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Named Series: Brain Mechanisms of Placebo

Brain mechanisms of expectation associated with insula and amygdala response to aversive taste: Implications for placebo

Issidoros Sarinopoulos, Gregory E. Dixon, Sarah J. Short, Richard J. Davidson, Jack B. Nitschke *

Waisman Laboratory for Brain Imaging and Behavior, Waisman Center, Departments of Psychiatry and Psychology, University of Wisconsin, 1500 Highland Avenue, Madison, WI 53705-2274, USA

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Abstract

The experience of aversion is shaped by multiple physiological and psychological factors including one's expectations. Recent work has shown that expectancy manipulation can alter perceptions of aversive events and concomitant brain activation. Accruing evidence indicates a primary role of altered expectancies in the placebo effect. Here, we probed the mechanism by which expectation attenuates sensory taste transmission by examining how brain areas activated by misleading information during an expectancy period modulate insula and amygdala activation to a highly aversive bitter taste. In a rapid event-related fMRI design, we showed that activations in the rostral anterior cingulate cortex (rACC), orbitofrontal cortex (OFC), and dorsolateral prefrontal cortex to a misleading cue that the taste would be mildly aversive predicted decreases in insula and amygdala activation to the highly aversive taste. OFC and rACC activation to the misleading cue were also associated with less aversive ratings of that taste. Additional analyses revealed consistent results demonstrating functional connectivity among the OFC, rACC, and insula. Altering expectancies of upcoming aversive events are shown here to depend on robust functional associations among brain regions implicated in prior work on the placebo effect.

Keywords: Expectancy; Aversion; Taste; Insula; Amygdala; Anterior cingulate; Orbitofrontal cortex; Dorsolateral prefrontal cortex

1. Introduction

The power of placebo has been reported for a wide variety of health problems such that understanding how placebos operate has become a critical scientific endeavor. A placebo is an inert or sham treatment used in clinical trials to determine if an active treatment is efficacious. People assigned to the placebo treatment often get better, a phenomenon known as the placebo effect. Recent work has demonstrated that placebo treatments induce biological and psychological effects in several distinct domains, including pain (Montgomery and Kirsch, 1997; Petrovic et al., 2002; Price et al., 1999; Voudouris et al., 1990; Wager et al., 2004; Zubieta et al., 2005), aversive visual stimulation (Petrovic et al., 2005), Parkinson's disease (Benedetti et al., 2004; de la Fuente-Fernández et al., 2001), and depression (Mayberg et al., 2002).

Historically, there have been two primary perspectives for approaching the mechanism underlying the placebo effect. Conditioning theorists have proposed that the placebo is a conditioned Pavlovian response, whereas others have advocated that the placebo is driven by expectancy (Haour, 2005; Stewart-Williams and Podd, 2004; Wager and Nitschke, 2005). Given that expectation is implicit in conditioned stimuli, the relative contributions of conditioning and expectancy to placebos are difficult to disentangle. In fact, all experimental and clinical literature on placebos can be explained in terms of expectations, including the extensive nonhuman animal research using conditioning paradigms to examine the placebo effect (for recent review, see Haour, 2005).

* Corresponding author.
E-mail address: jnitschke@wisc.edu (J.B. Nitschke).

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Although the exact mechanisms involved remain an enigma to scientists, recent functional imaging studies have started to elucidate the neural areas triggered by placebos and other forms of expectation to produce biological responses. Most of this work has focused on the neural networks in placebo analgesia and their effect on pain areas (Lieberman et al., 2004; Lorenz et al., 2005; Wager et al., 2004; Zubieta et al., 2005, 2006). This research has shown that activity within certain cingulate and prefrontal regions may modulate pain sensitive areas. Other studies have highlighted the role of these areas, especially the rACC and the OFC, in expectancy of a variety of emotional stimuli (Koyama et al., 2005; Nitschke et al., 2006; O'Doherty et al., 2002; Petrovic et al., 2005; Ploghaus et al., 1999, 2003; Wager et al., 2004). Moreover, Petrovic et al. (2005) have suggested that the rACC and lateral OFC participate in a generalized expectancy modulatory network that mediates placebo-related effects across different domains.

Given that prior research has established expectation as a central component of the placebo response (Amanzio and Benedetti, 1999; Benedetti et al., 1999; Benedetti et al., 2003; Colloca and Benedetti, 2005; Lanotte et al., 2005; Montgomery and Kirsch, 1997; Petrovic et al., 2005; Price et al., 1999; Zubieta et al., 2005), this rapid event-related fMRI study investigated the neural mechanisms of expectancy that predict behavioral and brain responses to aversion. In one condition, a veridical anticipatory cue informed subjects that they would receive a highly aversive taste. In a second condition, a misleading anticipatory cue informed subjects that they would receive a less aversive taste. In both conditions, a highly aversive bitter taste was invariably delivered (1.0 mM quinine hydrochloride). Previously, we have reported expectancy-related reductions in the insula and operculum (Nitschke et al., under review), areas that comprise the primary taste cortex and have been shown to respond to bitter aversive tastes (Rolls, 1999; Scott et al., 1986, 1999; Small et al., 1999, 2003; Yaxley et al., 1990; Zald et al., 2002). In this study, we probe the modulatory mechanism underlying such expectancy effects in the insula and the amygdala, another prime area activated by aversive tastes (O'Doherty et al., 2001; Small et al., 2003; Zald et al., 1998, 2002) as well as other forms of aversion (Büchel et al., 1998; Dalton et al., 2005; Davis and Whalen, 2001; LaBar et al., 1998; LeDoux, 2002; Nitschke et al., 2006). Our main hypothesis is that the attenuation of insula and amygdala responses to the highly aversive taste following the misleading cue will be predicted by rACC and OFC activation to the misleading cue itself. OFC and rACC activations to the misleading cue are also expected to predict perceptions of how aversive the highly aversive taste is.

2. Methods

2.1. Subjects

Subjects were students at the University of Wisconsin—Madison enrolled in Introduction to Psychology. Four men and seven women (ages 18–21 years, $M = 19.18$, $SD = .87$) were included in an initial part of the study to determine optimal taste concentrations. Participating in the fMRI portion of the study were 54 right-handed adults. Eleven subjects found the highly aversive bitter taste to be too aversive and were unable to complete the experiment. Forty-three people remained (24 men and 19 women), ranging in age from 18 to 22 ($M = 20.12$, $SD = .85$). All 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.

2.2. Materials and apparatus

Five taste solutions were delivered in this experiment: (a) highly aversive, 1.0 mM quinine hydrochloride ($C_{20}H_{24}N_2O_2$ HCl), (b) mildly aversive, 0.25 mM quinine hydrochloride, (c) neutral, distilled water, (d) mildly pleasant, and (e) highly pleasant. To examine current hypotheses about brain mechanisms for expectancy-related effects on the response to aversive taste, only data for the highly aversive tastant were analyzed for the present report. A 3.0 T GE Signa MRI scanner, equipped with a quadrature head coil, was used to image neural activation (GE Medical Systems, Waukesha, WI). A six-liter vacuum head pillow was used to immobilize subjects' heads (Par Scientific, Houston, TX), and a four-button response box (Current Designs, Philadelphia, PA) was used by subjects to rate the tastes. Infusion pumps (Razel Scientific, Stamford, CT) delivered the tastes via polyethylene tubing. Separate small tubes (.050-in. inside diameter) were used for each taste and were bound into a single straw using spiral wrap. As a result of this setup, subjects were unable to detect which of the small tubes delivered each taste. The lag between turning on the pump and the presentation of fluid was extremely short—approximately 200 ms—as the fluid was held at the tip of the straw in subjects' mouths. We determined during extensive piloting that there was no seepage once pumping was complete.

Visual stimuli were presented using a Silent Vision system (Avotec, Jensen Beach, FL), which consists of a MRI-compatible fiber-optic projection unit that is located in the scanner room and a monitoring unit located in the scanner control room. Subjects viewed visual stimuli through stereoscopic goggles that were mounted inside the head coil, suspended approximately 1.0–1.5 cm above the subject's eyes. E-Prime software (Psychology Software Tools, Pittsburgh, PA) was used for delivery of taste solutions, presentation of visual stimuli, and collection of rating data.

2.3. Procedure

Subjects took part in a simulated scan in the shell of an MRI scanner with no magnet to acclimate them to the scanning environment. We placed the subject in the mock scanner and fitted him or her with fiber-optic goggles, headphones, and a vacuum pillow to help immobilize his or her head. We then played an audio recording of an actual MRI scanner. Next, they tasted each of five tastes three times in the following order: highly pleasant, mildly pleasant, neutral, mildly aversive, and highly aversive (see below). They used a button response box to rate each taste delivered on a 9-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 the subject's motor response did not systematically vary with the strength of the taste.

During the simulation, subjects also viewed instructions that showed each of the cues and were told that "+" corresponded to "pleasant," that "✶" corresponded to "mildly pleasant," that "*0*" corresponded to "neutral," that "✶" corresponded to "mildy aversive," and that "−" corresponded to "aversive." They viewed each cue in this order and then tasted the corresponding taste solution. Subjects completed three practice trials for each of the conditions. In this way subjects learned which taste corresponded to each cue. Subjects were not misled during this training, and

they were not told that they might receive a misleading cue/taste combination.

Design limitations for this fMRI experiment did not allow more than one rating scale; consequently, this study cannot disambiguate intensity and valence aspects of taste (Small et al., 2003), nor were on-line ratings of expectation assessed. Immediately following the simulation, subjects took part in the fMRI session. Directly after the fMRI scan, subjects rated their reactions to the cues used in the fMRI experimental design described below and depicted in Fig. 1 along the following dimensions: Anxiety, Disgust, Aversion, Relief, and Enjoyment. Ratings were made on a 9-point Likert scale, with 0 labeled "Not At All," 4 labeled "Somewhat," and 8 labeled "A Lot."

2.4. Experimental design

The rapid event-related fMRI design had 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). As illustrated in Fig. 1, only the highly aversive and misleading aversive conditions are central to hypotheses tested here; therefore, the data for the remaining five conditions are not included in the present report. The seven conditions were presented in random order, and the order for each subject was randomized independently to eliminate the possible order effects that could have occurred due to aftertaste from one taste to the next. Interstimulus intervals were varied to facilitate deconvolution of overlapping hemodynamic responses corresponding to each event (Buckner, 1998; Ollinger et al., 2001; Serences, 2004). Intervals were varied in increments of whole seconds. Each trial began with an expectancy period lasting 2–5 s and consisting of a cue, presented for 2 s, followed by a black screen for the remainder of the period. This was immediately followed by 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 (see Section 2.3 above), and finally a delay period with a black screen lasting between 2–5 s.

2.5. fMRI data acquisition

The data acquisition protocol consisted of the following: (a) a three-plane, sagittal scan was first acquired 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; (b) a sagittal, T1-weighted, spin echo coplanar scan was acquired 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; (c) an axial, 3D T1-weighted inversion-recovery fast gradient echo sequence was acquired as a high-resolution anatomical image for fitting of 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 = 0.9 mm, 124 slices, slice thickness = 1.2 mm, gap = 0.0 mm, scan time = 7 min 29 s; (d) three or more axial scans were acquired 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 = 0.000 mm, flip angle = 60°, NEX = 1, matrix = 8192 × 64, 32 slices, slice thickness = 6.6 mm, gap = 0.0 mm, scan time = 9 s each; (e) a sagittal, echo planar imaging (EPI) scan was acquired, reconstructed on-line, and reviewed for image quality to verify the prescription for the experimental EPI scans, which were reconstructed off-line and 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; (f) field maps were acquired 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 (Cusack et al., 2003): TR = 2.0 s, TE was varied for each of the four scans so that $TE_1 = 30.0$ 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; (g) eight sagittal, T2*-weighted, blood oxygen-level dependent (BOLD) EPI scans were conducted for the experimental paradigm: 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 min 54 s each. These eight EPI scans were the functional runs used in our experiment.

Because fMRI data for the OFC and amygdala are prone to signal loss resulting from differential magnetic susceptibility coefficients at bone/air/tissue boundaries, we calculated signal-to-noise ratios (SNR) resulting from the above fMRI data acquisition parameters. SNR was determined independently for each voxel by dividing the mean time series signal by the standard deviation of that time series signal. Regional SNR estimates were obtained by averaging across voxels in each statistical ROI mask. As expected, there was some signal loss in the OFC and amygdala, which compromised our ability to detect effects there. SNR values ranged from 115 to 140 for clusters in the DLPFC (Table 1), a region with minimal signal loss. Those for the OFC clusters implicated in the present study ranged from 32 to 102, and those for the rACC clusters ranged from 64 to 102. The amygdala cluster had an SNR value of 81, and the left and right insula values were 150 and 125, respectively.

2.6. Data analysis

The functional image data were reconstructed with a Fermi spatial filter to the k-space data. Slice-timing correction was performed with

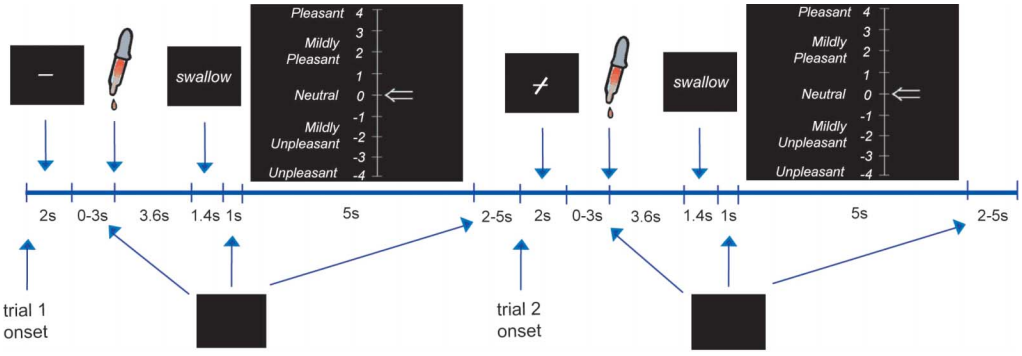


Fig. 1. Trial structure for the aversive and misleading conditions is shown. For the aversive condition (trial 1), a minus sign preceded a highly aversive taste (1.0 mM quinine). For the misleading condition (trial 2), a minus sign with a slash preceded the same highly aversive taste. Subjects viewed identical screens in both conditions, with the exception of the cue, which is illustrated for each condition separately.

Table 1
Activations derived from the aversive versus misleading contrast for the expectancy period showing correlations with activation in the left insula, right insula, and right amygdala clusters identified by the aversive versus misleading contrast for the taste period shown in Fig. 2

Brain region	Talairach Coordinates			Size (mm^3)	r
	x	y	z		
Left insula	−35	−1	5	90	$t = 3.34$
Left anterior medial OFC	−23	49	−4	215	−.81
Right anterior medial OFC	27	43	−9	76	−.81
Left lateral OFC	−41	36	−7	85	−.80
Left posterior OFC	−25	26	−9	358	−.87
rACC	2	38	14	188	−.82
Subgenual rACC	7	31	−2	672	−.82
Left DLPFC	−20	43	29	210	−.87
Right insula	37	2	4	100	$t = 3.14$
Left anterior medial OFC	−22	50	−1	101	−.75
Right anterior medial OFC	26	42	−10	76	−.76
Left anterior lateral OFC	−32	47	−2	746	−.85
Right anterior lateral OFC	39	46	−5	156	−.82
Left posterior OFC	−32	27	−11	113	−.70
rACC	3	39	12	104	−.76
Left DLPFC	−29	29	38	189	−.81
Right amygdala	24	6	−14	103	$t = 3.41$
Right anterior medial OFC	18	52	−11	81	−.76
Left anterior lateral OFC	−34	49	−8	76	−.72
Right anterior lateral OFC	30	41	−11	100	−.83
Left anterior lateral OFC/ventrolateral PFC	−37	46	−7	85	−.72
Right anterior lateral OFC/ventrolateral PFC	36	45	−5	368	−.75
Right DLPFC	26	44	27	162	−.78
Right DLPFC	25	44	18	142	−.80

R and t values are for entire cluster. OFC = orbitofrontal cortex. rACC = rostral anterior cingulate cortex. DLPFC = dorsolateral prefrontal cortex. PFC = prefrontal cortex. All areas are significant at $p < .05$ (corrected), except for the left insula ($p = .055$, corrected).

alignment to the first slice collected in each TR. Application of a high-pass temporal Fourier filter removed frequencies slower than 0.02 Hz (i.e., slower than double the longest trial length). Runs were then motion corrected using the image realignment algorithm in AFNI 2.40e (Robert Cox, National Institutes of Health). Four subjects with excessive movement were eliminated from all further processing, thus reducing the number of remaining subjects to 39. Minimal blurring was accomplished by applying a 2-mm Gaussian smoothing filter to the data (Friston et al., 1995; Nitschke et al., 2006). 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 β-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 (0–2 s), and the time lag selected was used for both the expectancy and taste periods. This variable onset allowed for sensitivity to varying blood perfusion rates across the brain, while fixing the time lag as the same for both expectancy and taste periods ensured that the two responses were properly separated and estimated. The resultant percentage signal change maps were transformed into the standardized Talairach space via identification of anatomical landmarks on the high-resolution inversion-recovery images. The following landmarks were identified manually: the superior edge of the anterior commissure, the posterior margin of the anterior commissure, the inferior edge of the posterior commissure, two mid-sagittal points, the most anterior point of the brain, the most posterior point of the brain, the most superior point of the brain, the most inferior point of the brain, the most left point of the brain, and the most right point of the brain.

Of note, the taste period included delivery of a tastant, the instruction to swallow, and the subsequent rating scale, which cannot be statistically separated with the current design. We designed trials in this manner to have subjects swallow at the same time in each experimental condition. Consequently, the associated motor activity should not vary systematically by condition. Similarly, the evaluative ratings were not expected to differentiate the conditions, because the rating scales were identical for all conditions and should elicit similar memory, comparison, and motor processes. In addition, the independence of expectancy-related activation and taste response activation is difficult to ensure in our paradigm due to the short interstimulus interval relative to hemodynamic lag (O'Doherty et al., 2002), although the above statistical modeling procedures were implemented to minimize the possibility of variance due to the taste being misattributed to the expectancy period and vice versa.

There were eight experimental scan runs, during which each of the taste conditions was presented four times during each run. Each condition occurred 32 times over the course of the entire experiment. Prior research has established that gustatory responses are characterized by rapid adaptation (Pfaffmann et al., 1971), which was also observed for the aversive condition in insula and amygdala areas reported here, as indicated by one-way repeated-measures MANOVAs for the aversive condition across the eight runs ($ps < .05$). Although effects across the eight runs did not attain significance for the taste ratings (Small et al., 2003) or for analogous MANOVAs conducted for the misleading condition, differences between conditions were reduced for both the insula and amygdala responses to taste and the taste ratings after our fourth experimental run. For that reason we limited our analyses to the first four runs, resulting in 16 presentations of each condition across the four runs.

To test our hypotheses characterizing the brain mechanisms governing expectancy-modulated responses to a highly aversive taste, we used stringent criteria for selecting only those subjects showing robust psychophysical differences between the misleading and aversive conditions, as indicated by their taste ratings. First, we used a median spilt method to select a group of high responders from the sample of 39. This resulted in a group of 18 subjects. We also wanted to exclude any subjects whose 95% confidence interval around the mean rating difference for the two conditions included 0. This was not the case for any of the 18 subjects. All

remaining subjects were excluded from statistical analyses. One additional subject was excluded for analyses on the amygdala, due to extreme MR signal values in the amygdala (3.5 *SD* above the mean).

To identify the brain regions in which suppression of the response to aversive taste occurred for the misleading condition, we conducted a voxel-wise t test comparing the aversive and misleading conditions across the four runs for the taste period. Since our hypotheses focused on insula and amygdala responses to aversive taste, we used anatomically defined masks to confine the search volume to within the insula and amygdala regions for this t test (Lancaster et al., 2000; Talairach and Tournoux, 1988). Using AlphaSim in AFNI, Monte Carlo simulations were run to correct for multiple testing. Considering the spatial correlation of the input data, we determined that an uncorrected p value threshold of 0.005 resulted in a minimum cluster size of 91 mm^3 to achieve a corrected map-wise $p < .05$. Statistical regions of interest (ROIs) were defined for any insula or amygdala clusters meeting the corrected p value criterion.

To test the hypothesis that OFC and rACC activation following the misleading cue would be associated with suppression observed in the insula and amygdala ROIs (misleading < aversive) derived from the above voxel-wise t tests, we first computed percent signal change difference scores comparing the aversive and misleading conditions for each of the statistically defined insula and amygdala ROIs for the taste period. We then performed a voxel-wise regression, regressing these percent signal change difference scores for the taste period in these insula and amygdala seed regions on the misleading versus aversive contrast brain map for the expectancy period. We restricted our search volume to bilateral OFC and rACC regions (Lancaster et al., 2000; Talairach and Tournoux, 1988), using the same method described above for the voxel-wise t test. Here we determined that an uncorrected p value threshold of .005 corresponded to a minimum cluster size of 72 mm^3 to achieve a corrected map-wise $p < .05$. Statistical ROIs were defined for OFC and rACC clusters meeting the corrected p value criterion. We then computed correlation coefficients between the difference scores derived from these ROIs for the expectancy period and the difference scores derived from the insula and amygdala ROIs for the taste period.

To test for associations between expectancy activation and subjects' ratings we first 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. A voxel-wise regression was employed to regress the rating difference scores on the misleading versus aversive contrast brain map for the expectancy period. We restricted our analyses to bilateral OFC and rACC regions using the 72 mm^3 minimum cluster size determined above. Statistical ROIs were defined for OFC and rACC clusters meeting the corrected p value criterion. We then computed correlation coefficients between the difference scores derived from the expectancy ROIs and the difference scores derived from the taste ratings.

To ascertain that OFC and rACC regions implicated in the above regressions for the aversive and misleading conditions were actually activated by the anticipation of aversive taste (O'Doherty et al., 2002), voxel-wise t tests compared aversive to neutral conditions and misleading to neutral conditions during the expectancy period. A conjunction map was constructed by taking the union of the resulting two statistical parametric maps (i.e., a voxel-level AND operation; Nichols et al., 2005). The joint probability threshold was set at a p value of .005. This conjunction procedure yielded a mask containing only those voxels that were significantly activated above $t = 1.83$ ($p = .0707$) in each of the two contrasts, such that the probability of finding a voxel that is independently significant in each and both contrasts (i.e., the joint probability) can be estimated by multiplying the probabilities for each contrast: $.0707 \times .0707 = p < .005$ (Allan et al., 2000; Cabeza et al., 2002; Dolcos et al., 2004; Liu et al., 2003; Nitschke et al., 2006). Overlapping thresholded regions that met the above criteria and fell within the anatomical boundaries of the OFC and rACC (Talairach and Tournoux, 1988; Lancaster et al., 2000), as implemented in AFNI, were the focus of this conjunction analysis (Ganis et al., 2004; Knutson et al., 2001; Nitschke et al., 2006).

In addition to the above regressions conducted across period to examine modulation of the insula and amygdala activations during the taste period by the OFC and rACC activations during the expectancy period, we also explored functional associations among these regions within the taste period. Specifically, we performed functional connectivity analyses on deconvolved time series estimates of the aversive and misleading conditions during the taste period using the insula and amygdala clusters identified in the voxel-wise t test above (Knight et al., 2005). This method allows the functional connectivity of different conditions to be assessed separately and is conceptually similar to what is attempted by effective connectivity analyses in SPM (Friston et al., 1997). All analyses were conducted using AFNI tools. First, we performed deconvolution analysis. This allowed us to extract time series data for the misleading and aversive taste conditions. Then, we used our statistically defined insula and amygdala ROIs from the voxel-wise t test as masks and extracted the average deconvolved time series estimates associated with each taste category (aversive and misleading). Deconvolved time series estimates for the aversive and misleading conditions were then used as seeds in a cross-correlation analysis across all OFC and rACC voxels for each subject's data. The degree to which resultant fit coefficient maps reflecting the synchrony of seed regions with OFC and rACC areas in the aversive and misleading conditions differed from zero was assessed with one-sample t tests. Finally, we used paired t tests to compare the fit coefficient data for the aversive and misleading conditions.

3. Results

During the fMRI experimental task, subjects rated the highly aversive taste following the aversive cue more unpleasant than the same taste following the misleading cue, $t(17) = 7.55$, $p < .001$, as would be expected based on our criteria for subject selection. Only subjects showing robust psychophysical differences between the aversive and misleading conditions, as indicated by taste ratings, were selected to test our hypotheses about the brain mechanisms governing expectancy-modulated responses to a highly aversive taste. Directly following the scan, subjects rated their reactions to the highly aversive cue as more unpleasant than their reactions to the mildly aversive cue used for misleading trials (and for veridical mildly aversive trials): anxiety, $t(17) = 5.72$, $p < .001$; disgust, $t(17) = 6.23$, $p < .001$; aversion, $t(17) = 3.71$, $p = .002$; relief, $t(17) = -3.11$, $p = .006$; and enjoyment, $t(17) = -1.71$, $p = .10$. Difference scores comparing the two cues for each of the five rating scales were not correlated with the difference score comparing the aforementioned on-line taste ratings for the aversive and misleading conditions ($ps > .40$), with the exception of a trend for relief, $r = .43$, $p = .08$.

To identify insula and amygdala areas where the misleading cue attenuates neural activation to aversive taste, we compared the BOLD responses to the highly aversive bitter taste preceded by the veridical cue with responses to the same taste preceded by the misleading cue using a voxel-wise contrast across the whole brain (thresholded at $p < .05$, two-tailed, corrected; see Section 2.6). When subjects expected a less aversive taste, decreased activation was observed in bilateral insula (Fig. 2A; Table 1). Both left and right activations were within the previously identified taste responsive regions (Nitschke et al., under review), consistent with a large literature on the primary

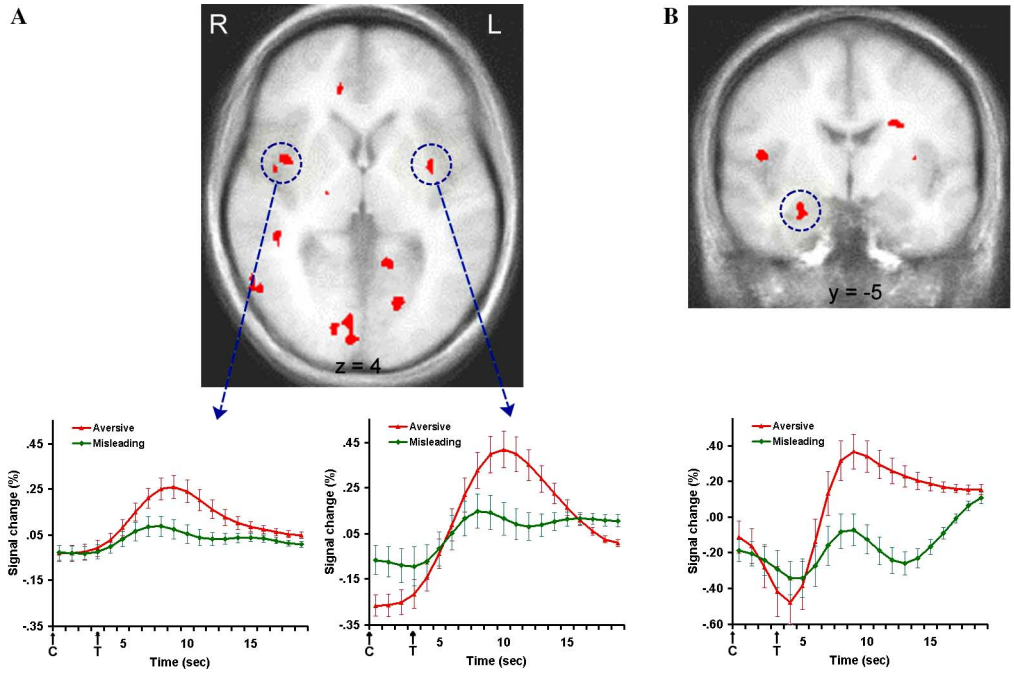


Fig. 2. Circled bilateral insula (A) and right amygdala (B) clusters were more strongly activated by the highly aversive condition versus the misleading condition during the taste period. The onset of the 2-s cue (C) preceded delivery of the tastant (T) by 2–5 s. Time series plots of the circled clusters illustrate average percentage signal change across all time points of the aversive (red) and misleading (green) trials and were derived from deconvolved timeseries estimates for the whole trial. Error bars are for standard error around the mean after adjusting for between-subject variance (Loftus and Masson, 1994).

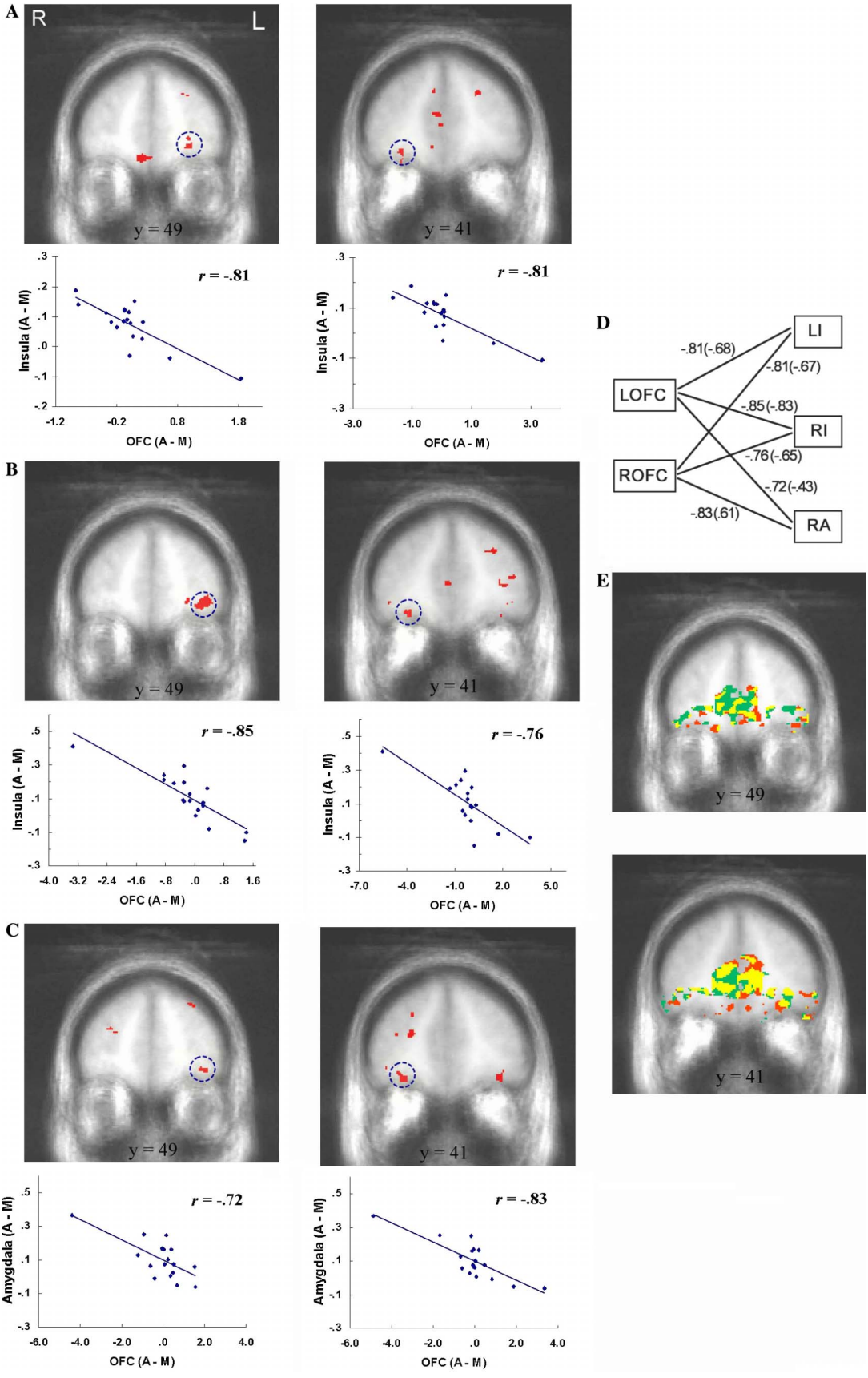
taste cortex (Rolls, 1999; Scott et al., 1986, 1999; Small et al., 1999, 2003; Yaxley et al., 1990). In addition, an attenuated response to the highly aversive taste following a misleading cue was observed in the right amygdala (Fig. 2B; Table 1), another area previously shown to respond to aversive tastes (O'Doherty et al., 2001; Small et al., 2003; Zald et al., 1998, 2002).

To evaluate our main hypothesis that expectancy-related activation in the rACC and OFC mediates the observed suppressed response to aversive taste, we regressed the magnitude of reduction in neural activity during the taste period (aversive > misleading) in the clusters identified above on the brain contrast map comparing activation to misleading versus the veridical cue (misleading > aversive) in the expectancy period (see Section 2.6). As illustrated in Fig. 3A, expectancy-related increases in OFC activation during the expectancy period were correlated with reduced activation to the highly aversive taste in the left insula for bilateral anterior medial, left lateral, and left posterior OFC (Table 1). Similar effects were observed for the right insula, including bilateral anterior medial, bilateral anterior lateral, and left posterior OFC (Fig. 3B; Table 1). The right amygdala also showed associations with the same areas, including right anterior medial and bilateral anterior lateral OFC, as well as bilateral anterior lateral OFC/ventrolateral PFC (Fig. 3C; Table 1). The predicted correlations between rACC increases during anticipation and attenuated activation to the highly aversive taste were observed for the left and right, and for the right insula, as shown in Figs. 4A and B. Correlations conducted without the outliers apparent in some of the plots in Figs. 3 and 4 revealed similar associations, as shown in Figs. 3D and Fig. 4D. Consistent with other reports on expectations of various forms of aversion (Koyama et al., 2005; Nitschke et al., 2006; O'Doherty et al., 2002; Petrovic et al., 2005; Ploghaus et al., 1999; Wager et al., 2004), these areas all show greater activation during the expectancy period for both conditions than for the neutral condition, as indicated by voxel-wise t tests (Figs. 3E and 4E).

Based on recent studies implicating the DLPFC in placebo analgesia (Wager et al., 2004; Zubieta et al., 2005), we examined the above voxel-wise regression maps for activations in the DLPFC. There were left DLPFC clusters for both the left and right insula, and two right DLPFC clusters correlated with the right amygdala area (Table 1).

We tested for correlations between self-reported expectancy effects in taste ratings (misleading < aversive) and fMRI activity during the expectancy period (misleading > aversive) in hypothesized regions. Regions within the rACC (235 mm^3) and right anterior OFC/ventrolateral prefrontal cortex (58 mm^3, $p = .11$ corrected) showed significant correlations, $r = -.78$ and $-.76$, respectively (Fig. 4C). Subjects reporting the largest decreases in taste aversiveness in the misleading compared to aversive condition showed greater activation in these rACC and OFC areas to the misleading cue. No significant clusters were observed in the DLPFC. Fig. 5 illustrates map-wise activity across the brain for all of the above regressions.

For the purposes of comparison, the above voxel-wise analyses were also conducted for the 21 subjects not


A
R
L
y = 49
y = 41
r = -.81
Insula (A - M)
OFC (A - M)
B
r = -.85
r = -.76
C
r = -.72
r = -.83
Amygdala (A - M)
D
LI
RI
RA
LOFC
ROFC
-.81(-.68)
-.81(-.67)
-.85(-.83)
-.76(-.65)
-.72(-.43)
-.83(.61)
E

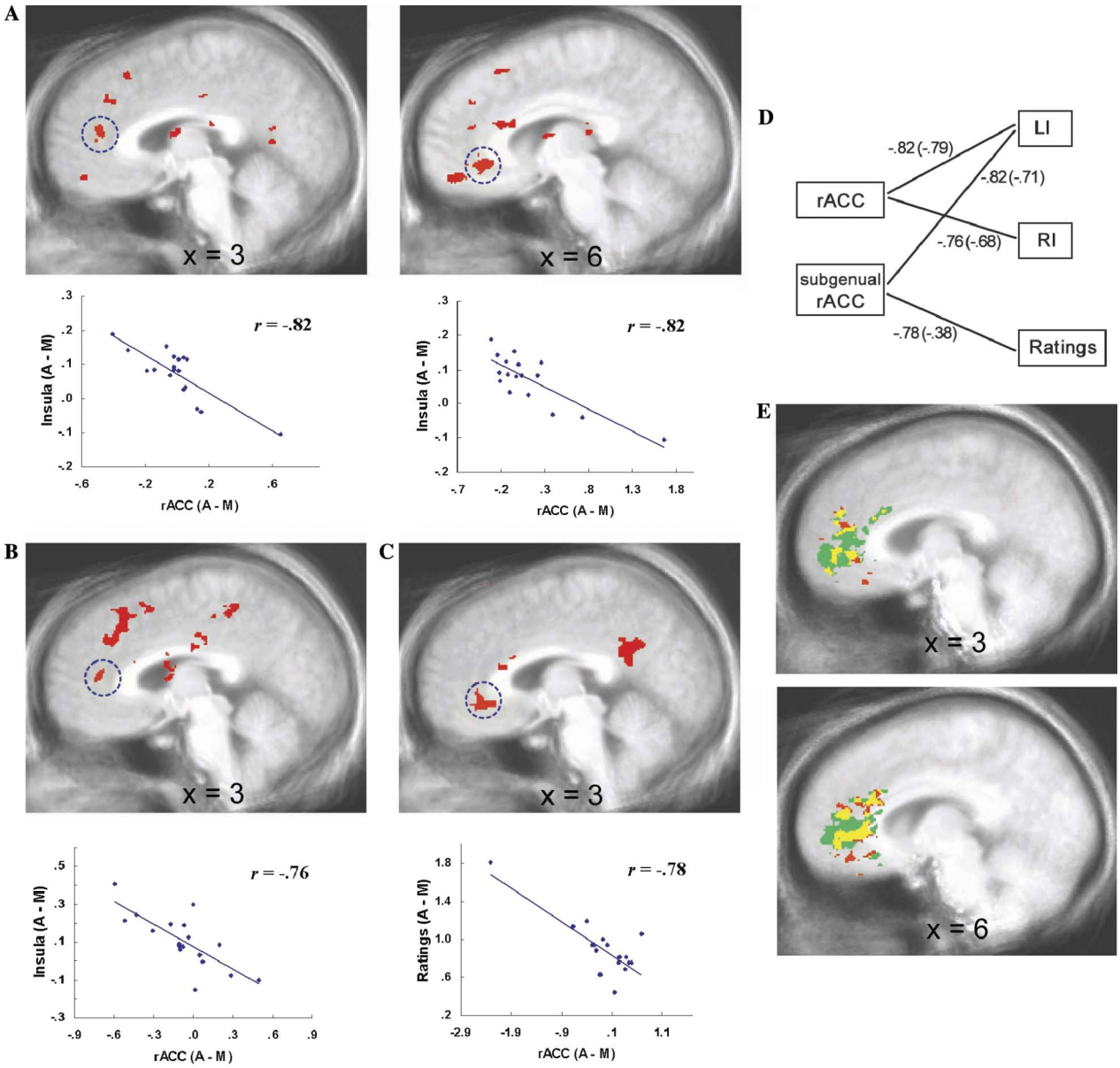


Fig. 4. Activation in circled rACC regions derived from the aversive versus misleading contrast for the expectancy period was correlated with activation in the left insula (A) and right insula (B) clusters identified for the aversive versus misleading contrast for the taste period shown in Fig. 2, and was correlated with taste ratings (C). Increased activation to the misleading cue during the expectancy period was associated with reduced activation in bilateral insula to the highly aversive taste following the misleading compared to the veridical cue. Individuals with higher activation to the misleading cue in the rACC cluster shown in C rated the highly aversive taste as less aversive when it followed the misleading compared to the veridical cue. (D) The diagram summarizes the correlations depicted in (A–C) and includes correlations without outliers in parentheses. (E) The conjunction map displays the results of voxel-wise *t* tests comparing aversive to neutral conditions and misleading to neutral conditions during the expectancy period. Illustrated are activations within the anatomical boundaries of the ACC and adjacent medial PFC (Lancaster et al., 2000; Talairach and Tournoux, 1988), as implemented in AFNI. Red areas showed greater activation during the expectancy period for aversive than neutral trials. Green areas showed greater activation during the expectancy period for misleading than neutral trials. Yellow areas showed greater activation during the expectancy period for both aversive and misleading trials than neutral trials. A=aversive condition. M=misleading condition. rACC=rostral anterior cingulate cortex. LI=left insula. RI=right insula.

Fig. 3. Activation in circled left and right OFC regions derived from the aversive versus misleading contrast for the expectancy period was correlated with activation in the left insula (A), right insula (B), and right amygdala (C) clusters identified by the aversive versus misleading contrast for the taste period shown in Fig. 2. Increased activation to the misleading cue during the expectancy period was associated with reduced activation in bilateral insula and right amygdala to the highly aversive taste following the misleading compared to the veridical cue. (D) The diagram summarizes the correlations depicted in (A–C) and includes correlations without outliers in parentheses. (E) The conjunction map displays the results of voxel-wise *t* tests comparing aversive to neutral conditions and misleading to neutral conditions during the expectancy period. Illustrated are activations within the anatomical boundaries of the ACC, OFC and adjacent ventral PFC (Lancaster et al., 2000; Talairach and Tournoux, 1988), as implemented in AFNI. Red areas showed greater activation during the expectancy period for aversive than neutral trials. Green areas showed greater activation during the expectancy period for misleading than neutral trials. Yellow areas showed greater activation during the expectancy period for both aversive and misleading trials than neutral trials. A=aversive condition. M=misleading condition. OFC=orbitofrontal cortex. LOFC=left orbitofrontal cortex. ROFC=right orbitofrontal cortex. LI=left insula. RI=right insula. RA=right amygdala.

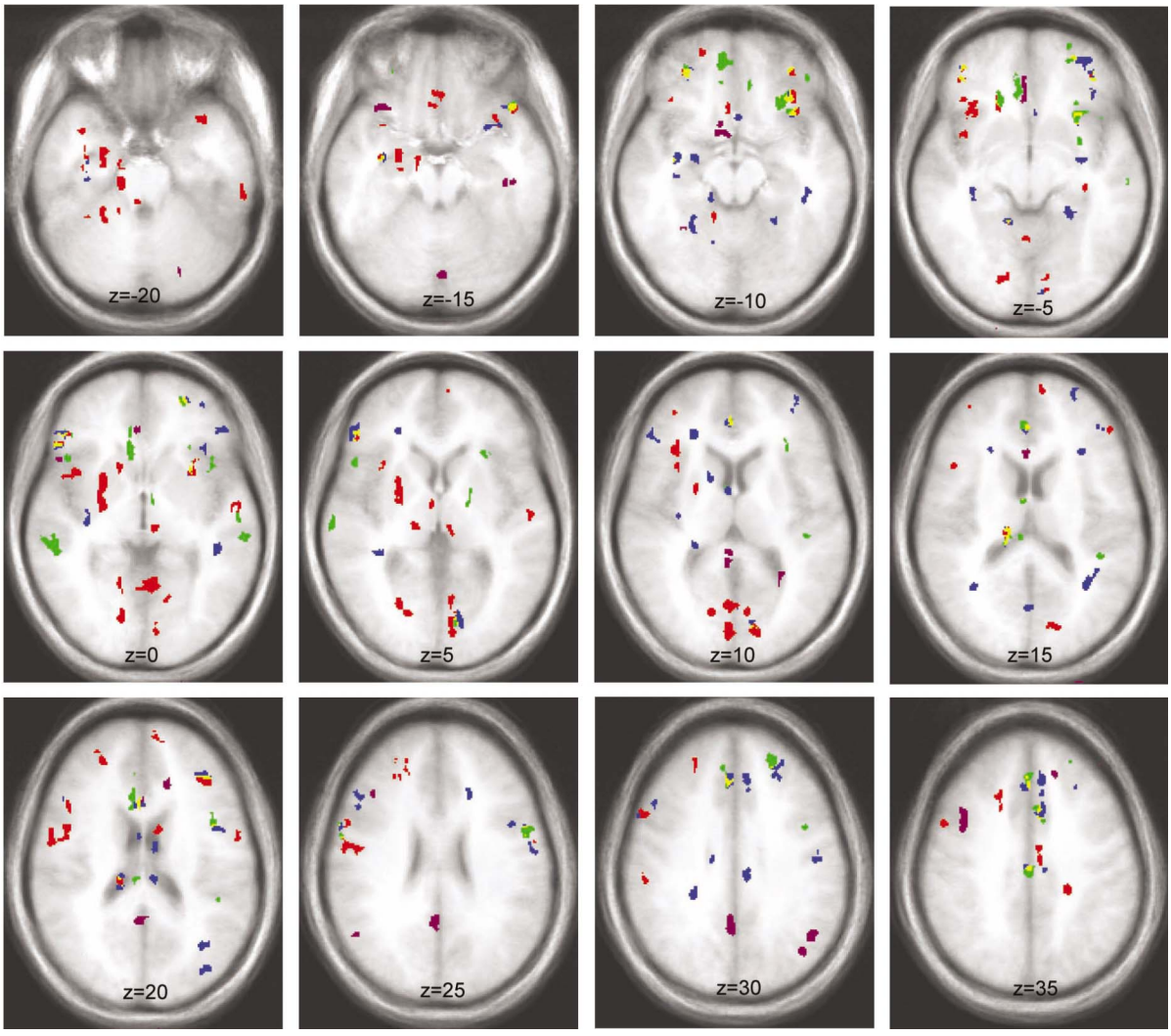


Fig. 5. Map-wise activity displaying the extent of overlap among the regions identified in regression analyses for which activations during the anticipation period were associated with taste ratings and with left insula, right insula, and right amygdala activations during the taste period (Figs. 3 and 4). Purple areas only showed greater activation to the misleading than aversive cue during the expectancy period that was associated with ratings of the highly aversive taste as less aversive when it followed the misleading compared to the veridical cue. Green areas only showed greater activation to the misleading than aversive cue during the expectancy period that was associated with reduced activation in the *left insula* to the highly aversive taste following the misleading compared to the veridical cue. Blue areas only showed greater activation to the misleading than aversive cue during the expectancy period that was associated with reduced activation in the *right insula* to the highly aversive taste following the misleading compared to the veridical cue. Red areas only showed greater activation to the misleading than aversive cue during the expectancy period that was associated with reduced activation in the *right amygdala* to the highly aversive taste following the misleading compared to the veridical cue. Yellow areas showed greater activation for any two or more of the regression analyses ($p < .005$, uncorrected).

showing the reliable psychophysical effect. The voxel-wise *t* test on the taste period revealed no insula or amygdala activations, unlike the findings for the 18 subjects who are the focus of this report. Using the insula and amygdala clusters identified for the 18 subjects, a voxel-wise regression on the misleading versus aversive contrast brain map for the expectancy period for the 21 subjects revealed no rACC or OFC activations. Similarly, the analogous voxel-wise regression for all 39 subjects revealed no rACC or OFC activations. For separate voxel-wise regressions on the 21 and 39 subjects, there were also no rACC or OFC areas showing the association with taste ratings observed for the 18 subjects in the present report.

Of relevance to the hypothesized role of the OFC and rACC in the down-regulation of the insula and amygdala to aversion, a voxel-wise *t* test comparing activation for misleading versus veridical conditions during the anticipation period was conducted as an ancillary analysis. Two small clusters showed more activation during expectancy following a misleading versus a veridical cue (misleading > aversive). A right anterior medial OFC region (58 mm^3) did not overlap with any of the regression clusters, and a rACC region (65 mm^3) showed moderate overlap with the rACC cluster implicated in the above regression on the left insula.

In addition, we examined whether deconvolved time series estimates within the above identified insula and amyg-

dala clusters were synchronous with deconvolved time series estimates in the OFC and rACC during the taste period. Fit coefficient fMRI maps, assessed with voxel-wise one-sample t tests (see Section 2.6), showed functional connectivity for the misleading condition between the right insula and right medial OFC and between the left insula and rACC ($p < .01$). Nearly identical clusters were identified for the voxel-wise paired t test comparing the misleading and aversive conditions. Post hoc comparisons showed that the fit coefficients for the misleading condition were significantly higher than the fit coefficients for the aversive condition in the right medial OFC region associated with the right insula, $t(17) = 2.87$, $p = .01$, and in the rACC region associated with the left insula, $t(17) = 2.95$, $p = .009$. These results indicate that insula responses to taste were more strongly associated with medial OFC and rACC regions in the misleading than the aversive condition, suggesting that the modulatory influence of the insula by these regions observed above for the expectancy period may extend into the taste period.

4. Discussion

In this study, we set out to investigate the expectancy-related brain mechanisms for altered neural responses to a highly aversive bitter taste. To maximize our ability to isolate these mechanisms, analyses were restricted to only those individuals showing the largest discrepancy in their psychophysical ratings of the same highly aversive taste following the misleading cue as compared to the veridical cue. Those individuals all rated the highly aversive taste markedly less aversive when it followed the misleading cue than when it followed the veridical cue. We found that these subjects showed a remarkably consistent pattern of rACC and OFC activation to the misleading cue predicting subsequent attenuation of bilateral insula responses to the highly aversive taste. Moreover, activation of the same bilateral OFC areas to the misleading cue also was associated with a smaller right amygdala response to the highly aversive taste. These findings are consistent with studies implicating the rACC and OFC in the placebo effect (Petrovic et al., 2005; Ploghaus et al., 2003; Wager et al., 2004). These same rACC and OFC areas also figure importantly in the anticipation of aversive as well as pleasant events (Koyama et al., 2005; Nitschke et al., 2006; O'Doherty et al., 2002; Petrovic et al., 2005; Ploghaus et al., 2003; Wager et al., 2004). In addition, the DLPFC findings are consistent with recent reports on placebo analgesia (Wager et al., 2004; Zubieta et al., 2005), lending further support to the relevance of PFC-mediated representations of expectancy-related information needed for cognitive control (Miller and Cohen, 2001; Wager et al., 2004).

These findings build on our recent report for the same paradigm with a larger sample of subjects with less stringent inclusion criteria (Nitschke et al., under review). There it was found that large areas of the bilateral insula and operculum that activated more to the highly aversive taste than a neutral taste also showed more activation to the same highly aversive taste when it followed the veridical cue than when it followed the misleading cue. That report included no analyses on the expectancy period. For that larger sample, analyses conducted here resulted in similar albeit smaller insula, rACC, OFC clusters that generally did not meet the spatial cluster threshold needed for attaining significance at a corrected $p < .05$ threshold, as was used in the present study.

Analyses of the expectancy data are central to the current report, with findings elucidating the rACC and OFC as brain regions that likely play a key role in the attenuated insula response to aversive taste. Moreover, just as right insula activation in response to the highly aversive taste was found to be associated with subjects' ratings of how aversive that taste was in Nitschke et al. (under review), rACC and right OFC activation to the misleading cue showed the same association with taste ratings in the present study. It is noteworthy that both of these regions overlapped with areas that predicted right insula responses to the highly aversive taste, given that the right insula was the locus of our previous findings for taste ratings and other previous research implicating a broader role of the insula, especially on the right, in interoceptive awareness (Adolphs, 2002; Adolphs et al., 2000; Craig, 2002, 2003; Craig et al., 2000; Critchley, 2004; Critchley et al., 2004; Damasio, 2003). Signals sent by the rACC and OFC to the right insula may serve as a precursor of right insula function in awareness and subjective experience (Petrovic et al., 2005; Ploghaus et al., 2003; Wager et al., 2005).

In addition, the present study examined expectancy effects on amygdala responses to aversive taste, based on findings in the literature for aversive tastes (O'Doherty et al., 2001; Small et al., 2003; Zald et al., 1998, 2002) as well as other forms of aversion (Büchel et al., 1998; Dalton et al., 2005; Davis and Whalen, 2001; LaBar et al., 1998; LeDoux, 2002; Nitschke et al., 2006). Here, we found that subjects with greater activation in the OFC (but not in the rACC) in response to the misleading versus veridical cue showed less activation of the amygdala to the highly aversive taste when it followed the misleading cue than when it followed the veridical cue. Although present findings are generally consistent with the literature, other reports have typically implicated more medial regions of the OFC (i.e., ventromedial prefrontal cortex) and the subgenual ACC (Kim et al., 2004; Phelps et al., 2004; Quirk et al., 2003; Rosenkranz et al., 2003; Wager et al., 2004). Those areas are difficult to image with fMRI due to signal loss resulting from differential magnetic susceptibility coefficients at bone/air/tissue boundaries, and our failure to detect activation in those regions likely reflects this problem.

The present data demonstrate that the network processing aversive taste may be suppressed by expectancy-related brain activity in specific regions of the rACC and OFC, as well as the DLPFC. Particularly important may be the bilateral anterior OFC sectors illustrated in Fig. 3 as showing the predicted association with modulation of

the insula and amygdala areas. Specifically, we found that decreased neural response in insula and amygdala regions to aversive taste was predicted by higher activation in the rACC, OFC, and DLPFC during an expectancy period. This suggests that activation in these brain areas may be an antecedent to the reduction in the network processing aversive taste. Activation in similar regions during an expectancy period was recently shown to predict attenuated activation in pain regions (Wager et al., 2004).

Capitalizing on the prior research establishing the importance of expectation in placebos (Amanzio and Benedetti, 1999; Benedetti et al., 1999, 2003; Montgomery and Kirsch, 1997; Petrovic et al., 2005; Price et al., 1999; Zubieta et al., 2005), this study directly manipulated expectancy, instead of utilizing an inert treatment, in order to investigate expectancy-related brain mechanisms that predict changes in subjects' experience of an aversive event and their neural responses to it. The rACC and OFC were found to be key substrates of the expectancy processes that predicted behavioral and brain responses to an aversive taste. These findings correspond well with other work documenting their importance in anticipatory processes and the down-regulation of brain areas such as the amygdala. The rACC and OFC regions identified in this study may partially constitute a generalized modulatory network that mediates the impact of induced expectancies upon sensory processing across different modalities (Petrovic et al., 2005). Thus, modulatory processes served by these regions in placebo may not be specific to placebo responses, but rather a part of the mechanisms involved in expectation-induced modulation and regulation of emotional processing more generally.

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