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Individual Differences in Prefrontal Cortex Activity during Perception of Bitter Taste Using fNIRS Methodology

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Abstract

Although bitter taste has a crucial role in nutrition by preventing the ingestion of toxic foods, there are few studies on bitter taste neuroimaging. To identify cortical areas involved in bitter taste perception and to determine if individual differences in taste sensitivity to 6-n-propylthiouracil (PROP) are represented in the brain by different cortical activation patterns, we examined 48 healthy volunteers using functional near-infrared spectroscopy. Participants rated the perceived intensity of filter paper disks impregnated with PROP and NaCl during the imaging procedure and were then classified as PROP tasters and nontasters. We monitored cortical activity in both the anterior and posterior regions of the dorsolateral prefrontal cortex (DLPFC) and in the ventrolateral prefrontal cortex (VLPFC). No activity was detected in the anterior DLPFC in any of the participants. However, during the administration of PROP, significant cortical activation was detected in the more posterior regions of the left DLPFC and in the left and right VLPFC but only in PROP tasters. PROP nontasters showed no cortical activity in these areas. These data suggest that the prefrontal cortex is involved in the conscious perception of the bitter taste of PROP and that the pattern of activity is consistent with individual differences in the ability to taste this compound. Thus, the PROP phenotype is associated with fundamental differences in cortical taste processing.

Key words: cortical activation, dorsolateral prefrontal cortex (DLPFC), 6-n-propylthiouracil (PROP), optical topography, ventrolateral prefrontal cortex (VLPFC)

Introduction

Plants produce a large number of structurally diverse, toxic bitter substances as protection against predation by insects and grazing animals (Kingsbury 1964; Fahey et al. 2001). Because many of these compounds are also bitter tasting to humans, bitter taste may serve as an important warning signal against the ingestion of poisonous and harmful substances in the diet (see Behrens and Meyerhof 2006).

Humans possess ~25 types of bitter taste receptors (Shi and Zhang 2009). Considerable progress has been made in linking specific classes of bitter compounds to their respective receptor molecules, but the vast number of bitter compounds that individuals might encounter in natural foods far exceeds the number of available receptors (Meyerhof et al. 2010). Functional expression studies suggest that some of these receptors are broadly tuned, whereas others recognize only a few compounds with common structural properties (Meyerhof et al. 2010). One of the most studied receptors is TAS2R38, which recognizes the thiourea (N-C = S) moiety of glucosinolates, a large family of antithyroid compounds that are widely distributed in plants of the *Brassica* sp. (Bufe et al. 2005). Individual differences in bitterness perception of *Brassica* vegetables such as broccoli, Brussels sprouts, and many dark leafy greens are well known (Drewnowski and Gomez-Carneros 2000). Genetic variation in the ability to

recognize and avoid overconsumption of dietary goitrogens might have conferred important survival advantages to human beings adapted to living in different local environments (Wooding et al. 2004).

In 1932, A.L. Fox first discovered that ~30% of individuals were taste blind to the synthetic compound phenylthiocarbamide (PTC) (Fox 1932), and a large number of subsequent studies confirmed the presence of "taster" and "nontaster" groups in different populations around the globe (Guo and Reed 2001). Positional cloning of the gene that encodes TAS2R38 revealed the presence of 3 single nucleotide polymorphisms in the protein at positions A49P, A262V, and V296I giving rise to PAV (Proline-Alanine-Valine; the taster variant) and AVI (Alanine-Valine-Isoleucine; the nontaster variant) (Kim et al. 2003). Nontasters of PTC and the related compound, 6-n-propylthiouracil (PROP), carry 2 copies of the insensitive allele (AVI/AVI), whereas tasters carry one or 2 copies of the sensitive allele (PAV/PAV or PAV/AVI) (Kim et al. 2003; Bufe et al. 2005). PAV/PAV individuals perceive extreme bitterness from PTC and PROP at suprathreshold concentrations and are typically referred to as "super-tasters" (Duffy et al. 2004; Bufe et al. 2005). The occurrence of other variants (AAV and PVI) is rare (Wooding et al. 2004) and is associated with intermediate sensitivities to these compounds (Timpson et al. 2007). Although genotype explains the majority (55–80%) of the variation in this trait (Kim et al. 2003; Prodi et al. 2005; Tepper et al. 2008), other factors such as age, gender, and taste bud density also contribute to the perceived bitterness of PTC/PROP (Prutkin et al. 2000; Essick et al. 2003; Hayes et al. 2008).

Sandell and Breslin (2006) reported that PAV/PAV individuals gave higher bitterness ratings to gluosinolate-containing vegetables but not to non-glucosinolate–containing vegetables, suggesting that the TAS2R38 receptor may be specific for this class of compounds in humans. However, numerous others studies suggest that the influence of PROP status on the perception of bitterness may be more generalized. Those who perceive PROP as more bitter give higher intensity ratings and lower hedonic ratings to a wide range of bitter-tasting foods and beverages, including fruits and vegetables, whole grains, and alcohol (reviewed in Tepper et al. 2008, 2009). Thus, obtaining a better understanding of this phenotype could shed additional light on why individuals select or avoid bitter-tasting foods that could impact their nutritional status and overall health.

Although genetic approaches are rapidly yielding new information about our sense of taste, there are fewer empirical data on bitter taste neuroimaging. Until now, the major human brain mapping techniques used for taste-related functions have been positron emission tomography (Kinomura et al. 1994), functional magnetic resonance imaging (fMRI) (Kringelbach et al. 2004; Ogawa et al. 2005), electroencephalography (Kobal 1985), and magnetoencephalography (Kobayakawa et al. 1996). Although these methods have made important contributions to the mapping of cerebral taste processing, they often require participants to be restricted in their movements, and this feature does not allow them to perform taste tests in a natural manner.

In this work, we used functional near-infrared spectroscopy (fNIRS) to explore cortical areas involved in bitter taste perception. fNIRS is an optical method based on the properties of hemoglobin to absorb near-infrared light. In fNIRS, the subject simply wears a set of optodes (emitters/detectors of near-infrared light) on his/her head; the method does not require the subject to be confined to a scanner (Strangman et al. 2002). fNIRS has been used to study cortical activity associated with visual and motor function (Kuboyama et al. 2004; Kashou et al. 2007), language (Watanabe et al. 1998), emotions (Herrmann and Ehlis 2003), cognitive conflict (Schroeter et al. 2004) and, more recently, taste function. fNIRS detects signals from lateral cortical surfaces, but it is not able to measure the activity of deeper gustatory areas, such as the insular and orbitalfrontal cortices, where the near-infrared light cannot reach. Nevertheless, recent studies suggest that the lateral prefrontal cortex is a crucial area for mediating higher cognitive processing of taste and other food-related behaviors (Kringelbach et al. 2004). Using fNIRS, Okamoto, Matsumani, et al. (2006) reported that the dorsolateral prefrontal cortex (DLPFC) was involved in memory formation of taste. The same research group also showed activation in the bilateral ventrolateral prefrontal cortex (VLPFC) when the task required participants to simply taste the stimuli, without memory processing (Okamoto et al. 2009).

The major aim of this research was to determine if taste responsiveness to PROP is differentially represented in the DLPFC and/or the VLPFC cortex of individuals classified as PROP tasters and nontasters. The study of brain areas involved in PROP sensory perception may add new insights into bitter perception as well as food acceptance.

Materials and methods

Participants

A total of 48 healthy adults were eligible to participate in the study. The participants were assigned to 2 subsamples that were monitored for different cortical areas (for details, see below "fNIRS recording" section). The first 24 participants recruited (13 females) were 25–40 years of age (32.0 mean ± 4.9 standard deviation). The next 24 participants (15 females) were 21–35 years of age (29.1 ± 4.2). Participants were excluded if they used medications that interfere with taste or if they had a history of neurological, sensory, or psychiatric disorders. They were all right handed as assessed by the Edinburgh Questionnaire, a standardized handedness inventory (Oldfield 1971). Written informed consent was obtained from each participant after full procedural and technical

explanation of the experiment. The ethics committee of the ''Burlo Garofolo'' Hospital in Trieste, where all the testing was conducted, granted permission.

PROP tasting protocol

PROP taste intensity was assessed using a standardized filter paper method developed and validated previously (Zhao et al. 2003). The method involves each subject tasting 2 filter paper disks; one is impregnated with 50 mmol/L 6-n-2-propylthiouracil (#P3755, Sigma-Aldrich) and the other is impregnated with 1.0 mol/L NaCl (VWR Scientific). Both compounds were presented in suprathreshold concentrations. The subject places each disk on the tip of the tongue until it is wet and rates the intensity of the taste using the labeled magnitude scale (LMS) (Green et al. 1996). The LMS is a 100-mm scale with label descriptors placed at quasi-logarithmic intervals along length of the scale from ''barely detectable'' to ''strongest imaginable.'' Instructions for using the scale were identical to those described by Green et al. (1996). Accordingly, strongest imaginable was defined as the most intense oral sensation an individual has experienced in everyday life. The ballot was translated into Italian and was used in a previous study (Tepper et al. 2008).

Based on previous work (Zhao et al. 2003), we used LMS numerical cutoff scores of >67 and <13 to identify supertasters and nontasters, respectively. Individuals who rated the intensity of PROP between these values were identified as medium tasters. NaCl ratings were used as a reference standard for classifying those who gave a borderline rating to PROP. This comparison is based on the rationale that super-tasters give higher ratings to PROP than NaCl, medium tasters give the 2 compounds similar ratings, and nontasters give higher ratings to NaCl than to PROP (Tepper et al. 2001).

fNIRS recording

Optical topography (OT) is a multichannel fNIRS system that allows the detection and localization of cortical activation by monitoring changes in cerebral blood flow (CBF) and cerebral blood volume (Maki et al. 1995). This spectroscopic method operates in the near-infrared region of the electromagnetic spectrum (Jöbsis 1977). Using optical fibers (or optodes) that emit and detect near-infrared light, fNIRS measures the localization of brain regional activation by directly detecting variations in optical signal. These signals reflect changes in the concentration of oxy-hemoglobin (HbO_2) and deoxy-hemoglobin (Hbb) due to their different specific spectra in the near-infrared range (between 650 and 1000 nm). The area between each adjacent emitter–detector fiber pair is called a channel (Ch). Variation in total hemoglobin concentration can be indirectly derived by summing changes in HbO_2 and Hbb. Typically, HbO_2 concentration changes are considered an estimate of CBF, and total hemoglobin concentration changes are considered an estimate of cerebral blood volume; there is more ambiguity in interpreting Hbb variations (Meek 2002).

The Hitachi ETG-100 OT system was used (Hitachi Medical Corporation). It records simultaneously from 24 Chs on the cortex, measuring vascular changes from the surface to a depth of 2 cm below the scalp (McCormick et al. 1992; Watanabe et al. 1998) that is sufficient to ensure cerebral penetration (Hebden and Delpy 1997; Villringer and Chance 1997). The ETG-100 emits infrared light at 2 wavelengths, 780 and 830 nm, and their intensities are modulated at different frequencies ranging from 1 to 6.5 kHz. The reflected light is sampled once every 100 ms and separated into 2 modulated signals, one for each wavelength, by a number of corresponding lock-in amplifiers. After analog-to-digital conversion, optical data are transferred to a computer. The modified Beer–Lambert law (Villringer and Chance 1997) is used to estimate changes in the concentration of HbO_2, Hbb, and total hemoglobin in response to stimulation. The unit of measurement is mM*mm, which is the product of the hemoglobin concentration expressed in millimolar and the optical path length expressed in millimeters (Maki et al. 1995).

Considering the previous literature on cortical neuroimaging of taste processing, we divided our participants in 2 subsamples basing on the region of interest we planned to monitor. For the first subsample, we referred to the fNIRS study of Okamoto, Matsunami, et al. (2006) and detected cortical activity in the more anterior and central portions of the DLPFC. We used a plastic holder (probe) to keep the optical fibers in place. The probe contained 16 optodes of 1 mm in diameter arranged in a 4×4 pattern. Eight of the optodes were emitters and 8 were detectors. They were placed 3 cm a part, providing 24 Chs. In order to have a reliable craniocerebral structural correlation, probes were placed using the international 10–20 electroencephalography system of electrode placement (Jasper 1958). Ch 23 was placed over the reference point, Fpz (Figure 1), maintaining the row of Chs also containing Chs 22 and 24 on the virtual line joining Fp1 and Fp2. For the localization of cortical areas covered by each Ch without using a 3D digitizer and to probabilistically register the fNIRS data to the standard Montreal Neurological Institute (MNI) space, we referred to the methods developed by Tsuzuki et al. (2007) and by Singh and colleagues (2005). The probabilistic localization for each ch is given in Table 1; cerebral labeling is based on the brain atlas constructed by Tzourio-Mazoyer et al. (2002).

For the second subsample, the regions of interest were the VLPFC and a more posterior portion of the DLPFC. These locations were based on studies by Kringelbach et al. (2004) and Okamoto, Dan, et al. (2006). In this case, we used 2 plastic holders to keep the optical fibers in place, each one containing 9 optodes of 1 mm in diameter arranged in a 3×3 pattern. Within each probe, 5 of the optodes were emitters and 4 were detectors. They were placed 3 cm a part,

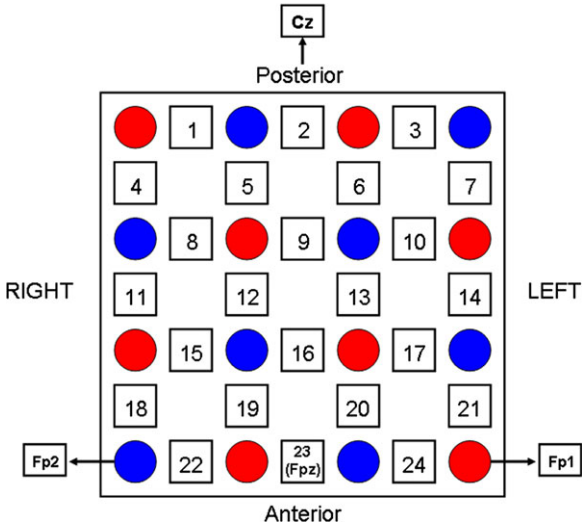

Figure 1 Schematic representation of how the probe was placed over the frontal lobes in the first subsample. Circles represent near-infrared emitters and detectors and squares chs. Chs and their respective positions on the head are shown. Also indicated are the reference points of the international 10–20 electroencephalography system of electrode placement, which were used for reliable probe positioning.

providing 12 Chs per hemisphere. One probe was placed over the right frontal lobe and the other over the left frontal lobe, keeping Ch 11 over F7 on the left side and Ch 24 over F8 on the right. In both cases, the inferior Ch row of probes was maintained on the virtual line joining T3 with F7 and T4 with F8 (Figure 2). We used the same methods and brain atlas cited above in order to localize and label Ch positions on the cerebral cortex. Table 2 shows the probabilistic localization for each Ch in this subsample. Our regions of interest in this subsample were cortical areas covered by Chs 9, 10, 11, and 12 on the left hemisphere and Chs 20, 21, 23, and 24 on the right hemisphere, namely left and right VLPFC/peri-Sylvian regions, respectively, which have recently been implicated in taste function (Okamoto et al. 2009). The rest of the Chs covered the left and right DLPFC. These locations were selected to exclude from our data activity in the motor areas during the task (Okamoto, Dan, et al. 2006).

The probes were positioned to ensure that the fibers touched the subject's scalp. The OT device automatically detected whether the contact was adequate to measure emerging photons.

Procedure

In order to measure participants' naïve reactions to PROP, OT data were acquired during the PROP tasting procedure. All testing was carried out in the morning, and participants were not allowed to eat or drink (except for water) for 1 h prior to the session. Participants were tested in a quiet room under soft white lights, sitting in front of an experimenter who passed them the stimuli (glasses of 100 mL of spring water and the filter paper disks impregnated with PROP and NaCl). They were given the following instructions: "I will hand you some objects, in particular 2 glasses of spring water and 2 different filter paper disks. Each time I give you a glass of water, you will drink it in one gulp. When I give you each filter paper disk, you will place it in the mouth, allow it to become wet with saliva and then remove it. You must pay attention to the intensity of taste that you perceive because after removing each disk from your mouth I will ask you to rate your taste perception by drawing a mark on the scale."

After giving instructions, the probes were placed in the correct position and the experiment was started. We administered the filter paper disk impregnated with PROP first and NaCl disk second. This was done so that responses to

Table 1 Probabilistic estimation of chs' cortical localization in MNI space and corresponding cortical labeling in the first subsample

Channel	MNI coordinates estimation (mm)				Anatomical label
	x	*y*	*z*	Standard deviation	
Ch 1	24	24	60	7.4	R frontal sup gyrus
Ch 2	2	28	59	7.9	R/L* frontal sup medial gyrus
Ch 3	–22	23	61	7.7	L frontal sup gyrus
Ch 4	37	30	50	6.9	R frontal middle gyrus
Ch 5	13	40	54	6.9	R frontal sup gyrus
Ch 6	–12	41	54	6.8	L frontal sup gyrus
Ch 7	–35	30	50	7.2	L frontal middle gyrus
Ch 8	25	47	44	6.1	R frontal sup gyrus
Ch 9	2	50	44	6.7	R/L* frontal sup medial gyrus
Ch 10	–22	47	43	6.7	L frontal sup gyrus
Ch 11	38	52	29	5.6	R frontal middle gyrus
Ch 12	14	61	34	6.1	R frontal sup gyrus
Ch 13	–12	60	35	5.7	L frontal sup gyrus
Ch 14	–36	51	29	6.4	L frontal middle gyrus
Ch 15	26	65	19	4.9	R frontal sup gyrus
Ch 16	3	66	22	7.1	R/L* frontal sup medial gyrus
Ch 17	–24	65	20	5.6	L frontal sup gyrus
Ch 18	39	63	4	4.9	R frontal middle gyrus
Ch 19	15	71	9	4.5	R frontal sup gyrus
Ch 20	–13	72	9	4.9	L frontal sup gyrus
Ch 21	–37	63	4	5.1	L frontal middle gyrus
Ch 22	28	68	–5	4.1	R frontal sup orb gyrus
Ch 23	3	68	–4	5.4	R/L* frontal mid orb gyrus
Ch 24	–24	68	–5	4.2	L frontal sup orb gyrus

R, right; L, left; Orb, orbital; sup, superior. *Chs located on the nasion–inion virtual line.

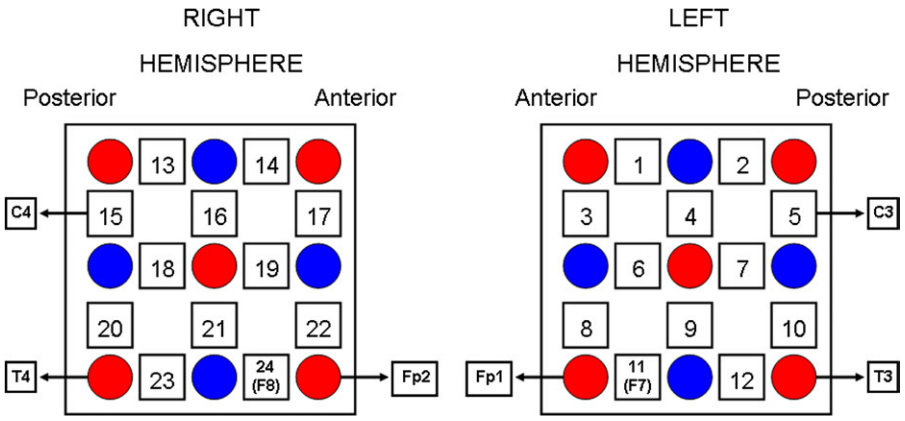

Figure 2 Schematic representation of how the probes were placed over the left and right lateral frontal lobes in the second subsample. Circles represent near-infrared emitters and detectors and squares chs. Chs numbers and their respective positions on the head are shown. Also indicated are the reference points of the international 10–20 EEG system of electrode placement, which were used for reliable probes positioning.

PROP would not be influenced by a previous stimulus in the mouth. All participants performed the tasting trials with the right hand. Each subject was tested according to the following timeline. First, the subject rinsed his/her mouth with 100 mL of spring water. After 35 s, the OT recording began. Recording consisted of 5 s for baseline data acquisition followed by 30 s during which time the subject placed the PROP disk on the tip of the tongue until it was wet and then removed it. This was followed by another 5 s for baseline data acquisition, when the subject rated PROP taste intensity using the LMS. The task ended with a second rinsing of the mouth with 100 mL of spring water and then a break of 3 min. The same procedure was followed for the NaCl rating. In normal adults, the cerebral vascular response takes about 10–12 s after stimulation to be completed (Wobst et al. 2001; Meek 2002). In our experiments, this period of time was extended to 30 s to allow completion of the tasting task and the return of cerebral hemoglobin to baseline levels. This time period produced satisfactory hemodynamic responses in all participants.

Data analysis

Our analyses focused on variations in HbO_2, which estimates changes in CBF of activated brain areas (Meek 2002). Components of the HbO_2 signal related to slow fluctuations of CBF and heartbeat noise were removed by bandpass filtering between 0.02 and 1 Hz. In order to prevent movement artifacts, a filter was used to remove detections with rapid changes in HbO_2, (signal variations > 0.1 mM*mm over 2 consecutive samples). All trials in all Chs were used for statistical processing.

For each Ch in each of the 2 conditions (PROP and NaCl), an arbitrary baseline was calculated as the mean relative change in HbO_2 in the 5 s before the onset of each stimulus and in the 5 s between the 30th and the 35th second after its onset. A period of 30 s was enough to reestablish the baseline. The hemodynamic response was calculated as the mean change in HbO_2 over the 30 s after the onset of the stimulus.

To identify the activated Chs, we performed one-tailed paired t tests comparing the baseline and the hemodynamic response for each Ch and each tasting condition. Separate analyses were conducted for taster and nontaster groups.

In order to control Type I error for multiple testing, we used a false discovery rate (FDR) approach (Genovese et al. 2002; Singh and Dan 2006) that controls the proportion of false positives among the Chs that are significantly detected. We selected a q value of 0.05, so that there were no more than 5% false positives (on average) in the number of Chs emerging with significant contrasts.

When appropriate, we used a nonparametric Mann–Whitney U test to compare heomodynamic responses between taster and nontaster groups across all 24 Chs to detect possible regional differences in cortical activity associated with PROP or NaCl taste intensity. A nonparametric test was preferred over its parametric counterpart (e.g., analysis of variance) because of the limited sample size and a predicted unbalanced distribution of participants between taster and nontaster groups. Statistical analyses were conducted using SPSS version 13.0 for Windows (SPSS Inc.).

In order to have an estimate of the Ch projections on a rendered brain, we used the "Spatial registration of NIRS chs location" function of the NIRS-SPM version 3.0 software, which is a SPM5 and MATLAB based software package freely downloadable from the Web site http://bisp.kaist.ac.kr/NIRS-SPM (Jang et al. 2009; Ye et al. 2009). Using the "Stand alone" option (without using MRI images), we produced a spatial representation of the Ch locations on a rendered brain, referring to the MNI coordinates reported in Tables 1 and 2.

Table 2 Probabilistic estimation of chs' cortical localization in MNI space and corresponding cortical labeling in the second subsample

Channel	MNI coordinates estimation (mm)				Anatomical label
	x	*y*	*z*	Standard deviation	
Left hemisphere					
Ch 1	–46	19	46	7.6	Frontal middle gyrus
Ch 2	–59	–9	45	7.6	Postcentral gyrus
Ch 3	–45	36	34	6.9	Frontal middle gyrus
Ch 4	–58	10	34	7.6	Precentral gyrus
Ch 5	–66	–18	32	7.4	Supramarginal gyrus
Ch 6	–55	27	21	6.4	Frontal inferior tri gyrus
Ch 7	–65	–1	21	6.0	Postcentral gyrus
Ch 8	–51	43	7	6.1	Frontal inferior tri gyrus
Ch 9	–60	16	9	6.9	Frontal inferior oper gyrus
Ch 10	–67	–14	1	6.3	Temporal sup gyrus
Ch 11	–54	34	–5	4.9	Frontal inferior orb gyrus
Ch 12	–65	–0	–14	4.6	Temporal middle gyrus
Right hemisphere					
Ch 13	60	–10	45	7.5	Postcentral gyrus
Ch 14	48	18	47	7.4	Frontal middle gyrus
Ch 15	68	–19	32	7.0	Supramarginal gyrus
Ch 16	61	8	34	6.9	Precentral gyrus
Ch 17	47	35	35	6.3	Frontal middle gyrus
Ch 18	66	–3	21	6.1	Postcentral gyrus
Ch 19	57	26	22	6.1	Frontal inferior tri gyrus
Ch 20	70	–15	3	4.6	Temporal sup gyrus
Ch 21	62	13	10	5.8	Frontal inferior oper gyrus
Ch 22	53	41	8	5.1	Frontal inferior tri gyrus
Ch 23	65	–1	–11	4.7	Temporal middle gyrus
Ch 24	56	32	–4	4.7	Frontal inferior orb gyrus

orb, orbital; sup, superior; tri, triangular.

Results

In the first subsample, we identified 4 nontasters (2 females), 18 medium tasters (9 females), and 2 super-tasters (1 female). In this subsample, no Ch survived the FDR threshold in any group of tasters, either during the administration of PROP or during the administration of NaCl. Thus, no activation of the anterior DLPFC was found with these stimuli (Supplementary Tables 1–4).

In the second subsample, we identified 7 nontasters (5 females), 17 medium tasters (10 females), and no super-tasters. In this subsample, 5 Chs survived the FDR threshold in the group of tasters ($n = 17$) during the administration of PROP (the largest P value below the threshold was $P = 0.010$), indicating significant cortical activation in the left DLPFC (Chs 7–8), in the left VLPFC (Chs 9–12) and in the right VLPFC (Ch 21) (Figure 3 and Supplementary Table 5). During the administration of NaCl, 5 Chs survived the FDR threshold (the largest P value below the threshold was $P = 0.007$), including Chs 16–22 located above the right DLPFC and Chs 21, 23, and 24 located above the right VLPFC (Figure 4 and Supplementary Table 6).

In the group of nontasters ($n = 7$), no Ch survived the FDR threshold during the administration of PROP, indicating the absence of identifiable prefrontal cortical activity in response to this stimulus (Supplementary Table 7). On the contrary, 2 Chs survived the FDR threshold during the administration of NaCl (the largest P value below the threshold was $P = 0.003$), one located above the left DLPFC (Ch 8) and one above the left VLPFC (Ch 11). Another 2 Chs did not pass the FDR threshold, but they showed a high statistical significance as well. They were Ch 22 ($P = 0.008$) and Ch 23 ($P = 0.0095$), both located above the right VLPFC (Figure 5 and Supplementary Table 8). Table 3 summarizes these results.

When we compared hemodynamic responses associated with the perception of PROP between taster and nontaster groups across all 24 Chs, we found 2 significant differences, both indicating higher cortical activity in tasters: Ch 12 ($Z = –2.45$; $P = 0.014$), located above the left VLPFC, and Ch 21 ($Z = –2.00$; $P = 0.045$), located above the right VLPFC (Figures 6 and 7). We did not found any significant difference in activation between groups following the NaCl stimulus (Figure 6).

Discussion

We studied prefrontal cortical activity associated with the taste of PROP, a bitter compound, whose perception is inherited as Mendelian trait (Blakeslee and Fox 1932; Kalmus 1958). Using fNIRS, we demonstrated activation in the DLPFC and VLPFC in participants shown to be differentially sensitive to the bitterness of PROP using a valid and reliable screening test (Zhao et al. 2003). In the first subsample, in which we monitored cortical activity in the more anterior and central portions of the DLPFC, as previously done by Okamoto, Matsunami, et al. (2006), we found no significant cortical activation or differences between tasters and nontasters. In the second subsample, the group of tasters showed significant activity in the left DLPFC, the left VLPFC, and the right VLPFC when tasting PROP. However, the nontaster group showed no significant activity in the prefrontal cortex. A post hoc comparison of the 2 groups for differences in regional cortical activation found that tasters showed higher activity in both left and right VLPFC following stimulation with PROP. Together, these data suggest that the prefrontal cortex is involved in the conscious perception of the bitter taste of PROP, and the pattern of activity is

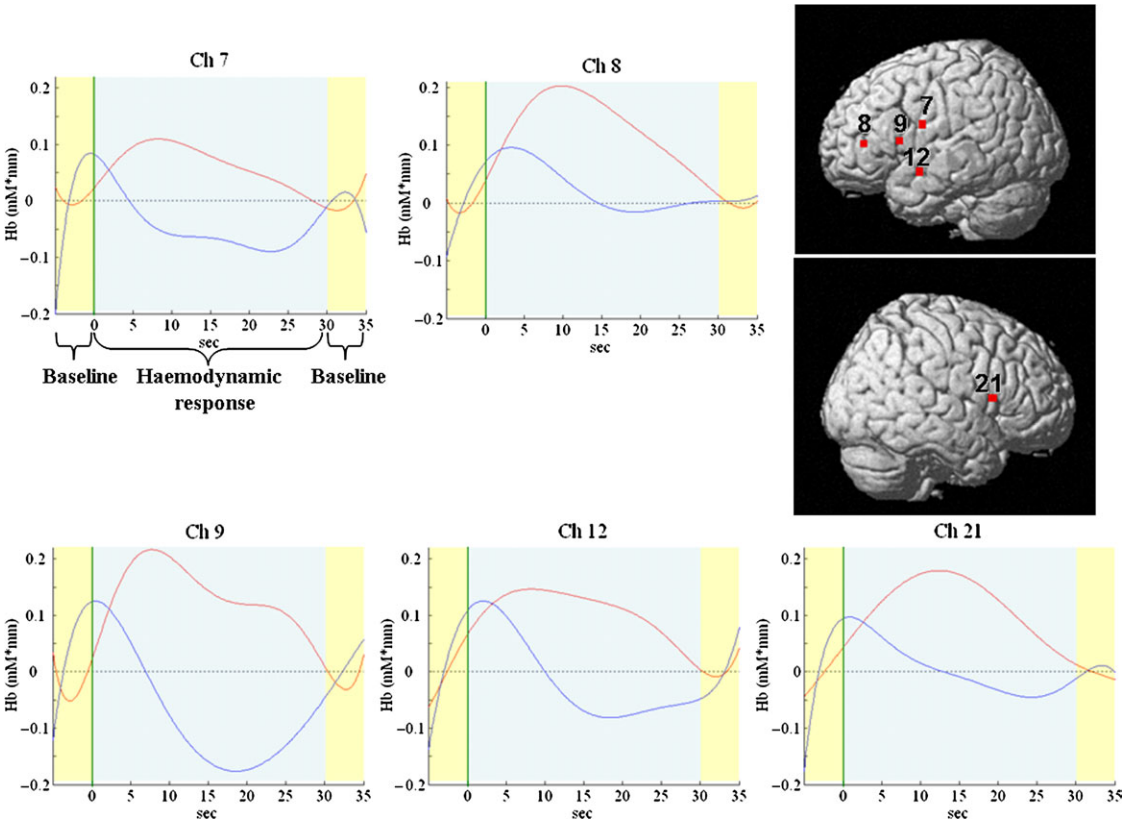

Figure 3 Time courses of variation in concentration of haemoglobin (Hb) in mM*mm during PROP administration in the taster group, HbO_2 significantly increased, showing cortical activation, in the left DLPFC (ch7–ch8), in the left VLPFC (ch9–ch12), and right VLPFC (ch21). (Curves indicate changes in HbO_2 and Hbb concentration. The vertical line shows when the filter paper disk touched the tip of the tongue). The right side of the figure shows the Chs cortical location on a rendered brain.

consistent with individual differences in the ability to taste this compound.

Neuroimaging techniques, such as positron emission tomography, fMRI, and magnetoencephalography, have been used to define the cerebral processes involved in taste sensation. These studies have shown that taste signals originating from the periphery project to the primary gustatory cortex, which includes the parietal operculum and superior posterior insula (Kobayakawa et al. 1996; Mizoguchi et al. 2002; Ogawa et al. 2005). Further evidence suggests that the orbitofrontal cortex functions as a secondary sensory area, where gustatory and oral somatosensory signals integrate into flavor (Small et al. 1997; de Araujo et al. 2003). fMRI data also demonstrate a crucial role for the lateral prefrontal cortex in processing the reward value of taste (Kringelbach et al. 2003) and flavor (Small et al. 2004). In particular, Kringelbach et al. (2004) showed that the left DLPFC plays a role in taste processing but is not associated with its affective or rewarding features, which are processed by the right DLPFC (Kringelbach et al. 2003). Other authors used fNIRS to study memory encoding of taste stimuli and found bilateral activity in the VLPFC, either with (Okamoto, Dan, et al. 2006; Okamoto, Matsunami, et al. 2006) or without a memory task (Okamoto et al. 2009). This activation was found in participants tasting mixtures of compounds or flavors that were neutral with respect to intensity and pleasantness.

Until now, no studies have examined the role of the cerebral cortex in PTC/PROP processing, with the except of Scott et al. (1998), who observed activity in the insula of the macaque following the administration of different bitter compounds, including PTC and PROP. Our results demonstrate recruitment of the lateral prefrontal cortex in the perception of the bitter taste of PROP. Specifically, only PROP tasters, who were able to perceive the bitterness of this stimulus, exhibited bilateral symmetry in taste representation of this compound in the VLPFC. Our taster group also showed activation of the left DLPFC to PROP stimulation. We did not ask the participants to rate the pleasantness of the stimuli. However, tasters (in contrast to super-tasters) typically rate the PROP-impregnated disks used here as moderately bitter (Zhao et al. 2003), and they anecdotally report that the taste sensation is not objectionable to them (Tepper BJ, unpublished data). If tasters experience PROP as a hedonically neutral, this could explain why we observed activity only in the left DLPFC that is associated with perceptual processing of the stimulus but not the right DLPFC that

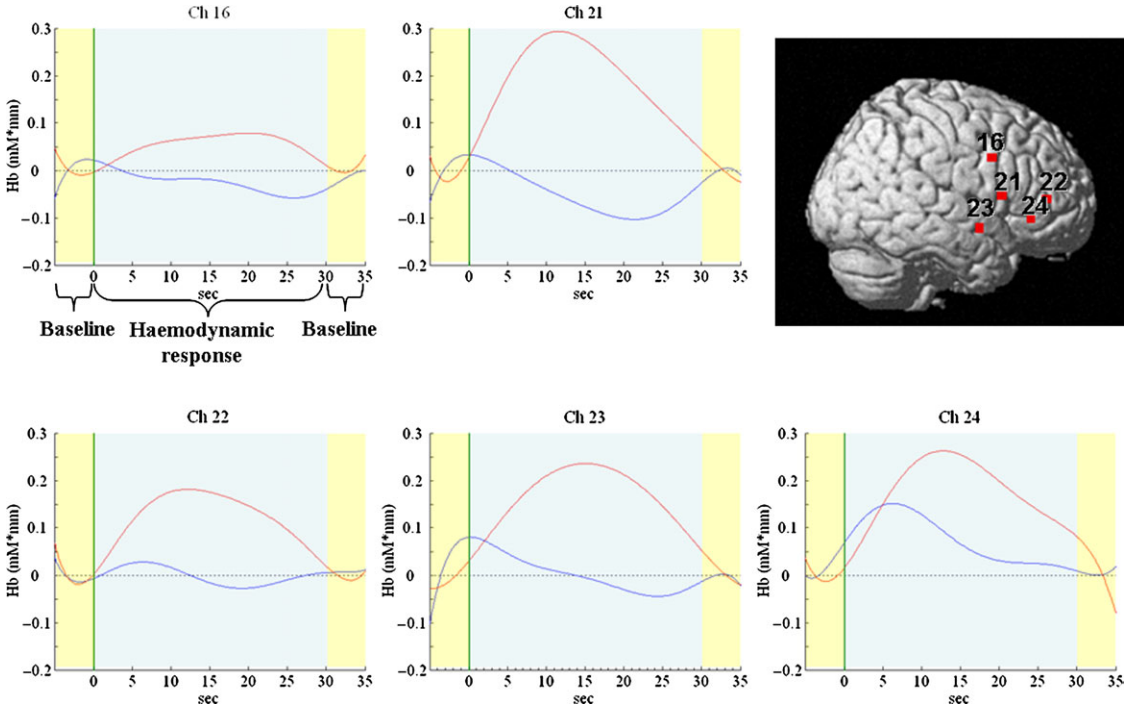

Figure 4 Time courses of variation in concentration of haemoglobin (Hb) in the unit of mM*mm during NaCl administration in the taster group. HbO_2 significantly increased, showing a cortical activation, in ch16 and ch22 located in the right DLPFC, and in ch21-ch23-ch24 located in the right VLPFC. (Curves indicate changes in HbO_2 and Hbb concentration. The vertical line shows when the filter paper disk touched the tip of the tongue). On the right, they are reported Chs cortical location on a rendered brain.

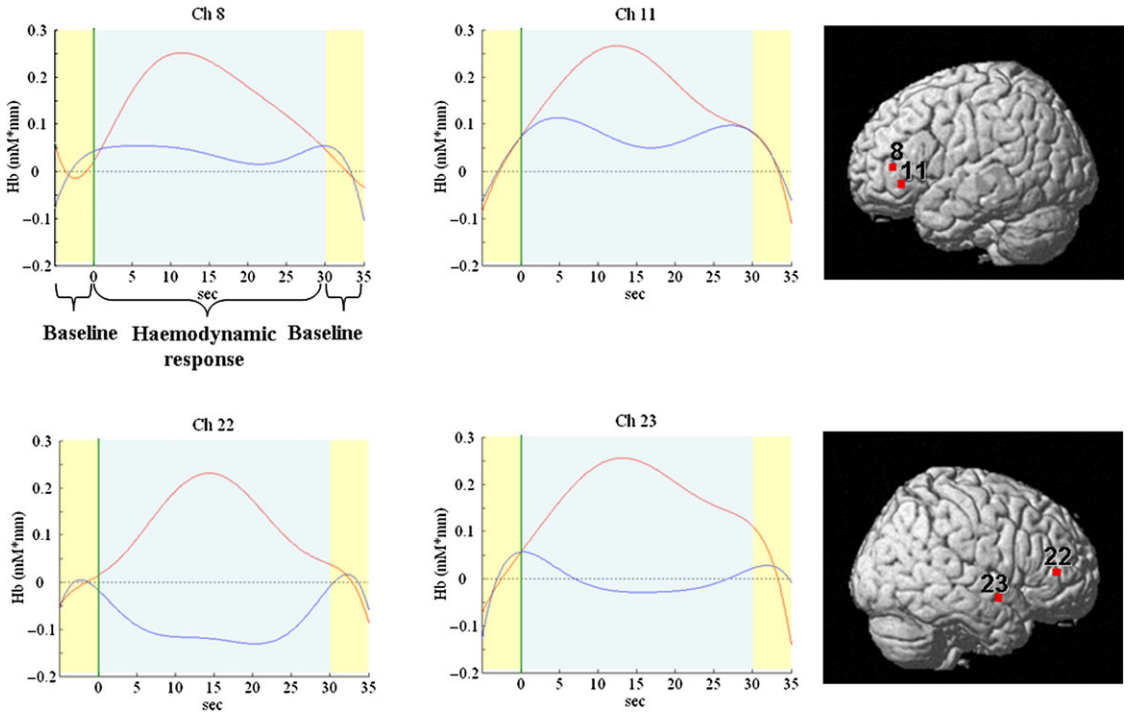

Figure 5 Time courses of variation in concentration of haemoglobin (Hb) in the unit of mM*mm detected in nontaster group during NaCl administration, HbO_2 significantly increased, showing a cortical activation, in the left DLPFC (ch8), in the left VLPFC (ch11), in the right DLPFC (ch22), and in the right VLPFC (ch23). (Curves indicate changes in HbO_2 and Hbb concentration. The vertical line shows when the filter paper disk touched the tip of the tongue). On the right, they are reported Chs cortical location on a rendered brain.

Table 3 Summary of the results

Group	Compound	Right VLPFC	Right DLPFC	Left VLPFC	Left DLPFC
Taster	Prop	Ch 21 (t_{16} = –3.45; P = 0.0015)	None	Ch 9 (t_{16} = –3.32; P = 0.002)	Ch 7 (t_{16} = –2.86; P = 0.0055)
				Ch 12 (t_{16} = –2.62; P = 0.0095)	Ch 8 (t_{16} = –5.25; P < 0.001)
	NaCl	Ch 21 (t_{16} = –3.42; P = 0.002)	Ch 16 (t_{16} = –3.29; P = 0.0025)	None	None
		Ch 23 (t_{16} = –3.52; P = 0.0015)	Ch 22 (t_{16} = –2.76; P = 0.007)		
		Ch 24 (t_{16} = –3.53; P = 0.0015)			
Nontaster	Prop	None	None	None	None
	NaCl	Ch 22 (t_6 = –3.31; P = 0.008)	None	Ch 11 (t_6 = –6.41; P < 0.001)	Ch 8 (t_6 = –4.10; P = 0.003)
		Ch 23 (t_6 = –3.17; P = 0.0095)			

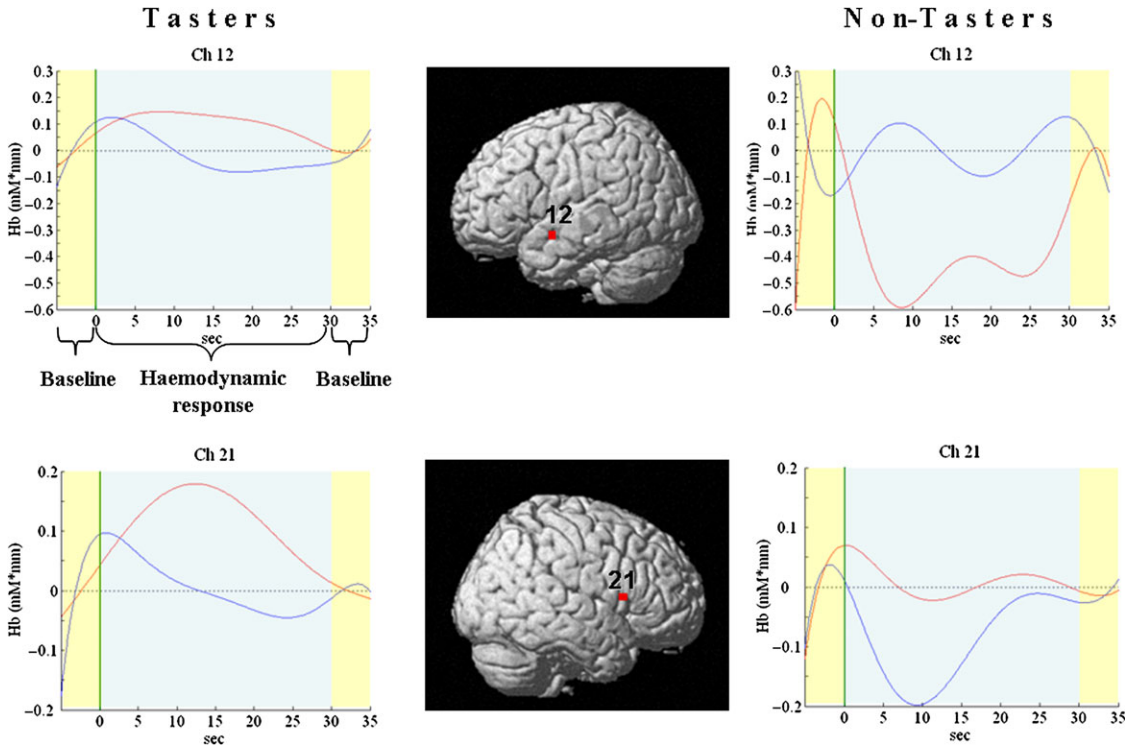

Figure 6 Time courses of variation in concentration of haemoglobin (Hb) in the unit of mM*mm representing the flow of statistical analysis, during PROP administration in the 2 groups. Respect to nontasters, taster group showed significant HbO_2 increases in the left VLPFC (ch12) and in the right VLPFC (ch21). (Curves indicate changes in HbO_2 and Hbb concentration. The vertical line shows when the filter paper disk touched the tip of the tongue). In the centre of the figure, they are reported Chs cortical location on a rendered brain. Although HbO_2 shows a large decrease, the change was not significantly different from baseline (t_6 = 1.42; P = 0.103).

is associated with reward processing (Kringelbach et al. 2004).

We also examined brain activation during NaCl administration in order to compare it with that associated with PROP. As we reported above for PROP, we observed no activation in the first subsample of participants when recording from the more anterior and central portions of DLPFC. However, we found significant activation in the DLPFC and the VLPFC in the second subsample of participants. Specifically, in the taster group, we found activation in the right DLPFC and the right VLPFC. In the nontaster group, cortical activity was found in the left DLPFC and in the left VLPFC, with a tendency for bilateralism in both areas (for details, see Results and Figure 5). In spite of this, the groups did not statistically differ in regional cortical activity associated with NaCl. Thus, nontasters and tasters showed activation to NaCl on different sides of the brain, but the reasons for this difference are presently unclear. More research is needed to further clarify this topic.

This study has some limitations. First, our protocol required participants to place and remove the impregnated

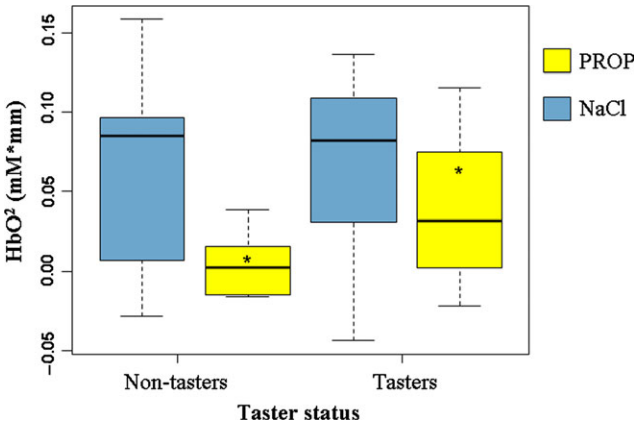

Figure 7 Box plot comparing overall HbO_2 concentration changes between tasters and nontasters in response to PROP or NaCl administration (*P = 0.045, nonparametric Mann–Whitney U test). Black horizontal lines inside the boxes represent the median. Box height is the interquartile range.

disks from the tongue. Thus, it was difficult to separate cortical activation due to physical movement associated with the tasting task from true taste-evoked activation. However, some physical movement occurs in all cortical activation studies making this a limitation in all studies. Second, we did not record at the moment each subject removed the filter paper disk from his/her mouth. These data would have allowed us to measure cortical activation at the time of onset of the stimulus, before the participants rated stimulus intensity. A future study adopting this procedure may clarify the possible influence of short-term memory on the cortical activations we identified. Again referring to the experimental design, the presentation of a neutral control stimulus (i.e., a tasteless filter paper disk) would have allowed us to isolate effects due to tactile sensations from the disk, but tactile effects alone could not have explained the different patterns of responses observed in the 2 groups of participants. Finally, although DNA was not collected in this study, we have previously shown good concordance between TAS2R38 genotypes and PROP status with the paper disk method (Tepper et al. 2008). Future fNIRS studies should include genetic analysis of TAS2R38 haplotypes to confirm the results of the phenotyping procedure.

In conclusion, we detected bilateral representation of the taste of PROP in the VLPFC and, unilaterally, in the left DLPFC. We also found that this neocortical activation is restricted to participants who perceived the bitterness of this compound as assessed by a paper screening test (Zhao et al. 2003); those who did not perceive PROP showed no significant cortical activation when it was administered. These data suggest that perceptual differences in the subjective experience of PROP project to the cortical level and can be reliably measured using fNIRS. Thus, neurophysiological variation in brain activation may be considered a further characteristic of the PROP phenotype.

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/.

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