Neuron, Vol. 42, 687–695, May 27, 2004, Copyright ©2004 by Cell Press

Remembrance of Odors Past: Human Olfactory Cortex in Cross-Modal Recognition Memory

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Summary

Episodic memory is often imbued with multisensory richness, such that the recall of an event can be endowed with the sights, sounds, and smells of its prior occurrence. While hippocampus and related medial temporal structures are implicated in episodic memory retrieval, the participation of sensory-specific cortex in representing the qualities of an episode is less well established. We combined functional magnetic resonance imaging (fMRI) with a cross-modal paradigm, where objects were presented with odors during memory encoding. We then examined the effect of odor context on neural responses at retrieval when these same objects were presented alone. Primary olfactory (piriform) cortex, as well as anterior hippocampus, was activated during the successful retrieval of old (compared to new) objects. Our findings indicate that sensory features of the original engram are preserved in unimodal olfactory cortex. We suggest that reactivation of memory traces distributed across modality-specific brain areas underpins the sensory qualities of episodic memories.

Introduction

Episodic memory involves the conscious retrieval of contextually unique events (Tulving, 1983). A cardinal feature of such memories is their multisensory quality. For example, the recollection of a seaside holiday may conjure up the sight of a beach umbrella, the sound of crashing surf, and the smell of brackish seaweed. Thus, remembering a prior episode frequently relies on the reactivation of associations that span multiple sensory domains.

How the sensory qualities of a prior episode are integrated into a unified mnemonic experience is poorly understood. Associative or relational theories of explicit memory postulate that the primary constituents of a memory engram are preserved in modality- and category-specific regions of the brain, with the hippocampus, as the final recipient of inputs from multiple sensory streams, binding these elements together into a coherent whole (Marr, 1971; Cohen et al., 1997; Mishkin et al., 1997; Norman and O'Reilly, 2003). In this way, the hippocampus compiles the various distributed representations that together form an episodic memory, without itself being their physical repository. Such an arrangement ensures the fidelity of the original trace and permits access to the entire engram via partial cues from different sensory channels (Mesulam, 1998).

Experimental support for a relational account of explicit memory has centered on the associative functions of the medial temporal lobe (Cohen et al., 1997). Animal studies indicate that the hippocampus (Dusek and Eichenbaum, 1997; Wood et al., 2000; Alvarez et al., 2001) and adjacent parahippocampal regions (Murray et al., 1993; Higuchi and Miyashita, 1996; Buckley and Gaffan, 1998) mediate the retrieval of associations between unrelated items. In humans with selective hippocampal damage, associative memory is impaired only when requiring access to cross-modal information (Vargha-Khadem et al., 1997; Mayes et al., 1999), and functional imaging studies in normal subjects show that the retrieval of cross-modal associations elicits hippocampal activity (Gabrieli et al., 1997; Stark and Squire, 2000). In light of these observations, it is worth noting that recent human imaging (Stark and Squire, 2001) and lesion (Stark et al., 2002) data also implicate hippocampus in item-based (nonassociative) retrieval, underscoring the likelihood that relational functions do not account for the entire range of hippocampal processes in memory.

However, while hippocampus provides a candidate locus for associative binding, there is less support for active participation of modality-specific brain regions during the retrieval of episodic memories. According to relational models, such areas maintain the sensory qualities of an episodic memory, bound together through reciprocal connections with hippocampus (Cohen et al., 1997). Recent experiments indicate that higher-order visual and auditory areas of the brain can be reactivated during memory for pictures and sounds (Nyberg et al., 2000; Wheeler et al., 2000; Vaidya et al., 2002). However, it is unknown whether unimodal regions specifically devoted to chemosensory processing can maintain representations of an episodic memory trace. Here, we describe a memory paradigm that allowed us to examine the participation of unimodal olfactory cortex in cross-modal retrieval processing. The design is similar to that previously used to study emotional contextual memory (Maratos and Rugg, 2001; Maratos et al., 2001; Smith et al., 2004a, 2004b), modified to incorporate an olfactory manipulation. In an initial study phase, subjects were presented with combinations of odors and pictures and instructed to form stories or associations between each pair of stimuli (Figure 1). In a subsequent test phase, only the pictures were presented, while subjects performed an object recognition task. Because primary olfactory (piriform) cortex is not known to receive direct verbal, visual, or semantic inputs, we hypothesized that

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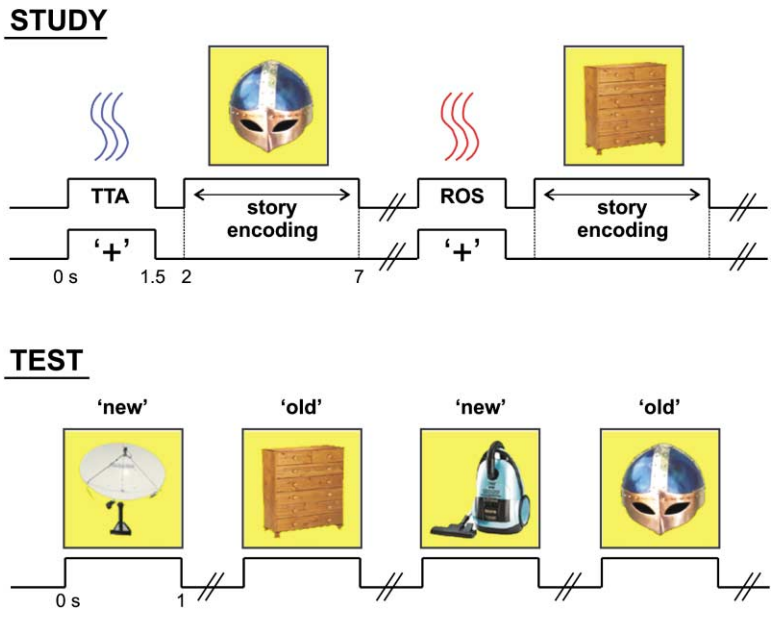

Figure 1. Experimental Paradigm

In the cross-modal study phase (top), each trial began with the appearance of a cross-hair, which cued subjects to sniff an odor for 1.5 s. After a 0.5 s delay, an object was presented for 5 s, during which time subjects imagined a story or link between the odor and object. TTA, trithioacetone; ROS, rose. In the test phase (bottom), objects were presented for 1 s in the absence of sniff cues or odor delivery, and subjects made an old/new recognition judgment regarding each item.

the detection of retrieval-related (object-cued) activity in piriform cortex would unambiguously signal its involvement in episodic memory processes. In addition, by manipulating odor valence, we tested the impact of emotional context on the neural substrates of memory retrieval, with specific emphasis on areas previously implicated in human affective processing (Dolan, 2002).

Results

Behavioral Data

Odor Valence Ratings

Subjective ratings of odor valence (−10 to +10) were as follows: positive odors, 6.2 ± 0.39 (mean ± SEM); neutral odors, 1.1 ± 0.66; negative odors, −6.8 ± 0.55 (Figure 2A). Across the three odor groups, there was a significant main effect of valence ($\chi^2 = 34.47$; df = 2; $p < 0.001$; Friedman test for related samples). Pairwise post hoc comparisons showed that the positive odors were rated as significantly more pleasant than the neutral and negative odors, and the neutral odors were rated as significantly more positive than the negative odors (all p's < 0.001; Wilcoxon signed-ranks test).

Memory Performance

Subjects performed well on the recognition memory task across all valence levels (Figure 2B; Table 1). Recognition accuracy was greatest for objects that had been paired with positive-smelling odors during the study phase. Repeated-measures ANOVA demonstrated a significant main effect of emotional context on correct hits [$F(1.5, 21.2) = 5.005$; $p < 0.05$; df adjusted using Greenhouse-Geisser correction]. The hit rate for objects that had been presented in the context of positively valenced odors was significantly higher than that for objects in either neutral [$F(1, 14) = 6.7$; $p < 0.05$] or negative [$F(1, 14) = 4.81$; $p < 0.05$] contexts. There were no significant differences in hit rates between neutral and negative contexts. Mean reaction times for hits did not differ significantly according to emotional context (Table 2).

Breathing

In a separate group of subjects (n = 15) studied outside the scanner, respirations were monitored during object recognition to determine whether breathing patterns systematically varied in a condition-specific manner. There were no significant differences between correct response types for the volume of first inspiration following event onset [$F(2.3, 31.4) = 1.398$; $p = 0.26$], peak amplitude of first inspiration [$F(2.1, 29.3) = 2.101$; p =

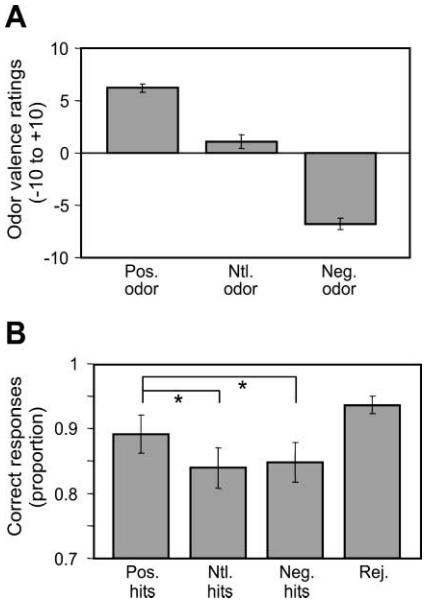

Figure 2. Behavioral Data

(A) Mean subjective ratings of odor valence for positive (pos.), neutral (ntl.), and negative (neg.) categories.

(B) Recognition accuracy during the test phase for hits (old objects) that had been associated at study with positive, neutral, and negative odor contexts and rejections (new objects). The proportion of positive hits was significantly higher than the proportions of neutral and negative hits. *$p < 0.05$.

Table 1. Memory Recognition Performance

	Positive Hits	Neutral Hits	Negative Hits	New
Accuracy (proportion)				
Mean	0.89	0.84	0.85	0.94
SEM	0.03	0.03	0.03	0.01
RTs (ms)				
Mean	838	853	852	919
SEM	18.8	22.9	24.4	26.7

0.14], latency to inspiratory peak [$F(2.3, 31.2) < 1$; $p = 0.63$], or mean level of respiration across the event duration [$F(2.6, 36.2) < 1$; $p = 0.59$] (repeated-measures ANOVA; df adjusted).

Neuroimaging Data

Object Recognition: Effect of Olfactory Context

Our primary focus was to determine whether there was evidence for the reactivation of sensory olfactory cortex during successful object recognition. The contrast of hits (collapsed across valence) versus correct rejections revealed enhanced neural activity in right posterior piriform cortex (Figures 3A–3D; Table 2). This activation was situated at the junction of frontal and temporal cortices and overlapped regions previously shown to be activated in human neuroimaging studies of basic olfactory processing (Zatorre et al., 1992; Savic et al., 2000; Sobel et al., 2000; Gottfried et al., 2002a). Crucially, this enhanced piriform activity was expressed in the absence of actual odor stimulation. A group signal time course from piriform cortex indicated that the hit-evoked response generally conformed to the predicted shape of the canonical hemodynamic response function and significantly differed from correct rejections at the peak centered around 6 s (Figure 3E). In the same contrast, we also detected a significant enhanced response in right anterior hippocampus (Figures 3B–3D). Plots of percent signal change from the peak piriform and hippocampal voxels show that positive, neutral, and negative odor contexts all contributed similarly to the recognition effects observed in these areas (Figures 3F and 3G). Thus, responses in these regions could not be explained by an interaction with valence (tested at $p < 0.05$ uncorrected). Moreover, the responses were still evident when inclusive masking between positive, neutral, and negative hits (each minus correct rejections) was used to limit activations to those common across all three valence contexts.

Because the cortical substrates of olfaction broadly overlap with those involved in memory, it is possible that the neural activity in piriform cortex might reflect a general property of memory retrieval, as opposed to specific reactivation of olfactory context. Therefore, in order to demonstrate odor specificity, we compared the present study to a nonolfactory fMRI data set (Smith et al., 2004b). This latter study involved visual-visual (instead of olfactory-visual) associative encoding but was otherwise identical in design and subject number ($n = 15$), providing a valid nonolfactory control. Briefly, at encoding, neutral objects were superimposed centrally on top of pleasant, neutral, or unpleasant background pictures, consisting mostly of standardized images taken from the International Affective Picture System (IAPS) (Lang et al., 1997). Subjects were instructed to imagine links between objects and backgrounds during this phase. A subsequent test phase was analogous to the olfactory paradigm and involved the presentation of the objects alone (for details, see Smith et al., 2004b). To test for the specificity of odor context on memory-related effects in piriform cortex, we performed a between-group ANOVA incorporating the factors of experiment (olfactory versus nonolfactory) and condition (hits versus correct rejections). This contrast revealed a significant interaction in right piriform cortex, which reflected greater activity in the olfactory study (Figure 3H). Peak activity was detected at the same voxel identified in the primary experiment (30, 3, −12; $Z = 3.17$), suggesting that this structure is more sensitive to retrieval in the odor condition.

In a supplementary analysis of the nonolfactory data set, we performed a region-of-interest analysis centered on piriform cortex. There was no evidence that responses to hits versus correct rejections were significantly enhanced in either right ($Z = 1.62$; $p > 0.5$) or left

Table 2. Regions Activated during Object Recognition

	MNI Coordinates (mm)			
Brain Region	x	y	z	Peak *Z*
Left inferior prefrontal cortex	−42	42	21	3.40
	−51	39	3	3.79
	−45	30	18	3.66
Right caudate	9	6	6	3.10
Right posterior piriform cortex[1]	30	3	−12	3.34
Left precentral gyrus	−51	0	15	3.09
Right anterior hippocampus	33	−24	−15	2.83
Left middle temporal gyrus	−51	−27	−12	3.42
Right cerebellum	24	−36	−27	3.60
Left superior temporal sulcus	−51	−51	27	3.50
Left intraparietal sulcus	−33	−60	36	4.07
	−27	−60	45	3.57
	−39	−60	48	3.53
Right intraparietal sulcus	39	−69	39	3.60
Right occipital cortex	18	−87	0	3.67

[1]Also identified in a direct comparison between olfactory and nonolfactory contexts.

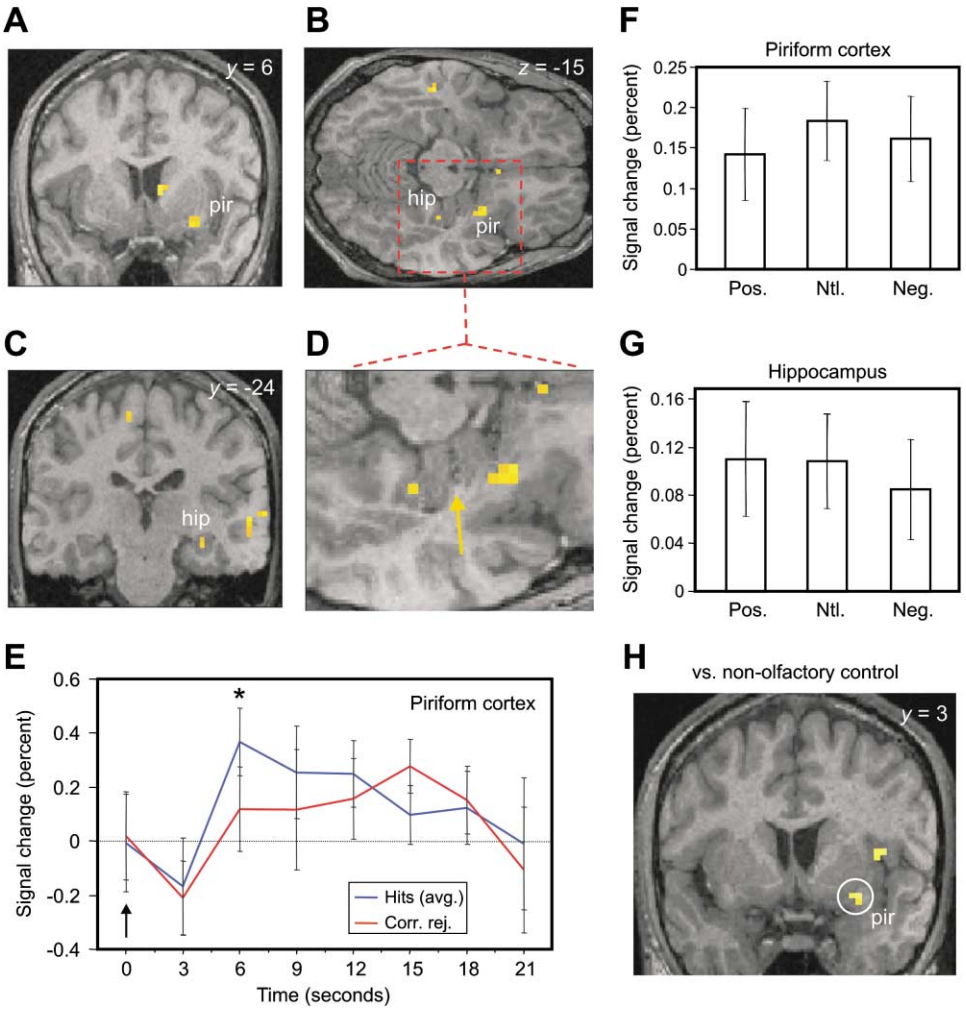

Figure 3. Effects of Odor Context on Object Recognition

(A–D) The contrast of hits versus correct rejections revealed significant activity in posterior piriform (pir.) cortex and anterior hippocampus (hip.). Activations are thresholded at $p < 0.005$ for display and overlaid on coronal (A and C) and horizontal (B and D) sections from the normalized T1-weighted anatomical scan from one subject. The area outlined in (B) is magnified in (D) to illustrate the anatomy more clearly. The yellow arrow points to the temporal horn of the lateral ventricle, which physically separates amygdala and hippocampus.

(E) A group signal time course from the peak voxel in piriform cortex is depicted for hits (collapsed across valence) and correct rejections, expressed in units of percent signal change. The arrow points to event onset. *Conditions differ significantly at $p < 0.05$.

(F and G) Contrasts of percent signal change from the activation maxima in piriform cortex (F) and hippocampus (G) indicate that positive, neutral, and negative hits (each minus correct rejections) similarly contributed to the effect.

(H) Direct comparison to a nonolfactory fMRI data set (Smith et al., 2004b) that only differed in the pairing of pictures (instead of odors) at encoding still revealed significant neural activity in right piriform cortex (between-group ANOVA; inclusively masked by olfactory hits versus correct rejections at $p < 0.005$).

($Z = 1.36$; $p > 0.6$) piriform cortex (p values corrected for multiple comparisons within the region of interest). Together, these findings imply that the responses observed in piriform cortex cannot be attributed to general effects of associative memory.

We also note additional brain regions (in the current olfactory paradigm) showing enhanced activity for hits versus correct rejections (Table 2). Of these areas, bilateral posterior parietal cortex and left inferior prefrontal cortex have commonly been reported in imaging studies of recognition memory (e.g., Henson et al., 1999; Konishi et al., 2000). Other regions that showed differential activity were right caudate nucleus, left precentral gyrus, left middle temporal gyrus, right cerebellum, left superior temporal sulcus, and right occipital cortex.

Object Recognition: Effect of Emotional Context

Direct comparisons between positive and negative hits revealed how encoding items in different emotional (olfactory) contexts influenced neural activity at the time of their retrieval. As the test phase involved the presentation of neutral objects alone, we avoided conflating effects of emotional memory with intrinsic emotional attributes of the actual test stimuli. The contrast of [positive hits − negative hits] revealed significant activation in left medial orbitofrontal cortex (OFC) (−15, 24, −12; $Z = 3.23$) (Figure 4A), right anterior insula (42, −33, 18; $Z = 3.66$), and midcingulate cortex (−6, −18, 36; $Z = 3.63$). The opposite contrast of [negative hits − positive hits] demonstrated activation in left lateral OFC (−33, 30, −6; $Z = 3.12$), abutting lateral prefrontal cortex (Figure 4B),

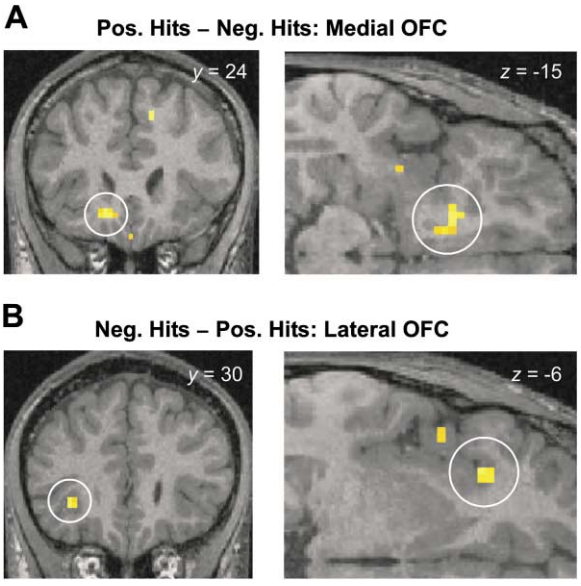

Figure 4. Effects of Emotional Context on Object Recognition

(A) The contrast of positive minus negative hits revealed significant responses in medial OFC. (B) By comparison, negative minus positive hits was associated with neural activity in more lateral aspects of OFC, extending into lateral prefrontal cortex. Activations are superimposed on coronal ([A and B], left side) and horizontal ([A and B], right side) sections from a T1-weighted scan (display, $p < 0.005$).

right hypothalamus (12, −6, −9; Z = 3.89), and left parahippocampal gyrus (−24, −27, −21; Z = 3.49).

Discussion

Our primary aim in this study was to establish whether a retrieval cue in one modality (visual) would elicit sensory-specific neural activity in a different modality (olfactory), following the explicit encoding of cross-modal (odor-object) associations. We show that successful recognition of old objects (compared to correct rejection of new objects) was associated with significant activation in piriform cortex.

The observed effect in piriform cortex was not influenced by emotional context, as responses were present across all levels of valence. Critically, we can be confident that piriform activity was not a result of direct odor stimulation, since object recognition occurred in the absence of odor, nor was it likely to be related to differential breathing patterns (in a complementary behavioral study). Furthermore, direct comparison to a nonolfactory fMRI data set (Smith et al., 2004b) demonstrated that the effect in piriform cortex was specific to association with olfactory content, rather than the retrieval of associative information per se. Finally, the absence of known visual or lexical inputs into olfactory cortex makes it improbable that object perception or verbal mediation could easily account for the data that are seen here. We suggest that retrieval-related responses in piriform cortex provide evidence for the incidental recovery of olfactory context (experienced at encoding). The implication is that representations of the original episode are preserved in modality-specific sensory brain regions. The retrieval of this trace is not contingent upon a necessity to recover actual sensory-specific information at test but may be incidentally reactivated.

Whether piriform cortex is critical to the recognition of objects (which had been encoded with odors) cannot be determined here. It is possible that piriform activation may reflect other aspects of retrieval processing (Rugg and Wilding, 2000) or even retrieval-related olfactory "imagery" (Elmes, 1998; Bensafi et al., 2003). Indeed, because odor imagery has been shown to elicit sniffing (Bensafi et al., 2003), which in turn can elicit activation in piriform cortex (Sobel et al., 1998), it is possible that the piriform activity could indicate odor imagery rather than odor reactivation. However, we think this is unlikely for two reasons. First, in a postscan debriefing, subjects consistently stated that successful object recognition was most commonly associated with the recall of the story (which sometimes led to secondary recollection of the odor name), but not with active odor imagery. No subject reported trying to conjure up actual smells consciously during the test phase. Second, respiratory measurements collected in a supplementary study outside of the scanner did not reveal any significant breathing differences between conditions. Thus, systematic variations in breathing (as a physiological index of odor imagery) probably do not underlie the activity pattern observed in piriform cortex. As such, we feel that a mnemonic role for piriform cortex is the most parsimonious interpretation of our results.

The idea that reactivation of sensory regions that are engaged at encoding underpins episodic retrieval is in keeping with models of episodic memory, whereby the hippocampus binds a distributed trace maintained across sensory-specific regions. Such a system preserves the integrity of the original engram and enables its access by partial or incomplete cues, lending flexibility and adaptability to the memory system (Cohen et al., 1997; Mesulam, 1998). Our results provide strong support for these theories, in so far as we show that the presentation of a partial retrieval cue (old object) is sufficient to trigger the hippocampus. The suggestion that the hippocampus then reconstructs the entire trace (odor-object) across sensory regions is supported by the activation of piriform cortex in the absence of a specific odor cue. As the major recipient of afferent input from olfactory bulb (Haberly, 1998), piriform cortex is a biologically credible repository of olfactory engrams and accords with the findings described here. In contrast, it is less likely that sensory elements of the original trace would be distributed in higher-order olfactory areas, such as OFC or cingulate cortex, where sensory fidelity is inevitably compromised through progressive synaptic convergence and divergence. This factor may help explain the corresponding absence of retrieval-related activity in these particular regions.

Piriform cortex frequently emerges as a candidate for odor learning and memory in animal models, and numerous studies have demonstrated learning-related plasticity in piriform cortex (Schoenbaum and Eichenbaum, 1995; Saar et al., 1999; Mouly et al., 2001). By comparison, the role of piriform cortex in human olfactory memory is not well characterized. Damage to medial temporal lobes disrupts odor memory in humans (Rausch et al., 1977; Jones-Gotman and Zatorre, 1993), but the diffuse nature of such lesions precludes a more precise localization. Recent neuroimaging approaches

have provided some clarification. In two PET studies, odor recognition memory was associated with enhanced piriform cortex activity when compared to odorless baseline scans (Savic et al., 2000; Dade et al., 2002), although the presence of odor during memory testing complicates data interpretation. More recently, an fMRI study of visual-olfactory associative learning showed that a conditioned visual cue, in the absence of odor stimulation, elicited neural activity in piriform cortex (Gottfried et al., 2003). Taken together, these findings imply that olfactory structures support learning-related processes and are in keeping with the present data.

Convergent evidence from animal and human studies increasingly shows that memory for nonchemosensory features might be represented within relevant sensory-specific areas. For example, in single-unit recordings from primate inferotemporal visual cortex, neuronal activity is related to long-term memory both for visual paired associates (Sakai and Miyashita, 1991) and for learned cross-modal associations between visual and auditory stimuli (Gibson and Maunsell, 1997). In humans, neuroimaging studies indicate that subsets of brain regions that are initially activated during the encoding of visual and auditory material are reactivated during retrieval (Nyberg et al., 2000; Wheeler et al., 2000; Vaidya et al., 2002), suggesting a functional overlap of encoding and retrieval processes within higher-order sensory-specific structures. The current results extend these findings to show that similar principles are applicable to the chemosensory domain and are instantiated at the level of primary olfactory (piriform) cortex.

Our paradigm also enabled us to study how emotional (and not just sensory) context influences the neural correlates of object recognition, in order to dissociate emotional memory effects from those related to online processing of affective attributes of the stimuli. Behaviorally, our results are in partial agreement with the idea that memory is enhanced for emotional items (Cahill et al., 1995; Hamann et al., 1999). Successful recognition was greater for positive items than for neutral items, but enhancement was not observed for negative hits. It is notable that similar behavioral profiles have been demonstrated in similar imaging studies that used visual emotional contexts (Erk et al., 2003; Smith et al., 2004a, 2004b). The differential impact of positive and negative emotions on recognition performance may reflect greater attentional capture on the part of highly arousing negative stimuli, detracting from the encoding of concurrently presented neutral stimuli. Alternatively, or in addition, the engagement of different neuromodulatory systems by positive and negative stimuli may have disparate effects on memory processing (see Smith et al., 2004a, 2004b).

The neural substrates of emotional contextual retrieval were primarily expressed in dissociable subregions of OFC. The contrast of positive (versus negative) hits was associated with activity in medial OFC, while negative (versus positive) hits elicited activity in more lateral OFC regions. Similar effects in medial OFC have also been identified in a visual version of this emotional memory paradigm (Maratos et al., 2001). Moreover, these valence-specific patterns have emerged in other studies that span a variety of modalities. Thus, the processing of smells (Gottfried et al., 2002a; Anderson et al., 2003), tastes (Small et al., 2001), faces (O'Doherty et al., 2003), and monetary reinforcers (O'Doherty et al., 2001) has revealed medial-lateral response differences in OFC that vary according to the degree of pleasantness. Similar relationships have been identified in the setting of appetitive and aversive conditioning (Gottfried et al., 2002b). Our findings agree with these observations and suggest that the retrieval of emotional context recruits the same brain regions that are associated with affective processing.

Episodic memory retrieval involves the recollection of information spanning sensory domains and levels of emotional complexity. The challenge for a representational memory system is to link these varied elements into a coherent whole while preserving the integrity of the individual constituents, an organization that may help optimize learning and behavior (Cohen et al., 1997; Mesulam, 1998). The current study allowed us to delineate the neural systems underlying the incidental retrieval of different components of a complex engram. Our findings lend credence to the idea that episodic memory processes rely on the interactions between medial temporal memory systems and domain-specific cortical regions of the brain.

Experimental Procedures

Subjects

Nineteen healthy, right-handed subjects (ten women; mean age, 25 years; range, 18–33 years) consented to participate in the experiment, which was approved by the joint Ethics Committee of the National Hospital for Neurology and Neurosurgery and the Institute of Neurology. No one reported any history of neurological, psychiatric, pulmonary, or ear-nose-throat problems or difficulty smelling. Four volunteers (two women) were eliminated, due to poor behavioral performance (two subjects), excessive head motion (one subject), or technical difficulties (one subject), leaving fifteen subjects for data analysis.

Stimuli

Visual stimuli consisted of 200 emotionally neutral, nonarousing objects preselected from prior studies (Smith et al., 2004a, 2004b). Objects came from a wide range of semantic categories (e.g., animals, tools, vehicles), but any pictures containing olfactory associations (e.g., foods, flowers) were excluded. Objects were presented within yellow boxes on a gray background and back-projected onto a headbox mirror within the scanner.

Olfactory stimuli were comprised of nine odors, three each of positive, neutral, and negative valence. The positive valence odors were orange (50% v/v in mineral oil [m.o]; Absolute Aromas, Alton, UK), rose maroc (undiluted; Aqua Oleum, Stroud, UK), and vanilla (vanillin; 8% w/v in propylene glycol; Sigma-Aldrich, Dorset, UK). The neutral valence odors were β-ionone (1% v/v in m.o.; Sigma), anisole (1% v/v in m.o.; Sigma), and isoamyl formate (1% v/v in m.o.; Sigma). The negative valence odors were trithioacetone (1% v/v in m.o.; Sigma), 4-methylpentanoic acid (1% v/v in m.o.; Sigma), and trimethylamine (1% v/v in distilled water; Sigma). Odors were presented using a ten-channel computer-controlled olfactometer that is suitable for the MRI environment and delivers odor pulses rapidly, without perceptible changes in tactile, auditory, or temperature features (Gottfried et al., 2002a, 2003). Airflow rates were set at 2 l/min. Stimulus delivery of odors and pictures was controlled using Cogent 2000 (Wellcome Department of Imaging Neuroscience, London), as implemented in Matlab 6 (Mathworks, Inc., Natick, MA).

Training

Prior to the main experiment, subjects were exposed to all nine odors and asked to provide verbal descriptors, in order to reduce odor differences in familiarity and nameability at the time of scan-

ning. Subjects were encouraged to think of several different labels (e.g., for orange odor, orange, tangerine, lemon, citrus, grapefruit), so that they might have a flexible semantic repertoire with which to construct stories. If there were difficulties labeling a particular odor, subjects were offered four or five potential names. Subsequently, subjects were given nine training trials, three for each valence level, to practice creating stories or links between odors and pictures, until they felt comfortable with the task. Subjects were instructed to be as inventive as possible by imagining vivid episodic associations that were rich in detail. For example, for the pairing of a picture of a duck and the smell of roses, one subject remarked, "Going to the park with my grandmother to feed the ducks."

Study Phase

Subjects participated in a cross-modal encoding task, during which time they smelled an odor, viewed an object, and imagined a link between the two stimuli. Each trial began with the onset of a central crosshair, which signaled the subject to sniff for 1.5 s (Figure 1A). Odor delivery coincided with the duration of the sniff. After a 0.5 s pause, a picture appeared for 5 s, while the subject thought of an associative link between the odor and the object. There were 135 trials (45 events per 3 valence levels), which recurred every 11.5 s. Thus, each object was presented once, and each odor was presented 15 times. No odor occurred more than once every four trials, to limit olfactory habituation, and no odor valence level occurred more than twice in a row. Odor-object pairs were randomly assigned and counterbalanced across subjects, with the constraint that each object was paired an equal number of times with each odor valence, to prevent any systematic relationships between particular pictures, odors, and valence levels. To reduce subject fatigue, scanning was divided into two sessions of 13 min each.

Test Phase

The object recognition task took place 5 min after the study phase. During each trial, subjects had to decide whether they were viewing a study (old) or a novel (new) object. Objects were each presented for 1 s, and subjects responded as quickly and accurately as possible, using a pushbutton (Figure 1B). Trials recurred every 3.2 s. The events comprised the 135 old pictures seen at study and 65 new pictures, for a total of 200 events. Object presentation order was randomized across subjects. The test phase was divided into two scanning sessions (5.5 min each). Airflow was shut off during this phase. Subjects were explicitly told that odors would not be delivered during this time, and that they would not need to make any more sniffs.

Behavioral Data

Mean accuracy and reaction times were computed from subjects' responses during the test phase. Post hoc valence ratings for the nine odors (-10, extremely unpleasant; $+10$, extremely pleasant) were also collected from every subject, and means were determined for each valence level. Subjects were also debriefed postscanning and questioned about task performance and strategy during the memory test.

In a supplementary behavioral study, respiratory data were collected from an additional group of 15 subjects. This took place outside the scanner but was otherwise identical to the primary fMRI experiment. Breathing was recorded online using abdominal and thoracic breathing belts that were coupled to a pressure sensor, sampled on computer, and analyzed offline (Gottfried et al., 2002a). Subject-specific respiratory waveforms were time locked to event onset, baseline corrected, and averaged across each condition type. The mean volume of the first inhalation following event onset, peak amplitude of inhalation, latency to peak inhalation, and average level of respiration over the event duration were each computed. For statistical analysis, the data corresponding to the four correct response conditions (positive, neutral, and negative hits and correct rejections) were entered into a series of repeated-measures ANOVAs, with adjustments for nonsphericity.

Image Acquisition and Preprocessing

Gradient-echo T2*-weighted echoplanar images (EPI) with blood oxygen level-dependent contrast were acquired using a Siemens Sonata 1.5 T MRI scanner (Erlangen, Germany). Images were acquired 30° to the bicommissural line (rostral > caudal), using z-shimming in the slice-selection direction to reduce signal dropout in orbitofrontal cortex (Deichmann et al., 2003). We collected 40 slices per volume, which provided near whole-brain coverage, apart from the vertex. Imaging parameters were as follows: TR, 3.6 s; TE, 50 ms; field of view, 192 mm; in-plane resolution, 3 mm; slice thickness, 2 mm; interslice gap, 1 mm. For each of the two study sessions, 225 volumes were acquired, as well as another 97 volumes for each of the two test sessions (all minus five "dummy" volumes to permit T1 equilibration). Spatial realignment (Friston et al., 1995a), slice timing, spatial normalization, and smoothing (8 mm), were performed using SPM2 (Wellcome Department, London, UK). Anatomical T1-weighted scans were also acquired and coregistered with each subject's normalized mean EPI image.

Data Analysis

The event-related fMRI data were analyzed with SPM2 (Wellcome Department, London), using the general linear model (Friston et al., 1995b). On the basis of each subject's responses at test, trials were classified into one of seven conditions: positive hits (correct old responses, where "positive" refers to an object that had been paired with a positive-valence odor at study), neutral hits (paired with a neutral-valence odor at study), negative hits (paired with a negative-valence odor at study), false alarms (incorrect old), correct rejections (correct new), misses (incorrect new), and no response. The onset times for the seven test conditions ($\times 2$ sessions) were modeled as stick (δ) functions, then convolved with a canonical hemodynamic response function (HRF) and its temporal and dispersion derivatives, which formed regressors of interest. Subject-specific movement parameters and a high-pass filter (128 s) were included as regressors of no interest. Condition-specific β values, corresponding to the HRF peak, were estimated at each voxel, after correction for serial correlations. Relevant contrasts of parameter estimates from all subjects (collapsed across sessions) were then entered into one-sample Student's t tests (random-effects analysis).

In a separate fMRI model, the condition-specific event onsets were convolved with finite impulse response (FIR) functions to provide estimates of the percent change in hemodynamic signal at successive 3 s intervals. This illustrative model was used to depict group signal time courses from the peak voxel in piriform cortex.

We report significant activations at a threshold of $p < 0.001$ uncorrected in regions predicted a priori, including memory-related areas in inferior prefrontal and posterior parietal cortices, primary olfactory (piriform) cortex, and emotion-related structures in OFC. Due to the recognized complications of recovering fMRI signals in medial temporal lobe (Ojemann et al., 1997), we set a threshold of $p < 0.005$ uncorrected in reporting hippocampal activations. An extent threshold was not applied ($k = 0$). Voxels are reported in Montreal Neurological Institute (MNI) coordinate space. For display, the right side of the image corresponds to the right side of the brain.

Acknowledgments

We thank J.S. Winston, J.M. Kilner, and H. Spiers for helpful comments. J.A.G. is supported by a Howard Hughes Physician-Postdoctoral Fellowship. The work was carried out under a Wellcome Trust Programme Grant to R.J.D.

Received: January 6, 2004
Revised: February 27, 2004
Accepted: April 13, 2004
Published: May 26, 2004

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