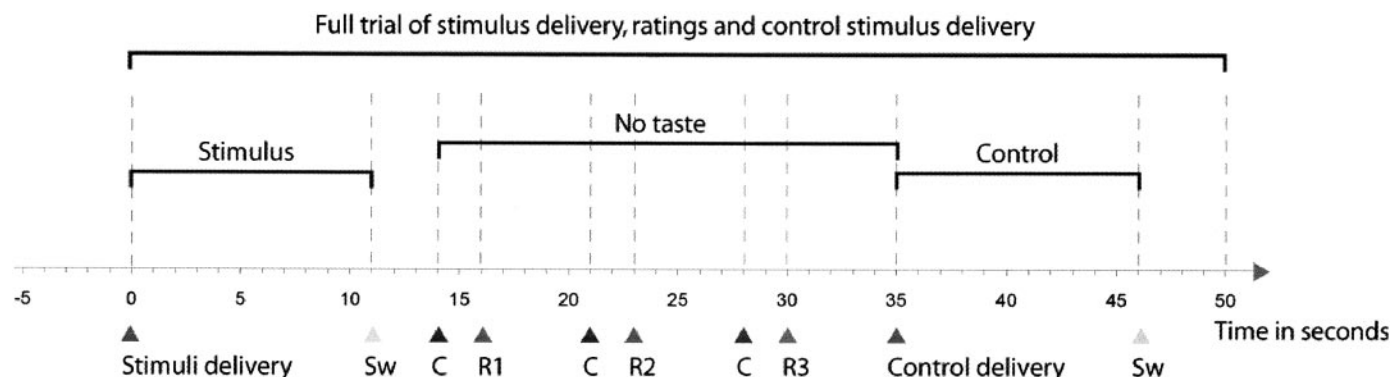


FIG. 1. Each full trial lasts 50 s as shown in the figure. The stimulus is delivered at time 0 and swallowing (Sw) is cued after 11 s and completed within a period of 3 s. The 3 subjective ratings are then taken during a nontaste period of 21 s. Each rating begins with a cue (C) which is on for 2 s, and each of the subjective ratings of pleasantness, intensity, and refreshment (R1, R2, and R3, respectively) are taken during a period of 5 s (subjects are pretrained). The tasteless control solution is delivered at time 35, and 11 s later swallowing is cued for a period of 3 s, which is followed by a 1-s inter-trial gap period.

frequency as possible (960 Hz), a short echo time of 25 ms, and local shimming for the inferior frontal area.

The matrix size was  $64 \times 64$  and the field of view was  $192 \times 192$  mm yielding  $3 \times 3$  mm in-plane resolution. Continuous coverage was obtained in 14 slices from +60 (A/P) to -38 (A/P). Acquisition was carried out during the task performance yielding 900 volumes in total for each session. A whole brain T2\* weighted EPI volume of the above dimensions and an anatomical T1 volume with slice thickness of 1.5 mm and in-plane resolution of  $1.5 \times 1.5$  mm was also acquired.

### fMRI data analysis

The imaging data were analyzed using SPM99 (Wellcome Department of Cognitive Neurology, Institute of Neurology, London). Pre-processing of the data used SPM99 realignment, reslicing with sinc interpolation, normalization to Montreal Neurological Institute (MNI) coordinates (Collins et al. 1994) (the coordinate system used throughout this paper), and spatial smoothing with a 10-mm full-width at half-maximum isotropic Gaussian kernel and global scaling. The time series at each voxel were high-pass and low-pass filtered with a hemodynamic response kernel. A general linear model was then applied to the time course of activation of each voxel and linear contrasts were defined to test the specific effects of each condition. Voxel values for each contrast resulted in a statistical parametric map of the  $t$  statistic which was then transformed into the unit normal distribution (SPM Z).

Comparisons were defined between each of the stimuli and its corresponding control stimulus within a trial. Conditions were thus defined for water delivery (*water*) and for the control trial-specific tasteless delivery following each water delivery (*control water*). This way the statistical parametric maps for effects of water could be defined by the comparison: [water - control water]. Similarly, comparisons were defined for *salt* and *glucose* with respect to their corresponding control conditions (*control salt* and *control glucose*). Thus the contrasts for each of these comparisons were mutually orthogonal. In addition, these conditions were defined for both the pre- and postsatiety periods.

Group effects were assessed by conjunction analyses across different stimulus conditions and second-level, random effects analysis. The main effects of taste were defined as the conjunction contrast: [water - control water] and [salt - control salt] and [glucose - control glucose]. Reported  $P$  values based on this group analysis for a priori regions of interest (e.g., the insula/operculum and the orbito-frontal cortex) were corrected for the number of comparisons made within each region using the small volume correction (SVC) procedure (Worsley et al. 1996). Checks were performed using the estimated motion as a covariate of no interest to rule out the possibility of the observed results being due to motion-related artifacts.

## RESULTS

### Behavioral data

The ratings given by subjects to the pleasantness of water during the first (presatiety) period are significantly higher than the ones given during the postsatiety period (mean  $\pm$  SE):  $0.76 \pm 0.18$  for the preperiod and  $0.1 \pm 0.15$  for the postperiod. A one-tailed pairwise Student's  $t$ -test performed on these ratings resulted in a  $t$ -value of  $t = 2.48$  ( $P < 0.03$ ,  $df = 10$ ). No significant differences were found with respect to the other stimuli used, that is, the pleasantness of the glucose ( $0.74 \pm 0.20$ ) and of the salt ( $-1.14 \pm 0.15$ ) were not altered by drinking water to satiety (Rolls et al. 1983).

To complement these pleasantness ratings, refreshment ratings were also taken throughout the experiment. As expected, the water was rated as more refreshing while thirsty than after

satiety. Indeed,  $t$ -test comparisons between presatiety and postsatiety refreshment ratings revealed that water was found to be significantly more refreshing before drinking water to satiety ( $0.90 \pm 0.14$ ) than during the post period ( $0.09 \pm 0.2$ ). A one-tailed pairwise Student's  $t$ -test performed on these ratings resulted in a  $t = 2.78$  ( $P < 0.02$ ,  $df = 10$ ). No significant differences were found with respect to the other stimuli used. (The refreshment ratings were  $+0.41 \pm 0.22$  for the glucose and  $-1.11 \pm 0.20$  for the NaCl.) We found that the refreshment ratings correlated highly with the pleasantness ratings ( $r = 0.89$ , calculated over all subjects and all stimuli), and also that the correlations with the brain activations (see Fig. 8) were similar for pleasantness and refreshment and therefore focus for the remainder of this paper on the pleasantness rather than the refreshment ratings.

No significant differences for intensity were found with respect to any of the stimuli, as expected (Rolls et al. 1983), indicating that the hedonic response to water is independent of any simple, perhaps peripheral, gating of the neural response, which would have altered the perceived intensity. (The intensity ratings were  $-1.15 \pm 0.14$  for water,  $+1.28 \pm 0.14$  for the glucose, and  $+1.38 \pm 0.16$  for the NaCl.)

### fMRI data analyses

To meet the aims of this investigation, the analyses described next included the following. First, we describe the cortical responses to water to compare them with those produced by prototypical tastants such as salt and glucose. Second, we describe the similarities and differences in the neural activity produced by water when thirsty and when satiated. Third, we correlated the subjective ratings of pleasantness, refreshment, and intensity with the activation produced in different cortical areas. Fourth, we compare the level of activity (that is independent of any stimulus being delivered) in different brain areas while thirsty and after drinking to satiety.

### The taste of water

To establish the cortical regions activated by water (including the 2 phases of the experiment), we initially performed a group analysis to reveal which voxels were significantly activated by the comparison [Water - Tasteless solution] including both the pre- and the postsatiety sessions. This analysis revealed activations illustrated in Fig. 2 bilaterally extending from the anterior insula/frontal opercular cortex (e.g., [37 22 3],  $Z = 4.98$ ,  $P < 0.05$  corrected for multiple comparisons; and [-33 22 3],  $Z = 4.67$ ,  $P < 0.05$  corrected for multiple comparisons; see coronal sections at top left of Fig. 2) to a more mid insular/opercular region (e.g., [-60 -2 18],  $Z = 5.05$ ,  $P < 0.05$  corrected for multiple comparisons; and [37 6 -4],  $Z = 4.84$ ,  $P < 0.05$  corrected for multiple comparisons; see horizontal sections in the bottom right of Fig. 2); in medial parts of the caudal orbitofrontal cortex (OFC) ([-1 35 -16],  $Z = 3.9$ ,  $P < 0.03$  SVC; and [9 19 -16],  $Z = 3.7$ ,  $P < 0.05$  SVC); and in the two parts of the rostral anterior cingulate cortex and adjoining cortex (e.g., [1 24 39],  $Z = 4.82$ ,  $P < 0.05$  corrected for multiple comparisons; labeled ACC in the upper left part of Fig. 2).

To determine if water does activate similar areas to those activated by the prototypical tastants glucose and salt, we



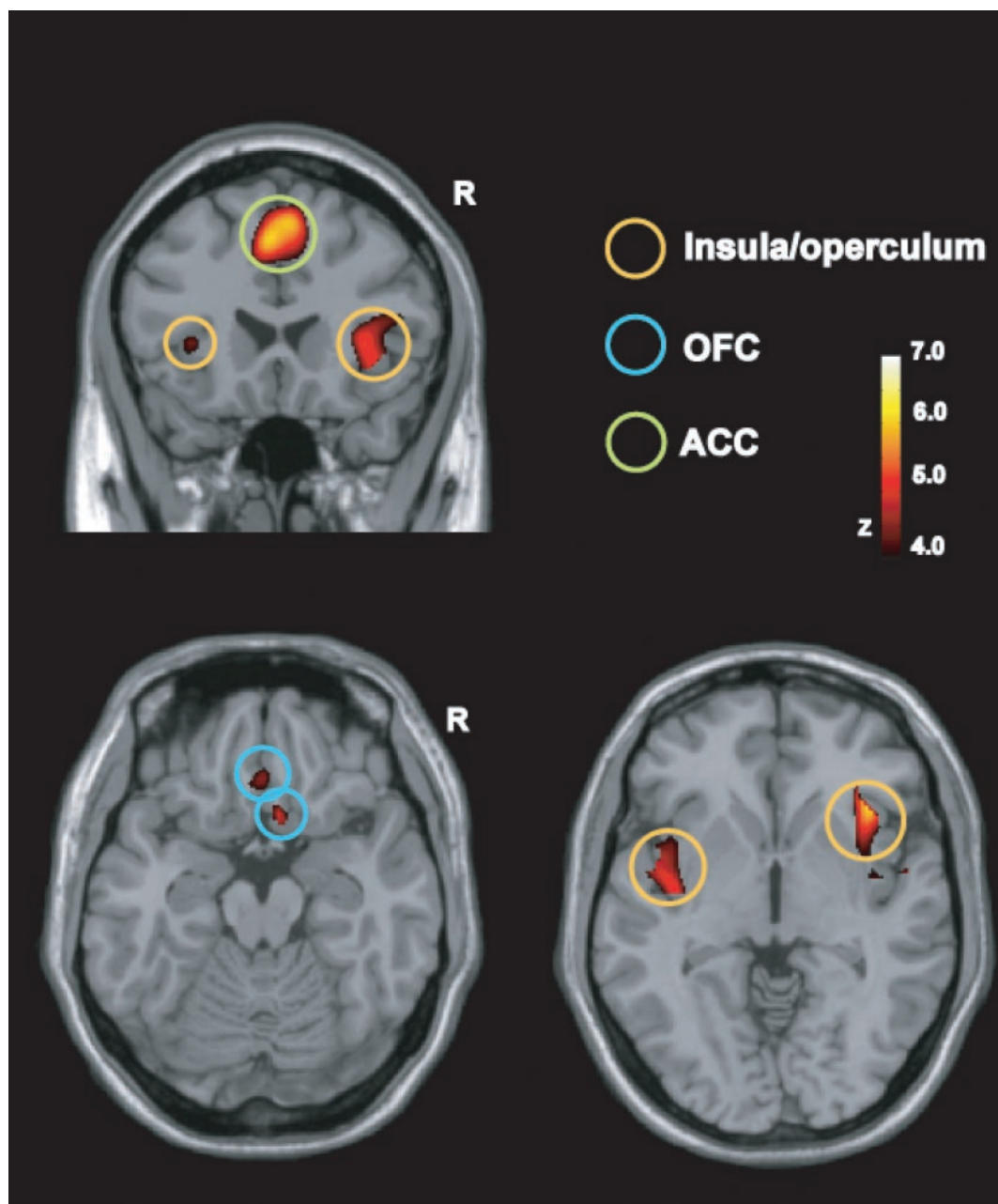


FIG. 2. Analysis showing the group main effect for the comparison (water – control – water). Significant activations were found in the insular/opercular cortex, the orbitofrontal cortex (OFC), and the anterior cingulate cortex (ACC) (see text for details). The activations were thresholded at  $P < 0.0001$ , uncorrected for multiple comparisons, to show their extent. The scale shows the Z scores (uncorrected for multiple comparisons). Values  $> 4.54$  are significant at  $P < 0.05$  corrected for multiple comparisons.

performed the conjunction analysis described in METHODS. This conjunction analysis revealed significant activations in the bilateral operculum/insula ([39 24 4],  $Z = 5.17$ ,  $P < 0.05$  corrected for multiple comparisons), caudal orbitofrontal cortex ([2 30 -18],  $Z = 4.53$ ,  $P < 0.05$  corrected for multiple comparisons), and in two parts of the rostral anterior cingulate and adjoining cortex ([2 40 16],  $Z = 7.17$ ,  $P < 0.05$  corrected for multiple comparisons; and [-2 32 37],  $Z = 4.47$ ,  $P < 0.05$  corrected for multiple comparisons). The results of this conjunction analysis are shown in Fig. 3. This conjunction analysis thus shows that water elicits responses in similar cortical areas to those activated by prototypical tastants such as glucose and salt. The time course of activation for an anterior right insula

cluster ([40 20 0]) for water, glucose, and salt are shown in Fig. 5b. The responses to the different stimuli are similar.

#### *Modulation of water in the mouth by the level of thirst*

Second, we investigated how the physiological state of the organism modulates the brain responses to the oral delivery of water. We first aimed to reveal which brain areas responding to water in the mouth are not modulated by the level of thirst. We thus performed a conjunction analysis across the conditions [water pre – tasteless pre] and [water post – tasteless post]. This revealed significant bilateral activations by water when both thirsty and satiated in the anterior part of the insular/

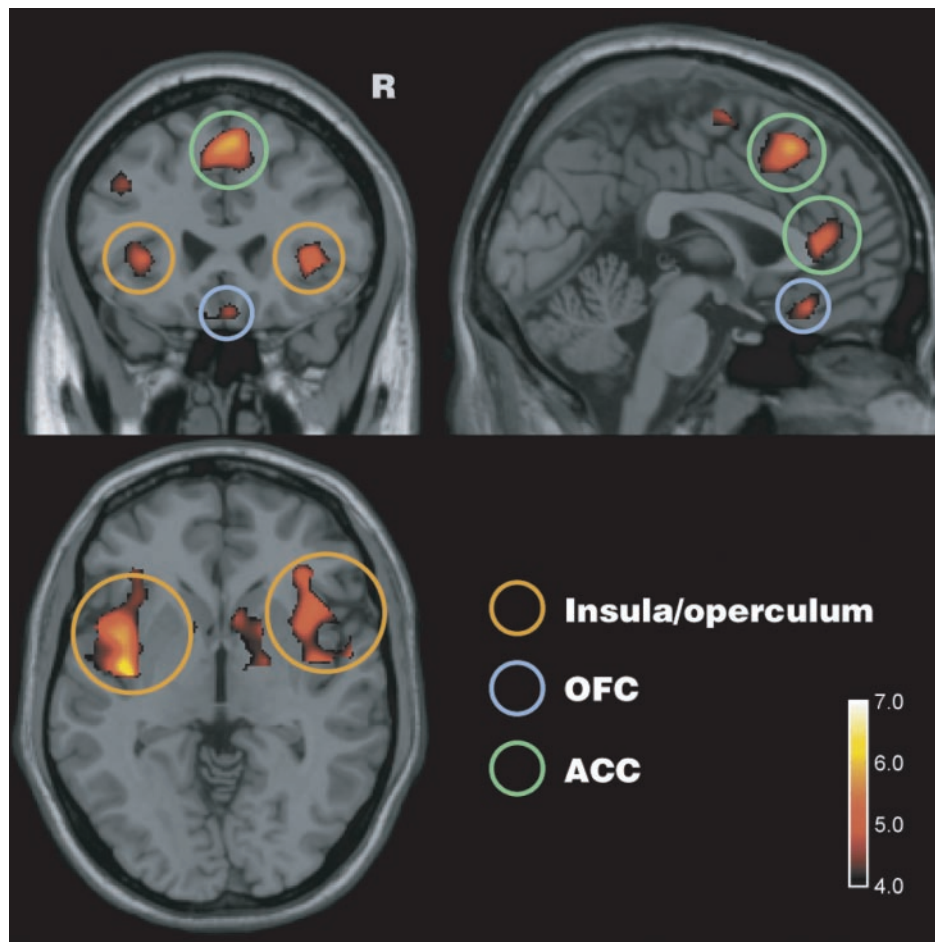


FIG. 3. Conjunction analysis revealing the main effects of taste across the comparisons (water – control water) AND (glucose – control glucose) AND (salt – control salt). Significant activations were found in the insular, opercular, anterior cingulate, and orbito-frontal cortices (see text for details). The activations were thresholded at  $P < 0.0001$ , uncorrected for multiple comparisons, to show their extent.

frontal opercular area (e.g.,  $[\pm 40 \ 20 \ 0]$   $Z = 4.8$ ,  $P < 0.05$  corrected for multiple comparisons). These activations are depicted in *blue* in Fig. 4*a*. The time courses of these activations produced by water for a cluster centered in the right anterior part of the insula, for both pre- and postsatiety periods, are shown in Fig. 5*a* and confirm that the activations produced by water are similar when thirsty and when satiated. As shown in Fig. 4*a*, this region extends anteriorly to include some voxels in the caudolateral part of the orbitofrontal cortex which adjoins the frontal operculum and insula.

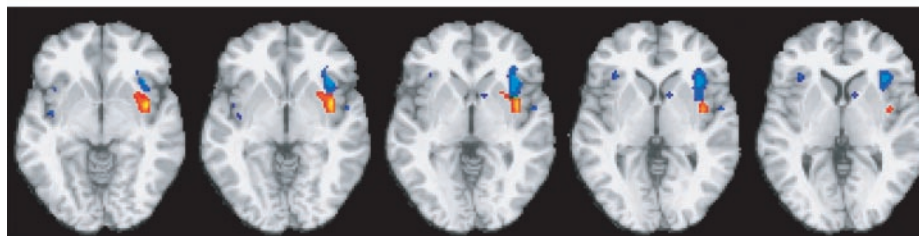
To uncover brain areas where the activation produced by water in the mouth is modulated by the level of thirst, the contrast [water pre – water post] was computed in each individual subject. A second-level, random effects analysis performed on these contrasts revealed a significant cluster of voxels in the caudomedial orbitofrontal cortex (areas 25/11,  $[6, 22, -7]$ ,  $P < 0.005$  uncorrected for multiple comparisons), as shown in Fig. 4*b*. This cluster adjoined and may have included some voxels in the subgenual anterior cingulate cortex. In addition, the second-level, random effects analysis performed on these contrasts revealed a significant cluster of voxels in the right mid insula ( $[34 -4 -4]$ ,  $Z = 3.55$ ,  $P < 0.05$  corrected with SVC), as shown in *red* in Fig. 4*a*. The corresponding time courses for this right mid insula region (centered at  $[34 -4 -4]$ ) are shown in Fig. 6. Thus in both the medial orbitofrontal cortex and a mid part of the right insular cortex, the oral delivery of water produced smaller effects when satiated than when thirsty.

To provide further evidence on the activations produced by placing water in the mouth before and after satiation with water, we show in Fig. 7 the BOLD signals (mean across subjects  $\pm$  SE) obtained from the overall time course of the activations in the reported main clusters. This analysis confirms what was found in the main random effects and conjunction analyses illustrated in Fig. 4. In particular, Fig. 7 shows that there was no significant reduction in the BOLD signal in the clusters illustrated in *blue* in Fig. 4*a* in the anterior insula/opercular taste area. On the other hand, there was a significant reduction in the activations produced by drinking water to satiety in the medial orbitofrontal cortex (the area illustrated in Fig. 4*b*); and also in the right mid insula region shown in *red* in Fig. 4*a*. This analysis helps to confirm that the anterior insula/opercular area behaves differently to the mid insular area in this paradigm. These conclusions were supported by a two-way ANOVA on the data shown in Fig. 7 (for the time period 4- to 12-s poststimulus delivery), with one factor dehydrated versus hydrated, and the other factor the brain region, and the interaction was significant ( $F[2,6] = 40.8$ ,  $P < 0.001$ ), providing further evidence that the activations produced by water in the different brain regions were differently affected by whether thirst was present.

#### Correlations with subjective ratings

We correlated brain activity with the subjective pleasantness ratings for the water stimulus taken across the whole experi-

A



B



FIG. 4. Comparison of the effects of delivering water in the mouth during the pre- (dehydrated) period against the post- (hydrated) period (water pre- - water postsatiation). *a*: axial sections through the insular cortex showing the main effects of water through conjunction analysis : [water pre - control water pre] AND [water-post - control post], in blue); and the results of [water pre - water post] saturation (in red) as revealed through second-order, random effects analysis. Activations were thresholded at  $P < 0.001$  uncorrected to show their extent. *b*: subgenual cingulate/medial orbitofrontal cortex activation in the [water pre - water post] comparison as revealed through second-order, random effects analysis. Activations were thresholded at  $P < 0.005$  uncorrected to show their extent.

ment. Most importantly we found (in a group random effects analysis) that a region of the medial orbitofrontal cortex [ $-3, 45, -16$ ;  $Z = 3.92$ ,  $P < 0.05$  SVC; and [ $16, 46, -16$ ;  $Z = 3.42$ ,  $P < 0.001$ ] was significantly correlated with the pleasantness ratings of water (see Fig. 8, *top left*), which is quite close to the region illustrated in Fig. 4*b*, which describes a related comparison. A scatter plot showing the values of the BOLD signal in the first medial orbitofrontal cluster of voxels described is shown in Fig. 8, *bottom*, with the regression line shown. This emphasizes the strong relation between the pleasantness ratings of water and the activation in this medial orbitofrontal cortex region. We also found that regions of the anterior cingulate cortex (ACC) at [ $-2, 19, 28$ ] and [ $-2, -5, 43$ ] were significantly correlated ( $P < 0.0001$ ) with the pleasantness ratings of water across the whole experiment (see Fig. 8). In addition (not shown in Fig. 8), inspection at lower thresholds showed that there was some correlation with the pleasantness rating for water in a part of the mid insula close to that illustrated in Fig. 4*a* in red [ $47, -1, 6$ ,  $Z = 2.9$ ,  $P < 0.005$ ]. No significant correlations were found with the intensity ratings. The finding that there is no correlation of the activation of any brain area in this study with the intensity ratings is important, for it shows that the correlations of activation of the medial OFC with pleasantness cannot be ascribed to a different factor such as intensity.

*Levels of brain activity that were different in the pre- and postsatiation state measured when stimuli were not being delivered*

We created a comparison between presatiation and postsatiation periods independent of stimulus delivery events. The analysis was performed for the period 14–35 s with respect to the onset of the stimulus in each trial, which is, as shown in

Fig. 1, a time when no stimuli were being delivered. This comparison revealed significant activity differences in the rostral anterior cingulate cortex (Brodmann area 32, [ $-8\ 46\ 16$ ],  $Z = 4.96$ ,  $P < 0.05$  corrected for multiple comparisons, see Fig. 9).

## DISCUSSION

The results show that the oral delivery of water activated a part of the human anterior insular and frontal opercular cortex (see Fig. 2). Moreover, the results shown in Fig. 3 indicate that the same region activated by water is also activated by the prototypical tastants glucose and NaCl. This brain region corresponds with the anterior insular and adjoining anterior opercular cortex of macaques which is the primary taste cortical area in that it receives direct projections from the taste thalamic nucleus, VPMpc (the parvocellular part of the ventro-postero-medial thalamic nucleus; Pritchard et al. 1986), and has taste-responsive neurons (Scott et al. 1986; Yaxley et al. 1990). On these grounds, the anterior insular and frontal opercular region shown in Figs. 2 and 3 is the putative human primary taste cortex. Also consistent with the macaque (Rolls et al. 1988; Yaxley et al. 1988) is the finding that the homeostatic need state, in this case thirst, does not modulate the responsiveness to oral water (as shown in Fig. 4*a* in blue). The representation in this primary taste cortical area in humans as well as in macaques appears to be of the identity and intensity of the oral taste stimulus, and not of the reward value or the correlated affective value, both of which decrease to zero when water is drunk to satiety, as confirmed in this study. Individual neurons in the macaque primary taste cortex are tuned to respond best to each of the prototypical stimuli sweet (glucose), salt (NaCl), bitter (quinine), and sour (HCl), and also to water (Rolls et al. 1988; Scott et al. 1986), with each neuron showing a distrib-



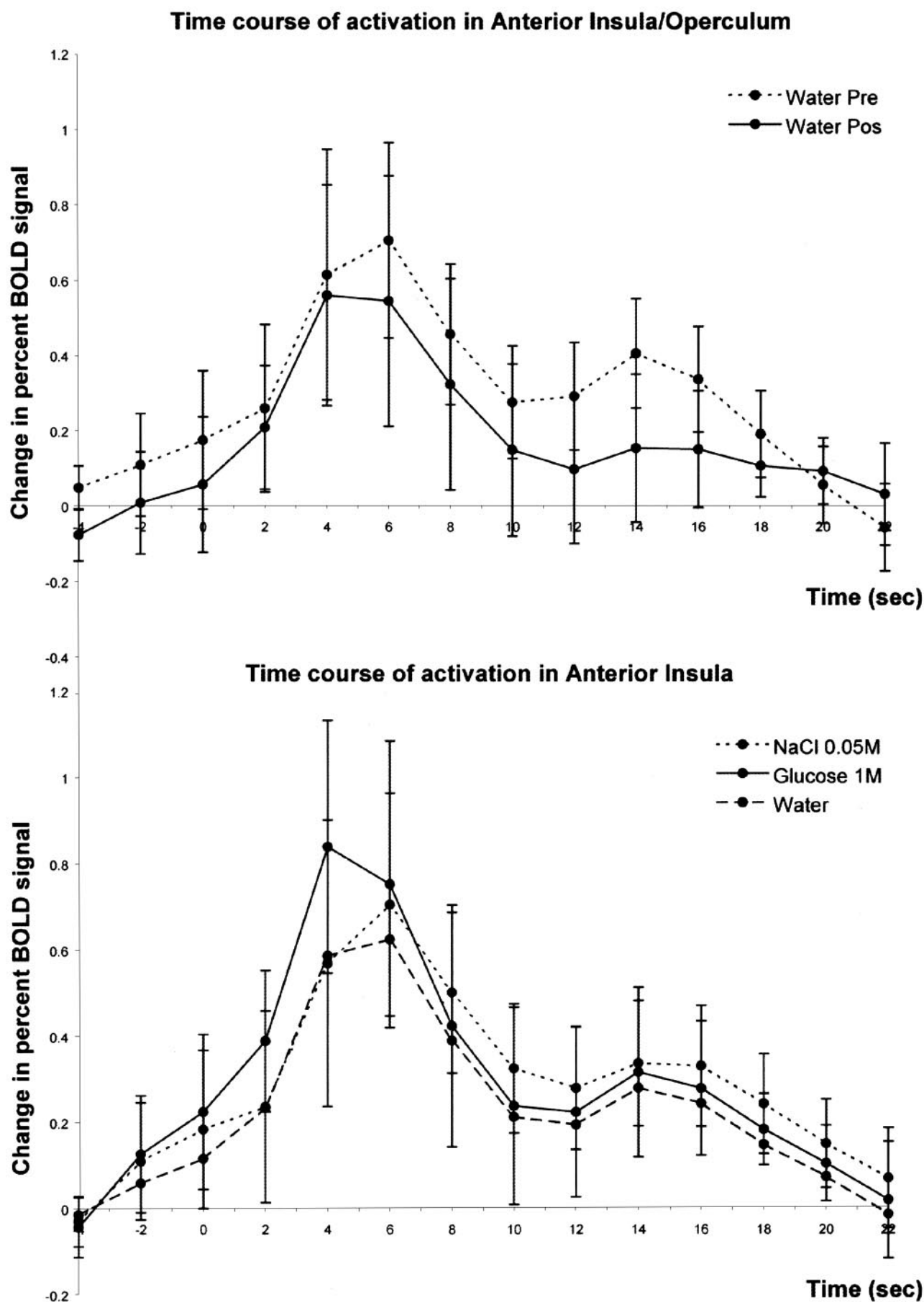


FIG. 5. Time courses of activation extracted from a cluster in the right anterior insula (MNI coordinates [40, 20, 0]) with respect to the delivery of (a) water, with the response shown separately for the presatiation and postsatiation states; and (b) water (throughout the experiment), salt, and glucose.

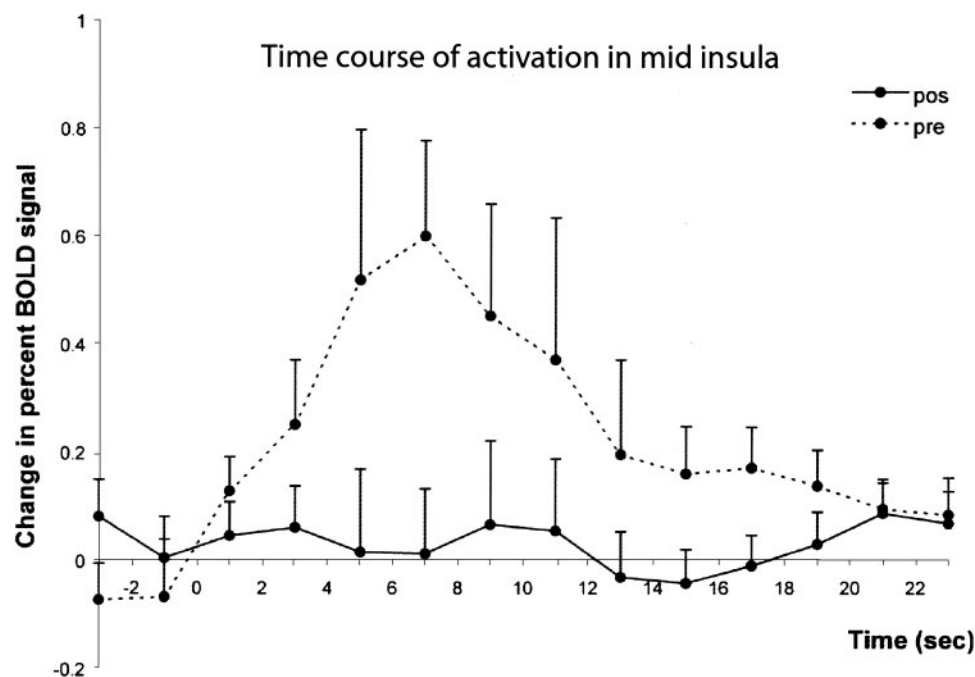


FIG. 6. Time courses of activation extracted from a cluster in the right mid insula (MNI coordinates [34, -4, -4]) with respect to the delivery of water, shown separately for presatiety and postsatiety states. This region ( $Y = -4$ ) was acquired coronally after 1 s from the start of volume acquisition, and this is reflected in the time axis scale.

uted representation in that each typically responds to several stimuli in the set. The finding that the same region of the human brain is activated by water, glucose, and NaCl is consistent with this evidence that water responses form a continuous part of what is represented in the primary taste cortex, although of course the fMRI methodology is too insensitive to reveal directly anything about the nature of the encoding used by the neurons. In humans, as in macaques, it is notable that this primary taste cortical area extends right to the anterior border of the anterior insula and adjoining frontal operculum into the transitional region which topologically might be described as a far caudal part of the orbitofrontal cortex but which architectonically can be classified as PrCO, Iai, and 12o (Carmichael and Price 1994; Öngür and Price 2000; Petrides and Pandya 1994). Although it has been shown previously that oral water activates the insular and opercular cortices in humans (Zald and Pardo 2000), that study did not use a tasteless control for nonspecific including somatosensory effects produced by placing a fluid into the mouth. The present finding constitutes

the first report that water activates taste-related cortical areas in humans when contrasted to a tasteless control solution, which includes the ionic components of saliva.

The results also show that a part of the caudomedial human orbitofrontal cortex is activated by water and by the taste stimuli sweet and salt (Fig. 3, OFC). In this region, the activation produced by the delivery of water is high when the subjects are thirsty, and decreases to baseline after the subjects have drunk water to satiety (Figs. 4b and 7). Thus the activation of this caudomedial part of the human orbitofrontal cortex is large when the subjects find the water rewarding (in that the subjects want to drink the water), and when they find its taste pleasant and refreshing as shown by the subjective ratings. Moreover, the same location shows activation that is correlated with the refreshing value and pleasantness of the water delivered into the mouth (Fig. 8). This region may thus be the human secondary taste cortex, corresponding to the macaque secondary taste cortex, defined by its projections from the primary taste cortex (Rolls and Baylis 1994), and which also contains single neurons that only respond to water delivered into the mouth when thirst is present (Rolls et al. 1989). The human caudomedial orbitofrontal cortex also shows a motivation-selective decrease in its responsiveness to oral stimuli, in that its activation to water but not to glucose or salt decreased after the subjects had consumed water to satiety. This directly parallels the motivation-selective decrease in the pleasantness of water but not glucose after water has been consumed to satiety, shown by Rolls et al. (1983) and confirmed in this investigation. It is notable that the part of the human orbitofrontal cortex showing these effects is far medial, whereas in the macaque the region is more lateral in the caudal orbitofrontal cortex (Rolls et al. 1989, 1990). However, in macaques, taste neurons are not confined to this lateral part of the orbitofrontal cortex, and indeed, are found in smaller numbers widely in the orbitofrontal cortex, mixed in with neurons responding to stimuli in other modalities, including olfactory and visual (Rolls and Baylis 1994). We suggest that to help better under-

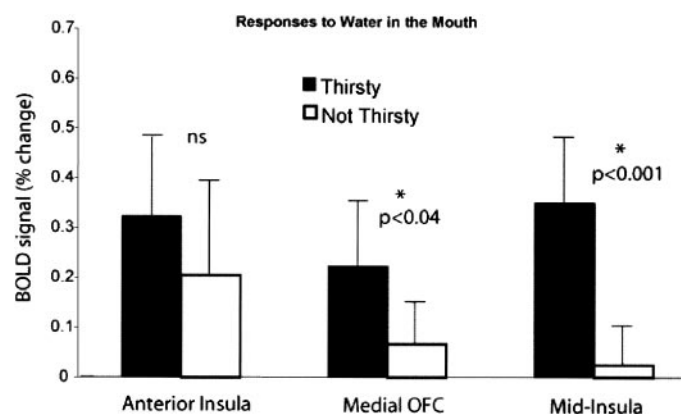


FIG. 7. The change in the BOLD signal produced by water in the mouth when thirsty, and when not thirsty following drinking water to satiety, in 3 different regions. The mean and SE across 11 subjects are shown, measured from the time course in the relevant clusters of voxels.



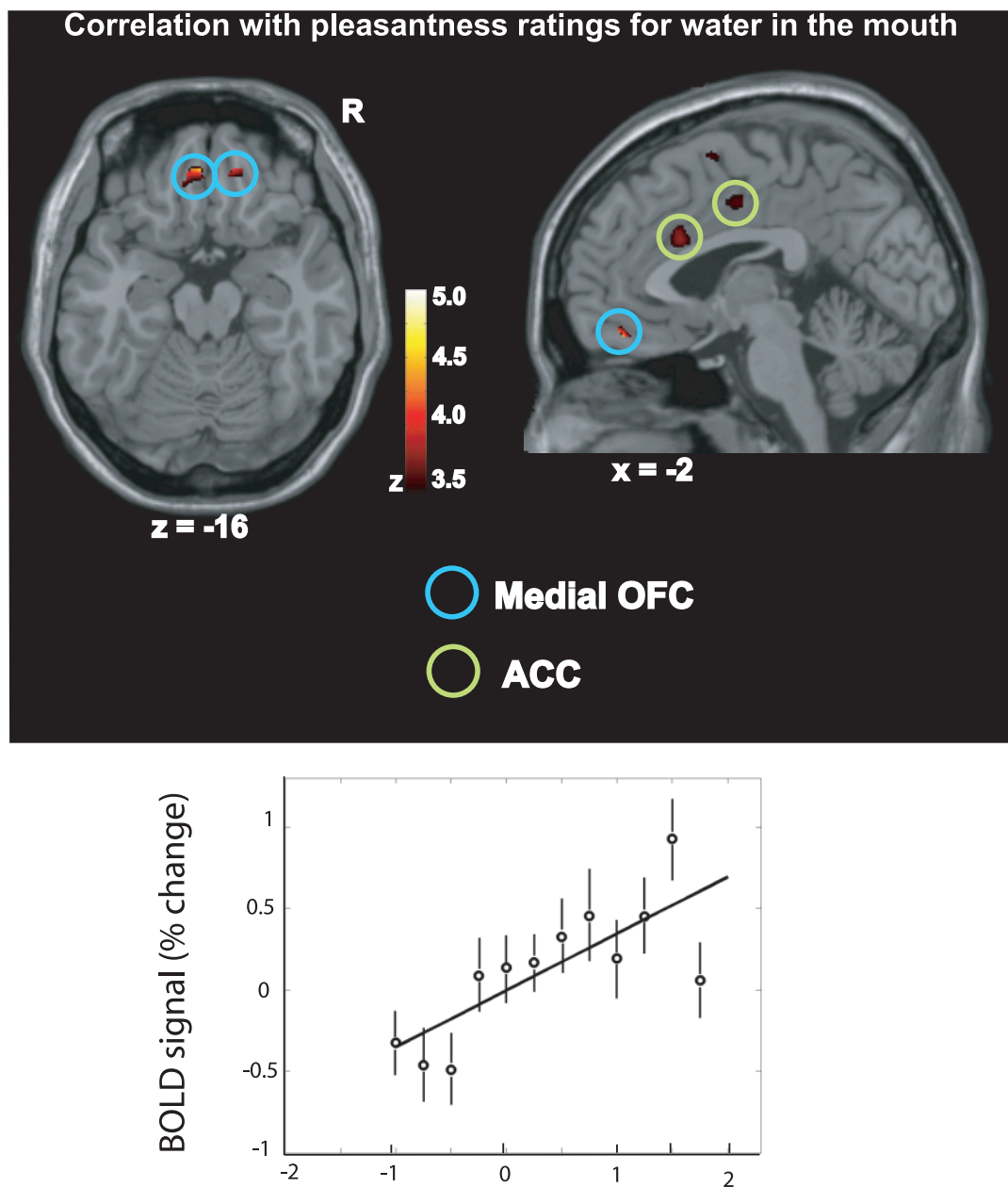


FIG. 8. Correlations with the pleasantness of the taste of water in a group random effects analysis. *Top left*: regions of the medial caudal orbitofrontal where the activation was correlated with the subjective pleasantness ratings of water throughout the experiment. *Bottom*: a scatter plot showing the values of the BOLD signal in the medial orbitofrontal cortex (mean across subjects  $\pm$  SE), with the regression line shown. *Top right*: regions of ACC where activation was correlated with the subjective pleasantness ratings of water given throughout the experiment.

stand the activation of the medial orbitofrontal cortex by water when thirst is present it would be of considerable interest to record from single neurons in this medial region in macaques, for the most medial part of the macaque orbitofrontal cortex has been relatively little explored with respect to taste and olfactory representations. The finding that pleasantness (and refreshment) ratings for water are correlated with activations in the medial orbitofrontal cortex (Fig. 9) is consistent with previous studies implicating the medial part of the orbitofrontal

cortex in the representation of the affective properties of primary and secondary reinforcers including pleasant touch, monetary reward, and pleasant odors (de Araujo et al. 2003; O'Doherty et al. 2001; Rolls et al. 2003a,b), and even face attractiveness (Aharon et al. 2001).

In this investigation, we found a region of the insula that is midway between the anterior and the posterior insula, and which we refer to as mid insula, which was activated by water but not by sweet and salt stimuli, and which further was only

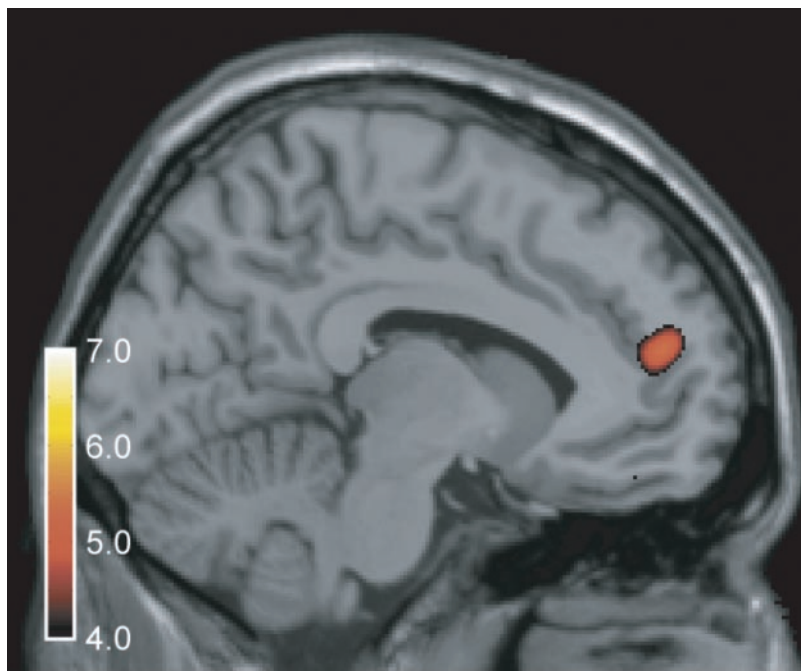


FIG. 9. Rostral anterior cingulate cortex activation revealed in the group effects analysis for the presatiety – postsatiety comparison, when the stimulus delivery events are not taken into account. The activations were thresholded at  $P < 0.05$ , corrected for multiple comparisons.

activated by water when thirst was present and not after satiation (see Fig. 4a, red). This region has not been investigated neurophysiologically in macaques. Previous functional neuroimaging studies in humans have demonstrated that regions located in the mid and posterior insular cortex are consistently activated by general visceral and homeostatic-related stimuli such as temperature (Craig 2002), nociceptive stimulation (Craig 2002), fasting (Tataranni et al. 1999), and isometric and dynamic exercise (King et al. 1999). Indeed, we have found activation by painful stimuli applied to the hand of a part of the mid insula which is quite close (Rolls et al. 2003b). However, we think it unlikely that exactly the same mid insula area is activated by pain and by water, because the activations we describe were larger when the water was rewarding and more pleasant than after drinking water to satiety when the water was less pleasant (Figs. 4a and 7). A similar region was shown to have activations that correlate with mean arterial pressure in humans (Critchley et al. 2000). Activations in the insula were also produced by hypertonic saline injection, leading to thirst (Denton et al. 1999). The functional segregation within the mid and posterior insular cortex suggested by the results of Craig and colleagues may be partially explained by the functional organization of the posterior thalamic nuclei in humans. In a cytoarchitectonic and immunohistochemical characterization of the ventral medial nucleus of the human thalamus, Blomqvist et al. (2000) have demonstrated different reactivity for the calcitonin gene-related peptide (CGRP, an important visceral-related modulator; Yasui et al. 1989) within posterior regions of this nucleus. In particular CGRP-positive fibers were observed in an intermediate region located adjacent, but distinct to, VMpc and the posterior ventral medial nucleus of the thalamus (VMpo), named by these authors the posterior nucleus (Po). In the rat (similar data are presently unavailable in nonhuman primates), CGRP-positive terminal fibers in the thalamus originate (preferentially) from the external medial parabrachial subnucleus and convey general visceral-related information (Blomqvist et al. 2000; Yasui et al. 1989), whereas

CGRP-negative terminals originate from the central medial parabrachial subnucleus, a gustatory relay receiving taste-related information from the NST. In addition, different thalamic regions in the macaque monkey appear to represent either general visceral or gustatory information (Pritchard et al. 1989). Taking also into consideration the nonpeptidergic structure of the taste pathway (Kruger et al. 1988), Blomqvist and colleagues (2000) thus conclude that a clear separation exists between gustatory and visceral-dedicated thalamic nuclei in humans as determined by the distinct immunoreactivity to CGRP in VMpc and Po. This separation could thus be further represented in the human insular cortex, in that more anterior regions seem to be dedicated preferentially to gustatory processing, whereas a more posterior region seems to respond to water delivered into the mouth when thirst is present. Given the lack of relevant neurophysiology on this region to date, we are not sure how to interpret the activation found in this study to water when thirst is present, but note that autonomic and endocrine responses will be elicited by water delivered under these conditions, and suggest that the activations we found in the mid insula could be related to the initiation of these autonomic and endocrine responses. Neurophysiological investigations in macaques are likely to be revealing with respect to this issue.

We also found that parts of the anterior cingulate and adjoining cortex showed activation to water and to the tastants sweet and salt (Fig. 3, ACC). Moreover, when no oral stimuli was being delivered, it was found that the level of activity in a part of the anterior cingulate cortex was higher when thirsty than when satiated (see Fig. 9). This is consistent with previous findings showing that the primate ACC is also involved in autonomic and emotional control (Devinsky et al. 1995), and in particular, is a target in rats of projections from circumventricular organs (McKinley et al. 2001) and has been shown in a PET study to have blood flow that correlates with thirst produced by hypertonic stimuli in humans (Denton et al. 1999).

The question arises about how water delivered into the

mouth produces its effects found at the neuronal level in the insular and orbitofrontal cortices (Scott et al. 1986; Yaxley et al. 1990), at the psychophysical level (Bartoshuk 1974), and at the neuroimaging level in humans as described in this paper. One possibility is that the water acts through a taste-sensitive system. In particular, salt-best neurons might decrease their firing rate below the spontaneous level when water is placed on the tongue. Such salt-best neurons may be sufficiently sensitive to respond to the decrease of a few mM in the concentration of  $\text{Na}^+$  (and other cations) when the saliva in the mouth is replaced by water. Another possibility is that water-best neurons in the cortex respond to a combination of fluid of a low viscosity in the mouth and the lack of taste neurons firing. Neurophysiological investigations are required to resolve this issue. With respect to human psychophysics, it is notable that whereas the subjects of Bartoshuk (1974) reported after adaptation to 10 mM NaCl that water in the mouth tasted bitter-sour, our subjects found that taste of water pleasant. The difference is almost certainly due to the fact that the pleasantness of water is high when subjects are slightly water-deprived as in the present study, and indeed the findings described in this paper underline the point that the pleasantness of water in the mouth is a function of need state.

In the context of the much discussed issue of hemispheric specialization (Davidson 1992), we found little evidence for lateralization of the taste effects described in this paper. For example, the activation of the anterior insular/opercular (primary) taste cortex by water (Fig. 2) and by other tastes (Fig. 3) is clearly bilateral. Also, the activation of the orbitofrontal cortex by water was to at least some extent bilateral (see Figs. 2 and 3), and the representation of the pleasantness of the taste of water in the medial orbitofrontal cortex was clearly bilateral (Fig. 9). The only indication of any laterality we found was in the contrast of the effects of water delivery when thirsty—the effects of water delivery when not thirsty, which did produce more signal on the right mid insula (see Fig. 4a, activations shown in red). Certainly in macaques the representation of taste is bilateral (see Rolls and Scott 2003 for review), and, when a representation of a part of the body surface is being considered, there is little theoretical advantage of lateralization (Rolls and Deco 2002). This compares with the suggestion corroborated by clinical findings (Pritchard et al. 1999) that gustatory processing may involve more the left side of the human brain (Craig 2002). The evidence in this study shows that taste is in general represented bilaterally in the insular/opercular cortex and in the orbitofrontal cortex.

Throughout this paper we have presented the results of the group analyses (including conjunction and second level random effects analyses), as this is statistically very powerful (Friston et al. 1999; Worsley et al. 1996). However, we note that all the effects described were present at the individual subject level.

In conclusion, the results provide evidence not only for where the stimulus of water in the mouth is represented in the brain, but also on the medial orbitofrontal cortex region where the activations are larger when thirsty than when satiated, and where the pleasantness and refreshing value of water are represented in the brain. As such, the investigation advances our understanding of where affect and emotion are represented in the brain (Rolls 1999). Moreover, the findings provide evidence that, as in macaques, in humans there are separate

representations of the identity of tastes and oral stimuli in the mouth (in the anterior insular/opercular cortex), and of their hedonic value (in the medial orbitofrontal cortex). Further, the findings provide new evidence on the functions of the mid part of the human insula and are consistent with the hypothesis that it may be related to autonomic function as part of an interoceptive network (Craig 2002). Finally, we note that the temperature of water may influence its pleasantness. We already know that some single neurons in the primate orbitofrontal cortex do respond to the temperature of liquids in the mouth (Kadohisa, Verhagen, and Rolls, unpublished data), and we believe that the same could be true in humans.

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## DISCLOSURES

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