Supporting Online Material

Materials and methods

Subjects

Fourteen healthy right-handed volunteers (mean age, 22 years; range, 18-32 years) took part in the study and provided full informed consent. Participation was limited to those subjects who enjoyed eating both vanilla ice cream and peanut butter sandwiches. No one reported any history of neurological, psychiatric, ear-nose-throat, or respiratory problems. Subjects were asked to fast for 6 hours prior to their scheduled arrival time at the laboratory, but were permitted to drink water, tea, or coffee in moderation. One volunteer was discarded from the analysis due to poor behavioral performance during scanning, leaving a total of 13 subjects. The study was approved by the joint National Hospital for Neurology and Neurosurgery/Institute of Neurology Ethics Committee.

Stimuli

The 2 unconditioned stimuli (UCS) consisted of 2 food-based odors: vanilla (pure vanilla extract, $n = 6$ subjects; or vanillin 8% w/v in propylene glycol, $n = 7$ subjects) and peanut butter. These odors were chosen on the basis of their close perceptual correspondence to their food counterparts, and because they could be easily distinguished from each other. For each subject, one odor was designated the target (to-be-devalued) UCS and the other was designated the non-target UCS. The assignment of odors as target or non-target UCS was counterbalanced across subjects. Stimuli were presented using a multi-channel computer-controlled olfactometer,

suitable for the MRI environment and capable of rapidly delivering odor pulses in the absence of tactile, thermal, or auditory variations. This device was modified from its initial design (*S1, S2*) by exchanging the original odor chambers with 60-cc plastic cylinders, filled with 4 cc of liquid odor (or distilled water, in the case of the control tube). Air entered through a hole in the top of the cylinders, via Teflon tubing that terminated below the liquid level, and the resulting odor-saturated air was conducted through a second hole in the cylinder top toward the subject. In this manner odorized air was constantly refreshed throughout the experiment. Airflow rates were set at 2.0 L/min.

Three arbitrary fractal color images comprised the 2 conditioned stimuli (target CS+ and non-target CS+) and the non-conditioned stimulus (CS-). These pictures were back-projected from a PC computer toward the subject lying inside the scanner, who was fitted with a mirror atop the headbox coil. Identities of the CS+ and CS- stimuli were also counterbalanced across subjects. Both odor and picture delivery were controlled using Cogent 2000 software (Wellcome Dept. of Imaging Neuroscience, London, UK) as implemented in Matlab 6.0 on a PC computer.

Two different foods were used to elicit sensory-specific satiety and reinforcer devaluation. A given subject received either vanilla ice cream or open-faced peanut butter sandwiches, depending on the identity of the previously determined target UCS, for consumption between pre- and post-satiety scanning sessions. Subjects did not know in advance which food they would be given.

Task

Subjects participated in a simple spatial-discrimination task. They were not explicitly informed about the CS/UCS contingencies, but were merely told that the pictures would occasionally

appear in combination with the 2 odors. At baseline, and between successive trials, a green fixation cross-hair appeared centrally. At trial onset (t = 0), a fractal image was presented to the left or right of the cross-hair, for a duration of 1000 msec, and subjects were asked to respond by pushbutton ("left" or "right") as quickly as possible. At t = 750 msec, the cross-hair turned into a red asterisk, which signalled subjects to make a medium sniff. This sniff cue persisted for 1500 msec, then reverted to the green cross-hair (t = 2250 msec). Trials occurred every 8.1 sec. Note that the picture CS and odor UCS overlapped for 250 msec, and that subjects sniffed on each and every trial, for the duration of the sniff cue, regardless of odor presence.

Experiment

Appetitive olfactory conditioning followed a 50% partial-reinforcement schedule, in which only one-half of all CS+ picture items was paired with their corresponding UCS odors. This resulted in 6 different conditions: 1) target CS+, paired with target UCS ("**Tgt CS+p**"); 2) target CS+, unpaired ("**Tgt CS+u**"); 3) non-target CS+, paired with non-target UCS ("**nTgt CS+p**"); 4) non-target CS+, unpaired ("**nTgt CS+u**"); 5) target CS- ("**Tgt CS-**"); and 6) non-target CS- ("**nTgt CS-**"). In this way, by comparing unpaired CS+ and CS- conditions, learning-related responses could be isolated from mere stimulus properties of the UCS. Inclusion of non-target events controlled for general session effects, subjective feelings of fullness, and visceral or autonomic changes that might otherwise modulate hemodynamic activity. Note that the CS- was randomly divided into "target" and "non-target" CS- conditions to provide orthogonal baselines for purposes of data analysis (see below), but were otherwise experimentally identical.

Scanning was divided into 3 sessions: training, pre-satiety, and post-satiety. Subjects participated in the same spatial decision task throughout. First, an 8-minute "training" phase

introduced the subjects to the different stimuli and permitted parallel learning of both target and non-target stimulus-reinforcement contingencies. This phase ensured that in the subsequent sessions, the neural responses induced by reward anticipation were not confounded by learning *per se*. Each event type was repeated 10 times, except for the CS- condition, which was repeated 20 times, for a total of 60 events. After a 60-90 s delay, a 14-minute "pre-satiety" session was performed, which resembled the first session, except that each event type was repeated 16 times (for CS-, 32 times), totalling 96 events. This phase established a pre-satiety, post-learning baseline. Reinforcer devaluation of the target UCS, by means of sensory-specific satiety, then followed this session. Subjects were removed from the scanner and fed a meal of the target food. They were asked to eat as much as they could, until they no longer found the target food palatable, short of becoming completely full. It was our aim to devalue the rewarding properties of the target reinforcer, while preserving the reward value of the non-target reinforcer. Finally, subjects were returned to the scanner for the third and final session. This "post-satiety" phase was identical in format to the pre-satiety phase and lasted 14 minutes. For all 3 sessions, stimulus presentation was randomized, with the constraint that each condition was equally distributed per each ¼ of the experiment, to minimize the effect of olfactory habituation and differences in learning rates.

Behavioral measurements

Behavioral ratings, collected before and after eating, included hunger level (0, full; +10, starving), pleasantness of the target food (-10, very unpleasant; +10, very pleasant), and both valence (-10, very unpleasant; +10, very pleasant) and intensity (-10, undetectable; +10, very strong) of target and non-target odors. For odor valence, ratings were also taken on-line after ¼,

½, and ¾ of the pre- and post-satiety sessions. Here, subjects had 8 s to indicate pleasantness for each odor by using the keypad to move a vertical bar along a visual analog scale. Mean odor valence scores were then averaged across 6 pre-feeding and 5 post-feeding ratings. Statistical analysis of the ratings data was performed using non-parametric Wilcoxon signed-ranks tests in SPSS for Windows. Significance was set at $P < 0.05$ (two-tailed).

Reaction times (RTs) were acquired on-line during scanning and analyzed in Matlab 6. The RTs were subjected to log transformation prior to statistical analysis in order to render a normal response distribution. Subject-specific mean RTs for each condition were then computed across the first and second halves of each experimental session. The mean RTs for target and non-target CS+ conditions were averaged across paired and unpaired events for comparison to CS-. Data were entered into a repeated-measures ANOVA for statistical analysis.

Respiratory measurements were also monitored on-line in all subjects, though the data from 2 subjects was lost due to technical difficulties. Subjects were equipped with a pair of breathing belts affixed around the chest and abdomen. As the subject breathed, the resulting pressure changes within the belts could be detected by a piezo-resistive differential transducer (0-1 psi) positioned outside the scanner. The analog signal was then sampled at 100 Hz and digitized on a PC computer. Subject-specific mean peak sniff amplitudes were calculated for each condition, then normalized to the mean CS- amplitude, for each session, to permit group statistical analysis (repeated-measures ANOVA). Sniff latencies corresponding to the time-to-peak were also computed.

Data acquisition and analysis

We acquired T2*-weighted echoplanar images (EPI) with blood oxygen-level dependent (BOLD) contrast on a Siemens Sonata 1.5 T scanner. A total of 681 volumes (slices/volume, 35; repetition time, 3.15 s) was collected over the 3 sessions, plus 5 "dummy" volumes at the start of each session. Signal dropout in basal frontal and medial temporal structures due to susceptibility artifact was reduced by using a tilted plane of acquisition (30^{o} to the anterior commissure-posterior commissure line, rostral > caudal) and performing *z*-shimming in the slice-selection direction (*S2*). Imaging parameters were: echo time, 50 ms; field-of-view, 192 mm; in-plane resolution, 3 mm; slice thickness, 2 mm; interslice gap, 1 mm. High-resolution T1-weighted structural scans from 12 subjects were coregistered to their mean EPI images and averaged together to permit anatomical localization of the functional activations at the group level.

Data pre-processing of the functional scans, including spatial realignment (*S3*), slice-time correction, normalization to a standard EPI template, and smoothing with a 6-mm (full-width half-maximum) Gaussian kernel, was accomplished with Statistical Parametric Mapping (SPM2). The event-related fMRI data was then analysed by constructing a set of delta (stick) functions corresponding to the event-onset times for each of the 6 conditions (Tgt CS+p, Tgt CS+u, nTgt CS+p, nTgt CS+u, Tgt CS-, nTgt CS-). These regressors were convolved with a canonical hemodynamic response function (HRF) and its temporal derivative to accommodate latency differences. Subject-specific movement parameters, a high-pass filter (cutoff, 128 s), and the 16-s on-line ratings periods (in pre- and post-satiety sessions) were also modelled as covariates of no interest. Olfactory learning in the training phase was estimated using condition x time interactions, by convolving the regressors with an exponential time-constant equal to ¼ of the session length (*S4*). Condition-specific estimates of neural activity (betas), corresponding to

the height of the HRF, were computed independently at each voxel for each subject, using the general linear model (*S5*).

Using a random-effects analysis, we entered the relevant contrasts of parameter estimates from the 13 subjects into a series of one-way *T*-tests or repeated-measures ANOVA with non-sphericity correction, where appropriate. We focused on 4 contrasts. 1) Olfactory learning during the training phase was estimated in a conjunction of target CS+u and non-target CS+u, each minus their respective CS- baselines, using condition x time interactions. This tested for incremental responses that were common to both appetitive cues and increased over the course of learning. 2) The effect of selective satiation was examined in the contrast of [pre-Tgt CS+u – post-Tgt CS+u] – [pre-nTgt CS+u – post-nTgt CS+u], which highlighted brain regions showing decreased responses to the target CS+ from pre- to post-satiety, relative to non-target CS+ responses. Contrasts of parameter estimates were plotted after adjusting for the CS- baselines. 3) Areas sensitive to reinforcer devaluation that overlapped neural representations of odor UCS were examined in a conjunction between (a) the effects of satiety (contrast 2 above) and (b) a contrast comparing CS+p – CS+u (training phase only), which isolated odor-specific responses while cancelling out effects unrelated to olfactory processing (*S2*). This latter contrast reflected a conjunction between target and non-target conditions. 4) Areas evoked by olfactory learning that were also sensitive to selective satiation were determined in a conjunction of contrasts 1 and 2 above. In *a priori* brain regions previously identified in neuroimaging studies of olfactory processing and appetitive conditioning (*S2, S6, S7*), including piriform cortex, amygdala, OFC, ventral striatum, midbrain, hypothalamus, insula, and cingulate cortex, we report activations surviving a threshold of $P < 0.001$ uncorrected. Reported voxels conform to MNI (Montreal

Neurological Institute) coordinate space. For display the right side of the image corresponds to the right side of the brain (so-called neurological convention).

Supporting tables

Table S1. Neural activations evoked by olfactory learning

Brain region	Peak MNI coordinates			*Z* value
	x	*y*	*z*	
Left posterior dorsal amygdala	-24	-12	-12	4.19
Right hypothalamus	6	-3	-3	4.02
Left ventral midbrain	-12	-21	-21	3.75
Left piriform cortex	-18	0	-15	3.42
Right anterior insula	39	3	0	3.34
Left rostromedial OFC	-12	51	-18	3.17

Table S2. Neural activations selectively modulated by reinforcer devaluation

Brain region	Peak MNI coordinates			*Z* value
	x	*y*	*z*	
Left dorsal amygdala*	-15	-6	-18	4.11
Right anterior cingulate cortex	6	12	42	3.76
Left ventral striatum	-9	15	0	3.64
	-15	12	-3	3.10
Right anterior insula	39	18	-6	3.62
Left rostral OFC	-33	42	-12	3.19
Right caudal OFC*	24	33	-12	3.16

*, central representations of odor UCS were also evident in these regions

Table S3. Learning-evoked activations that are also sensitive to reinforcer devaluation

Brain region	Peak MNI coordinates			*Z* value
	x	*y*	*z*	
Left posterior dorsal amygdala	-24	-12	-12	4.83
Left dorsal amygdala	-15	-6	-18	4.42
Left piriform cortex	-18	0	-15	4.58
Anterior cingulate cortex	0	9	45	4.12
Right rostral OFC	21	51	-15	3.58
	33	54	-15	3.12
Left rostral OFC	-33	42	-18	3.54
	-12	54	-18	3.12
Right hypothalamus	6	-6	-3	3.50
Right anterior insula	39	3	-9	3.44

References

S1. T. S. Lorig, D. G. Elmes, D. H. Zald, J. V. Pardo, *Behav. Res. Methods Instrum. Comput.* **31**, 370 (1999).

S2. J. A. Gottfried, R. Deichmann, J. S. Winston, R. J. Dolan, *J. Neurosci.* **22**, 10819 (2002).

S3. K. J. Friston et al., *Hum. Brain Mapp.* **2**, 165 (1995).

S4. C. Buchel, J. Morris, R. J. Dolan, K. J. Friston, *Neuron* **20**, 947 (1998).

S5. K. J. Friston et al., *Hum. Brain Mapp.* **2**, 189 (1995).

S6. J. A. Gottfried, J. O'Doherty, R. J. Dolan, *J. Neurosci.* **22**, 10829 (2002).

S7. J. O'Doherty, R. Deichmann, H. D. Critchley, R. J. Dolan, *Neuron* **33**, 815 (2002).