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Neural Correlates of Tasting Concentrated Quinine and Sugar Solutions

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Zald, David H., Mathew C. Hagen, and José V. Pardo. Neural correlates of tasting concentrated quinine and sugar solutions. *J Neurophysiol* 87: 1068–1075, 2002; 10.1152/jn.00358.2001. Behavioral, ethological, and electrophysiological evidence suggests that the highly unpleasant, bitter taste of a concentrated quinine hydrochloride (QHCL) should activate the human amygdala. In the present study, healthy subjects tasted 0.02 M QHCL or water while regional cerebral blood flow (rCBF) was assayed with $H_2{}^{15}O$ PET. Subjects were also studied while tasting a pleasant sucrose solution and resting with eyes closed (ECR). Tasting QHCL significantly increased rCBF within the left amygdala relative to control conditions of tasting water and ECR. Sucrose and water caused small to moderate rCBF increases in the amygdala relative to ECR, but sucrose did not significantly increase activity within either amygdalae relative to water. In the frontal lobe, QHCL and sucrose both activated the right posterior orbitofrontal cortex (OFC) relative to water, but portions of the anterior OFC and inferior frontal pole showed valence specific responses to QHCL. These data indicate that the left amygdala responds robustly to QHCL and more moderately to nonaversive sapid stimuli, both pleasant and unpleasant gustatory stimuli activate the right posterior OFC, and the left inferior frontal pole/anterior OFC demonstrates valence-specific responses to aversive gustatory stimuli.

INTRODUCTION

Neuroimaging studies increasingly indicate that exposure to stimuli with aversive properties produces robust increases in amygdala activity. Studies in the olfactory (Birbaumer et al. 1998; Zald and Pardo 1997), gustatory (Zald et al. 1998a), visual (Irwin et al. 1996; Lane et al. 1997; Taylor et al. 1998), and auditory modalities (Zald and Pardo 2000a) all demonstrate the ability of highly aversive stimuli to induce increased activity within the amygdala. Several lines of evidence suggest that the taste of high concentrations of quinine hydrochloride (QHCL) should make a particularly good stimulus for inducing increases in amygdala activity. QHCL represents the prototypical stimulus for producing the perception of bitterness. Most mammals consistently reject QHCL and other bitter-tasting substances as unpalatable (Glendinning 1994), and humans experience the taste of high concentrations of QHCL as extremely aversive. Indeed, some theorists have suggested that the perceived unpalatability of bitter substances evolved to facilitate the rejection of naturally occurring poisons (almost all of which taste bitter) (Brieskorn 1990; Glendinning 1994). Thus there may exist quantitative or qualitative differences in brain responses to bitter substances relative to other tastes. Given its role in recognizing and responding to potentially threatening stimuli, the amygdala represents a likely site for such differences to emerge.

Clinical and electrophysiological evidence also suggests a link between bitter tastes and amygdala activation. Gustatory hallucinations induced by seizures in or near the amygdala most frequently involve bitter or novel unpleasant tastes (Falconer and Cavanagh 1959; Hausser-Hauw and Bancaud 1987). Electrophsyiological evidence from studies with rodents similarly suggests a unique link between bitter tastes and amygdala activity. First, the amygdala possesses a greater proportion of cells tuned to bitter-tasting QHCL than is seen in other parts of the gustatory system (Nishijo et al. 1998). Second, these QHCL-tuned cells demonstrate substantially higher spike rates than amygdala cells that are tuned to other gustatory stimuli (Nishijo et al. 1998). To test the responsivity of the human amygdala to aversive QHCL, we exposed healthy human subjects to a high-concentration QHCL solution while regional cerebral blood flow (rCBF) was assayed with positron emission tomography (PET).

Electrophsiological data from nonhuman primates and rodents also indicate that pleasant gustatory stimuli should also activate the amygdala (Azuma et al. 1984; Nishijo et al. 1998; Scott et al. 1993). Indeed, studies of primates indicate that as many or more amygdala cells are tuned to sweet stimuli (Scott et al. 1993) as are tuned to bitter stimuli. However, in a previous PET study of human gustatory hedonics, we failed to observe significant increases in amygdala activity during pleasant gustatory stimulation with chocolate relative to tasting water (Zald et al. 1998a). Chocolate was utilized in this initial study because of its highly positive hedonic qualities. However, three problems limit interpretation of this earlier study. First, the chocolate (presented in solid form) was not well matched with water in terms of its somatosensory features. Second, the perception of chocolate involves both olfactory and gustatory processing. Third, it is possible that water itself produces modest activations of the amygdala, which might obscure the ability to detect increases induced by a pleasant gustatory stimulus (Zald and Pardo 2000a). This latter possibility finds support from a recent PET study indicating that water and sucrose are both capable of producing moderate rCBF increases in the amygdala when contrasted with a non-

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sapid control condition (Frey and Petrides 1999). Furthermore, a recent fMRI study reported amygdala activation in subjects tasting sucrose relative to tasting artificial saliva (O'Doherty et al. 2001). To examine the relative effects of sucrose and water on amygdala activity, we additionally asked subjects to taste a sucrose solution, "taste" water, or rest with their eyes closed (ECR) while undergoing PET imaging. This allowed examination of the effects of tasting a pleasantly valenced, pure gustatory stimulus that is well matched with water in terms of somatosensory features.

METHODS

Subjects and stimulation paradigm

Nine healthy subjects (4 right-handed females; 2 right-handed males, and 3 left-handed males) with an average age of 24 yr (range: 18–34 yr) were studied with PET while tasting a 0.02 M solution of QHCL, tasting deionized distilled water, and during ECR. All subjects completed written informed consent approved by the Minneapolis Veterans Affairs Medical Center Human Subjects Committee and Radioactive Drug Research Committee. Subjects were informed that they would receive an unpleasant taste during one scan condition but were blind to the scan number for that condition, the identity of the stimulus, and the degree of unpleasantness.

The 0.02 M QHCL represents a highly concentrated solution of QHCL. Most electropsychophysical studies use stimuli that are at least 1 log unit lower in concentration. Such a high concentration was selected for the present study because it produced consistently strong hedonic and intensity ratings on pilot testing, whereas lower concentrations produced less consistently robust ratings. Deionized distilled water served as the primary control condition so as to control for the somatosensory and motor processes associated with intraoral stimulation. One subject reported that the deionized distilled water tasted slightly bitter. Spring water was substituted for deionized distilled water for this subject because she perceived the spring water as tasteless. No other subjects reported detecting a taste other than water during exposure to the deionized distilled water.

Prior to fluid injection, 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 fluid in your mouth, swish it around a couple of times, and then allow your tongue to rest. If you start to feel that there is too much fluid in your mouth, briefly raise your hand and I will stop injecting the fluid." In the QHCL and water conditions, subjects held a small plastic cannula between their teeth. An initial 3 ml fluid was injected into the mouth synchronous with the start of scan acquisition. In the QHCL condition, an additional 2–3 ml was slowly injected into the subject's mouth over the next 40 s. In the water condition, subjects received an additional 3–6 ml over the course of the scan to ensure that they perceived the water beyond the initial stimulation period.

After each condition, subjects rated the stimulus for pleasantness-unpleasantness (11-point visual analog scale with anchors at 0-extremely unpleasant, 5-neutral, and 10-extremely pleasant) and intensity (11-point visual analog scale with anchors at 0-undetectable and 10-extremely intense). Subjects were additionally asked to rate the extent to which they experienced fear or anxiety during the gustatory stimulation and were queried as to the identity of the solution they had tasted. Because QHCL leaves a lingering aftertaste in the mouth, it was not possible to apply a counterbalanced design. Thus in all cases, subjects received the QHCL condition after the water and ECR conditions.

To test whether a nonaversive gustatory stimulus would activate the amygdala relative to water, we also asked subjects to taste a sweet fluid (30% sucrose solution). To ensure that subjects had robust experiences of intensity and normal hedonic ratings, we applied an a priori inclusion criteria for perceptual ratings (pleasantness rating >5, intensity rating >5). This caused two subjects to be excluded, leaving 7 subjects with QHCL, water, sucrose, and ECR conditions. On initial data analysis, the results from the sucrose condition relative to water appeared quite weak. To ensure that this did not simply reflect a statistical power issue, we included data from an additional three subjects who met the perceptual rating criteria outlined in the preceding text. Thus for contrasts between sucrose and water, and sucrose and ECR, a total of 10 subjects participated (6 right-handed females, 2 right-handed males, and 2 left-handed males; mean age = 23, range = 18–34). Stimulus administration was identical for sucrose and water (see description of water administration in the preceding paragraph). The sucrose and water conditions were counterbalanced.

Imaging and analysis

rCBF was estimated from normalized (1,000 counts) tissue radioactivity using an ECAT 953B camera (Siemens, 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), and a 90-s scan acquisition beginning on radiotracer arrival into the brain. Subjects were placed in the scanner to maximize visualization of ventral frontal and temporal lobe regions. Images were reconstructed with a three-dimensional (3-D) reconstruction algorithm with a 0.5 cycles/pixel Hanning filter (Kinahan and Rogers 1989) with attenuation correction using a two-dimensional transmission scan. All scans were normalized for global activity, coregistered, and nonlinearly warped to a reference stereotactic atlas (Talairach and Tournoux 1988) with automated software (Minoshima et al. 1992–1994). Images were blurred with a 3-pixel (6.75 mm) 3-D Gaussian filter producing a final image resolution of ~10 mm full-width at half-maximum.

Effect sizes are reported as *Z* scores (rCBF change at the peak pixel/global SD of all intracerebral pixels) (Fox et al. 1988; Worsley et al. 1993). Primary analyses utilized a threshold of $P < 0.0005$ (equivalent to a *Z* score = 3.3) for the evaluation of statistical significance. This threshold is slightly more conservative than the $P < 0.001$ cutoff frequently used in pixel-wise analyses of PET studies and is derived from a bootstrapping analysis of the rate of false positive foci emerging due to chance (Zald et al. 1998a). Follow-up analyses that only examined the amygdala utilized a *Z*-score criteria of 2.88 ($P < 0.005$) that is equivalent to an overall significance of $P < 0.05$ corrected for the number of resolution elements in the amygdala bilaterally (Worsley et al. 1993).

RESULTS

Psychoperceptual ratings

Subjects rated the QHCL as highly aversive (mean = 1.7; range, 0–3) and highly intense (mean = 8.5; range, 7–10). Most of the subjects described the QHCL as "disgusting," "gross," or "horrible." All subjects reported increased muscle tension on tasting QHCL. Two subjects also reported feeling moderately anxious during the QHCL condition. Interestingly, QHCL was experienced as highly novel. All of the subjects failed to identify the QHCL by name, and several had difficulty describing it except when queried with a forced choice format of the four basic tastes. By contrast, all subjects correctly identified the sucrose solution. Those subjects meeting the inclusion criteria rated the sucrose as moderately pleasant (mean, 7.2; range, 6–8) and intense (mean, 6.9; range, 5–9).

PET results

QHCL–WATER. Table 1 displays the location of rCBF maxima in the contrast between the QHCL and water conditions.

TABLE 1. *Locations of increased rCBF in the contrast between the conditions of tasting QHCL and of tasting pure water*

Area	*x*	*y*	*z*	*Z* Score
Left anterior OFC/frontomarginal gyrus (BA 10/11)	−24	50	−11	4.6
Left amygdala	−18	−10	−14	4.5
Left dorsal cingulate (BA 24)	−17	−15	43	4.4
Right orbitofrontal cortex (BA 11)	26	26	−18	3.9
Right basal forebrain region	8	1	−11	3.9
Right inferior temporal gyrus (BA 20)	39	−17	−20	3.5
Cerebellar vermis	−3	−58	−27	3.3

Stereotactic coordinates (mm) identify the location of the rCBF maxima according to the atlas of Talairach and Tournoux (1988). *x*, medial-lateral position relative to the midline (+ = right hemisphere); *y*, anterior-posterior position relative to the anterior commissure (+ = anterior); and *z*, inferior-superior position relative to the intercommissural plane (+ = superior). Only areas reaching statistical significance are displayed in each table.

QHCL significantly activated the left amygdala (see Fig. 1*A*). The focus fell within a medial band of the amygdala, covering a large extent of its anterior-posterior axis. Region of interest analysis (ROI) of the left amygdala (4.5-mm sphere placed on the left amygdala maxima) revealed that seven of nine subjects showed a >4% rCBF increase in the left amygdala. In contrast, only two subjects showed >4% rCBF increase in a similarly placed ROI in the right amygdala, and both of these subjects also had large rCBF increases in the left amygdala. These data indicate that the amygdala activation by QHCL is largely lateralized to the left amygdala.

Several additional areas demonstrated rCBF increases in the comparison of tasting QHCL and tasting water. In the frontal lobe, a focus localized to the right posterior orbitofrontal cortex (OFC) (see Fig. 1*B*). In monkeys, a secondary gustatory region localizes to the caudolateral OFC. The focus in the current study lies close to the cytoarchitectually homologous region in humans (Small et al. 1999). More anteriorly, a strong focus arose in an extreme anterior portion of the inferior frontal lobe (see Fig. 1*B*). This anterior focus encompassed a large volume of cortex and included portions of the anterior orbital gyrus and the frontomarginal gyrus.

A significant focus localized to the basal forebrain region (see Fig. 1*B*). The location of this activation appears most consistent with the nucleus accumbens or the underlying olfactory tubercle. However, the complex and heterogeneous topography of the basal forebrain makes a precise labeling of this focus difficult. The emergence of a basal forebrain focus conforms with previous data implicating this area in the hedonic processing of gustatory stimuli (Small et al. 1997a; Wilson and Rolls 1990). Significant activations also surfaced in the dorsal anterior cingulate, cerebellar vermis, and the right inferior temporal gyrus.

Finally, bilateral rCBF increases also emerged in the dorsal anterior insula/opercular region during the QHCL condition relative to water but failed to reach the pixel-wise criteria for statistical significance ($x = 30, y = 18, z = 16, Z$ score = 3.2, $P < 0.001$ and $x = -28, y = 18, z = 16, Z$ score = 3.1, $P < 0.001$). These foci fall near the anterior boundary of the primary gustatory area in humans (Small et al. 1999). Because some data suggest that handedness may affect the laterality of insular responses to tastes (Faurion et al. 1999), we performed a post hoc analysis excluding the left-handed subjects in the study. This exclusion raised the left hemisphere response slightly (to Z score = 3.3), while slightly lowering the right hemisphere response (to Z score = 2.9).

SUCROSE–WATER. Tasting sucrose produced a far more restricted pattern of activation than tasting QHCL. The strongest focus in the contrast between the sucrose and water conditions localized to the right OFC ($x = 21, y = 21, z = -16, Z$ score = 3.3) at coordinates that resembled the focus in the contrast between QHCL and water. No other foci reached statistical significance in this comparison. Only a weak focus emerged in the left anterior insula in this condition ($x = 33, y = 14, z = 11, Z$ score = 2.3). A larger magnitude focus emerged in the

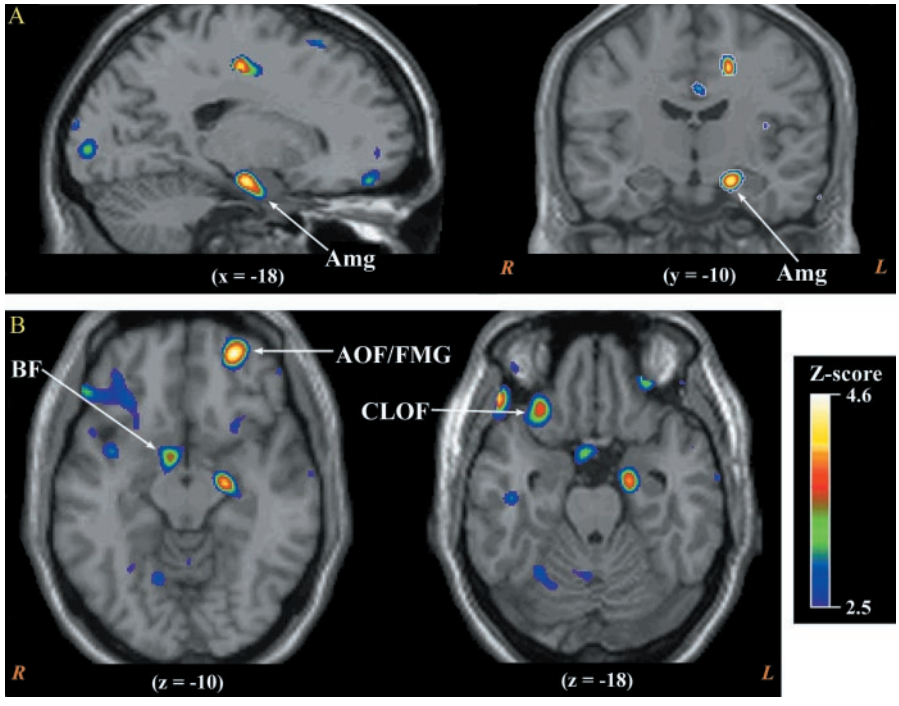

FIG. 1. *A* and *B*: sagittal ($x = -18$) and coronal ($y = -10$) slices displaying the left amygdala activation (denoted Amg) in the contrast between tasting aversive quinine hydrochloride (QHCL) and tasting water. The dorsal cingulate activation can be seen in both slices, and a nonsignificant increase can also be seen in the left insula in the coronal slice. *B*: transverse slices through $z = -10$ and $z = -18$, displaying activations in the left anterior orbitofrontal/frontomarginal gyrus region (AOF/FMG), the right caudolateral orbitofrontal cortex (CLOF) and basal forebrain (BF). The superior and inferior aspects of the amygdala activation can also be seen along the medial wall of the left temporal lobe. In all figures, regional cerebral blood flow (rCBF) data were thresholded to only display activations exceeding a Z score of 2.5 ($P < 0.005$). Effect size magnitude is color coded according to the color bar appearing at the right of the figure. In all figures, activations are displayed on a Talairach-warped, high-resolution, T-1 weighted, magnetic resonance imaging (MRI) template.

TABLE 2. *Locations of increased rCBF in the contrast between the conditions of tasting QHCL and tasting sucrose*

Area	*x*	*y*	*z*	*Z* Score
QHCL > sucrose				
Right medial orbital gyrus (BA 11)	19	35	−20	4.1
Left cerebellum	−15	−49	−14	3.6
Left anterior orbital gyrus (BA 11)	−24	44	−16	3.5
Left frontomarginal gyrus (BA 10)	−24	55	−11	3.5
Left cerebellum	−19	−67	−16	3.5
Right entorhinal cortex (BA 28)	19	−6	−27	3.4
Left cerebellum	−30	−69	−22	3.3
Sucrose > QHCL				
Left inferior parietal lobule (BA 40)	−39	−37	36	−3.4
Left posterior temporal white matter	−33	−33	4	−3.4
Brain stem	6	−24	−27	−3.4
Left inferior temporal gyrus (BA 38)	−35	−6	−27	−3.3
Left inferior temporal gyrus (BA 38)	−33	−8	−22	−3.3

Negative *Z* scores indicate greater activity in the sucrose condition relative to the quinine hydrochloride (QHCL) condition.

left anterior insula ($x = 42, y = 19, z = 7, Z$ score = 2.9) when the analysis was restricted to right-handers, but this still failed to reach more rigorous levels of statistical significance.

QHCL VERSUS SUCROSE. Table 2 displays the peak maxima in the contrast between the QHCL and sucrose conditions for the seven subjects completing both conditions. QHCL caused significantly greater rCBF in the left anterior OFC/frontomarginal gyrus region (see Fig. 2). The frontomarginal gyrus in the right hemisphere also showed increased rCBF relative to the sucrose condition but fell below the threshold for statistical significance ($x = -35, y = 55, z = -7, Z$ score = 3.0, $P = 0.001$). Significant foci also emerged in the right anterior entorhinal cortex, the cerebellum, and the right OFC. A small magnitude focus also arose in the left amygdala but failed to reach full statistical significance ($x = -19, y = -1, z = -20, Z$ score = 2.1, $P < 0.05$).

Although sucrose caused little activation relative to water, several areas demonstrated significantly greater activity during the sucrose condition than during the QHCL condition. These included the inferior parietal lobule, the brain stem, an area of posterior temporal white matter, and part of the left anterior inferior temporal gyrus (see *bottom* of Table 2 for details).

ANALYSIS OF AMYGDALA ACTIVITY DURING SAPID STIMULATION RELATIVE TO RESTING BASELINE. In the preceding analyses, water served as a control condition for both QHCL and sucrose. However, amygdala responses to water have not been widely explored in humans. It is possible that water activates the amygdala to an extent that obscures activations induced by nonaversive gustatory stimuli (Frey and Petrides 1999). To test this possibility, we examined rCBF within the amygdala during the tasting of water relative to the ECR condition. To further characterize amygdala activity during the processing of sapid stimuli, we similarly contrasted the sucrose and QHCL conditions with the ECR condition. All subjects meeting inclusion criteria for the QHCL or sucrose condition were included in this analysis, providing 12 subjects for the water condition, 10 for the sucrose condition, and 9 for the QHCL condition.

As can be seen in Table 3, QHCL, sucrose, and water all caused at least moderate activations within the amgydaloid region relative to a resting baseline. During the water condition, two discrete but nonsignificant foci (both involving <4 pixels with $P < 0.005$) localized to the right amygdala and left perimagydalar regions. Slightly greater magnitude foci arose in both amygdalae in the contrast of the sucrose and the ECR conditions (see Fig. 3*A*). However, neither sucrose nor water produced as robust activation as QHCL. QHCL-induced substantial activation of the left amygdala relative to ECR (see Fig. 3*B*). This activation extended along the anterior-posterior axis of the amygdala, with the peak falling more anterior than the maxima in the contrast of QHCL and water. In contrast, no discrete foci mapped to the right amygdala (although foci did emerge significantly lateral to the right amygdala at $x = 37, y = -10, z = -22$, and $x = 37, y = -1, z = -16$).

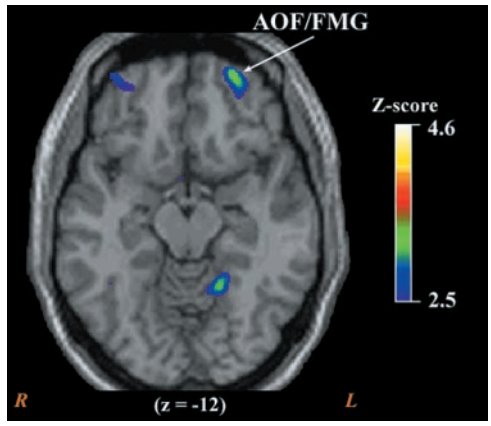

FIG. 2. Transverse slice through $z = -12$ displaying the left anterior orbitofrontal/frontomarginal (AOF/FMG) activation in the contrast of QHCL and sucrose. Note the magnitude of the focus is reduced relative to the contrast with water. More posteriorly, the superior edge of the left cerebellar focus can be seen. In this slice, it cannot be clearly seen whether the focus lies in the cerebellum or posterior-medial temporal lobe. However, the peak (which falls on a more inferior slice) appears more clearly within the cerebellum.

ANALYSIS OF INSULAR ACTIVITY DURING SAPID STIMULATION RELATIVE TO RESTING BASELINE. Although the insula was not a primary focus of this investigation, its relatively weak level of activation in contrasts of tastants with water is striking. No statistically significant peaks arose in the insula during the contrast of sucrose and water, and restriction of analyses to right handed subjects produced only a slight increase in the magnitude of these foci. We have previously proposed that cortical activations induced by water may obscure rCBF caused by gustatory stimuli (Zald and Pardo 2000b). If so, contrasts of sapid gustatory stimuli should induce far larger rCBF increases in the insula when they are compared with a resting scan. However, it must be noted that activations relative to ECR do not exclusively reflect gustation but include the effects of somatosensory and thermosensory features of the stimuli and motoric responses such as tongue movement and swallowing (Zald and Pardo 1999).

TABLE 3. *Amygdala activations during tasting QHCL, sucrose, and water relative to resting with eyes closed (ECR)*

Condition	*x*	*y*	*z*	*Z* Score
QHCL	−20	−1	−18	4.7
Sucrose	−19	−4	−11	3.0
	26	−1	−16	3.0
Water	−15	−1	−9	2.5*
	24	1	−11	2.5

* The peak lies in the periamygdaloid cortex.

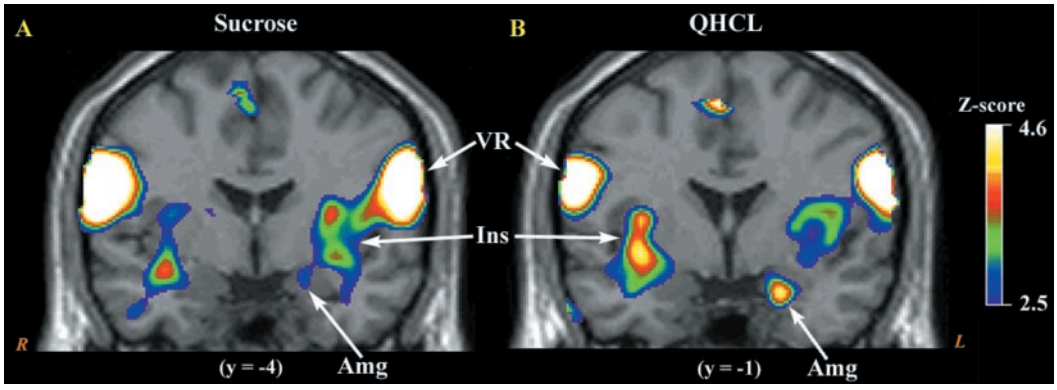

FIG. 3. rCBF increases in the left amygdala (denoted Amg) during tasting sucrose (*A*) and QHCL (*B*) relative to eyes closed resting. A modest rCBF increase localizes to the left amygdala in the sucrose condition with a more substantial increase arising in the QHCL condition. A small modest intensity peak also localizes to the right amygdala 3 mm anterior to this slice (it appears as a lateral extension to the ventral insular/claustrum activation that can be seen impinging on the lateral amygdala in the coronal section displayed in this figure). Note the dramatic insular (Ins) and ventral Rolandic (VR) activations that were not present (or were only weakly present) when sucrose and QHCL were contrasted with water. Insular and ventral Rolandic foci occur bilaterally (although arrows only point to them unilaterally to avoid cluttering the figure). Also note that distinct dorsal and ventral insular regions emerge in these contrasts.

Table 4 displays the location of insular peaks in the contrast of QHCL and sucrose with ECR scans. As can be seen from Table 4 and Fig. 3, robust and widespread insular activity emerges in these contrasts. This includes both anterior dorsal regions consistent with primary gustatory cortex as well as more ventral areas not traditionally associated with gustation.

DISCUSSION

Amygdala

The present study demonstrates the ability of aversively experienced, concentrated QHCL to activate the human amygdala. This finding converges with our previous report of amygdala activation during gustatory stimulation with aversive saline (Zald et al. 1998a) and a similar finding by O'Doherty et al. (2001) using fMRI.

Several caveats are necessary in interpreting the amygdala response (as well as responses in other brain regions). First, these data cannot tease apart the extent to which the perception of bitterness or the aversive nature of the stimulus led to the response. Single-cell recordings in nonhuman primates suggest that the patterns of firing in the amygdala are too nonspecific to provide much information about taste quality (bitter vs. sour for instance) but instead primarily reflect the emotional valence of the stimulus (Scott et al. 1993). Previous observations of amygdala activation during exposure to a different aversive taste (saline) clearly indicate that other aversive tastes are capable of activating the amygdala. Nevertheless, it remains possible that the taste quality of QHCL produced stimulus specific influences on the magnitude or laterality of the responses.

TABLE 4. *Insular/opercular activations during tasting QHCL and sucrose relative to ECR*

Area	*x*	*y*	*z*	*Z* Score
QHCL				
Right (Mid) insula	33	−4	−4	4.5
Right dorsal insula	35	−6	14	4.2
Left dorsal insula	−33	−4	9	4.2
Right ventral anterior insula	30	14	−7	3.9
Left (Mid) insula	−37	−4	2	3.6
Sucrose				
Right dorsal insula	30	−10	14	4.3
Left (Mid) insula	−33	1	−4	4.2
Right ventral insula	33	−1	−9	4.0

Second, the 0.02 M concentration of QHCL used in this study represents a higher concentration than is used in most electrophysiological and psychoperceptual studies. This high level was selected to ensure strong hedonic responses. However, subjects' difficulty describing the taste suggests that they perceived the QHCL as more than just bitter. Furthermore, this concentration may have produced reflexive muscular responses. Future studies using lower concentrations of QHCL (which are strictly perceived as bitter, but are likely to produce lower emotional responses) will be necessary to determine the extent to which bitter perception per se activates the amygdala.

The high concentration of QHCL may have also influenced amygdala activity because subjects experienced the taste as novel. Studies in animals suggest that the amygdala plays a role in the neophobic response to novel gustatory stimuli (Borsini and Rolls 1984; Nachman and Ashe 1974). Small and colleagues (Small et al. 1997a) observed activation of the left amygdala in humans tasting novel flavors. However, the novel flavors in that study were experienced as unpleasant, making it difficult to distinguish whether the novelty, the unpleasantness or both factors contributed to the amygdala response. Novelty does not appear to be an essential requirement for tastes to activate the amygdala because aversive saline (which is easily recognized) activates the right amygdala (Zald et al. 1998a). Nevertheless, novelty may affect the laterality of the amygdala response, with greater left amygdala activity developing when tastants are experienced as novel.

The lateralized activation pattern is of interest given the complex influences of right anterior-medial temporal lesions on taste processing. Lesions of the right anterior-medial temporal lobe have been reported to enhance intensity ratings of QHCL (Small et al. 2001), while leaving ratings of sucrose unchanged (Small et al. 2001) and impairing detection thresholds of citric acid (Small et al. 1997). These findings suggest that the right anterior-medial temporal lobe exerts taste-specific

influences on gustatory perception. Perhaps, lesions of the right amygdala (or other right anterior-medial temporal structures) produce a release from inhibition in the processing of QHCL, thus allowing a left amygdala response to dominate. In support of this possibility, Henkin et al. (1977) reported elevated recognition thresholds for bitter tasting urea in patients with left temporal lesions. However, this finding still awaits replication and Small et al. (2001) did not observe significant alterations in intensity perception of QHCL in patients with left temporal lesions relative to normals or patients with right temporal lesions. There may also exist lateralization differences in the hedonic coding of specific tastes, distinct from any changes in perception of the sensory features of tastes, but this possibility has never been formally tested. In summary, although the neuroimaging and lesion literature converge in identifying lateralized effects in the anterior-medial temporal processing of specific tastes, a full understanding of these effects remains elusive.

The failure of sucrose to activate the amygdala relative to water converges with our previous finding that tasting chocolate does not activate the amygdala more than tasting water (Zald et al. 1998a). However, the present result must be interpreted in light of sucrose's and water's ability to cause small to moderate rCBF increases in both amygdalae relative to a resting baseline. The magnitude of these foci in the present study appears highly consistent with that observed by Frey and Petrides (1999) in contrasts of sucrose and water with nonsapid tongue stimulation.

Perhaps the lack of distinction between sucrose and water reflects the fact that sucrose and water both are capable of acting as positive appetitive reinforcers. Electrophysiological studies clearly indicate that as many, or more, cells in the amygdala respond to sweet tastes than bitter tastes (Nishijo et al. 1998; Scott et al. 1993). Indeed, O'Doherty et al. (2001) observed at least moderate activations in the left amygdala in five of seven subjects in an fMRI study contrasting glucose with artificial saliva. Thus the reinforcing features of water, or other features of water (such as its different osmolarity from saliva), appear to cause a bias against observing activations by sweet solutions. An alternative hypothesis involves the possibility that sucrose and water produce more brief transient responses to sucrose and water, which is more detectable with fMRI than PET, whereas aversive tastes produce more sustained activity during an extended exposure making it more amenable to detection with PET. Future fMRI studies examining the temporal pattern of responses to different tastes, water and artificial saliva will hopefully illuminate this issue.

An additional limitation of the present study involves the difference in the psychoperceptual ratings of QHCL and sucrose. Sucrose was neither experienced as intensely nor with the same level of hedonic strength as QHCL. Unfortunately, it is difficult to simultaneously match sucrose and QHCL in terms of both perceptual intensity and hedonic strength. Indeed, attempting to increase the concentration of sucrose may lower or even reduce its hedonic strength. Even at the concentration utilized in this study, we excluded several subjects because they perceived the sucrose as unpleasantly sweet. Moreover, increasing the positive hedonic strength of the stimulus probably would not dramatically increase the activation induced by sucrose. Chocolate (which is experienced as far more pleasant than sucrose) also fails to significantly activate the amygdala relative to water (Zald et al. 1998a). Moreover, a recent analysis of brain responses during successive exposures to chocolate showed no indication of a correlation between ratings of pleasantness and amygdala activity (Small et al. 2001).

Inferior frontal cortex

The left frontomarginal gyrus in the inferior frontal pole and the adjacent anterior OFC showed the largest activation in the contrast between the QHCL and water conditions. It is, of course, difficult to determine the extent to which this response to QHCL represents a stimulus-specific effect, or an effect of valence, intensity, or novelty. Nevertheless, both areas showed a preferential activation relative to sucrose. We have previously observed a similar left anterior OFC area in contrasts between saline and water ($x = -24$, $y = 41$, $z = -7$), and saline and chocolate ($x = -21$, $y = 39$, $z = -7$) (Zald et al. 1998a), suggesting a preferential response to aversive relative to pleasant tastes in this region. It must be noted, however, that O'Doherty et al. (2001) have observed an anterior OFC area that responds to glucose relative to artificial saliva. The location of the area observed by O'Doherty and colleagues appears relatively close to the area observed to selectively respond to aversive QHCL and saline. This suggests that at least portions of the anterior OFC are not exclusively responsive to aversive tastes. In contrast, the frontomarginal response observed in the present study appears more unique because it has not previously emerged in other studies of taste. Interestingly, activity in the frontomarginal gyrus correlates with perceptual ratings of aversiveness during exposure to unpleasant odorants (Zald et al. 1998b). Thus its emergence in the present study suggests that the frontomarginal gyrus may be commonly activated during exposure to highly aversive chemical stimuli.

Rolls and colleagues (Baylis et al. 1995; Rolls et al. 1990) refer to the caudolateral OFC as secondary gustatory cortex based on single-cell recordings and its afferents from the insula. The posterior OFC foci in the present study lie close to (although slightly medial to) a region in humans that shares similar anatomical features to this caudolateral gustatory region in monkeys (Small et al. 1999). This likely represents an earlier stage of processing than the anterior areas showing responses to tastes. Indeed, the anterior regions largely lack direct gustatory and amygdala projections but likely receive information from these areas secondary to more posterior OFC areas (Carmichael and Price 1996; Zald and Kim 2001).

Insula

The dorsal insula and neighboring operculum are frequently described as primary gustatory cortex on the basis of anatomical, electrophsyiological, and lesion evidence (Norgren 1990). Numerous neuroimaging studies support their role in gustatory processing (Faurion et al. 1999; Francis et al. 1999; Frey and Petrides 1999; Kinomura et al. 1994; Small et al. 1997b, 1999; Zald et al. 1998a). However, the responses in this area in the present study were relatively weak during contrasts with water, only reaching high magnitudes in contrasts with a nonsapid condition. These data support the argument that gustatory responses in the insula may be partially obscured by activity induced by water (Zald and Pardo 2000b) with substantially

larger insular responses emerging during contrasts with nonsapid stimuli (Frey and Petrides 1999). The human insula contains topographically large, nongustatory, intraoral representations (Zald and Pardo 1999). Indeed, electrophysiological data in monkeys indicate that a far greater proportion of insular cells respond to nongustatory stimulation than gustatory stimulation (Scott et al. 1986; Smith-Swintowsky et al. 1991). Thus the overall effect of taste coding may appear small relative to the effect of other intraoral coding in this region. Furthermore the ability to observe taste-induced rCBF changes in the insula may be limited by the high proportion of cells with inhibitory responses to tastes (Katz et al. 2000). Given these factors, it is actually quite impressive how successful neuroimaging studies have been at teasing out gustatory responses.

Conclusion

In summary, tasting aversive QHCL activates the amygdala and several additional cortical regions. The data also indicate that intraoral stimulation with nonaversive sapid stimuli can produce modest activations within the amygdala.

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