nature neuroscience

Altering expectancy dampens neural response to aversive taste in primary taste cortex

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The primary taste cortex consists of the insula and operculum. Previous work has indicated that neurons in the primary taste cortex respond solely to sensory input from taste receptors and lingual somatosensory receptors. Using functional magnetic resonance imaging, we show here that expectancy modulates these neural responses in humans. When subjects were led to believe that a highly aversive bitter taste would be less distasteful than it actually was, they reported it to be less aversive than when they had accurate information about the taste and, moreover, the primary taste cortex was less strongly activated. In addition, the activation of the right insula and operculum tracked online ratings of the aversiveness for each taste. Such expectancy-driven modulation of primary sensory cortex may affect perceptions of external events.

The insula and adjacent operculum comprise the primary taste cortex in humans and nonhuman primates. Neurons in the anterior insula and frontal operculum of nonhuman primates respond differently to different tastants[1–6]. Similarly, neuroimaging research with humans indicates that these regions, as well as the posterior insula and parietal operculum, distinguish tastants that differ in hedonic value[7–14], which is consistent with research on humans with brain lesions[15–18] and research using electrical stimulation[19,20]. In nonhuman primates, neuronal response to taste in primary taste cortex is not modulated by motivational state (for example, hunger), suggesting that these insula and operculum regions respond solely to the objective qualities of a tastant[1,2,21,22]. However, expectancies can change both the perceptions of events in other modalities and the neural responses to these events[23,24]. Such findings led us to predict that if subjects believed that a highly aversive tastant would be less aversive than it actually was, not only would they perceive the taste differently but, in addition, their primary taste cortex would activate less strongly.

In this study, we measured subjects' responses using fMRI while they tasted solutions that were highly aversive (1.0 mM quinine), mildly aversive (0.25 mM quinine), neutral (distilled water), mildly pleasant (0.5 M glucose) or highly pleasant (1.2 M glucose). Each solution was preceded by a unique cue: a minus sign for highly aversive, a minus sign with a slash for mildly aversive, a circle for neutral, a plus sign with a slash for mildly pleasant and a plus sign for highly pleasant (**Fig. 1**). After the delivery of each solution, subjects rated how aversive or pleasant the taste was (Methods). After extensive instruction about the cue and taste contingencies and three practice trials for each of the conditions, an additional two conditions were introduced during the fMRI session without explicitly informing the subjects. In one condition, a mildly aversive cue preceded the highly aversive taste. Thus, we could compare activation induced by the identical highly aversive tastant following accurate versus misleading cues (Methods). We predicted that subjects would rate the highly aversive bitter taste following the misleading cue as less aversive than the same taste following the accurate cue. We also tested whether the primary taste cortex—insula and operculum—would activate less strongly in response to the highly aversive bitter taste following the misleading cue than in response to the same taste following the accurate cue. For the sake of comparison, we included the analogous misleading pleasant condition (that is, a mildly pleasant cue that preceded a highly pleasant taste).

In addition to replicating previous reports that bitter taste activates the insula and operculum areas that constitute primary taste cortex[6–9], we found that a simple manipulation of expectancy modulated neural activity in the primary taste cortex in response to the bitter taste. The highly aversive (bitter) taste preceded by a misleading cue, which signaled that the upcoming taste would be mildly aversive, activated bilateral primary taste cortex less strongly than the same taste when it was preceded by a veridical cue that indicated that the upcoming taste would be highly aversive. The misleading cue also changed behavior: subjects rated the subsequent taste as less aversive than the same taste when it followed the veridical cue. Moreover, the right insula and operculum tracked this altered behavior, corresponding to the perceived aversiveness of the taste. The analogous manipulations with pleasant tastes did not reveal any regions within the insula or operculum that significantly differentiated between the taste conditions.

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Received 21 December 2005; accepted 18 January 2006; published online 5 February 2006; doi:10.1038/nn1645

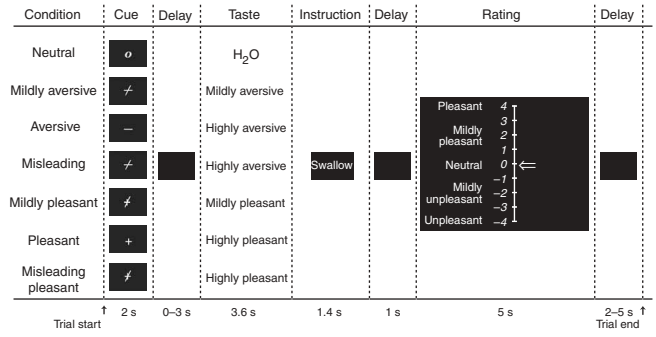

Figure 1 Experimental design. Trial structure for the seven conditions. For the neutral condition shown at the top, a circle cue invariably preceded a neutral taste (distilled water). Aversive taste solutions were delivered in three conditions: for the mildly aversive condition, a minus sign with a slash preceded a mildly aversive taste (0.25 mM quinine); for the aversive condition, a minus sign preceded a highly aversive taste (1.0 mM quinine); and for the misleading condition, the minus sign with a slash preceded the highly aversive taste (1.0 mM quinine). Pleasant taste solutions were delivered in the remaining three conditions: for the mildly pleasant condition, a plus sign with a slash preceded a mildly pleasant taste (0.5 M glucose); for the pleasant condition, a plus sign preceded a highly pleasant taste (1.2 M glucose); and for the misleading pleasant condition, a plus sign with a slash preceded a highly pleasant taste (1.2 M glucose). Subjects viewed identical screens across the conditions, with the exception of the cue, which is illustrated for each condition separately. The only other difference among the seven conditions was the taste delivered, as indicated for each condition.

RESULTS

Aversive tastes

As hypothesized, taste ratings (made immediately after the taste solution was delivered on each trial) indicated that subjects perceived the highly aversive taste preceded by the aversive cue to be more aversive than the same taste preceded by the mildly aversive cue ($t_{42} = -7.94$, $P < 0.001$, $\eta^2 = 0.60$; **Fig. 2**). To assess the orderings of the ratings across the four conditions, we conducted a one-way repeated-measures multivariate analysis of variance (MANOVA) with the condition (neutral, mildly aversive, misleading, aversive) as a within-subjects factor. The condition effect was highly significant ($F_{3,40} = 353.22$, $P < 0.001$, $\eta^2 = 0.96$) and the means for the four conditions were in the predicted order, as confirmed by a linear trend ($F_{1,42} = 985.39$, $P < 0.001$, $\eta^2 = 0.96$). *Post-hoc* analyses indicated that all possible pairwise comparisons were significant ($P < 0.001$).

Of the above 43 subjects who completed the study, 4 had excessive movement and thus their data could not be used. Nine additional subjects were classified as nonresponders based on their rating data for aversive and pleasant tastes following misleading cues (Methods).

Activation in primary taste cortex

To identify the brain areas activated by the highly aversive taste, we compared responses to the extremely bitter and neutral tastes that were preceded by veridical cues using a voxelwise contrast across the whole brain (thresholded at $P < 0.05$, two-tailed, corrected; Methods). The aversive taste activated bilateral insula and operculum more than the neutral taste did (**Fig. 3**). A large middle insula and frontal operculum cluster (2,293 mm^3) and a smaller posterior insula and parietal operculum cluster (443 mm^3) were activated on the right, and homologous regions were activated on the left (869 and 283 mm^3, respectively). The extensive insula and operculum activation observed here is consistent with previous studies[7–10]. Because of baseline differences at cue onset (**Fig. 3**) that were probably due to carry-over from the previous trial, we defined baseline activity as the percent signal change averaged across the first 3 s of each trial and then partialed it out from the percent signal change during the taste period (Methods). The resulting adjusted activation values were used in all subsequent analyses for these insula and operculum regions. Results remained the same for analyses conducted on the taste period activation without partialing out the baseline activity.

To determine whether the activation of the primary taste cortex was attenuated when subjects expected a less aversive taste, we next compared activation for the two conditions with the same highly aversive taste. Each of the four insula and operculum clusters identified by the aversive versus neutral whole-brain contrast (above) were represented in the repeated-measures group (responders, nonresponders) × condition (misleading, aversive) × hemisphere (left, right) × region (middle, posterior) MANOVA. The key group × condition interaction was significant ($F_{1,37} = 4.18$, $P = 0.048$, $\eta^2 = 0.101$). Simple effects analyses revealed an effect of condition for the responders ($F_{1,29} = 16.82$, $P < 0.001$, $\eta^2 = 0.367$) but not for the nonresponders ($F_{1,8} = 0.20$, $P = 0.67$, $\eta^2 = 0.025$). The responders showed less activation to the highly aversive taste when the tastant was preceded by the (misleading) mildly aversive warning symbol than when it was preceded by the (accurate) aversive cue in all four insula and operculum clusters: left middle, $t_{29} = 2.86$, $P = 0.008$, $\eta^2 = 0.220$; left posterior, $t_{29} = 6.97$, $P < 0.001$, $\eta^2 = 0.626$; right middle, $t_{29} = 2.84$, $P = 0.008$, $\eta^2 = 0.217$; and right posterior, $t_{29} = 3.39$, $P = 0.002$, $\eta^2 = 0.283$. This pattern was not observed for the nonresponders in any of the four insula and operculum regions (all $P > 0.41$). There were no other effects involving group, and the only other effect involving condition was a main effect of condition ($F_{1,37} = 5.47$, $P = 0.025$, $\eta^2 = 0.129$): namely, less activation for the misleading condition than the aversive one, as observed for the responders above.

To assess the orderings of the insula and operculum activation across the four conditions for the 30 responders, we conducted a repeated-measures condition (neutral, mildly aversive, misleading, aversive) × hemisphere (left, right) × region (middle, posterior) MANOVA. As observed for the taste ratings above, the condition effect was highly significant ($F_{3,27} = 185.46$, $P < 0.001$, $\eta^2 = 0.954$) and the means for the four conditions were in the predicted order, as confirmed by a linear trend ($F_{1,29} = 433.32$, $P < 0.001$, $\eta^2 = 0.937$). Simple effects analyses

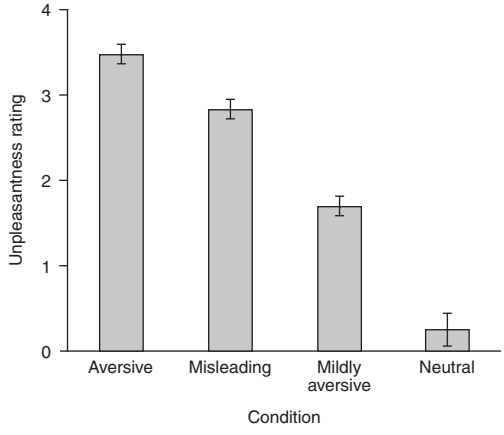

Figure 2 Subjects' online ratings of the unpleasantness (with the sign reversed to make values positive) of the tastes for the aversive, misleading, mildly aversive and neutral conditions while in the scanner ($n = 43$). Error bars are for confidence intervals (± 95%; ref. 49) around the mean after adjusting for between-subject variance[50].

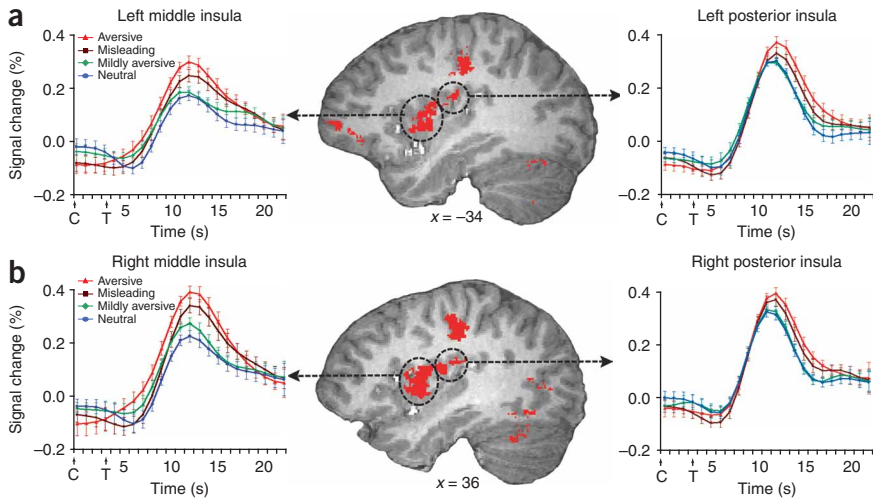

Figure 3 Circled middle insula and frontal operculum clusters and posterior insula and parietal operculum clusters that were more strongly activated by the highly aversive taste than by the neutral taste. (**a**,**b**) The left and right insula and operculum clusters shown here were also more strongly activated by the highly aversive taste following the highly aversive cue than by the same taste following the misleading (mildly aversive) cue. Time courses illustrate mean percent signal change across all time points of the trials in each of the four conditions: highly aversive taste following aversive cue (red), highly aversive taste following misleading mildly aversive cue (brown), mildly aversive taste following mildly aversive cue (green) and neutral taste following neutral cue (blue). The onset of the 2-s cue (C) preceded delivery of the tastant (T) by 2–5 s. Error bars are for confidence intervals (± 95%; ref. 49) around the mean after adjusting for between-subject variance[50].

revealed that the condition effect and linear trend were observed for each of the four insula and operculum regions (all $P < 0.001$). Collapsing across the four regions, all pairwise comparisons among the four conditions were significant ($P < 0.001$).

An analogous MANOVA for the nine nonresponders revealed that the condition effect was significant ($F_{3,6} = 51.55$, $P < 0.001$, $\eta^2 = 0.963$), as was the condition linear trend ($F_{1,8} = 166.52$, $P < 0.001$, $\eta^2 = 0.954$). Simple effects analyses revealed that the condition effect and the linear trend were observed for each of the four insula and operculum regions (all $P < 0.001$). Collapsing across the four regions, the critical comparison between the aversive and misleading conditions was not significant ($F_{1,8} = 0.20$, $P = 0.67$, $\eta^2 = 0.025$), whereas all remaining pairwise comparisons among the four conditions were significant ($P < 0.02$).

Representation of taste ratings in primary taste cortex

To test whether the activation of any areas of the insula or operculum tracked subjects' ratings of the unpleasantness of the highly aversive bitter taste, we conducted a voxelwise regression across the whole brain for all 39 subjects (Methods). More specifically, we calculated difference scores between unpleasantness ratings for the highly aversive taste following the misleading (mildly aversive) cue and for the same taste following the accurate cue; these scores were then regressed on the brain contrast map comparing activation in response to the highly aversive taste in these two conditions.

A region of the right insula and operculum (190 mm^3) spanning the two right insula and operculum clusters identified by the aversive versus neutral whole-brain contrast (above) was correlated with subjects' online ratings of taste unpleasantness. This was the case for the zero-order correlation ($r = 0.66$, $P < 0.001$; **Fig. 4**) and for the partial correlation removing the variance associated with baseline differences for the first 3 s of each trial ($r = 0.62$, $P < 0.001$). The greater the reduction in activation in response to the highly aversive taste following the mildly aversive compared to the aversive cue, the less unpleasant were the ratings for that taste following the mildly aversive compared to the aversive cue. We did not observe this pattern elsewhere in the insula or operculum, except for one smaller cluster (63 mm^3) in the right insula.

Anatomical specificity of insula and operculum activation

Because the anatomical boundaries of the primary taste cortex in humans have not been definitively determined, we conducted ancillary analyses assessing taste responses throughout the insula and operculum. The human primary taste cortex may extend more posteriorly and ventrally[7–10,16,19] than the rostrodorsal insula and frontal operculum locus of the primary taste cortex in nonhuman primates[2]. The results from our principal analyses (above) are consistent with this suggestion. To further address the anatomical specificity of our findings, we examined activation in subregions of the insula and operculum. This was accomplished by deriving anatomical regions of interest (ROIs) corresponding to each of the five principal gyri in the human insula[25]: the anterior short gyrus, middle short gyrus, posterior short gyrus, anterior long gyrus and posterior long gyrus. We also created anatomical ROIs for the frontal operculum, parietal operculum and temporal operculum, and further subdivided the larger frontal operculum and temporal operculum into anterior and posterior sectors[26] (**Supplementary Note** online). Also relevant to anatomical specificity, we processed all our data with minimal spatial smoothing (Methods).

We observed the robust responses to each of the tastes throughout the insula (**Fig. 5** and **Supplementary Fig. 1** online) and operculum (**Fig. 6**). These results suggested that the primary taste cortex is more extensive in humans than in nonhuman primates[8,10]. We conducted a region (anterior short gyrus, middle short gyrus, posterior short gyrus, anterior long gyrus, posterior long gyrus) × hemisphere (left, right) × condition (misleading, aversive) repeated-measures MANOVA on the

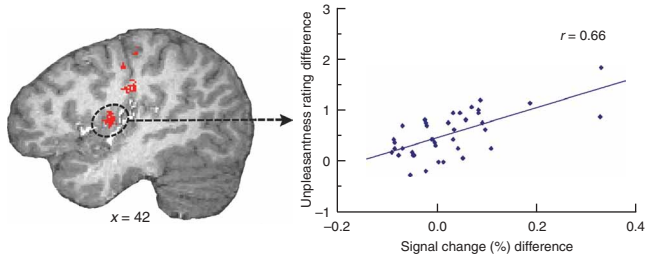

Figure 4 Circled right insula and operculum cluster for which activation was correlated with taste ratings. This taste was rated as less unpleasant when it followed the mildly aversive cue than when it followed the highly aversive cue by individuals who had greater reductions in activation of this area for the highly aversive taste following the mildly aversive cue compared to when the same taste followed the highly aversive cue. The partial correlation removing the variance associated with baseline differences for the first 3 s of each trial was 0.62.

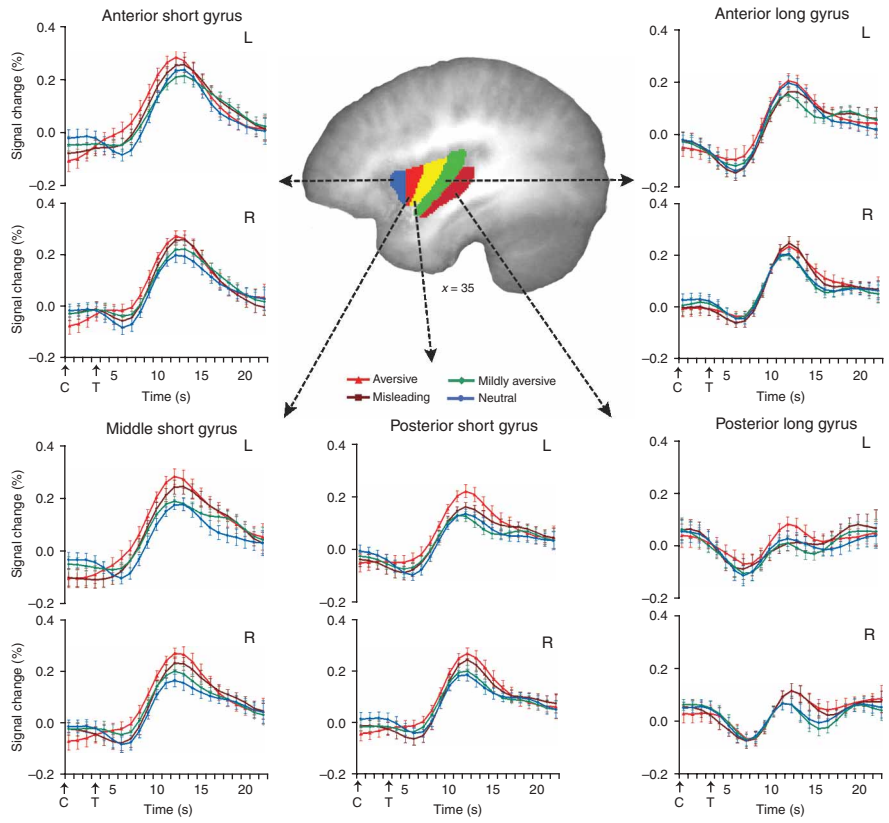

Figure 5 Insula responses to tastes in each of the four conditions. Red, highly aversive taste following aversive cue; brown, highly aversive taste following misleading mildly aversive cue; green, mildly aversive taste following mildly aversive cue; and blue, neutral taste following neutral cue. Time courses illustrate mean percent signal change across all time points of the trials for each of the five primary insula gyri in humans for both the left and right insula: anterior short gyrus, middle short gyrus, posterior short gyrus, anterior long gyrus and posterior long gyrus. The onset of the 2-s cue (C) preceded delivery of the tastant (T) by 2–5 s. Error bars are for confidence intervals (± 95%; ref. 49) around the mean after adjusting for between-subject variance[50]. Similar responses were observed in dorsal and ventral aspects of each gyrus (**Supplementary Fig. 1**). L, left; R, right.

activation in the five insula gyri adjusted for baseline activity averaged across the first 3 s of each trial, as we did for the four insula and operculum regions above. A region × condition interaction ($F_{4,26} = 6.29$, $P = 0.001$, $\eta^2 = 0.492$) was qualified by a region × condition quadratic trend ($F_{1,29} = 15.35$, $P < 0.001$, $\eta^2 = 0.346$; **Fig. 7a**). Follow-up simple effects analyses indicated greater activation on aversive trials than misleading trials for middle short, posterior short and anterior long gyri (all $P < 0.05$). Similarly, a region (anterior frontal operculum, posterior frontal operculum, parietal operculum, anterior temporal operculum, posterior temporal operculum) × hemisphere (left, right) × condition (misleading, aversive) repeated-measures MANOVA on activation in the five operculum sectors revealed a region × condition interaction ($F_{4,26} = 12.81$, $P < 0.001$, $\eta^2 = 0.663$), also qualified by a region × condition quadratic trend ($F_{1,29} = 33.76$, $P < 0.001$, $\eta^2 = 0.538$; **Fig. 7b**). Follow-up simple effects analyses indicated greater activation on aversive trials than on misleading trials for posterior frontal, parietal and anterior temporal opercula (all $P < 0.003$).

Pleasant tastes

Similar to the ratings for the highly aversive taste, subjects rated the highly pleasant taste preceded by the pleasant cue as being more pleasant than the same taste preceded by the mildly pleasant cue ($t_{42} = 8.20$, $P < 0.001$, $\eta^2 = 0.62$; **Supplementary Fig. 2** online). For the one-way, repeated-measures MANOVA with condition (neutral, mildly pleasant, misleading pleasant, pleasant) as a within-subjects factor, the condition effect was highly significant ($F_{3,40} = 187.46$, $P < 0.001$, $\eta^2 = 0.93$), and the means for the four conditions were in the predicted order, as confirmed by a linear trend ($F_{1,42} = 553.28$, $P < 0.001$, $\eta^2 = 0.93$). *Post-hoc* analyses indicated that all possible pairwise comparisons were significant ($P < 0.001$).

We compared the absolute values of taste ratings for the highly aversive condition with those for the highly pleasant condition to determine if there was a difference in the magnitude or intensity of ratings for the two extreme taste conditions. This analysis revealed a significant difference ($t_{42} = 3.84$, $P < 0.001$), indicating that subjects rated the highly aversive taste as being more unpleasant than the highly pleasant taste was pleasant.

A voxelwise contrast across the whole brain (thresholded at $P < 0.05$, two-tailed, corrected; Methods) comparing highly pleasant and neutral tastes that were preceded by veridical cues resulted in no clusters in the insula or operculum. We also conducted a repeated-measures group (responders, nonresponders) × condition (misleading pleasant, pleasant) × hemisphere (left, right) × region (middle, posterior) MANOVA on the above four insula and operculum clusters identified for the voxelwise contrast comparing highly aversive and neutral tastes (**Supplementary Fig. 3** online). Unlike the corresponding aversive conditions, the group × condition effect was not significant ($F_{1,37} = 0.03$, $P = 0.86$, $\eta^2 = 0.001$). Moreover, simple effects analyses indicated that the condition effect was not significant for the responders ($F_{1,29} = 1.22$, $P = 0.28$, $\eta^2 = 0.040$) or nonresponders ($F_{1,8} = 0.77$, $P = 0.41$, $\eta^2 = 0.087$). Finally, analogous to the voxelwise regression conducted for the aversive conditions, we computed difference scores between pleasantness ratings for the highly pleasant taste following the misleading cue and for the same taste following the accurate cue; these scores were then regressed on the brain contrast map comparing activation in response to this taste for these two conditions. No insula or operculum clusters were found.

DISCUSSION

These data show that neural responses to taste in the primary taste cortex are modulated by expectations and not solely by the objective qualities of taste[1,2,21,22]. Insula and operculum responses to a highly aversive taste were reduced when expectancies were manipulated to mislead subjects into believing that the taste would be less unpleasant than it actually was. The findings that expectancies altered both neural activity and perception in response to aversive taste are consistent with research on other modalities[23,24].

Primary taste cortex in humans covers a larger expanse of the insula and operculum[7–10,16,19] than has been reported for nonhuman primates[2–4,21,22]. We found that this area of the brain responded not

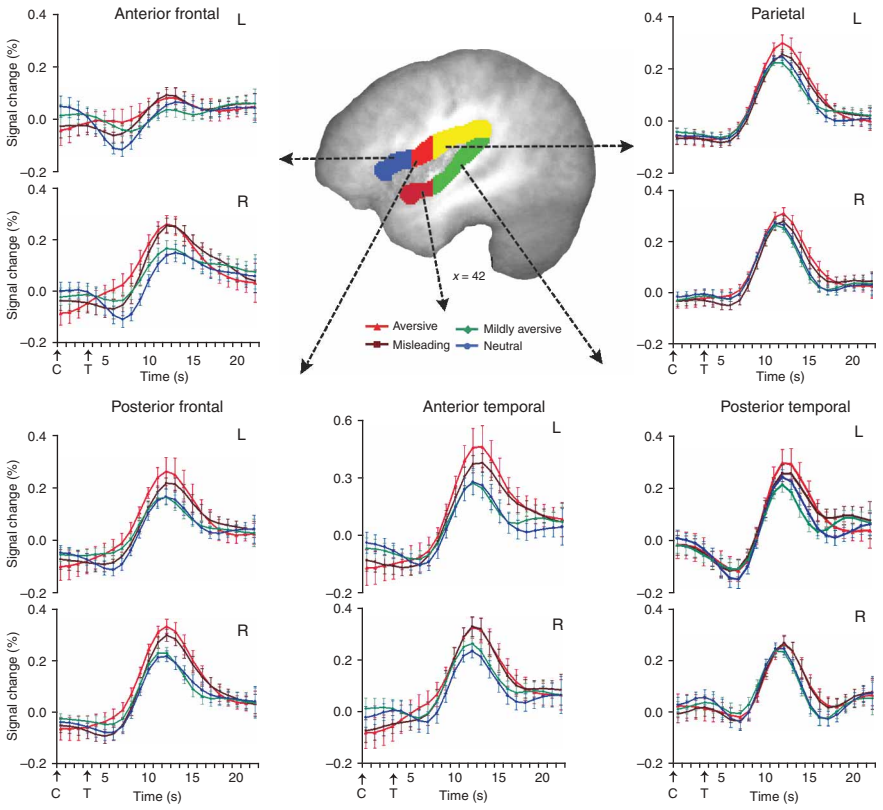

Figure 6 Operculum responses to tastes in each of the four conditions. Red, highly aversive taste following aversive cue; brown, highly aversive taste following misleading mildly aversive cue; green, mildly aversive taste following mildly aversive cue; and blue, neutral taste following neutral cue. Time courses illustrate mean percent signal change across all time points of the trials for the anterior frontal, posterior frontal, anterior temporal, posterior temporal and parietal opercula in humans for both the left and right hemisphere. The onset of the 2-s cue (C) preceded delivery of the tastant (T) by 2–5 s. Error bars are for confidence intervals (± 95%; ref. 49) around the mean after adjusting for between-subject variance[50]. L, left; R, right.

only to aversive tastes but also to water, as reported elsewhere[9,11,13]. Thus, the insula and operculum are activated not only by the aversiveness of the stimuli but also by its gustatory properties. The impact of expectancy on the neural response to the highly aversive taste was not uniform across the primary taste cortex; rather, it was most apparent for the central gyri of the insula—middle short, posterior short and anterior long gyri—and the adjacent posterior frontal, anterior temporal and parietal opercula.

The observed insula activation is also important in the context of other research that implicates the insula in various forms of aversion, including pain[23,27,28], fear conditioning[29,30], facial expressions of disgust[31,32], disgusting odorants[32], unpleasant pictures[33,34] and unpleasant tastes[10,14]. This is consistent with intracerebral recordings in humans and nonhuman primates, which show that only a fraction (approximately 10%) of the insula and operculum neurons in the primary taste cortex respond to tastes[3,4,19–22,35]. Recent work has emphasized a broader role of the insula, especially on the right, in interoceptive awareness relevant to various emotional reactions, aversive and otherwise[28,36–39]. The association with taste ratings observed here for the right insula and operculum is in line with the lateralization of interoceptive awareness.

We believe that the failure to detect significant changes in the insula and operculum as a function of the pleasant taste is probably a consequence of our design in which taste trial types were fully randomized within subjects. The highly aversive taste was rated as significantly more unpleasant than the highly pleasant taste was pleasant. It is possible that carry-over effects from the aversive tastes obscured the response to the pleasant tastes. A plausible alternative explanation is that neural responses for the highly pleasant taste habituated more rapidly than those for the highly aversive taste. However, this was not the case (Methods). Future research examining a potent pleasant taste that is more salient than other tastes delivered in the same experiment[10] is needed to determine conclusively whether expectancies modulate insula and operculum responses to pleasant tastes.

Leading one to believe that something is less aversive results in the downregulation of the insula, which may provide a crucial neural mechanism for the potent impact of expectancy manipulation in psychotherapy, hypnosis and the placebo effect[23,24,40–44]. In the present study, a simple cue altered behavioral and brain responses to a highly aversive taste in the same way that has been reported for an inactive salve in placebo analgesia[23] or for a sugar pill in medication trials[45].

These data document that changing what a person expects can modulate neural activity in gustatory cortex. Moreover, these findings reveal that the primary taste cortex does not solely map the physical characteristics of taste stimuli but also tracks the subjective experience of taste hedonics. Such expectancy-driven alterations in neural activity in primary sensory cortex may help the organism prepare for upcoming sensory input and may be part of the neural machinery that is used to predict changes in the external world. In humans, these neural changes seem to be associated with systematic changes in the subjective hedonic experience of sensory stimuli.

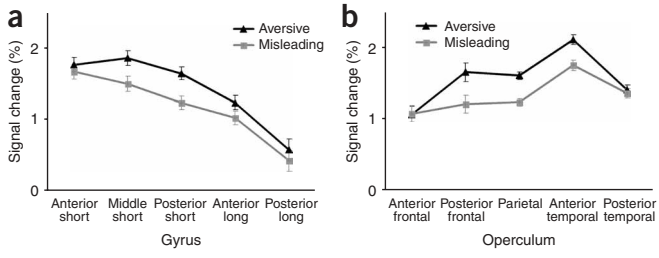

Figure 7 Insula and operculum responses to highly aversive taste following aversive and misleading mildly aversive cues. (**a**,**b**) Mean area under the curve (in percent signal change) across all time points of the trials across both hemispheres for the insula and operculum. Error bars are for confidence intervals (± 95%; ref. 49) around the mean after adjusting for between-subject variance[50].

METHODS

Subjects. Subjects were students at the University of Wisconsin, Madison who were taking the 'Introduction to Psychology' course. Four men and seven women were included in an initial part of the study, in which we determined

optimal taste concentrations. These subjects were 18–21 years of age (mean = 19.18, s.d. = 0.87). We had 54 right-handed adult participants in the fMRI portion of the study, of which 11 found the highly aversive, bitter taste to be too aversive and were unable to complete the experiment. The remaining 43 subjects (24 men and 19 women) were 18–22 years of age (mean = 20.12, s.d. = 0.85). Subjects gave informed consent according to a human subjects protocol of the Health Sciences Institutional Review Board of the University of Wisconsin, Madison and were paid for their participation.

Experimental procedure. Subjects took part in a simulated scan in the shell of an MRI scanner with no magnet in order to acclimate them to the scanning environment. We placed subjects in the mock scanner and fitted them with fiber optic goggles, headphones and a vacuum pillow to help immobilize the head. We then played an audio recording of an actual MRI scanner. Next, subjects viewed instructions that showed each of the cues and were told that '+' corresponded to 'pleasant', '≠' to 'mildly pleasant', '0' to 'neutral', '≠' to 'mildly aversive' and '–' to 'aversive'. They viewed each cue in this order and then tasted the corresponding taste solution (see Materials below). Subjects completed three practice trials for each of the conditions. They were not misled during this training and were not told that they might receive a misleading cue-taste combination. They used a button response box to rate each taste delivered on a nine-point Likert scale (–4 = unpleasant, –2 = mildly unpleasant, 0 = neutral, 2 = mildly pleasant, 4 = pleasant). A pointer was positioned randomly each time subjects used the rating scale. When they pressed a button on the response box, the pointer incremented one place up the scale. When the pointer went past the top of the scale, it started over at the bottom. By positioning the pointer randomly and having it only move in one direction, we ensured that subjects' motor response did not systematically vary with the strength of the taste. Functional MRI experimental design limitations did not allow more than one rating scale; consequently, this study cannot disambiguate intensity and valence aspects of taste[14]. Immediately following the simulation, subjects took part in the fMRI session.

fMRI protocol. We used an event-related fMRI design with seven conditions: highly aversive (highly aversive cue preceding highly aversive taste), misleading aversive (mildly aversive cue preceding highly aversive taste), mildly aversive (mildly aversive cue preceding mildly aversive taste), neutral (neutral cue preceding neutral taste), mildly pleasant (mildly pleasant cue preceding mildly pleasant taste), misleading pleasant (mildly pleasant cue preceding highly pleasant taste) and highly pleasant (highly pleasant cue preceding highly pleasant taste). The seven conditions were presented in random order, and the order for each subject was randomized independently so as to eliminate the possible order effects that could have occurred if an aftertaste from one taste interfered with the next. Interstimulus intervals were varied to facilitate deconvolution of overlapping hemodynamic responses corresponding to each event. Intervals were varied in increments of whole seconds. There were two epochs—an expectancy period, followed by delivery of the taste. The expectancy epoch lasted between 2 and 5 s and consisted of a cue presented for 2 s, followed by a black screen for the remainder of the period. The taste epoch lasted between 13 and 16 s and consisted of the delivery of 400 µl of the taste solution over 3.6 s, an instruction to swallow for 1.4 s, a black screen for 1 s, a rating period for 5 s (Experimental procedure above) and finally a delay period with a black screen lasting between 2 and 5 s.

Materials. Taste solutions had varying concentrations of quinine hydrochloride ($C_{20}H_{24}N_2O_2$ HCl) or glucose. The tastes ranged from highly aversive to highly pleasant as follows: (i) highly aversive, 1.0 mM quinine; (ii) mildly aversive, 0.25 mM quinine; (iii) neutral, distilled water; (iv) mildly pleasant, 0.5 M glucose; and (v) highly pleasant, 1.2 M glucose.

A 3.0-Tesla MRI scanner (GE Signa), equipped with a quadrature head coil, was used to image neural activation (GE Medical Systems). Infusion pumps (Razel Scientific) delivered the tastes via polyethylene tubing. Stimuli were presented using a Silent Vision system (Avotec), which consisted of an MRI-compatible fiber-optic projection unit located in the scanner room and a monitoring unit located in the scanner control room. Stimuli were presented to the subject via fiber-optic, stereoscopic goggles. The goggles were mounted inside the head coil, suspended approximately 1.0–1.5 cm above the subject's eyes. We used E-Prime software (Psychology Software Tools) to deliver stimuli and to collect rating data. A 6-liter vacuum head pillow was used to immobilize subjects' heads (Par Scientific). A four-button response box (Current Designs) was used by subjects to rate the tastes.

Image acquisition. We first acquired a 3-plane, sagittal scan for localization purposes (repetition time (TR) = 32.1 ms; echo time (TE) = 1.9 ms; field of view (FOV) = 24 cm; flip angle = 30°; NEX = 1; matrix = 256 × 256; voxel size = 0.9 mm; 9 slices; slice thickness = 5.0 mm; gap = 5.0 mm; scan time = 1 min, 39 s). Then we obtained a sagittal, T1-weighted, spin-echo coplanar scan to generate coordinates for the functional scans (TR = 500.0 ms; TE = 18.0 ms; FOV = 24 cm; flip angle = 90°; NEX = 1; matrix = 256 × 256; voxel size = 0.9 mm; 30 slices; slice thickness = 4.0 mm; gap = 1.0 mm; scan time = 2 min, 24 s). Next we acquired an axial, three-dimensional (3D) T1-weighted, inversion-recovery, fast gradient echo sequence as a high-resolution anatomical image for fitting the functional data to an anatomical atlas (TR = 8.9 ms; TE = 1.8 ms; FOV = 24 cm; flip angle = 10°; NEX = 1; matrix = 256 × 256; voxel size = .9 mm; 124 slices; slice thickness = 1.2 mm; gap = 0.0 mm; scan time = 7 min, 29 s). This was followed by three or more axial scans for high-order autoshimming to homogenize the MRI field gradient (TR = 1.5 s; TE = 7.0 ms; *x*-FOV = 28 cm; *x*-voxel size = 0.034 mm; *y*-FOV = 0.0 cm; *y*-voxel size = 0.0 mm; flip angle = 60°; NEX = 1; matrix = 8; 192 × 64; 32 slices; slice thickness = 6.6 mm; gap = 0.0 mm; scan time = 9 s each). Subsequently, a sagittal, echoplanar imaging (EPI) scan was acquired, reconstructed online and reviewed for image quality to verify the prescription for the experimental EPI scans, which were reconstructed offline and were not visualized during image acquisition (TR = 2.0 s; TE = 30.0 ms; FOV = 24 cm; flip angle = 90°; NEX = 1; matrix = 64 × 64; voxel size = 3.8 mm; 60 slices; slice thickness = 4.0 mm; gap = 1.0 mm; scan time = 2 s). Next, we acquired field maps via four sagittal scans to correct warping of the experimental EPI scans around tissue-air interfaces such as the forehead, the brainstem and the sinuses (TR = 2.0 s; a different TE for each of the four scans: TE_1 = 30.0 ms, TE_2 = 31.0 ms, TE_3 = 33.0 ms and TE_4 = 36.0 ms; FOV = 24 cm; flip angle = 90°; NEX = 1; matrix = 64 × 64; voxel size = 3.75 mm; 30 slices; slice thickness = 4.0 mm; gap = 1.0 mm; scan time = 2 s each). Finally we conducted eight sagittal, T2*-weighted, blood oxygen level–dependent (BOLD) EPI scans for the experimental protocol (TR = 2.0 s; TE = 30.0 ms; FOV = 24 cm; flip angle = 90°; NEX = 1; matrix = 64 × 64; voxel size = 3.75 mm; 30 slices; slice thickness = 4.0 mm; gap = 1.0 mm; scan time = 8 m, 54 s each).

Image processing. The functional image data were reconstructed with a Fermi spatial filter to the k-space data. Slice timing was corrected with alignment to the first slice collected in each TR. We applied a high-pass temporal Fourier filter to remove frequencies lower than 0.02 Hz (that is, lower than twice the longest trial length). Runs were then motion corrected using the image realignment algorithm in AFNI 2.40e (Robert Cox, US National Institutes of Health). Four subjects with excessive movement were eliminated from all further processing, thus reducing the number of remaining subjects to 39. We did not apply a Gaussian smoothing filter to further blur the data. An averaged hemodynamic response function was used in a least-squares general linear model (GLM) fit to an ideal hemodynamic response function, and the resultant beta-weights were converted to percentage signal change. During the GLM fit, the time-to-onset of response was allowed to vary independently for each voxel for 0 to 2 s, and the time lag selected was used for both the cue and taste delivery epochs. This variable onset allowed for sensitivity to varying blood perfusion rates across the brain; moreover, by fixing the time lag as the same for both the cue and the taste periods, we were able to ensure that the two responses were properly separated and estimated. The resultant percentage signal change maps were transformed into the standardized Talairach space by means of the identification of anatomical landmarks on the high-resolution inversion recovery images[46].

Statistical analysis. As a manipulation check, we averaged subjects' ratings for each of the taste conditions. Six subjects did not rate the misleading aversive condition (mildly aversive cue preceding highly aversive taste) as less unpleasant than the highly aversive condition (highly aversive cue preceding highly aversive taste). Three additional subjects did not rate the misleading pleasant condition (mildly pleasant cue preceding highly pleasant taste) as less pleasant

than the highly pleasant condition (highly pleasant cue preceding highly pleasant taste). These three subjects also showed minimal rating differences between the misleading aversive and highly aversive conditions. Accordingly, the responders are the primary focus of analyses here, although analyses for nonresponders are included for the purposes of comparison.

There were eight experimental scan runs, during each of which every taste condition was presented four times. Thus, each condition occurred 32 times over the course of the entire experiment. Gustatory responses are characterized by rapid adaptation[47], which was also observed for the aversive condition in the insula and operculum areas reported here. We computed a run × hemisphere (left, right) × region (middle insula and operculum, posterior insula and operculum) repeated-measures MANOVA for the aversive condition to examine the main effect for run. For this analysis, we averaged pairs of successive runs to obtain a sufficient number of events so that the 3 s of baseline activity could be used as a covariate, as we did for all other analyses reported here. This MANOVA revealed effects for run ($F_{3,36} = 41.68$, $P < 0.001$, $\eta^2 = 0.776$) and a run linear trend ($F_{1,38} = 47.43$, $P < 0.001$, $\eta^2 = 0.555$; **Supplementary Fig. 4** online). This pattern of habituation to the highly aversive taste was also observed for analyses conducted on the taste period activation without partialing out baseline activity. No habituation was observed for the analogous MANOVA for the pleasant condition, which actually indicated a slight increase in insula and operculum response to the pleasant taste across the experiment, with effects for run ($F_{3,36} = 33.30$, $P < 0.001$, $\eta^2 = 0.735$) and a run linear trend ($F_{1,38} = 4.27$, $P = 0.05$, $\eta^2 = 0.101$). Because our focus was on responses to the unpleasant tastes, we limited our analyses to the first half of the experiment (four runs), resulting in 16 presentations of each condition across the four runs.

To identify the brain regions activated by the highly aversive taste, we conducted a voxelwise run (1, 2, 3, 4) × condition (neutral, aversive) repeated-measures ANOVA across the whole brain. Using AlphaSim in AFNI, we then ran Monte Carlo simulations to correct for multiple testing. Specifically, the spatial correlation of the input data and an uncorrected P value threshold of 0.005 resulted in a minimum cluster size of 70 mm^3 to achieve a corrected mapwise $P < 0.05$. Statistical ROIs were defined for any insula or operculum (that is, primary taste cortex) clusters meeting the corrected P value criterion. Ancillary analyses were also conducted for anatomical ROIs according to the major subdivisions of the insula and operculum (**Supplementary Note**). We then computed percent signal change (PSC) means for each ROI for each subject and assessed study hypotheses with MANOVAs and paired t-tests comparing the aversive and misleading conditions within the ROIs. When appropriate, we conducted follow-up analyses for MANOVA effects via simple effects analyses and computed *post-hoc* pairwise comparisons using paired t-tests. We visually inspected the distributions of all the data and further assessed normality by the Kolmogorov-Smirnov, Shapiro-Wilk, skewness and kurtosis statistics. Mild positive skews were observed for some of the data distributions, but no gross violations of normality were present. The main statistical tests used were ANOVAs, repeated-measures MANOVAs and t-tests, which are robust to moderate violations of normality for balanced designs. All statistical tests were two-tailed.

To test for associations between the activation of the primary taste cortex and subjects' ratings of the tastes, we derived rating contrasts by subtracting each subject's mean rating for the highly aversive taste in the aversive condition from the mean rating for the same taste in the misleading condition. We created PSC contrasts by subtracting the PSC during the misleading condition from the PSC during the aversive condition. We performed a voxelwise regression, regressing the rating contrast on the PSC contrast. To test the significance of the *a priori* hypothesis in the insula and operculum regions, we applied a correction for multiple comparisons using Monte Carlo simulations (AlphaSim in AFNI) within an ROI defined by the anatomical boundaries of these regions[25,26,46,48]. The spatial correlation of the input data and an uncorrected P value threshold of 0.01 resulted in a minimum cluster size of 41 mm^3 to achieve a corrected $P < 0.05$. We then computed correlation coefficients for the taste ratings and the PSC means for each cluster.

Finally, our main analytic strategy involved conducting the analyses described above after partialing out estimates of baseline activity. Baseline estimates were computed by averaging the first 3 s of the deconvolved time series data for the whole trial averaged across four runs. In addition to the MANOVAs and t-tests for PSC in the statistical ROIs described above, the same analyses were conducted on adjusted PSC values after partialing out the baseline estimates. In addition to the aforementioned zero-order correlations with taste ratings, we computed partial correlations removing the variance associated with these baseline estimates.

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

We acknowledge A. Alexander, M. Anderle, M. Carew, R. Fisher, T. Johnstone, R. Koch, A. Lakshmanan, K. Mackiewicz, H. Schaefer and E. Steege for their contributions to this project. J.B.N. was supported by a US National Institute of Mental Health Career Development Award (K08-MH63984), a Training Program in Emotion Research National Institute of Mental Health grant (T32-MH18931) and a HealthEmotions Research Institute fellowship. G.E.D. was supported by a National Science Foundation Graduate Fellowship. R.J.D. was supported by National Institute of Mental Health grants (MH40747, P50-MH52354, MH43454) and a National Institute of Mental Health Research Scientist Award (K05-MH00875). The research reported in this publication was also supported by the Mind Brain Body and Health Initiative, funded by the John D. and Catherine T. MacArthur Foundation, the Rockefeller Family and Associates and the Kohlberg Foundation, and by a core grant to the Waisman Center from the National Institute of Child Health and Human Development (P30 HD03352). Parts of this work were presented at the 32nd annual meeting of the Society for Neuroscience in San Diego, California, October 2004.

AUTHOR CONTRIBUTIONS

All authors except I.S. and S.J.S. contributed to discussions about the experimental design. J.B.N., G.E.D., S.J.S. and R.J.D. contributed to data collection. J.B.N., G.E.D., I.S. and R.J.D. contributed to data analysis. All authors except S.J.S. contributed to discussions about the interpretation of the results. J.B.N., G.E.D., I.S. and R.J.D. were primarily responsible for manuscript preparation and revision, and other authors provided comments on drafts.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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