Chem. Senses 34: 513–521, 2009

doi:10.1093/chemse/bjp026
Advance Access publication May 28, 2009

# The Role of the Human Hippocampus in Odor–Place Associative Memory

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## Abstract

Hippocampal lesions in rodents impair both object–place and odor–place associative memory. Subjects with hippocampal damage have impaired associative memory such as object–place memory. Whereas studies have investigated some types of associative memory, no investigation has specifically examined odor–place associative memory in subjects with well-defined amnesia. It is unknown whether amnesic subjects with hippocampal damage would be impaired on an odor–place associative task. We investigated the effect of hippocampal damage in amnesic subjects with hippocampal atrophy on odor–place associative memory and recognition memory tasks. Amnesic and healthy comparison subjects matched for age and education were tested on an odor–place associative task, an odor recognition task, and a place recognition task. The odor–place associative task required subjects to associate 6 odors with 6 spatial locations on a board. The recognition tasks required subjects to identify the 6 odors and the 6 locations that were presented during the associative task. Amnesic subjects were impaired for odor–place memory and place recognition, but not odor recognition compared with comparison subjects. These results suggest that the human hippocampus is necessary for odor–place associative memory and spatial recognition memory. These data provide support for the idea that odor–place associative memory is mediated by the hippocampus in both humans and rodents, suggesting an evolutionary continuity in cognitive function assigned to the hippocampus.

**Key words:** amnesia, associative memory, hippocampus, odor–place paired associate, recognition memory

## Introduction

Animal and human studies have shown that critical structures involved in memory include the hippocampus and adjacent cortical structures including the perirhinal, entorhinal, and parahippocampal cortices (Squire and Zola-Morgan 1991). There are numerous theories of hippocampal function and some agreement regarding the mnemonic functions of the hippocampus in terms of the nature of memory representation and the processes that support these memory representations. According to one theory, the hippocampus (*Cornu Ammonis* fields, dentate gyrus, and subiculum) supports mechanisms of associative learning and memory that bind features connected with an event into an integrated memory trace by linking neuronal activation from multiple sensory modalities; sight, sound, smell, etc. (Eichenbaum 2000; Kesner et al. 2000; Brown and Aggleton 2001; O'Reilly and Rudy 2001; Davachi et al. 2003).

Behavioral studies have examined the effects of hippocampal lesions on the formation of arbitrary associations using associative learning paradigms. For example, associative memory is important for remembering where things are located in the world around us (object–place associations). In humans, nonhuman primates, and rodents, hippocampal damage

impairs acquisition of object–place associations (Kessels et al. 2001; Gilbert and Kesner 2004; Crane and Milner 2005; Rolls et al. 2005; Gilbert et al. 2008). Further, studies in humans find that an intact hippocampus is required for face–house (Stark et al. 2002), word–word (Davachi and Wagner 2002; Meltzer and Constable 2005), object–context (Goh et al. 2004), and face–name (Chua et al. 2007) associative memory. However, information regarding the contribution of the human hippocampus in associative memory is incomplete, as studies to date have used item pairs that involve only pictorial, verbal, and/or spatial information.

Until recently, the neural substrates of olfactory memory have not been widely studied in humans. It is known that rodents with hippocampal lesions have impaired olfactory memory (Eichenbaum 1998; Ergorul and Eichenbaum 2004). Functional magnetic resonance imaging (fMRI) studies show activation of a complex network of brain regions during an olfactory recognition memory task. The active regions include the hippocampus, prefrontal cortex, fusiform/parahippocampal gyrus, lateral, and medial parietal areas (Cerf-Ducastel and Murphy 2006). Olfactory memory deficits have been observed in elderly populations and in patients with Alzheimer's disease with concomitant hippocampal dysfunction (Murphy et al. 1991, 1997, 2002, 2003). The data in elderly populations suggest that impaired memory for odors may be an early indicator of cognitive impairment and increased risk for neurodegenerative disease in nondemented older adults (Devanand et al. 2000; Gilbert and Murphy 2004; Wilson et al. 2007; Djordjevic et al. 2008; Gilbert et al. 2008). Whereas patients with Alzheimer's disease have impaired odor memory, these individuals have damage to the hippocampus plus temporal lobe and other brain regions, so the contribution of the hippocampus to odor memory remains unclear.

In rats, studies have assessed olfactory information in addition to spatial and object information as part of the to-be-remembered item pairs in associative memory tasks. Rodents with hippocampal lesion have impaired odor–place paired-associative memory (Gilbert and Kesner 2002). Gilbert et al. (2008) assessed memory for odor–place and object–place associations in healthy older adults. The older adults committed more errors during an odor–place associative memory task than during an object–place task. The older adults had intact immediate recognition memory for the individual odors and spatial locations used in the associative memory task. These data suggest that odor–place associative memory, like odor memory in general, may be sensitive to age-related memory decline (Gilbert et al. 2008).

To date, only a few studies have examined olfactory memory deficits in patients with circumscribed damage to the hippocampus. Levy et al. (2004) assessed odor recognition memory in amnesic subjects with hippocampal damage. The amnesic subjects had impaired odor recognition memory at a 1-h retention delay, but not at a 5-min retention delay, suggesting that odor recognition memory depends on the integrity of the hippocampus (Levy et al. 2004). It is not fully known to what extent the human hippocampus mediates olfactory memory, particularly in terms of associative memory (e.g., odor–place memory).

The hippocampus is known to be particularly vulnerable to damage following anoxia, and structural imaging and postmortem studies demonstrate relatively selective bilateral neuropathology of the hippocampus following an anoxic brain injury (Gadian et al. 2000; Kesner and Hopkins 2001; Manns, Hopkins, Reed, et al. 2003; Manns, Hopkins, and Squire 2003; Di Paola et al. 2008). Such individuals generally display a "pure" amnesic syndrome, with dense memory impairments but relative sparing of nonmnemonic functions such as intelligence and attention (Manns, Hopkins, and Squire 2003; Hopkins et al. 2004). Because amnesic subjects with hippocampal damage have impaired associative memory for certain types of information (Mayes et al. 2002; Giovanello et al. 2004; Turriziani et al. 2004; Holdstock et al. 2005), these subjects represent an ideal circumstance to explore odor–place associative memory, a type of associative memory previously not assessed in humans.

The aim of the present study was to explore odor–place associative memory in amnesic subjects with selective bilateral hippocampal damage compared with healthy comparison subjects. Because the parameters of the odor–place associative memory task (Gilbert et al. 2008) are similar to that used with rats, the current study provides comparison of associative memory across species using an analogous task. To that end, odor–place associative memory was examined in amnesic subjects who, other than their amnestic deficits, were nondemented and cognitively intact with normal intellectual function. Given that rodents with hippocampal lesions have impaired odor–place paired-associative learning (Gilbert and Kesner 2002), we hypothesized that humans with selective hippocampal damage would have impaired odor–place associative memory.

## Materials and methods

### Subjects

Amnesic subjects with hippocampal damage and healthy comparison subjects matched for age, gender, and education level were included in this study. There were 4 amnesic subjects, 3 males and 1 female, and 4 comparison subjects, 3 males and 1 female. The mean age of the amnesic subjects was 44.25 ± 5.17 years (range 30–54 years) with a mean educational level of 12.75 ± 0.25 years (range 12–14 years). The mean age of the comparison subjects was 38.5 ± 6.34 years (range 24–60 years; $t = 0.16$, $P = 0.88$) with a mean educational level of 13.0 ± 0.52 years (range 12–13 years; $t = 0.05$, $P = 0.94$). Neither the amnesic nor the comparison subjects had prior neurological disorders, alcohol or drug abuse, or psychiatric disorders. The amnesic subjects, as shown by their performance in other memory studies, have stable

nonprogressive cognitive deficits. This study was approved by the Brigham Young University Institutional Review Board and conformed to institutional and federal guidelines for the protection of human subjects. Written informed consent was obtained prior to behavioral testing in all subjects.

### Magnetic resonance imaging

magnetic resonance (MR) images were acquired at General Electric 3.0-T Scanner (GE Medical Systems) using standard clinical protocols. Sagittal $T_1$-weighted (repetition time/echo time [TR/TE]/excitations = 500/11/2) images were acquired and used for localization with a 24-cm field of view. With the midsaggital image as a reference, axial followed by contiguous axial proton density (TR/TE = 2500/15) and contiguous $T_2$-weighted (TR/TE = 5253/93.6) spin-echo images were acquired, with a slice thickness of 5 mm. Images were acquired on a 256 × 256 matrix with a 22-cm field of view for the axial images. Contiguous $T_1$ coronal images were acquired (TR/TE = 13/4.47), 1.2-mm thick, followed by coronal contiguous $T_2$-weighted images (TR/TE = 3500/114), 1.5-mm thick. Images were acquired with a field of view of 25.6 cm on a 256 × 256 matrix. A neuroradiologist rated all scans for gross lesions or other abnormalities.

### Volumetric image analysis

Proton density and $T_2$ axial dual-echo images were quantified as described by Blatter et al. (1995) using the software ANALYZE 5.0/6.0 (Biomedical Imaging Resource). The original 16-bit images were converted to 8-bit images in ANALYZE file format. A multistep volume analysis was then performed using several image-processing tools available in ANALYZE. Regions of cerebral spinal fluid (CSF), white matter, and gray matter were defined by the user and plotted in a 2-dimensional feature space. Quantitative MR analyses of the temporal lobe gyri were performed as per the methods described previously (Bigler et al. 1997, 2002).

Quantitative MR analyses of brain structures were performed on all amnesic subjects as per the methods described previously (Bigler et al. 1997, 2002). Volumes of the following brain structures were determined by using the region of interest (ROI) feature of ANALYZE that yields a count of gray matter, white matter, and CSF: lateral ventricles, third ventricle, fourth ventricle, temporal horns, total brain volume, and CSF.

Volumes of the following brain structures were determined by using the ROI feature: hippocampus, parahippocampal gyrus, fusiform gyrus, inferior temporal gyrus, middle temporal gyrus, and superior temporal gyrus (Bigler et al. 2002). The volumes of the temporal horn of the lateral ventricle, rhinal sulcus, inferior temporal lobe sulcus, and sylvian fissure were quantified as well. Temporal lobe volumes encompassed portions of Brodmann areas 20, 21, 22, 25, 27, 28, 34, 36, 37, 38, 41, and 42. For gyral volumes, the total number of gray matter and white matter pixels within the ROI for each section were summed together and multiplied by the voxel dimension; the CSF pixels were used to determine sulcal and temporal horn volumes. Tracing was done in the coronal plane, and all 3 planes were used to cross-check anatomical markers. We followed a previously published protocol for the temporal lobes (Bigler et al. 2002). Intrarater and interrater reliability exceeded 0.90.

Hippocampal volumes were measured in the coronal slices (Bigler et al. 1997). The hippocampal formation was manually traced in a posterior to anterior direction. The starting slice was identified using the following anatomical landmarks: 1) good separation of the lateral ventricles, 2) the appearance of the pulvinar of the thalamus, and 3) the appearance of the corpora quadrigemina. Measurement of the hippocampal formation was discontinued when the temporal horn of the lateral ventricle extended more than halfway across the width of the hippocampus. Intrarater and interrater reliability exceeded 0.90.

### Neuropsychological tests

Amnesic and comparison subjects were administered standardized neuropsychological tests to assess memory, general intellectual ability, and visual–motor function. Memory function was assessed with the Wechsler Memory Scale-III (Wechsler 1999), and intellectual function was assessed with the Wechsler Abbreviated Scale of Intelligence (Wechsler 1997). Visual–motor function and spatial memory was also assessed with the Rey–Osterrieth Complex Figure test (Meyers J and Meyers K 1995).

### Behavioral procedures

#### *Odor–place associative memory task*

The odor–place paired-associate task (Gilbert et al. 2008) used a blackboard (60 cm wide × 75 cm long) with 12 white circles (0.5 cm diameter) randomly positioned to define 12 arbitrary spatial locations. Hinged doors were attached to each side of the board, which allowed the board to be hidden from the view of the subjects. There were 12 salient odors that included the following: baby powder, Brut cologne, chocolate, cinnamon, coconut, coffee, dill, garlic, peppermint, pine, soap, and Vicks VapoRub . The odors used in the present study were selected from a published set of odors used in numerous studies by Murphy and colleagues (Murphy et al. 1997; Nordin and Murphy 1998; Hamilton et al. 1999; Cerf-Ducastel and Murphy 2006). The odors used in these published studies were found to be highly identifiable in both young and older adults; therefore, the odors were selected for use in the present study. The associative memory task consisted of a presentation and test phase. During the presentation phase, each subject was presented with 6 randomly selected odors, in a different order to each subject. Odors were presented in small opaque glass jars with a filter paper top that was permeable to the odor but hid the contents of the jar from view. Due to the saliency of odors, the odors could be verbally

identified, but such identification was not required in the present study. Subjects were given full disclosure of the demands of the odor–place paired-associate task. Each subject was given 5 s to smell the odor, after which the jar containing the odors was randomly placed on one of the white circles for 5 s (target location). Of the 12 white circles, 6 were randomly chosen as target locations and the other 6 circles were distractor locations. The jar was then removed from the board, and the hinged doors were raised to hide the board from view of the subjects. Otherwise, the subjects would be able to see and rehearse the spatial location during the interstimulus interval (ISI). Following a 30-s ISI, a second randomly selected odor was presented for 5 s and then paired with a different target location for 5 s. This procedure was followed until all 6 odors were presented and paired with 6 differing target locations. Following the last 30-s ISI, the hinged doors were opened again revealing the blackboard with 12 white circles. The subject was randomly given 1 of the 6 odors and then asked to place the odor in its paired location. Odors were presented randomly and in a different order as they were presented previously in order to eliminate the recency and primacy effect. For example, we randomly presented 6 odors (e.g., A, B, C, D, E, and F) during the presentation phase and then again randomized the order of odors during the test phase (e.g., B, D, F, E, C, and A). Once the subject had placed the jar on 1 of the 12 white circles, the location was recorded and the jar was removed from the board. Then the next odor was presented, and the subject was asked to place the odor in its paired location. This procedure continued until all 6 odors had been individually presented to the subject and the subject had placed each odor to its paired location.

The responses of the subjects were recorded as a correct pair, incorrect pair, or location error. An odor placed in its correctly paired location was recorded as a correct pair, an odor placed in a target location where a different odor was paired was recorded as an incorrect pair, and an odor placed in 1 of the 6-distractor locations where no odor was paired was recorded as a location error. In addition to the total number of correct responses, incorrect pairs and location errors were summed to obtain total errors. For our statistical analyses, we used error type (incorrect pair and location error) as our dependent variable, in order to assess what type of errors subjects were making. Thus, the correct responses are not included in an analysis of errors.

### *Recognition memory*

After the odor–place paired-associative task and without prior knowledge, subjects completed an odor recognition task and location recognition task. Subjects were not told that they would be given a recognition memory task after the associative memory task. The order of the odor and place recognition tasks was counterbalanced across subjects. For the odor recognition task, subjects were randomly and individually presented 12 odors (6 odors were previously presented during the associative task and 6 odors were new) and asked to identify whether each odor was presented during the odor–place paired-associative task (yes/no answer). The dependent variable was percent correct of odors correctly identified as previously presented and odors correctly identified as new (not previously presented). For the location recognition task, the entire board was presented to the subjects. For each dot, the subject was asked if that dot was a location where an odor was or was not presented (yes/no answer). The dependent variable was the percent correct—locations correctly identified as having an odor and locations that were not paired with an odor correctly identified.

For the odor recognition task, each response was recorded as a hit (i.e., correctly responded yes), miss (i.e., wrongly responded no), correct rejection (i.e., correctly responded no), or false positive (i.e., wrongly responded yes). Percent correct was calculated by adding together the hits and the correct rejections and dividing by 12, the total number of odors used in the recognition task.

For the place recognition task, each response was recorded as a hit (i.e., correct yes response), miss (i.e., wrong no response), correct rejection (i.e., correct no response), or false positive (i.e., wrong yes response). Percent correct was calculated by adding together the hits and correct rejections and dividing by 12, the total number of possible locations.

Alternatively, for the place recognition task, the location in which an odor was paired changed based upon the subjects' odor–place pairings if an incorrect pair or location error was made. Therefore, were subjects recognizing the locations they paired with an odor or were they recognizing the locations that actually had an odor pairing? The first place recognition analysis (see above paragraph) calculated responses based upon original and actual odor–place associations (i.e., those presented by the experimenter). This second place recognition analysis calculated responses based upon subjects' odor–place associations whether the associations were a correct pairing, an incorrect pairing, or a location error (i.e., the locations reported by the subjects, whether correct or incorrect). In other words, each response was also recorded as a hit (i.e., correct yes response), miss (i.e., wrong no response), correct rejection (i.e., correct no response), or false positive (i.e., wrong yes response) according to the subjects' responses during the test phase of the associative memory test. Percent correct was calculated by adding together the hits and correct rejections and dividing by 12, the total number of locations.

## Results

### Quantitative magnetic resonance imaging

Clinical brain magnetic resonance imaging (MRI) reports read by a neuroradiologist indicated no evidence of extrahippocampal lesions or other structural abnormalities for any of the amnesic subjects in that whole brain and ventricular volumes did not significantly differ from comparison subjects

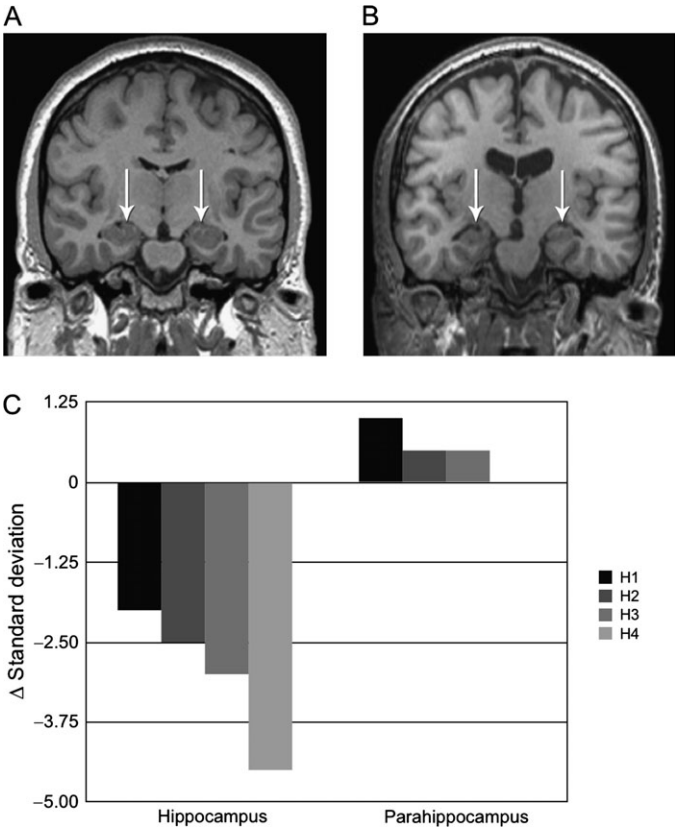


**Figure 1** Clinical brain MRI reports by a neuroradiologist for the amnesic subjects indicated no evidence of lesions or other structural abnormalities other than within the hippocampus. Shown are brain scans from **(A)** a healthy comparison and **(B)** an amnesic subject with bilateral hippocampal damage. Amnesic subjects had hippocampal volumes reduced by an average of 20%. **(C)** For each amnesic subject, graphed is the number of standard deviations away from the MRI comparison subjects' hippocampus and parahippocampal gyrus means. H4 parahippocampal volume was 0 standard deviations from the MRI comparison subjects and therefore does not appear on the graph.

(see Figure 1). The mean hippocampal atrophy for all amnesic subjects was 22.25 ± 0.11%. Using quantitative MRI, all 4 of the amnesic subjects had significant hippocampal atrophy as the right (mean = 1.91 ± 0.22 $cm^3$) and left (mean = 1.92 ± 0.25 $cm^3$) hippocampal volumes were more than 1 standard deviation below the normal MRI comparison subjects (right hippocampus mean = 2.55 ± 0.13 $cm^3$ and left hippocampus mean = 2.48 ± 0.13 $cm^3$). The amnesic subjects had significant hippocampal atrophy compared with normal MRI comparison subjects for the right hippocampus ($t = 6.47$, $P < 0.0001$) and for the left hippocampus ($t = 4.79$, $P < 0.001$), respectively. There were no differences in any temporal lobe gyri volumes for the amnesic subjects compared with comparison subjects.

## Neuropsychological tests

The results of the amnesic and comparison subjects' neuropsychological tests are shown in Table 1. The amnesic subjects' verbal intelligence quotient and performance intelligence quotient, and full scale intelligence quotient were within normal range. The amnesic subjects had memory impairments on all memory indices except for the auditory recognition index and working memory index, which is a measure of frontal lobe function. The amnesic subjects' memory scores were at least 1 standard deviation lower than the standardized mean on the immediate and general memory indices of the WMS-III but were within normal range on the working memory index. The amnesic subjects' visual–motor function on the Rey–Osterrieth Complex Figure test was within average range, but spatial memory was below average. Based on these tests, amnesic subjects exhibited impaired memory with spared intellectual function, working memory, and visual–motor function.

**Table 1** Neuropsychology test results for the amnesic and comparison subjects

| Neuropsychology test and subtest | Amnesic Mean ± SD | Comparison Mean ± SD | *P* value |
|---|---|---|---|
| WASI | | | |
| Full scale IQ | 101.5 ± 5.7 | 110.4 ± 10.5 | 0.18 |
| Verbal IQ | 98.3 ± 5.9 | 105.2 ± 8.0 | 0.19 |
| Performance IQ | 104.3 ± 13.6 | 112.8 ± 16.1 | 0.43 |
| WMS-III | | | |
| Immediate memory index | 73.0 ± 22.0[a] | 101.8 ± 13.7 | 0.046 |
| General memory index | 80.5 ± 25.6[a] | 113.0 ± 14.2 | 0.045 |
| Working memory index | 95.3 ± 10.8 | 112.0 ± 10.5 | 0.055 |
| ROCFT | | | |
| Copy | 33.3 ± 2.6 | n/a | n/a |
| Immediate recall | 9.9 ± 8.7[a] | n/a | n/a |
| Delayed recall | 11.0 ± 9.2[a] | n/a | n/a |

[a]Scores are more than 1 standard deviation (SD) below the standardized mean. WASI: Wechsler Abbreviated Scale of Intelligence; WMS-III: Wechsler Memory Scale-III; ROCFT: Rey–Osterrieth Complex Figure; n/a: not available.

## Behavioral procedures

### *Odor–place paired-associative task*

The results of the odor–place paired-associate task are shown in Figure 2A. A 2 × 2 analysis of variance (ANOVA) with errors (incorrect pair and location error) as the within factor and groups (comparison and amnesic) as the between factor found a significant error effect [$F(1,12) = 4.91$, $P = 0.05$], a significant group effect [$F(1,12) = 34.91$, $P < 0.0001$], and a significant error by group interaction [$F(1,12) = 8.73$, $P = 0.01$]. Amnesic subjects also made more incorrect pairs ($P < 0.0001$), but not location errors ($P = 0.12$) compared with comparison subjects. Within the amnesic group, subjects made more incorrect pairs than location

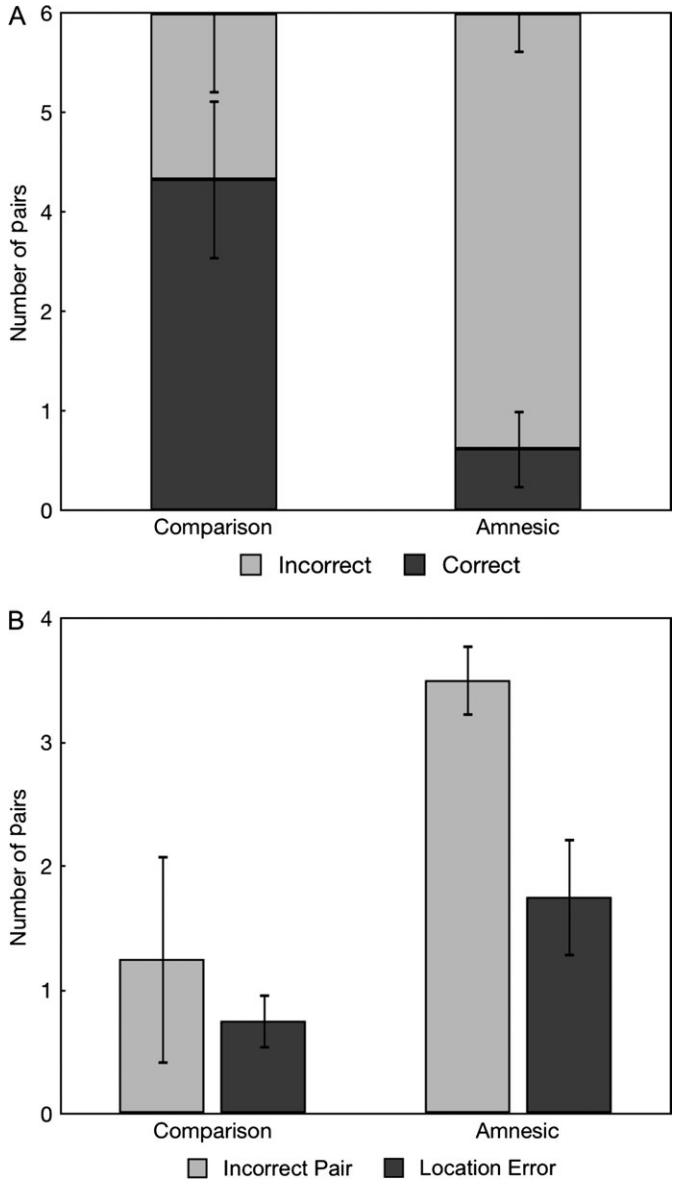


**Figure 2** **(A)** The figure shows the mean ± standard deviation (SD) number of correct and incorrect odor–place pairs (6 total pairs) in comparison subjects and amnesic subjects. Amnesic subjects were impaired compared with comparison subjects ($P < 0.05$) for odor–place associative memory. **(B)** The figure shows the mean ± SD number of total errors, incorrect pairs, and location errors in comparison subjects and amnesic subjects. Overall, amnesic subjects made more incorrect pairings than location errors compared with comparison subjects.

errors ($P = 0.02$). Within the comparison group, subjects made similar numbers of incorrect pairs and location errors ($P = 0.54$). Overall, amnesic subjects were impaired compared with comparison subjects making more incorrect pairs than location errors (Figure 2B).

### *Recognition memory*

The results of the odor recognition task are shown in Figure 3. The amnesic and comparison subjects were able to correctly identify the odors that were used during the paired-associative task, 80% versus 92%. An ANOVA with the percent correct as the within factor and groups (comparison and amnesic) as the between factor found no difference in performance between comparison and amnesic groups [$F(1,6) = 1.06$, $P = 0.33$]. Therefore, the amnesic subjects' performance for odor recognition memory was the same as the comparison subjects.

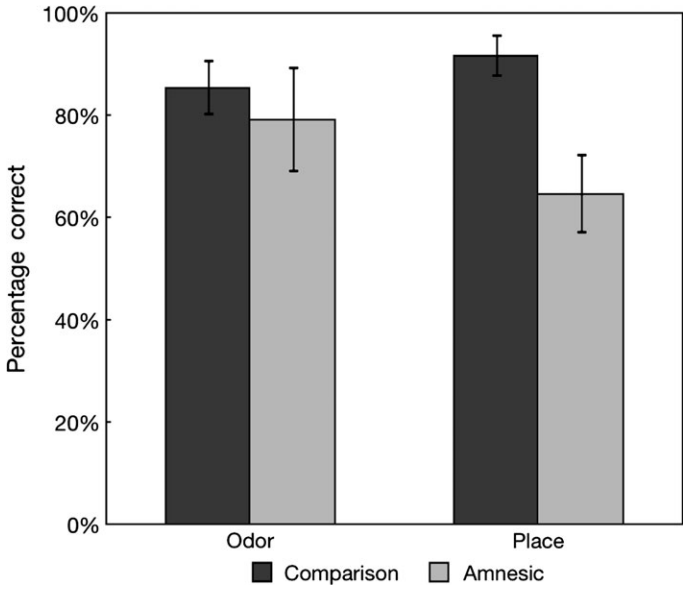


**Figure 3** The figure shows the performance of comparison subjects and amnesic subjects on the odor and place recognition tasks. Amnesic subjects had impaired place recognition ($P = 0.03$), but not odor recognition, compared with comparison subjects ($P = 0.33$).

The results of the place recognition task are shown in Figure 3. An ANOVA with the percent correct as the within factor and groups (comparison and amnesic) as the between factor found a significant difference [$F(1,6) = 2.90$, $P = 0.03$] for place recognition memory, indicating that comparison subjects outperformed the amnesic subjects. Amnesic subjects were impaired on the place recognition task compared with comparison subjects, 65% versus 92%, respectively.

An alternative place recognition analysis calculated responses based upon subjects' odor–place associations whether the associations were a correct pairing, an incorrect pairing, or a location error (i.e., the locations reported by the subjects, whether correct or incorrect). A 1-way ANOVA with the percent correct as the within factor and groups (comparison and amnesic) as the between factor found a significant difference for place recognition memory [$F(1,6) = 5.86$, $P < 0.0001$], indicating that comparison subjects outperformed the amnesic subjects. Thus, the amnesic subjects were impaired on the place recognition task compared with comparison subjects, 81% versus 98%. Amnesic subjects made fewer place recognition errors when responses were analyzed using their associations and not the original associations, 81% versus 65%.

## Discussion

Although impairments in associative memory have been well documented for pictorial, verbal, and/or spatial information, this is the first study to demonstrate that amnesic subjects with hippocampal damage have impaired odor–place associative memory. The majority of errors made by all subjects (comparison and amnesic) on the odor–place paired-associative task involved placing the odor in a target location where a different odor had been paired, instead of placing the odor in 1 of the 6-distractor locations where no odor was paired. This finding suggests that both groups were familiar with the specific odors, and spatial locations presented in that amnesic subjects were more likely to place odors in a wrong target location rather than in a distractor location. Overall, amnesic subjects were unable to associate which odor went with each specific location.

Our findings are similar to those observed in rodents with hippocampal lesions that were significantly impaired in learning an odor–place associative task but were able to discriminate both the odors and the spatial locations (Gilbert and Kesner 2002). Human studies corroborate these findings that the hippocampus supports associative memory. For example, Stark et al. (2002) showed that retrieval of cross-modal associations elicits hippocampal activity in an fMRI. Further, the human hippocampus is required for face–house (Stark et al. 2002), word–word (Davachi and Wagner 2002; Meltzer and Constable 2005), object–context (Goh et al. 2004), and face–name (Chua et al. 2007) paired-associative memory. Thus, the hippocampus in both rats and humans appears to be critically involved in memory in associative memory including odor–place associations.

Our amnesic subjects' odor recognition memory did not differ from the comparison subjects. The amnesic and comparison subjects were able to correctly identify the odors that were used during the paired-associative task, 80% versus 92%. Although, we did not formally assess olfactory sensitivity, previous research shows that several of our amnesic subjects have intact olfactory sensitivity (Levy et al. 2004). Amnesic and comparison subjects were able to discriminate between the odors, at least at relatively short delays (5–10 min). Therefore, the observed deficits in the amnesic subjects on the odor–place paired-associate task were not due to an inability to discriminate odors. Similar findings suggest that rats with hippocampal lesions are able to discriminate (e.g., recognize) odors using an analogous task (Gilbert and Kesner 2002). Rodent studies indicate that the hippocampus does not mediate short-term memory for odors (Otto and Eichenbaum 1992; Dudchenko et al. 2000). This finding is supported by human amnesic subjects with hippocampal damage who had impaired odor recognition at a 1-h retention delay, but not at a 5-min retention delay (Levy et al. 2004). The delay between the odor–place paired-associate task and the odor recognition task in the current study was approximately 5–10 min, which is much shorter than the 1-h retention deficit but similar to the 5-min retention delay findings reported by Levy et al. (2004). Another possible explanation for the spared odor recognition may be the amount of hippocampal atrophy in our amnesic subjects. Our subjects had approximately 25% hippocampal atrophy compared with comparison subjects, and no subject had complete hippocampal damage. Squire and colleagues (Broadbent et al. 2004; Gold and Squire 2005) suggest that when hippocampal damage is less than 40% atrophy, some aspects of memory, in particular recognition memory, may be spared despite documented hippocampal damage.

Amnesic subjects were impaired on the place recognition task compared with comparison subjects, 65% and 92%, respectively. Even if place recognition was calculated based on the subjects' recognition of places from their odor–place pairings (whether a correct pairing, an incorrect paring, or location error) and not the original odor–place pairings, amnesic subjects were impaired for place recognition compared with comparison subjects, 81% versus 98%. The amnesic subjects had spared odor recognition memory but impaired place (e.g., spatial) recognition memory. Impairments in recognition memory are dependent on the nature of the task, its complexity, and extent of medial temporal lobe damage (Squire et al. 2007). Thus, the spatial recognition impairment in our amnesic subjects may be due to the nature of the stimuli (e.g., spatial location). It is well documented that the human hippocampus supports spatial memory (Maguire et al. 1997; Barrash 1998; O'Keefe et al. 1998; Kessels et al. 2001). Nunn et al. (1999) found that memory for object location postively correlated with the amount of preserved hippocampus in patients with right medial temporal lobe resection. Further, amnesic subjects with bilateral hippocampal damage have impaired item and order recognition memory for spatial locations (Hopkins et al. 1995). These findings suggest that the hippocampus is important for spatial recognition memory. Altogether our findings support the idea that the hippocampus may be required for spatial recognition memory but other neural substrates may subserve odor recognition memory.

The results of the present study differ from the study of Gilbert et al. (2008), which found declining odor–place associative memory in older versus younger adults but spared recognition memory for both odors and spatial locations, which is different from our finding of impaired spatial recognition memory. As stated previously, hippocampal atrophy is implicated in age-related pathologies, and therefore, we expected similar results to the study of Gilbert et al. (2008). However, the older adults in the study of Gilbert et al. (2008) were healthy and presumably without pathological hippocampal damage. One possible explanation for impaired place recognition but spared odor memory in our amnesic subjects is that recognition memory may relate more to spared neocortical function even in the presence of hippocampal damage, especially when entorhinal and perirhinal cortical areas are intact, as appears to be the case in our

subjects. Decreased olfactory recognition memory is associated with increased severity of Alzheimer's disease, likely due to progressive damage to temporal lobe structures in addition to the hippocampus (Moberg et al. 1987). The progressive neuropathology in Alzheimer's disease involves temporal cortices, including damage to the entorhinal and perirhinal cortices and hippocampus, and is associated with increasingly severe memory impairment (Esiri and Wilcock 1984; Price et al. 1991; Braak H and Braak E 1997). As noted above, impaired recognition memory is dependent on the extent of medial temporal lobe damage (Squire et al. 2007). Thus, the impaired place recognition memory but spared odor recognition memory in our amnesic subjects is likely due to the intact temporal cortices and restricted hippocampal damage.

There are important limitations to the present study. First, all odors were verbally identifiable due to the saliency of the odors. Because there was no way to prevent subjects from verbalizing the odors, we cannot entirely discredit the possibility of our subjects using additional associations besides odor–place associations such as word–place, word–odor, etc. to solve the task. However, odor identification was not part of the current study. We also recognize that the odor–place associative memory deficits in the amnesic subjects may be secondary to impaired spatial memory. Until amnesic subjects with focal hippocampal damage complete an odor–object associative memory task, such claims cannot be made with certainty. Finally, our study involved a small sample size, but this is due to the rarity of amnesic subjects with pathology restricted to the hippocampus. However, the uniqueness of the current amnesic subjects is also the major strength of this study. These amnesic subjects represent an ideal circumstance to explore the role of the human hippocampus in odor–place associative memory. Another strength of our study is that we used an analogous odor–place paired-associative memory task that was used in rodents (Gilbert and Kesner 2002). Our findings indicate hippocampal damage results in impaired odor–place associative memory in humans, which is similar to that observed in rats using an analogous task.

The present study is the first to assess odor–place associative memory performance of amnesic subjects with damage restricted to the hippocampus. Our findings suggest that the human hippocampus is necessary for odor–place associative memory and spatial recognition memory. These data provide support for the idea that odor–place associative memory is mediated by the hippocampus in both humans and rodents, suggesting an evolutionary continuity in cognitive function assigned to the hippocampus.

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*Accepted April 30, 2009*