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Cholinergic plasticity in hippocampus of individuals with mild cognitive impairment: Correlation with Alzheimer's neuropathology

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Abstract. Several recent studies indicate that activity of cholinergic enzymes in the cortex of people with mild cognitive impairment (MCI) and early Alzheimer's disease (AD) are preserved. We correlated levels of hippocampal choline acetyltransferase (ChAT) activity with the extent of AD lesions in subjects from the Religious Order Study, including cases with no cognitive impairment (NCI), MCI, and with mild to moderate AD. Hippocampal ChAT activity levels were also determined in a group of end-stage AD patients who were enrolled in the University of Pittsburgh Alzheimer's Disease Research Center. MCI subjects were characterized with increased hippocampal ChAT activity. This elevation was no longer present in mild AD cases, which were not different from NCI subjects. Severe AD cases showed markedly depleted hippocampal ChAT levels. In NCI, MCI, and mild-moderate AD, there was a positive correlation between hippocampal ChAT activity levels and progression of neuritic plaque pathology in entorhinal cortex and hippocampus. A significant elevation of hippocampal ChAT in the MCI group was found selectively in the limbic (i.e., entorhinal-hippocampal, III/IV) Braak stages. We hypothesize that cholinergic changes in the hippocampus of MCI subjects reflect a compensatory response to the progressive denervation of the hippocampus by lost entorhinal cortex input. Moreover, the present findings suggest that the short-term memory loss observed in MCI is not caused by cholinergic deficits; it more likely relates to disrupted entorhinal-hippocampal connectivity.

1. Introduction

It has long been accepted that dysfunction of the cholinergic basal forebrain (CBF) system underlies some of the cognitive deficits seen in Alzheimer's disease (AD). Studies beginning in the mid to late 1970's, documented substantial reductions in cortical and hippocampal cholinergic markers in end stage AD [1–12]. These studies generally examined people in more advanced disease stages; more recent research has found stable levels of cortical and hippocampal cholinergic enzyme activity in early AD [13–15]. In addition, levels of choline acetyltransferase (ChAT), the acetylcholine-synthesizing enzyme, are increased in the hippocampus of subjects with mild cognitive impairment (MCI), indicating that the CBF-hippocampal projection system undergoes compensatory changes at early stages of the disease process [15].

Recently, it was proposed that the transition from normal aging to the pathological state of AD is associated with decreased neuroplasticity potential in the brain [16]. Since both MCI and early clinical AD

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cases are characterized by varying and often substantial, amounts of AD pathologic changes [13,15,17–20], particularly in the extent of neurofibrillary pathology, we determined whether increased ChAT activity in the hippocampus of MCI subjects correlated with the development and progression of these lesions during the clinical course of the disease. In AD, neurofibrillary tangles (NFTs) are thought to develop first in the entorhinal-transentorhinal area, then progressively spread in an orderly and predictable manner through the hippocampus and into the neocortex [21,22]. Based on the spread of NFT pathology, three main neuropathological stages can be distinguished, including "transentorhinal" (I/II), "limbic" (or "hippocampal", III/IV), and "isocortical"(V/VI) stage [21]. With the transition to each pathologic stage, more neuronal circuits are disrupted [23,24]. The disconnection between the entorhinal cortex and the hippocampus, which is hypothesized to be a primary event in AD pathology, has been well studied in animal models. Entorhinal cortex lesion deprives the hippocampus of its main excitatory glutamatergic input (i.e., the perforant pathway), inducing the sprouting of cholinergic medial septal excitatory projections into denervated hippocampal subfields in the rat [25]. Interestingly, similar hippocampal plasticity changes have also been observed in AD, supporting the concept that disease-related disruption of the perforant pathway initiates a compensatory cholinergic sprouting response in the hippocampus [26,27]. Therefore, the purpose of the present study was to determine whether hippocampal cholinergic up-regulation correlates with the distribution and quantity of NFT pathology determined by Braak staging.

2. Materials and methods

2.1. Subjects

The present investigation examined two groups of subjects (Table 1). The first group included virtually the same cases used previously in a study of cortical and hippocampal ChAT activity in MCI and early AD [15]. These subjects were enrolled in a longitudinal clinical-pathologic study of aging and AD of older Catholic nuns, priests and brothers (Religious Orders Study, ROS) [18,28–31]. The hippocampal ChAT activity measurements of this group have been reported previously [15]. The present investigation included 22 subjects with NCI (mean age $\pm$ SD $=$ 82 $\pm$ 6.8 years; mean MMSE $=$ 28 $\pm$ 1.6), 17 people with MCI (mean age $=$ 84.2 $\pm$ 5.8; mean MMSE $=$ 26.1 $\pm$ 2.6), and 14 subjects with mild to moderate AD (mean age $=$ 85.8 $\pm$ 6.6; mean MMSE $=$ 16.8 $\pm$ 7.7). These cases were derived from the first 60 consecutive cases to come to autopsy out of the more than 900 subjects enrolled in the ROS cohort; the investigators were blinded to the selection process. These experiments were approved by the human Investigations Committee of Rush-Presbyterian-St. Luke's Medical Center.

The second group of subjects included 16 patients, examined for the first time in this study, enrolled into the University of Pittsburgh Alzheimer's Disease Research Center (ADRC) patient cohort. On their last evaluation, performed within the same time frame as the ROS (i.e., within 12 months of death) their mean MMSE score was 8.1 ± 5.6; this was significantly lower than that in any of the 3 ROS groups ($p < 0.01$). Thus, the ADRC group represents clinically severe to end stage AD. Accordingly, the Pittsburgh ADRC sample was neuropathologically more advanced than the ROS diagnostic groups ($p < 0.01$); the majority of cases were judged neuropathologically as Braak stages V/VI. The ADRC AD cases also had a higher frequency of apolipoprotein E4 (Table 1), as is usually seen in specialty clinic populations. There were no differences in gender representation or duration of post-mortem interval in the ADRC and ROS groups. The mean age of ADRC AD subjects (78.9 ± 5.2) was the lowest among the four groups (overall $p = 0.019$), however, it was not significantly different from the NCI or MCI subjects in the ROS cohort (see Table 1). The Human Investigations Committee of the University of Pittsburgh approved this aspect of this study.

2.2. Clinical evaluation

Descriptions of the clinical evaluations in the ROS and University of Pittsburgh ADRC have been published previously [15,18,28–33]. In the ROS, neuropsychological tests, including the MMSE [34], were administered by trained neuropsychology technicians. The results of these tests were used by a board-certified neuropsychologist, blind to subjects' demographic and clinical data other than education, occupation and information about sensory or motor deficits and effort, to summarize impairment in cognitive domains as not present, possible, or probable. Clinical diagnosis was made by a board-certified neurologist with expertise in evaluation of the elderly. The diagnosis of dementia and AD followed the recommendations of the joint working group of the National Institute of Neurologi-

Table 1
Clinical, demographical, and pathological characteristics of the ADRC (late AD) and ROS subjects; natural logarithm of hippocampal ChAT levels

		Clinical Diagnosis					P-value
		NCI (N=22)	MCI (N=17)	Mild AD (N=14)	Late AD (N=16)	Total (N=69)	
Age (years) at death:	Mean ± SD (Range)	82.0 ± 6.8 (66–92)	84.2 ± 5.8 (75–97)	85.8 ± 6.6 (70–95)	78.9 ± 5.2 (72–90)	82.6 ± 6.5 (66–97)	0.019[a]
Number (%) of males:		13 (59.1%)	8 (47.1%)	9 (64.3%)	9 (56.3%)	39 (56.5%)	0.81[b]
Years of education:	Mean ± SD (Range)	18.2 ± 4.1 (8–25)	17.6 ± 3.5 (8–22)	16.0 ± 4.1 (6–21)	[N/A] (6–25)	17.5±3.9	0.25[a]
Number (Post-mortem interval (hours):	Mean ± SD (Range)	8.5± 7.3 (2.2–29)	6.2 ± 3.7 (3–13.9)	6.1 ± 2.9 (3–12)	5.6 ± 4.8 (2–18)	6.8 ± 5.3 (2.2–29)	0.31[a]
Mini mental state exam:	Mean ± SD (Range)	28.0 ± 1.6 (25–30)	26.1 ± 2.6 (20–30)	16.8 ± 7.7* (0–25)	8.1 ± 5.6 (2–23)	20.7 ± 9.3 (0–30)	< 0.0001[a]
Distribution of Braak scores:	0	2	0	0	0	2	
	I-/II	9	5	4	0	18	0.0002[b]
	III-/IV	9	9	6	3	27	
	V-/VI	2	3	4	13	22	
Log (HPC ChAT level):	Mean ± SD (Range)	0.9 ± 0.8 (−0.7, 2.5)	1.5 ± 0.6 (0.9, 2.5)	0.8 ± 0.6 (−0.4, 1.4)	−0.1 ± 0.7 (−1.6, 0.8)	0.8 ± 0.9 (−1.6, 2.5)	< 0.0001[a]

[a]One-way ANOVA.
[b]Fisher's exact test.
*One subject had missing MMSE.

cal and Communicative Disorders and the Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) [35]. The criteria for clinical classification of MCI were similar to those in the literature; these patients were not cognitively intact, however, they could not meet accepted criteria for dementia [20,36–41]. The present MCI population was defined as those rated as impaired on neuropsychological testing by the neuropsychologist, but who were not found to have dementia by the examining neurologist [15,18,20,28–32]. For all ROS subjects, a post-mortem interview was conducted to identify medical conditions that occurred during the interval between the last clinical evaluation and death. A summary clinical diagnosis was assigned by consensus of neurologists and neuropsychologists who reviewed all available clinical data, including records from each evaluation, the post-mortem interview, medical records and neuroimaging studies. This consensus diagnosis was made blind to all post-mortem data.

For the 16 cases enrolled in the University of Pittsburgh ADRC, clinical diagnosis of AD was made by neurologists, psychiatrists, and neuropsychologists, following a standardized ADRC evaluation at a Consensus Conference, utilizing NINCDS/ADRDA [35] and DSM-III-R [42] (now DSMIV) criteria. Neuropathological confirmation of definite AD was made by the ADRC neuropathologists using established criteria [43,44].

2.3. Pathological evaluation and tissue preparation

The procedure for brain tissue collection and processing was identical for ROS and ADRC subjects as described previously [15,18,28–31,45,46]. Cases were excluded if the brain exhibited significant non-AD pathology such as brain tumors, encephalitis, large strokes, multiple lacunar infarctions, etc. Following removal of the brain from the calvarium, one-cm thick slabs were either snap-frozen in liquid nitrogen or placed in 4% paraformaldehyde/0.1 M phosphate buffer. Hippocampal tissue was dissected in the coronal plane at the level of lateral geniculate nucleus (dissections were performed on dry ice to prevent tissue thawing) and stored at -80°C until assayed. From the immersion fixed slabs, select brain regions were dissected, paraffin embedded, cut at 8 μm, and stained with hematoxylin and eosin, modified Bielschowsky, thioflavin-S, and with a polyclonal anti-ubiquitin antibody (1:3000 dilution; Eastacreas Biologicals, Southbridge, MA).

A board-certified neuropathologist performed histopathological analysis, determined a Braak score [21], and made a final pathological diagnosis based upon both CERAD and NIA/Reagan diagnostic Criteria. The CERAD criteria are based on semi-quantitative estimation of neuritic plaque (NP) density, an age-adjusted plaque score, and presence or absence of dementia [43]. The NIA/Reagan [47] criteria are based upon an esti-

mation of cortical neuritic plaque density in combination with Braak stage. All cases were scored according to the Braak staging of neuropathologic changes. For this study, semi-quantitative estimates of diffuse plaques (DP), neuritic plaques, and NFTs were obtained for entorhinal cortex and hippocampus. The severity of these lesions was graded as follows: 0 (none), 1 (sparse), 2 (sparse/moderate), 3 (moderate), 4 (moderate/frequent), and 5 (frequent). The specific guidelines followed for determining the level of severity have been reported previously [48].

2.4. ChAT activity assay

Measurements of ChAT activity levels in the 16 ADRC subjects were performed following the same protocol described in our previous study [15]. Several ROS subjects underwent repeated measurements together with the ADRC cases, to control for possible variations in the assay procedure. Briefly, frozen hippocampal tissue samples were dissected of white matter and processed for ChAT activity assay by a modification of the Fonnum method [49,50]. Radioactive carbon-14 labeled acetyl Co-A (New England Nuclear, Boston, MA) was used for this assay. All samples, including controls and blanks, were run in triplicate, by a technician blinded to clinical diagnosis of ROS and ADRC subjects. BCA protein assay kits (Pierce, Rockford, IL) were used to determine protein content of the samples. ChAT activity values were expressed as μmol/hr/g protein.

2.5. Statistical analyses

Summary statistics are presented in mean $\pm$ SD, range, frequency, and percentage. The level of statistical significance was set at 0.05 (two-sided). For the comparison of clinical, demographical, and pathological characteristics among diagnostic groups (Table 1), one-way ANOVA and Fisher's exact test were used, as appropriate. The hippocampal ChAT levels were log-transformed and also compared among diagnostic groups using ANOVA. The ROS subjects were further stratified by Braak staging and their ChAT levels compared among diagnostic groups by the non-parametric Kruskal-Wallis test, as was the comparison of ChAT levels across Braak stages for each of the diagnosis category. If an overall significant result was found, additional post-hoc pair-wise comparisons were performed, adjusting for multiple comparisons by Tukey studentized range test in ANOVA analyses, or Bonferroni correction in Fisher's exact test or Kruskal-Wallis test. Spearman rank correlation was used in examining the relationship between neuropathological variables and ChAT levels.

3. Results

3.1. ChAT activity and clinical diagnosis

Hippocampal ChAT activity levels in the group with severe AD from our ADRC cohort were compared to those derived from our previous study of ROS subjects with NCI, MCI, and mild AD [15]. There was a significant difference in ChAT activity among the four groups (overall $p < 0.0001$, Table 1). The severe AD cases showed significantly lower ChAT activity relative to all three ROS diagnostic groups, and the MCI cases exhibited *higher* levels of ChAT compared to any of the other groups ($p < 0.05$).

3.2. Braak staging and clinical diagnosis

Within each clinical diagnostic group, there was heterogeneity of the extent of neuropathological changes. Each group was divided into four main pathological subgroups with Braak scores of "0" (no pathology), I/II (transentorhinal/ entorhinal pathology), III/IV (entorhinal/ hippocampal, or limbic), and V/VI (entorhinal/ hippocampal/ neocortical pathology; see Table 1) [21]. Only in the NCI group were there any subjects spared of pathology (stage "0", see Table 1). The NCI group also had the fewest cases with the highest level of NFTs pathology (stage V/VI), in contrast to the severe ADRC cases, which were mainly at the V/VI stage. The ROS clinical diagnostic groups with MCI and mild AD showed similar Braak staging (e.g., the Braak III/IV stage was more represented than stages I/II and V/VI). No MCI or AD cases were found *without* substantial (trans)entorhinal pathology (stage "0", see Table 1).

3.3. ChAT activity and Braak staging

To examine whether the up-regulation of ChAT activity in the MCI group related to a specific Braak stage, we compared hippocampal ChAT activity levels among the NCI, MCI, and mild AD cases with similar neuropathological changes (Table 2, Fig. 1). Statistically significant differences were found only in the III/IV stage ($p = 0.008$); the MCI cases showed significantly higher levels of hippocampal ChAT activity compared to either NCI or mild AD subjects at this level of pathology.

Table 2
Summary of natural logarithm of hippocampal ChAT levels, by ROS disease category and Braak stage

		Clinical Diagnosis			
		NCI (N = 20)*	MCI (N=17)	Mild AD (N=14)	P-valuea
Braak I/II					
	N	9	5	4	
	Mean ± SD	0.8 ± 0.9	1.4 ± 0.7	0.4 ± 0.6	0.20
	(Range)	(-0.7, 2.5)	(0.9,2.5)	(−0.1, 1.2)	
Braak III/IV					
	N	9	9	6	
	Mean ± SD	0.7 ± 0.7	1.6 ± 0.6	0.7 ± 0.6	0.008
	(Range)	(−0.7,1.6)	(1.0,2.5)	(−0.4,1.1)	
Break V/VI					
	N	2	3	4	
	Mean ± SD	1.3 ± 0.0	1.5 ± 0.5	1.1 ± 0.4	0.54
	(Range)	(1.3,1.4)	(0.9,1.9)	(0.5,1.4)	
P-valuea[a]		0.38	0.65	0.16	

[a]Kruskal-Wallis test.
*Two subjects with Braak stage = 0 excluded.

3.4. *ChAT activity and pathology in entorhinal cortex and hippocampus*

Combining all three ROS clinical diagnostic groups (see Table 3), we found a significant positive correlation between hippocampal ChAT activity and entorhinal NP ($r = 0.38$, $p = 0.004$) as well as entorhinal DP ($r = 0.27$, $p = 0.050$), but not entorhinal NFT ($r = 0.22$, $p = 0.107$). In all three ROS clinical diagnostic groups, increasing density of NP in entorhinal cortex was associated with significantly higher hippocampal ChAT activity ($p = 0.03$). In contrast, there was a trend for a correlation between hippocampal ChAT activity and density of entorhinal cortex DP ($p = 0.06$). However, when controlling for DP, ChAT activity levels were similar in NCI and mild AD, while they were significantly higher in MCI ($p = 0.043$).

As observed for entorhinal cortex NP, we found that increasing numbers of hippocampal NP correlated with higher hippocampal ChAT levels ($r = 0.32$, $p = 0.017$). Since there were only a few subjects with hippocampal DP, no significant statistical relationship could be discerned. There were no statistically significant relationships between hippocampal ChAT activity and hippocampal NFTs ($r = 0.08$, $p = 0.56$, see Table 3).

4. Discussion

The goal of this study was to examine whether hippocampal ChAT activity correlated with the degree of neurofibrillary pathology as defined by Braak scores. In cases diagnosed clinically with MCI and early AD we found similar proportions of the three main Braak neuropathological stages. In a previous study we showed that these diagnostic groups display comparable neuropathological diagnoses based on the NIA/Reagan Criteria, as well [15]. These findings are consistent with other studies of MCI showing that the underlying pathology was most similar to that found in AD [20,31, 51]. Taken together, these observations suggest that the pathology of the disease process occurs prior to the onset of clinical AD and progresses over many years [52]. The relationship between pathologic progression and changes in ChAT activity at the transition from MCI to AD remains a question of great significance for clinical diagnosis and early treatment of the disease.

When comparing NCI, MCI, and mild AD cases in each of the three Braak neuropathological stages, MCI subjects showed significantly higher ChAT levels only in the III/IV (entorhinal/hippocampal, or "limbic") stage. Although no statistically significant ChAT increases were found for Braak I/II and V/VI stages, there appeared to be a trend for higher ChAT values in MCI. The number of subjects in some of the ROS diagnostic groups was modest in these stages, and it is possible that a larger sample size would reach statistical significance. However, the present data suggest that individuals who manifest MCI are uniquely capable of a cholinergic compensatory response. This cholinergic up-regulation peaks at the time when the entorhinal cortex, which provides the major innervation to the hippocampus, is substantially disrupted in AD [31, 52]. Therefore, hippocampal cholinergic up-regulation may be stimulated by entorhinal-hippocampal discon-

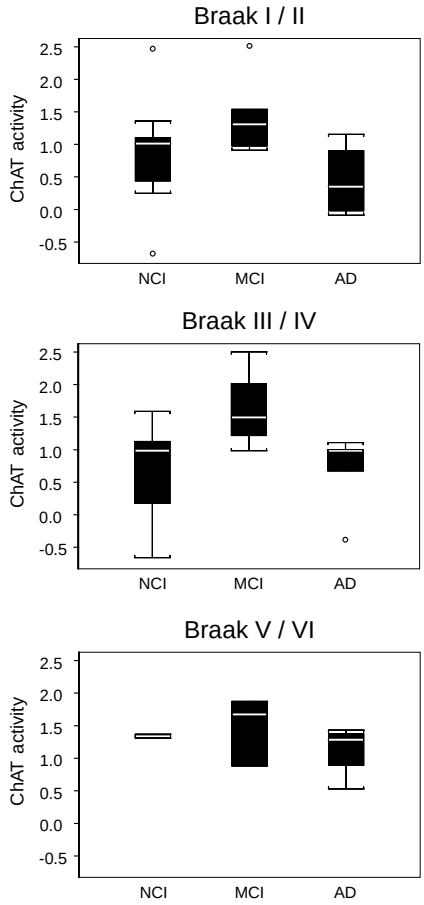

Fig. 1. Box plots showing logs of hippocampal ChAT activity measurements (μmol/hr/g protein), by clinical diagnosis (NCI, MCI, and AD) and neuropathological staging by Braak (I/II, III/IV, and V/VI). The horizontal white line in the middle of the black box indicates the median, while the top and bottom borders of the black box mark the 75th and 25th percentiles, respectively. The vertical lines above and below the black box extend to the farthest observation (denoted by the bracket) that is within 1.5 times the distance between the quartiles (inter-quartile range). Circles denote outliers, or observations falling outside of this range.

nection. This hypothesis is supported by studies in rats, showing that entorhinal cortex lesions induce sprouting of septal-hippocampal cholinergic fibers into hippocampal regions vacated by lost entorhinal innervation [25]. Similarly, compensatory sprouting of cholinergic hippocampal afferents occurs in AD [26,27]. Substantial reductions of layer II entorhinal-hippocampal projection neurons have been found not only in late AD, but also in subjects with MCI and early AD [31, 41,53]. These observations lend further support to our hypothesis that this initial entorhinal-hippocampal disconnection, and not cholinergic dysfunction, underlies the short-term memory deficits seen in MCI. It will be important to determine whether other components of the cholinergic system are altered in MCI and early AD including the high affinity choline uptake system or nicotinic receptors. Findings from these types of studies will also have to be correlated with cognitive change across clinical groups.

The up-regulation of ChAT in the hippocampus of people with MCI is most likely derived from the cholinergic neurons of the septal/diagonal band complex. Within the CBF, the neurons that project to the hippocampus are located more anteriorly than cholinergic neurons of the nucleus basalis of Meynert (NBM) [54]. Previous studies indicated that these CBF subfields show differential neuronal vulnerability in AD [55,56]. Neurons in the NBM complex, which provide the major cholinergic innervation to virtually all neocortical areas [57], undergo severe degeneration in late AD [55, 58]. Septal cholinergic neurons are more stable than their NBM counterparts, even in the later stages of AD [55,56]. Such greater vulnerability of cholinergic NBM cells could result from some early changes that precede both cell loss and clinical symptoms of AD dementia. These changes, which are more profound in the posterior NBM [55], include age-related down-regulation of the GluR2 receptor subunit [59, 60]. The loss of this subunit from the AMPA receptor complex can lead to increased Ca^{2+} permeability and excitotoxicity-related neurodegenerative changes (reviewed in [61]). In addition, age-related loss of calcium-binding proteins (CBP), such as calbindin-D28k, is believed to exacerbate CBF neuronal vulnerability to the disease process [62]. Collectively, these and other changes can contribute to NBM neuronal dysfunction. Future studies should examine changes in GluR2 and CBP expression levels in CBF of subjects with MCI and early AD.

Recent observations support the concept that NBM neurons do not undergo frank degeneration in MCI and mild AD, although phenotypic changes in their expression of different proteins occur during the early stage of the disease process. For example, in MCI and early AD there are significant reductions in NBM neurons containing the high (trkA) and low ($\mathrm{p75}^{\mathrm{NTR}}$) affinity receptor for nerve growth factor (NGF), but not ChAT [28–30]. These NGF receptor changes indicate that retrograde transport and/or signal transduction mechanisms could be impaired early in the disease. At present, there are no studies of septal cholinergic hippocampal-projection neurons in MCI and early AD, although this projection system undergoes cholinergic reorganization after entorhinal cortex lesions in rodents

Table 3
Correlations between hippocampal ChAT activity and semi-quantitative estimates of entorhinal and hippocampal AD pathology

Region/ AD pathology	Spearman rank correlation coefficient (r)	p-value
Entorhinal cortex		
Diffuse plaques	0.27	0.05
Neuritic plaques	0.38	0.004
Neurofibrillary tangles	0.22	0.107
Hippocampus		
Diffuse plaques	0.08	0.57
Neuritic plaques	0.32	0.02
Neurofibrillary tangles	0.08	0.56

and AD [25,26]. We propose that the septal cholinergic projections are capable of synaptic reorganization and increased synthesis of ChAT protein in response to entorhinal-hippocampal disconnection. However, as the disease process continues, this compensatory response subsequently fails, leading to the decline in hippocampal ChAT levels as seen in people with mild AD [15]. Continued impairment of such a putative compensatory mechanism then results in further decreases of hippocampal ChAT activity, as demonstrated in our current cohort of end-stage AD cases. Thus, the initial up-regulation of hippocampal ChAT activity could characterize the MCI state (marking the loss of entorhinal input and clinical memory loss), while the later down-regulation of cholinergic activity may mark the transition to AD. This scenario is consistent with Mesulam's proposal that AD is the result of plasticity mechanisms reaching the point of exhaustion over a prolonged period of time [16].

The specific biological mechanisms related to the up-regulation of cholinergic hippocampal activity in MCI most likely represent a complex interaction of cholinergic enzymes, amyloid precursor protein (APP) and Aβ, as well as growth factors [63]. APP production is increased in the denervated zone of the hippocampus after entorhinal cortex lesion [64], suggesting that early neuropathological changes in entorhinal cortex could exacerbate plaque development in the hippocampus. APP, which is contained in amyloid plaques, might play a role in neuritic outgrowth into plaques [65–67]. Thus, the aberrant sprouting response in plaques could be yet another stimulus for ChAT changes in the hippocampus. Such aberrant sprouting can be associated with abnormal and prolonged APP processing and increased Aβ production in AD [67,68]. Interestingly, experiments using mice over-expressing APP and presenilin 1, which result in amyloidosis in the hippocampus, show increased numbers of cholinergic/p75$^{\mathrm{NTR}}$ containing neurons in the medial septum and an increase in hippocampal innervation [69]. These findings suggest that amyloid deposition may act as a trophic substance for septal cholinergic neurons. This trophic-like response to amyloid may, in part, stimulate the up-regulation of hippocampal ChAT seen in people with MCI. Future immunohistochemical experiments in MCI hippocampus, using ChAT, APP, and Aβ antibodies, will be undertaken to further investigate this question. In addition, it remains to be demonstrated that the sprouting axons are capable of establishing appropriate contacts that would result in functional cholinergic synapses. If not, although counter-intuitive, it should be considered that increased hippocampal ChAT might reflect actual loss of cholinergic transmission in MCI.

In conclusion, our observations indicate that hippocampal ChAT up-regulation is specific for MCI; it is lost at the transition into early AD, and late AD. Only the latter state is characterized by a profound loss of ChAT activity. The present results suggest that the increase in hippocampal ChAT in MCI reflects a compensatory response to neuropathological lesions during the progression of AD. While there was a positive correlation between hippocampal ChAT and semi-quantitative estimates of regional neuritic plaques, no such relationship was observed for NFT. However, levels of NFTs were high in most of our cases, and the sample size in diagnostic groups may not have been large enough to detect significant differences. Future studies in our laboratory will employ unbiased stereological analysis in greater numbers of subjects to examine possible correlations between changes in hippocampal ChAT levels and numbers of entorhinal/hippocampal NFT. The present study also demonstrates that ChAT up-regulation in MCI hippocampus is specific for those cases affected with entorhinal/hippocampal pathology (Braak III/IV). Perhaps this compensatory response could predict clinical diagnosis needed to initiate early treatment strategies. Finally, the present findings suggest that early cognitive changes are not due to a loss of ChAT activity as commonly believed. Instead, mild

memory difficulties in MCI are more likely the result of a progressive loss of entorhinal input to hippocampus, depriving it of the cortical input necessary for normal memory function.

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