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Left hippocampal volume loss in Alzheimer's disease is reflected in performance on odor identification: A structural MRI study

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Abstract

The very high sensitivity and specificity of odor identification tasks in discriminating between Alzheimer's patients and normals suggests that they reflect the presence of underlying neuropathology. Significant neuropathological changes are seen in areas critical to processing olfactory information, even in the early stages of Alzheimer's disease (AD). The current study was designed to investigate whether performance on olfactory tasks (odor threshold and odor identification) was related to volumetric MRI measures of mesial temporal areas central to olfactory information processing and important in the neuropathology of AD. Participants were 8 male and 5 female patients with probable AD, and 10 male and 12 female normal age-matched controls, diagnosed at the UCSD Alzheimer's Disease Research Center. The study investigated correlations between volumetric measures of hippocampus, the parahippocampal gyrus and the amygdala, and the psychophysical measures of olfactory function. Robust relationships were observed between mesial temporal lobe volumes and olfactory functional measures. The finding of a strong relationship between left hippocampal volume and performance on the odor identification task ($r = .85$) is compatible with a left-hemisphere superiority for verbally mediated olfactory tasks. The findings suggest a neural substrate for the breakdown in functional performance on verbally mediated odor identification tasks in Alzheimer's disease and suggest the utility of quantitative MRI measures and psychophysical performance in the assessment of AD. These results support the potential clinical utility of inclusion of odor identification tests in diagnostic batteries for detecting AD. (*JINS*, 2003, *9*, 459–471.)

Keywords: MRI, Olfaction, Mesial temporal lobe, Amygdala, Parahippocampal gyrus

INTRODUCTION

Alzheimer's disease (AD) is a progressive, debilitating, neurodegenerative disease that afflicts more than 5 million Americans, most of them elderly. The unremitting neural degeneration is accompanied by deterioration of cognitive function, memory, language, and the essence of the person. Accurate diagnosis of AD is achieved only at autopsy or biopsy when the characteristic neuritic plaques and neurofibrillary tangles are identified in the brain. The difficulty in identifying individuals who are developing the disease has stimulated appreciable research on the functional expression of the underlying neurodegeneration. Efforts to develop pharmaceutical agents to prevent or retard the degenerative process are keen, and when successful will mandate a means of assessing functional impairment in those undergoing neurodegenerative processes. Identification of preclinical markers and an early diagnosis of AD are critically important, since early detection will help ensure early medical and social intervention for the patient and family.

Neurofibrillary tangles and neuritic plaques appear in entorhinal and transentorhinal areas of the brain during the silent preclinical stages of Alzheimer's disease (Braak & Braak, 1994; 1997; Price et al., 1991). Neuropathy then proceeds to other temporal lobe structures which include the hippocampus and the amygdala. Also affected relatively early in the disease is the frontal cortex, including the orbital frontal area. The entorhinal cortex is a target of in-

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coming olfactory information from the olfactory bulb, *via* the lateral olfactory tract. Entorhinal cortex supplies important input to the hippocampus CA1 neurons, with feedback from the hippocampus to the mesial entorhinal cortex. In the case of olfactory input to the hippocampus, the entorhinal cortex represents the direct and substantial link between incoming olfactory input and the hippocampus. The entorhinal cortex is multimodal and its integrity is critical for the flow of olfactory information. In addition to hippocampal projections, entorhinal cortex projects to orbital frontal cortex and receives input from frontal cortex and amygdala, areas important for olfactory function (Carmichael et al., 1994). Thus, areas that evidence early neuropathy in Alzheimer's disease, particularly in the mesial temporal lobe, are areas that, in addition to their well-known importance for memory function (Squire, 1992), are also critical for the processing of olfactory information.

This early vulnerability of the olfactory system to the neuropathology of AD has motivated our strategy to use olfactory functional testing to investigate Alzheimer's disease and to explore the potential for olfactory tests to contribute to detection of incipient dementia (Morgan et al., 1995; Murphy et al., 1990; Nordin & Murphy, 1996). Compared with young adults, normal older people show losses in odor sensitivity, odor intensity perception, odor identification, and odor memory (Murphy, 1983, 1993; Murphy et al., 1991, 1997; Schiffman, 1997). Recordings of brain activity with the olfactory event-related potential show slower processing of odor information (Morgan et al., 1997, 1999; Murphy et al., 1994b, 1997, 2000). Even compared with these age-related losses in function, patients with Alzheimer's disease show dramatic impairment in olfactory function (Murphy, 1999). Patients with probable AD show very poor sensitivity to odor (Murphy et al., 1990), severe deficits in odor identification (Doty et al., 1987; Knupfer & Spiegel, 1986; Koss, 1986; Morgan et al., 1995; Murphy et al., 1998; Serby et al., 1985; Waldton, 1974), impaired odor fluency (Bacon et al., 1999), poor odor recall and recognition memory (Murphy et al., 1987; Niccoli-Waller et al., 1999; Nordin & Murphy, 1996), and slower processing of olfactory information reflected in event-related potentials (Morgan & Murphy, 2002). The results of this research strongly support the use of olfactory measures in the assessment of Alzheimer's disease. Particularly high sensitivity and specificity for detecting Alzheimer's patients from controls has been demonstrated with odor identification or odor naming tests (Morgan et al., 1995; Morgan & Murphy, 2002; Murphy, 1999).

Reduced volume of the mesial temporal lobe, including the hippocampal formation and the parahippocampal area, has been detected in AD patients using magnetic resonance imaging (MRI) (Fennema-Notestine et al., 1997; Jack et al., 1998; Jernigan et al., 1991b). The hippocampus and associated medial temporal lobe areas, particularly entorhinal cortex, are critical for memory function. Impaired memory function is a critical feature in the diagnostic criteria for probable Alzheimer's disease (McKhann et al., 1984). Hippocampal atrophy in AD, demonstrated with structural MRI, is associated with impaired performance on both verbal and visual memory tasks (Cahn et al., 1998; Deweer et al., 1995; Fama et al., 1997; Petersen et al., 2000), including the memory subtests of dementia status tests (Deweer et al., 1995; Fama et al., 1997; Stout et al. 1996). Data from Jernigan's laboratory (Stout et al., 1999) showed significant correlations between the volume of mesial temporal lobe and the immediate recall measures, as well as recognition discriminability, on the California Verbal Learning Test (CVLT; Delis et al., 1987) in demented patients. Language and memory tasks with verbal or naming components have been lateralized to the left hemisphere and recently, stronger associations have been reported in Alzheimer's patients for performance on verbal memory tasks and the volume of the left hippocampus (Cahn et al., 1998; Toledo-Morrell et al., 2000).

Evidence that olfactory function is severely compromised in AD and that there are early neuropathological changes in mesial temporal lobe structures, particularly entorhinal cortex, in patients with AD (Braak & Braak, 1992, 1994, 1997; Price et al., 1991), has prompted the examination of the relationship between olfactory dysfunction and structural changes in the mesial temporal lobe in AD. Kesslak et al. (1991) found evidence for an association in AD patients between performance on a test of olfactory match-to-sample and structural MRI of the hippocampal formation and the parahippocampal gyrus, although the relationship was weaker than reported for verbal and visual tasks in the studies cited above. The non-verbal nature of this olfactory task may have mitigated against a strong relationship. An olfactory task that challenges the patient to identify an odor would seem best suited to detecting a relationship between hippocampal atrophy and olfactory dysfunction. The verbal component of such a task would also suggest lateralization to the left hemisphere. Thus, a significant relationship between the volume of the left hippocampus and performance on an odor identification task is hypothesized. In spite of considerable evidence in support of odor identification deficits in Alzheimer's disease, a significant relationship between performance on these tests of olfactory function and degeneration in the brain of AD patients has yet to be demonstrated *in vivo*.

The present study investigated the relationship between volumetric measures of mesial temporal lobe structures in AD patients and age-matched controls and performance on specific tasks designed to target these areas of neuropathology. Regions of the MTL examined include the hippocampus, parahippocampal gyrus, and the amygdala (Jernigan et al., 2001a, 2001b). The hypotheses were (1) that tests involving odor and visual identification would correlate strongly with volumetric measures of the hippocampal area; (2) that these associations would be stronger with left hippocampal volumes than with right hippocampal volumes; and (3) that left hippocampal volume would correlate more strongly with odor identification than with odor threshold.

METHODS

Research Participants

Participants were 8 male and 5 female patients with probable AD, and 10 male and 12 female normal elderly controls. Groups were matched for age (see Table 1 for demographics). Both probable AD patients and normal controls were participants in a longitudinal study at the Alzheimer's Disease Research Center (ADRC), University of California, San Diego. The diagnosis was made by two independent senior neurologists at the ADRC, according to the NINCDS–ADRDA criterion for probable AD (McKhann et al., 1984) and the DSM–IV criterion for dementia (American Psychiatric Association, 1994). The DSM–IV criterion requires memory impairment as well as impairment in one or more of the disturbances of aphasia, apraxia, agnosia, and disturbance in executive functioning that cannot be explained by other medical or neurological factors. Alternative causes of dementia (e.g., thiamin deficiency, thyroid dysfunction) were ruled out with extensive laboratory testing (e.g., blood tests, urinalysis). Additional screening tests (e.g., CSF, EEG, EKG, CT scan, MRI) were performed when appropriate. Exclusionary criteria included a history of alcoholism, psychiatric illness, cerebral vascular accident, head trauma, or other significant neurological condition. The diagnosis of probable AD required impairment in two or more areas of cognition that could not be explained by other medical/neurological factors. The assessed cognitive areas included attention, abstraction/problem solving, motor, verbal/language, perceptual/constructional memory, and orientation (for details on tests, see Nordin & Murphy, 1996). The controls were recruited from the patients' spouses and from advertising within the community. Controls were genotyped for the apolipoprotein e4 allele (Wenham et al., 1991). Half were e4+ and half were e4−. Mean scores on the following dementia diagnostics are found in Table 1: the Mini-Mental State Examination (MMSE; Folstein et al., 1975), and the Dementia Rating Scale (DRS; Mattis, 1976). The mean DRS score was 117.9 ($SE = 2.8$), suggesting that AD patients' dementia severity was mild to moderate.

Table 1. Demographics and dementia characteristics for normal controls and patients with Alzheimer's disease

	Normal controls		Alzheimer's patients	
Variable	*M*	(*SE*)	*M*	(*SE*)
Age	72.45	(1.78)	73.08	(2.19)
Education	15.5	(.67)	13.69	(.71)
DRS	141.10	(.50)	117.92	(2.77)
MMSE	29.68	(.12)	22.85	(1.04)

Note. *SE* in parentheses. MMSE: Mini-Mental State Exam; DRS: Dementia Rating Scale.

Testing Procedures

Odor threshold test

Detection thresholds were obtained monorhinically for butanol by a two-alternative (odorant and blank), forced-choice, ascending method of limits (see Murphy et al., 1990). The subject was presented with two bottles, one containing the odorant and the other a blank consisting of deionized water. The spout of the bottle was inserted into the nostril of interest. The subject was asked to squeeze the bottle in order to generate a puff of air. The subject did this sequentially with both bottles. Then his task was to identify the bottle containing the strongest odor. Subjects began at the lowest dilutional step (Step 9) in order to avoid adaptation (Ekman et al., 1967). Incorrect choices led to presentation of a higher concentration. Correct choices led to continued presentation of the same concentration to a criterion of five successive correct responses. The presentation of the odorant and blank were randomized for each trial. The nostril to be tested first was also randomly determined. There were approximately 45 s between trials in order to allow enough time for recovery of the olfactory system and to allow enough time for odorant concentration to equilibrate in the head space of the bottle.

Taste threshold test

Taste thresholds were measured with a two-alternative (tastant and blank), forced-choice procedure (see Murphy et al., 1990). The subject was presented with two cups, one containing a concentration of sucrose and the other containing deionized water. The order in which the blank and stimulus were presented to the subject was randomized. The stimuli were tested using the "sip and spit" method. The subject was instructed to rinse first with deionized water, taste the contents of the cup, retain it in the mouth for approximately 5 s, and then expectorate. After completing the same procedure for the two samples, the subject was asked to judge which one of the two cups contained the stronger taste. Thus, in both the olfactory and taste threshold tests the subject's task was identical: they were asked to choose the stronger of the two stimuli.

San Diego Odor Identification Test

The San Diego Odor Identification Test (Murphy et al., 1994a) employs a series of eight common natural odors (e.g., coffee, chocolate, peanut butter) presented in opaque, odorless, glass jars. Odors are presented individually for 5 s with a 45-s interstimulus interval to minimize adaptation (Ekman et al., 1967). The participant smells each odor, with the eyes closed to prevent visual cues, and then attempts to name it with the aid of a cue sheet that contains line drawings of the items as well as distractors. If the subject was not able to identify an odorant, she was asked to guess. The test takes less than 10 min to complete.

Boston Naming Test

The Boston Naming Test (Kaplan et al., 1983) employs a series of outline drawings of objects (e.g., *bench, rhinoceros*). Each item is presented one at a time, the participant examines each drawing and then attempts to name it. If a subject has difficulty in naming an object, a stimulus or phonemic cue is provided. Performance is evaluated by the number of spontaneous and cued correct responses.

Imaging protocol

Three whole-brain image series are collected for each subject. The first is a gradient-echo (SPGR) T1-weighted series with TR = 24 ms, TE = 5 ms, NEX = 2, flip angle = 45°, field of view of 24 cm, section thickness of 1.2 mm, no gaps. The second and third series are fast spin-echo (FSE) acquisitions yielding two separate image sets: TR = 3000 ms, TE = 17 ms, ET = 4 and TR = 3800 ms, TE = 102 ms, ET = 8. For all series, the field of view is 24 cm. Section thickness for the FSE series is 4 mm, no gaps (interleaved).

Image analysis

The image-analytic approach is similar to that used in our previous anatomical studies (Jernigan et al., 1990, 1991a, 1991b; Jernigan & Ostergaard, 1993), but represents a significant elaboration of these methods as described in Jernigan et al. (2001a, 2001b). Trained anatomists who are blind to subject diagnosis, age, gender or any other identifying information subject each image dataset to the following image analysis procedures: (1) interactive isolation of intracranial regions from surrounding extracranial tissue, (2) three-dimensional digital filtering of the matrix of pixel values representing brain voxels to reduce inhomogeneity artifact, (3) reslicing of the volume to a standard orientation, (4) tissue segmentation using semi-automated algorithms, and (5) neuroanatomical region-of-interest analysis.

Brain is first isolated from extracranial areas in the image, namely, from surrounding tissue that is in some instances contiguous with brain tissue and similar in signal value. This process results in a new volume within which the positions of brain voxels are coded, that is, a mask. The reproducibility of the stripping method was assessed by performing the stripping operations independently on six pairs of image volumes and comparing the within-pair discrepancies. Each pair represented two FSE volumes obtained on different occasions in the same individual. Discrepancies in brain volume were small, ranging from .03% to 1.25% with a mean of .54%.

Filtering is applied to reduce nonbiological signal drift across the field of view, which is presumably due to field inhomogeneity and susceptibility effects. A three-dimensional high-pass filter is applied, with two iterations, separately to the "stripped" proton density weighted and T2-weighted FSE image volumes. First, a roughly cubic near-neighbor averaging filter is applied to produce a smoothed dataset; then the original volume is divided by the smoothed dataset on a voxel-by-voxel basis; and finally each voxel value is multiplied by the mean voxel value of the original dataset. The dimensions of the cubic smoothing filter were chosen by subjective evaluation of the results obtained with a series of filter sizes and were set at approximately 30 mm. That is, the set of voxels averaged to create each voxel value in the smoothed dataset spans 33 voxels in the *x* and *y* directions, and 7 voxels in the *z* direction (i.e., it measures 31 mm × 31 mm × 28 mm). In constructing the smoothed datasets, near-neighbor averages are produced only for positions within the volumes coded as brain. Similarly, only the values for near-neighbors that are also brain voxels are averaged.

The tissue classification procedure is an interactive, supervised process. Operators manually designate the positions of three sets of tissue samples, one for each of the target tissues (gray, white, and CSF). The goals are to obtain samples in standard anatomical locations, within regions of homogeneous tissue; and to avoid artifacts and tissue abnormalities (such as ischemic damage). Samples are selected in locations that appear to be homogeneous and free of signal abnormalities both in the section to be sampled and in the adjacent sections. In most cases the operators select samples in six gray matter locations (bilaterally in the caudate nucleus, putamen, and the pulvinar of the thalamus); in four white matter locations (bilaterally in the suprasylvian white matter at the level of the pulvinar, and in similar locations at the level of the caudate/putamen); and in four locations within CSF-filled structures (2 samples are taken within the frontal horns, and two more posterior samples are taken at approximately the level of the trigones of the cerebral ventricles). The sample voxel values are then analyzed using simple regression techniques to separate first all brain parenchymal voxels from CSF voxels, and then gray matter voxels from white matter voxels. The regression formulae obtained in these simple analyses are then applied to classify each voxel within the volume as most similar to CSF, gray matter, or white matter. Interoperator reliabilities for estimated tissue volumes (for independent tissue classification by two anatomists) were estimated using 11 brain datasets, and were .92 for white matter, .95 for gray matter, and .99 for CSF.

In order to facilitate anatomical region definition, resectioned data sets are aligned to a standardized stereotactic space defined relative to the decussations of the anterior and posterior commissures and the structural midline. This improves the reliability of boundary determination, facilitates reference to standard brain atlases, and makes it possible to identify small structures more consistently. Registration of the T1-weighted and spin-echo data sets is accomplished so that registered sections from all three data sets are available to the operators when attempting to resolve anatomical boundaries. Anatomists circumscribe regions on tissue-segmented images. Standardized rules are applied for delineating a set of subcortical structures and cortical regions. Subcortical structures include the cerebral ventricles, the caudate nucleus, the nucleus accumbens, the lenticular nucleus, the thalamus, the substantia nigra, and a

region referred to as basomesial diencephalon (which includes septal nuclei, mamillary bodies and other hypothalamic structures, the bed nucleus of the stria terminalis, and the diagonal band of Broca). Cortical regions include the temporal lobe, frontal lobe, parietal lobe, occipital lobe, cingulate cortex, and insular cortex. Separate measures are obtained of three mesial temporal lobe structures: the hippocampus, the amygdala and adjacent entorhinal/perirhinal cortex, and parahippocampal gyrus. The four major cortical lobes are drawn to include cortical gray matter, underlying white matter, and CSF. Volumes of each tissue are estimated separately within each lobe, and white matter and CSF volumes are also measured in a deep subcortical zone not within any of the cortical lobes. Gray matter and adjacent CSF of the cingulate cortex and insular cortex are defined separately. ROI analysis of 10 brain datasets was performed independently by two anatomists. Interoperator reliability for estimated volumes of the 15 primary gray matter structures ranged from .85 to .99, with reliability for most measures exceeding .95.

For the present study the specific measures examined were volume estimates for gray matter regions: the hippocampus, the amygdala region, and the parahippocampal gyrus.

The boundaries of the mesial temporal lobe structures, arguably of particular importance in the present study, are defined as follows (and illustrated in Figure 1): The mesial temporal lobe subregions include the amygdala area (MTL-A), the parahippocampal region (MTL-P), and the hippocampal region (MTL-H). The hippocampal and parahippocampal regions extend posterior to the pulvinar of the thalamus where they lie inferior to the corpus callosum. These two regions extend anteriorly to (but not including) the section immediately posterior to the section in which the long columns of the fornix appear; that is, the anterior boundary is defined in part stereotactically. The transition to the amygdala region occurs in this (immediately posterior) section behind the long columns of the fornix and the region extends anteriorly to and including the section at which the temporal pole is entirely separated from the frontal lobe by the lateral sulcus. Within the posterior zone, the parahippocampal region includes entorhinal, parahippocampal, and some lingual gyrus. The inferior boundary is the collateral sulcus and the superior boundary is defined by

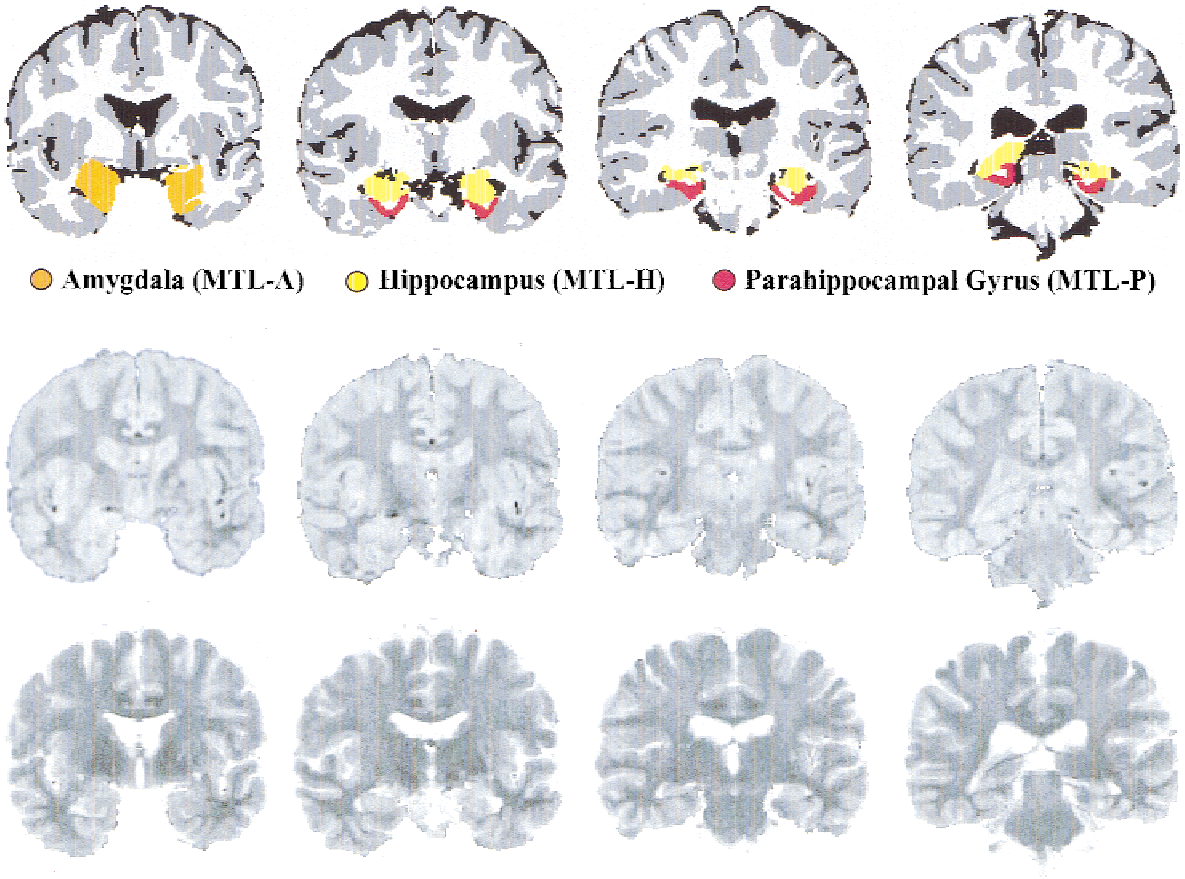

Fig. 1. Four representative, fully processed images from anterior (leftmost image) to posterior (rightmost image) with mesial temporal gray matter voxels color coded to illustrate the structural boundaries for the hippocampal (MTL–H), parahippocampal (MTL–P), and amygdala (MTL–A) regions that are critical to this study. See methods for full description of anatomical boundaries. All CSF is coded in black, all gray matter in dark gray, and all white matter (including abnormal signal) in light gray.

following the white matter through the bend in the parasubicular region, separating the subiculum (hippocampal region) from the entorhinal cortex. The more superior hippocampal region is primarily the hippocampal formation and retrosplenial gyri. In posterior sections where the temporal horns of the cerebral ventricles are seen, the hippocampal region includes the tail of the hippocampus, the fasciola cinerea, and the gyrus fasciolaris. The amygdala region includes amygdala, some very anterior hippocampus, contiguous entorhinal cortex, and the uncus (which includes perirhinal cortex). Representative, fully processed images from a normal brain, illustrating the boundaries of the measured brain structures are shown in Figure 1. The PD- and T2-weighted resliced images are also shown to aid in interpretation of the anatomical designations.

RESULTS

Behavioral Assessment

AD patients showed significantly impaired performance on the San Diego Odor Identification Test ($M = 27\%$ correct) relative to controls ($M = 62\%$), replicating earlier work (Morgan et al., 1995). The patients also showed poorer performance on the Boston Naming Test ($M = 77\%$) relative to controls ($M = 96\%$). A 2 (AD, NC) $\times$ 2 (San Diego Odor Identification Test, Boston Naming Test) mixed ANOVA showed a significant effect of diagnosis with performance better for NC than for AD patients [$F(1,31) = 21.31$, $p < .0001$]. There was a significant effect of task with performance on the odor task poorer than on the visual task [$F(1,31) = 91.33$, $p < .0001$]. The interaction approached significance with AD patients performance on the odor task showing a tendency to be poorer than their performance on the visual task [$F(1,31) = 3.40$, $p = .075$; see Figure 2].

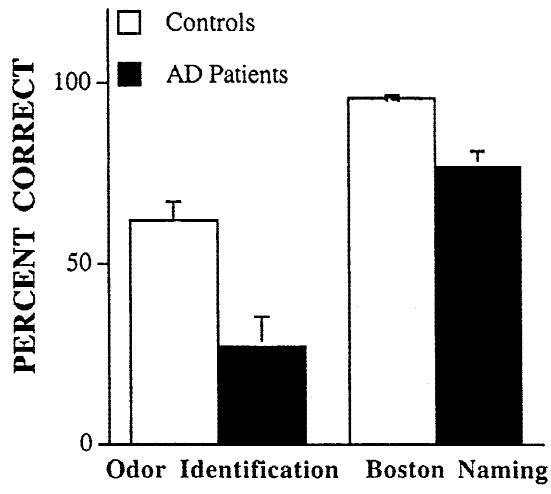

Fig. 2. Mean (and *SE*) difference in performance on the San Diego Odor Identification Test and the Boston Naming Test in Alzheimer's patients and controls.

A 2 (AD, NC) $\times$ 2 (left-, right-nostril odor threshold) mixed ANOVA indicated that Alzheimer's patients show some impairment in odor threshold sensitivity relative to normal controls but the difference in odor threshold did not reach statistical significance in this sample [$F(1,32) = 1.28$, $p < .27$]. Odor threshold sensitivity was poorer in the left nostril than in the right [$F(1,32) = 4.17$, $p < .05$]. The interaction did not reach statistical significance although there was some tendency for the mean threshold to be poorer in the left than the right nostrils for the AD patients ($M = 4.5$ and 5.4, respectively). Figure 3 shows the mean performance for patients and controls for odor threshold. Note that higher numbers indicate better ability to detect the stimulus. Taste threshold was not significantly different in the AD patients and the controls [$F(1,32) = .004$, $p > .95$].

Brain Measures

Differences between patients and controls for left and right mesial temporal lobe gray matter were assessed. In addition mesial temporal lobe was assessed separately for the hippocampal area, parahippocampal gyrus and amygdala. The units of volume measurements are a proportion of the total number of voxels in a volume to the number of voxels in the supratentorial cranial vault. The size of each voxel is .9375 $\times$ .9375 in plane, 4 mm thick.

Significant reduction in the mesial temporal lobe volumetric measures was found in the AD patients, replicating previous findings (Jernigan et al., 1991a). Table 2 indicates the differences in volume between patients and controls for the hippocampal gray, the parahippocampal gray and the amygdala. Note that the volumentric differences are shown separately for the left and the right hippocampal areas.

Brain–Behavior Relationships

Hippocampal region (MTL–H)

The results of the analysis of the relationships between olfactory measures and the volume of the hippocampus in the AD patients were different for the left and right hippocampus. These are displayed in Table 3, separately for the two hemispheres, for the AD patients and contrasted with relationships for the normal controls without genetic risk for AD. AD patients showed a highly significant relationship between volume of the left hippocampal area and identification of odor stimuli ($r = .85$, $p < .006$), with Bonferroni correction. This relationship was also confirmed with the nonparametric Spearman rank-order correlation [$rs = .69$, $p < .05$], and is illustrated in Figure 4. Thus, patients with smaller left hippocampal volumes were able to identify a smaller percentage of the odor stimuli. The relationship between left hippocampal volume and scores on the Boston Naming Test was $r = .74$, $p < .05$ (nonparametric Spearman $rs = .47$). When BNT and odor identification performance in the AD patients were considered as predictors of left hippocampal volume in a simultaneous regression, Beta

Table 2. Volume of the left and right hippocampus (MTL–H), amygdala (MTL–A), and parahippocampal gyrus (MTL–P), in normal controls and patients with Alzheimer's disease

	Normal controls		Alzheimer's patients		
	M	(*SE*)	*M*	(*SE*)	Significance test
Hippocampus (MTL–H)					
Left	0.0068	(.00023)	0.0050	(.00028)	$t = 5.0$, $p < .00003$
Right	0.0070	(.00031)	0.0052	(.00037)	$t = 2.8$, $p < .01$
Amygdala (MTL–A)					
Left	0.0055	(.00024)	0.0042	(.00034)	$t = 2.5$, $p < .02$
Right	0.0059	(.00024)	0.0044	(.00037)	$t = 3.9$, $p < .001$
Parahippocampal (MTL–P)					
Left	0.0038	(.00014)	0.0031	(.00017)	$t = 2.4$, $p < .02$
Right	0.0035	(.00014)	0.0031	(.00012)	$t = 3.4$, $p < .005$

Notes. SE in parentheses. (MTL–H): Posterior hippocampus, fasciola cinerea, gyrus fasciolaris; (MTL–A): amygdala, anterior hippocampus, contiguous entorhinal cortex, uncus; (MTL–P): parahippocampal gyrus, entorhinal cortex, lingual gyrus.

weights were .18 for BNT ($T = .78$, $p > .45$) and .73 for odor identification ($T = 3.30$, $p < .009$). When odor identification was entered first in a step-wise regression on left hippocampal volume, odor identification remained significant (Beta = .84, $T = 4.98$, $p < .0006$) when BNT was added, but BNT was not significant (Beta = .61, $T = .79$, $p > .45$, n.s.). However, when BNT was entered first into a step-wise regression (BNT: Beta = .64, $T = 2.61$, $p < .03$), BNT was no longer significant (Beta = .17, $T = .79$, $p > .45$) when odor identification was entered on Step 2, but odor identification was significant (Beta = .73, $T = 3.30$, $p < .009$). When left and right hippocampal volumes were considered as predictors of odor identification in AD patients in a multiple regression, the left hippocampus entered the model (Beta = .93, $T = 4.14$, $p < .0024$) but the right hippocampus was not significantly associated with odor identification (Beta = $-.13$, $T = -.60$, $p > .56$). Thus, the relationship between left hippocampal volume and odor identification in AD was confirmed with multiple regression techniques. In the AD patients, left hippocampal volume was less strongly associated with odor threshold than with odor identification (Table 3). There was little association between left hippocampal volume and taste threshold. The correlation of hippocampal volume to odor identification in AD may primarily reflect a correlation of both to AD severity, rather than a direct relation between hippocampus and odor identification. The correlation between left hippocampal volume and DRS was $r = .59$, $p < .05$, and between DRS and odor identification the correlation was $r = .65$, $p < .05$.

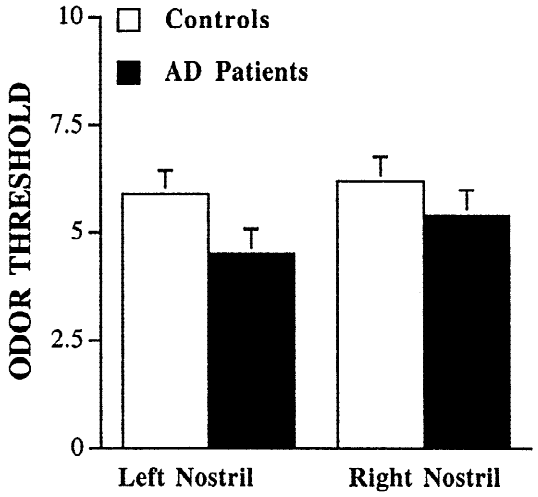

Fig. 3. Mean (and *SE*) difference in performance on odor threshold, determined separately in the left and right nostrils, in Alzheimer's patients and controls.

Amygdala region (MTL–A)

The AD patients showed significant correlations between odor threshold and right amygdala volume. That is, those who showed larger amygdala volumes had better olfactory threshold sensitivity. In the AD patients the right amygdala volume was more highly correlated with odor threshold than with odor identification. Correlations between odor threshold and left amygdala volume were not significant. The results of this analysis are shown in Table 3. Significant relationships observed between amygdala volume and olfactory threshold in the normal controls without genetic risk for AD are also shown in Table 3. Odor threshold and left amygdala volume were highly correlated; odor threshold and right amygdala volume were moderately correlated. Brain behavior relationships in those at risk for AD were complex and will be discussed elsewhere.

Parahippocampal region (MTL–P)

The results of the analysis of the brain–behavior relationships for the parahippocampal region (MTL-P), including entorhinal cortex, parahippocampal gyrus and some lingual

Table 3. Pearson product-moment correlations (r) between MRI volumes and behavioral tests

	Normal controls (E4−)		Alzheimer's patients	
MRI volume/test	Left	Right	Left	Right
(MTL–H): Posterior hippocampus, fasciola cinerea, gyrus fasciolaris				
San Diego Odor Test	.17	.23	.85***	.54*
Boston Naming Test	−.29	−.47	.74**	.33
Odor threshold left	−.14	−.02	.39	.62*
Odor threshold right	.03	.20	.55*	.66*
Odor threshold average	−.05	.09	.50	.68*
Odor threshold better	−.10	.01	.50	.73**
Odor threshold worse	−.01	.17	.47	.59*
Taste threshold	−.34	−.59	.28	.32
(MTL–A): Amygdala, anterior hippocampus, contiguous entorhinal cortex, uncus				
San Diego Odor Test	.69**	.52*	.08	.11
Boston Naming Test	.36	.31	−.44	−.09
Odor threshold left	.86***	.53*	.16	.59*
Odor threshold right	.89***	.59*	.32	.45
Odor threshold average	.89***	.57*	.25	.55*
Odor threshold better	.86***	.52*	.46	.63*
Odor threshold worse	.90***	.61*	.05	.44
Taste threshold	.04	−.04	.52	.60*
(MTL–P): Parahippocampal gyrus, entorhinal cortex, lingual gyrus				
San Diego Odor Test	.39	.40	−.03	.14
Boston Naming Test	−.03	−.14	−.48	−.35
Odor threshold left	−.08	.13	.41	.45
Odor threshold right	−.03	.35	.55*	.52
Odor threshold average	−.05	.24	.50	.51
Odor threshold better	−.10	.15	.62*	.65*
Odor threshold worse	−.01	.37	.37	.36
Taste threshold	−.21	−.56	.36	.39

Statistical significance: *$p < .05$, **$p < .01$, ***$p < .001$.
All *** correlations are significant at Bonferroni critical value of $p < .006$.

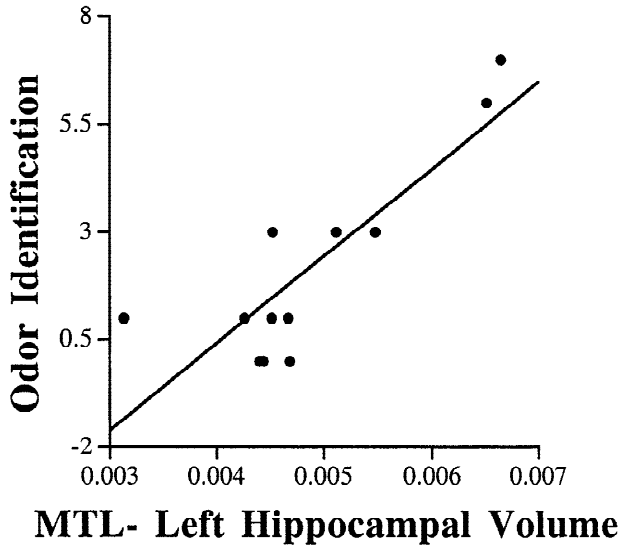

Fig. 4. Association between left hippocampal volume and performance on odor identification in Alzheimer's patients.

gyrus, supported a moderate association between brain volume and olfactory threshold in Alzheimer's patients, and these relationships are illustrated in Table 3. The patients' threshold performance was moderately associated with both left and right parahippocampal volumes, with threshold for the better nostril more highly correlated with brain volume than threshold for the poorer nostril. Threshold for the right nostril was more highly associated with brain volume than threshold for the left nostril. There was some tendency for the poorer threshold to be recorded in the left nostril in the AD patients, although the difference between means (M = 4.5 and 5.4, respectively) was not statistically significant (see results for olfactory assessment above). In contrast to the results for the left hippocampal measure where brain volume was highly correlated with performance on the San Diego Odor Identification Test, the parahippocampal measure was moderately correlated with the Boston Naming Test, but not with the San Diego Odor Identification Test. Thus the San Diego Odor Identification Test appears to be more affected by left hippocampal volume loss in AD.

DISCUSSION

The results of the present study revealed robust brain–behavior relationships. The Alzheimer's patients showed a highly significant relationship between volume of the left hippocampus (MTL–H) and identification of odor stimuli. AD patients also showed moderate relationships between hippocampus (MTL–H), amygdala (MTL–A), particularly on the right, the parahippocampal gyrus (MTL–P), and odor threshold. Normal controls without genetic risk for AD had negligible relationships between the hippocampal or parahippocampal gyrus and odor identification or odor threshold, but did evince significant relationships between performance on odor tasks, particularly odor threshold, and volume of the amygdala. The results support a relationship between neurodegeneration and olfactory function in AD.

It is of interest that the relationship of threshold with the amygdala is greater in normal controls with no dementia and no genetic risk for AD, and that the relationship between odor identification performance and left hippocampal volume is greater in AD patients. It is important to note that the odor threshold task in this study is the detection threshold, not the recognition threshold, and that the subject needed only to detect and not to name the odor in this task. This contrasts with the odor identification task in which the subject was required to name the odor. The relationship with amygdala in the normal controls presumably reflects the effects of normal aging on brain volume. The correlation of hippocampal volume to odor identification in AD may primarily reflect a correlation of both to AD severity, rather than a direct relation between hippocampus and odor identification. Because of the dissociation from incoming input to and the deterioration in the hippocampus in AD we would expect a stronger volumetric relationship with olfactory measures that include a memory component (odor identification) than with measures that are more sensory (odor threshold). The fact that the odor threshold is more impaired than taste threshold in AD supports the olfactory contribution to impaired odor identification. Less deterioration in the hippocampus and in odor identification performance would be expected in normal aging.

Volume of the left and right Hippocampal area (MTL–H), Amygdala region (MTL–A), and Parahippocampal gyrus (MTL–P), was significantly reduced in patients with Alzheimer's disease. The cortical volumes measured from the *in vivo* MRI data in these subjects are within the range of post-mortem volume measures in the brains of 19 individuals studied by Hubbard and Anderson (1981). Hubbard and Anderson expressed cortical gray matter as a proportion to total cranial vault and found a mean proportion of .42 (range .36–.47). The comparable value from in the present study for the elderly normal control subjects is a mean proportion of .42 (range .36–.47). The value from the AD patients in the present study is a mean proportion of .37 (range .33–.43), also similar to the proportion found in Hubbard and Anderson (1981) for senile dementia (mean proportion of .36 for 7 patients).

In the present study the measure "amygdala" includes amygdala, anterior hippocampus, contiguous entorhinal cortex, and the uncus, and thus is less anatomically specific than in fMRI studies, but probably within the resolution range of PET. Nevertheless, highly significant correlations were observed in the present study between odor threshold and volume of the amygdala in normal elderly persons (see Table 3). Only rarely in neuroimaging studies of olfaction has activation been detected in mesial temporal areas of amygdala (Cerf-Ducastel & Murphy, 2001; Savic et al., 2000; Small et al., 1997; Sobel et al., 2000; Zald & Pardo, 1997, 2000), entorhinal cortex (Cerf-Ducastel & Murphy, 2001; Levy et al., 1997; Zald & Pardo, 2000), parahippocampal gyrus, or hippocampus (Cerf-Ducastel & Murphy, 2001; Levy et al., 1997; Small et al., 1997; Zald & Pardo, 2000); although electrophysiological and anatomical studies indicate that the anterior cortical nucleus of the amygdala, the periamygdaloid area and the lateral entorhinal cortex receive direct projections from the olfactory bulb through the lateral olfactory track (Biella & De Curtis, 2000; Carmichael et al., 1994; Price, 1985; 1987). The entorhinal area also receives olfactory projections from the amygdaloid area and the piriform cortex. The hippocampus is activated by olfactory stimulation, receives a projection to CA1 from the lateral entorhinal area, and projects to the mesial entorhinal area. Certainly there is ample evidence of the role of these mesial temporal areas in the processing of olfactory information from human lesion studies (Jones-Gotman & Zatorre, 1993) and behavioral, anatomical and electrophysiological studies in lower mammals (Eichenbaum, 1998). Thus, the inconsistent findings regarding activation in fMRI of these areas in response to odor stimulation may reflect the challenges of reflecting olfactory processing with fMRI. As pointed out by a number of authors (e.g., Cerf-Ducastel & Murphy, 2001; Yousem et al., 1997), signal loss due to inhomogeneity artifacts may have decreased the detectable activation in primary olfactory areas in fMRI experiments, although similar artifacts do not occur with PET. Rapid adaptation in the olfactory system may also contribute to poor detectability of activation in primary olfactory areas since odors have typically been presented for long periods in PET and fMRI studies published to date. Certainly in fMRI studies where the stimulus protocol was designed to minimize adaptation, activation occurred in amygdala, hippocampus, entorhinal cortex or in regions that included these areas (Cerf-Ducastel & Murphy, 2001). The current approach using structural MRI and behavioral testing to examine brain–behavior relationships represents an alternative effective strategy for assessing brain–behavior relationships in the olfactory system.

In the present study the relatively large spatial extent of the areas of mesial temporal lobe included in the volumetric measures is likely to have underestimated relationships between functional measures and volumetric measures of some of the areas in mesial temporal lobe likely to be affected in early AD. The parahippocampal region (MTL–P) contained entorhinal cortex and parahippocampal cortex,

but also some lingual gyrus. The amygdala region (MTL–A) contained not only amygdala, but also anterior hippocampus, contiguous entorhinal cortex and uncus. The hippocampal area (MTL–H) included both the hippocampal formation and the retrosplenial gyri. Interestingly, all of these areas, with the possible exception of the lingual gyrus, receive olfactory projections (Carmichael et al., 1994; Cerf-Ducastel & Murphy, 2001; Price et al., 1991). It should be appreciated that the primary olfactory areas are more anatomically discreet than those relatively large regions of interest investigated in the present study. Nevertheless, highly significant correlations between the MTL subregions investigated and behavioral measures of olfactory function were observed. This work lays the foundation for fMRI studies addressing the functional specificity of more discreet anatomical regions of interest and encourages approaches to analysis of brain behavior relationships with structural MRI that are more anatomically specific than those employed in the present study. Voxel based methods may prove useful in exploratory analyses of the volume of individual structures and a combination of approaches may best characterize the degeneration in the AD brain (Andreasen et al., 1994; Rombouts et al., 2000; Sowell et al., 1999a, 1999b).

The AD patients showed a significant relationship between odor identification scores and hippocampal volume. This relationship was greater between odor identification and the left hippocampal gray volumes than between odor identification scores and the volume of the right hippocampal region. The lateralization is of interest given the recent neuroimaging findings of greater brain activation during verbally mediated tasks on the left side of the brain and to nonverbal tasks on the right (Cohen et al., 1999; Martin, 1999). The degree to which the hippocampus and surrounding areas (entorhinal and parahippocampal areas) contribute to episodic and semantic memory processes is a topic of interest and discussion (Squire, 1992; Tulving et al., 1999). Dissociating episodic and semantic processes has proven difficult. Because most patients with mesial temporal lobe lesions sustain neuropathological insult to surrounding tissue it is difficult to arrive at conclusive evidence from neuropsychological evaluation of patients. It is also difficult to differentially localize PET activation within these mesial temporal lobe structures. Neuropsychological studies tend to support hippocampal involvement in encoding rather than in retrieval and in episodic rather than in semantic memory. In contrast, in neuroimaging studies activation in the left hippocampus has been observed both during encoding and during retrieval, the latter in fewer studies and for verbal but not nonverbal tasks. For example, Nyberg et al. (1996) observed more hippocampal system activation for words encoded semantically than for words encoded perceptually, greater recall for semantically encoded words, and a strong positive correlation between recall performance and hippocampal activation. Cohen et al. (1999) have argued that the two types of evidence, neuropsychological and neuroimaging, rather than being in conflict, tend to support a larger role for the hippocampus and indeed the parahippocampal area in relational memory processing, binding together multiple streams of information. The present findings would be consistent with the hypothesis that performance on odor identification tasks is, in part, verbally mediated and that the deficit that is tapped in the AD patients by odor identification tasks reflects their impairment in odor encoding, verbal memory, and associative binding of odor and verbal information required for successful performance.

Lateralization of olfactory function has presented an inconsistent set of findings derived from different methodological approaches. Psychophysical reports of odor threshold in normal subjects suggest a right hemisphere advantage (Youngentob et al., 1982). Loss of brain tissue in the right hemisphere is associated with greater impairment in odor recognition memory in temporal lobectomized patients (Jones-Gotman & Zatorre, 1993), although Eskenazi et al. (1986) reported greater deficits on the side of the lesion, regardless of lesion location. Imaging studies have tended to show bilateral activation in primary olfactory cortex (piriform cortex) and greater activation in the right than in the left orbital–frontal cortex (Sobel et al., 2000; Zatorre & Jones-Gotman, 1992), prompting Zatorre and Jones-Gotman (2000) and others to conclude that the primary sensory response appears to be bilateral while higher processing preferentially involves the right orbital–frontal cortex. These studies have tended to use a passive odor detection task. In the case of MEG studies, Kettenmann et al. (1997) have reported greater activation in the right hemisphere than in the left during passive odor task. More recent PET findings have noted some hemispheric specialization based on the familiarity of odors. Royet et al. (1999) demonstrated activation in the left inferior frontal lobe while subjects rated odor familiarity. Interestingly, unfamiliar odors have tended to evoke greater activation on the right cerebral hemisphere while familiar odors have tended to evoke bilateral activation (Savic & Berglund, 2000). The odors in the present study were all familiar odors and one might expect that familiarity played a positive role in identification (Murphy et al., 1991; Niccoli-Waller et al., 1999). The present findings would suggest that laterialization of function will depend on the subject's task, the region of interest and the extent to which the region of interest is intact. Although the entorhinal cortex is clearly multimodal, its integrity will be critical for processing olfactory information that relies on memory because it represents the direct and substantial link between incoming olfactory input and the hippocampus. Thus degeneration of the entorhinal cortex and the resulting disconnection from the hippocampus (Braak & Braak, 1997) might be expected to affect activation in hippocampus during odor tasks with memory components.

The current results are consistent with existing literature demonstrating correlations between hippocampal volume loss and a number of visual and verbal performance measures (Cahn et al., 1998; Deweer et al., 1995; Fama et al., 1997; Petersen et al., 2000), including the memory subtests of dementia status tests (Deweer et al., 1995; Fama et al., 1997; Stout et al. 1996). Stronger associations have been

reported in AD patients for verbal memory performance and left hippocampal volume (Cahn et al., 1998; Toledo-Morrell et al., 2000). Indeed, Fama et al. (1997) showed correlations between left hippocampal volumes and overall score on the DRS that were in the same range as the correlation for the Boston Naming Test, but below the correlation between left hippocampal volume and the odor identification scores, in the present study.

CONCLUSION

The present study found highly significant brain–behavior relationships in Alzheimer's patients between volumetric measures of the mesial temporal lobe, particularly in the left hippocampus, and performance in identification tests. The finding of a stronger relationship between left hippocampal volume and performance on the verbally based odor identification task is compatible with a left-hemisphere superiority for verbally mediated tasks. The findings suggest a neural substrate for the breakdown in functional performance on odor identification tasks in Alzheimer's disease. These results also suggest the potential clinical utility of inclusion of odor identification tests in diagnostic batteries for detecting Alzheimer's disease.

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