Increased Metabolic Activity in Nucleus Basalis of Meynert Neurons in Elderly Individuals With Mild Cognitive Impairment as Indicated by the Size of the Golgi Apparatus

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Abstract
In this study, we examined the metabolic activity of nucleus basalis of Meynert (NBM) neurons in individuals clinically diagnosed with no cognitive impairment (NCI, n = 8), mild cognitive impairment (MCI, n = 9), and subjects with moderate Alzheimer disease (AD, n = 7). We used Golgi apparatus (GA) size as a measure of neuronal metabolic activity. Subjects with MCI showed increased NBM metabolic activity; they had significantly more neurons with larger GA size as compared with NCI and AD subjects. In contrast, more NBM neurons with extremely small GA sizes, indicating reduced metabolic activity, were seen in AD. When these cases were classified according to their AD pathology (Braak I–II, III–IV, or V–VI), Braak III–IV subjects showed significantly increased GA sizes, comparable with the increase in clinically diagnosed MCI, whereas in Braak V–VI, GA sizes were dramatically reduced. Of all MCI and NCI subjects with similar Braak III–IV pathology, the MCI subjects again had significantly larger GA sizes. The larger NBM neuronal GA size seen in MCI suggests increased metabolic activity, associated with both the clinical progression from NCI to MCI, and with the early stages of AD pathology.

Key Words: Alzheimer disease, Braak stage, Cholinergic, Golgi apparatus, Metabolism, Mild cognitive impairment, Nucleus basalis of Meynert.

INTRODUCTION
Alzheimer disease (AD) is a multifactorial disease for which age and the apolipoprotein E (ApoE) ε4 allele are important risk factors. Furthermore, AD is a progressive neurodegenerative disorder that is characterized by a gradual decline of numerous cognitive processes, culminating in dementia. Mild cognitive impairment (MCI) is a relatively broad clinical condition involving a slight memory deficit, which in many cases represents a transitional state between normal cognition and AD (1–3). Neuropathology studies of normal elderly and subjects with clinically recognizable MCI show that both the entorhinal cortex and hippocampus, structures important for memory function, are particularly vulnerable to neurofibrillary tangle (NFT) pathology and neuronal loss (4, 5). MCI is increasingly recognized as an important public health problem, because it is associated with significant morbidity and is considered to be a prodromal stage of AD (6). Subjects with MCI progress to AD at rates of 10% to 15% per year (7–9). Examining the neurobiologic mechanisms underlying MCI is crucial for the development of early diagnostic tools and therapeutic strategies.

Cholinergic neurons of the nucleus basalis of Meynert (NBM) or Ch4 area provide the major cholinergic innervation to the cerebral cortex and the amygdala (10). In late-stage AD, the basal cortical cholinergic projection system undergoes a severe reduction in levels of expression of choline acetyltransferase (ChAT) mRNA per NBM cholinergic neuron, reduced NBM ChAT activity, reduced number of ChAT-immunopositive NBM neurons, and also reduced cortical ChAT activity (11, 12). These robust changes in the cholinergic basal cortical system led to the development of cholinesterase inhibitors as the main treatment approach for AD. However, the efficacy of these drugs has been modest at best and only provides symptomatic benefit (13).

During the past few years, there has been great interest in determining whether the molecular pathology seen in end-stage AD is an early event in the pathogenesis of the disease. To this end, clinical pathologic investigations have been undertaken using data derived from people diagnosed with MCI without dementia of the AD type. These MCI studies revealed that cholinergic basal cortical deficits do not occur early on in the process, and, more surprisingly, that ChAT activity was increased in the hippocampus and superior frontal cortex (14). The upregulation of cortical ChAT activity suggests that there may be
increased metabolic activity within the cholinergic neurons of the NBM during the prodromal stages of AD.

Neuronal atrophy is an indicator of decreased cellular metabolism, which has been extensively studied as a contributing factor to AD. An altered size of the Golgi apparatus (GA), part of the protein processing and targeting machinery, has been used as a sensitive marker for changes in neuronal metabolic activity (15) (see “Materials and Methods”). We have previously found significantly smaller GA sizes in NBM neurons in patients with late-stage AD (Braak [16] V–VI), suggesting a decline in metabolic activity in these neurons (17–19). In contrast to these severe AD cases, we found more neurons with increased GA sizes in normal, aged subjects determined to be in the early pathologic stages of AD (Braak I–II) (17). This finding, together with the increase in cortical and hippocampal ChAT activity (14, 20), suggests that during the early pathologic stages of AD, cholinergic neurons are responsive to the disease process. Therefore, the purpose of the present study was to examine the metabolic activity of NBM neurons postmortem in people who had been clinically diagnosed with MCI premortem.

MATERIALS AND METHODS

Subjects

We evaluated tissue samples from the NBM of 24 individuals who were participants in the Religious Order Study (ROS) (21), a longitudinal clinical–pathologic study of aging and AD in older Catholic nuns, priests, and brothers (Table). Each participant agreed to an annual detailed clinical evaluation and to brain donation at the time of death. For all subjects, cognitive testing scores were available for the last year of life. Subjects were clinically categorized premortem with no cognitive impairment (NCI; n = 8, mean age = 81.0 ± 2.1 years, mean Mini Mental State Examination [MMSE] [22] = 29.0 ± 0.27), MCI insufficient to meet criteria for dementia (n = 9, age = 85.9 ± 1.0 years, mean MMSE = 26.3 ± 0.82), or moderate/severe AD (n = 7, age 83.9 ± 1.6 years, mean MMSE = 15.2 ± 3.62). The Human Investigations Committee of Rush University Medical Center approved the study.

Clinical Evaluation: Religious Order Study Population

Details of the ROS clinical evaluation have been published elsewhere (21). Briefly, a team of investigators performed a complete annual clinical evaluation that included assessments for stroke and parkinsonian signs. Trained neuropsychologists administered the MMSE and a battery of 19 tests measuring performance in five cognitive domains: orientation, attention, memory, language, and perception. An impaired domain score required impairment on multiple tests. A board-certified clinical neuropsychologist used these results to summarize impairment in each of the five cognitive domains as 1) not present, 2) possible, or 3) probable. After review of all clinical data from that year and examination of the participant, a board-certified neurologist with expertise in the evaluation of the elderly made a clinical diagnosis. The diagnosis of dementia and AD followed the recommendations of the joint working group of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS/ADRDA) (23). As an assessment of the subjects’ overall cognitive functioning, the global cognitive score (GCS) was determined. The GCS is a composite z-score based on the 19 cognitive tests. GCS >0 indicates that, compared with a reference population (first 82 deceased ROS cases), the subject’s cognitive function is above average, whereas GCS <0 indicates a below average score.

There are no consensus criteria for the clinical classification of MCI. The present MCI population was defined as those persons rated “impaired” on neuropsychologic testing by the neuropsychologist but who were not found to have dementia by the examining neurologist (21). These criteria are similar to, or compatible with, those used by others in the field to describe persons who are cognitively below the norm but do not meet the established criteria for dementia (1–3). A postmortem review was conducted at the time of death to identify any medical conditions that occurred during the interval between the last clinical evaluation and death. Finally, a consensus conference of neurologists and neuropsychologists reviewed all available data and formed a clinical diagnosis.

Pathologic Evaluation and Tissue Preparation

Brains were processed at autopsy as described previously (24). Briefly, brains were sectioned into 1-cm-thick slabs using a Plexiglas cutting apparatus and hemisected. One hemisphere was immersion-fixed in 4% paraformaldehyde. From the opposite hemisphere, selected brain regions, including the basal forebrain, were dissected, paraffin-embedded, and cut at 8 μm. Sections were stained with hematoxylin and eosin for cytoarchitectonic evaluation with a modified Bielschowsky silver procedure, thioflavin-S, and with an ubiquitin antibody (25) to facilitate a complete pathologic diagnosis by a neuropathologist blind to the clinical diagnosis. Subsequently, characterization of “normal” (with respect to AD or other dementing processes), “possible AD,” “probable AD,” or “definite AD” was based on Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) criteria, semiquantitative estimation of neuritic plaque density, age-adjusted plaque score, and presence or absence of dementia (26). All cases received Braak scores (16). Based on the spread of NFT pathology, cases were staged as follows. In Braak I–II, NFTs are mainly in the “transentorhinal” and “entorhinal” cortices, which correspond to the preclinical stage of AD (27). In Braak III–IV, the subsequent “limbic” or “hippocampal” stages, NFTs also include the hippocampus and amygdala. In Braak V–VI, nearly all neocortical association areas are involved, which corresponds to the clinical picture of fully developed AD. The subjects categorized as NCI ranged from Braak stages I–IV (four subjects Braak I–II and four subjects Braak III–IV), those categorized as MCI ranged from III to V (eight subjects Braak III–IV and one subject Braak V–VI) and the AD subjects ranged from I to VI (one subject Braak I–II, 2 subjects Braak III–IV, and 4 subjects Braak V–VI; see Table).
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<th>ApoE e4 Genotype</th>
<th>Sex</th>
<th>PMI (hours)</th>
<th>Brain Weight (g)</th>
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ApoE, apolipoprotein E; GCS, Global Cognitive Score; MMSE, Mini Mental State Examination (22); NIA-Reagan Diagnostic Criteria, National Institute on Aging (28); NK, not known; PMI, postmortem interval; ROS, Religious Order Study; SEM, standard error of the mean; Braak stage according to Braak and Braak (16).
In addition, each case was assigned an NIA-Reagan Diagnostic Criteria (28) based on an estimation of cortical neuritic plaque density in combination with Braak stage. The subjects did not display Lewy body pathology in any brain region, including the NBM. ApoE genotyping was performed as previously described (29) (Table).

**Golgi Size as a Measure for Neuronal Metabolic Activity**

The mature Golgi complex consists of stacks of flattened membrane cisternae, through which newly synthesized proteins pass when traveling from the endoplasmic reticulum to their final destinations in the cell (30). The exact mechanisms that control shape, size, number of cisternae and their molecular composition, and what significance these have for GA functioning are still unresolved (31). However, measurements of alterations in the size by light and electron microscopy have been used frequently as a marker for changes in metabolic activity of neurons, in different cell types, and in different species. For instance, in the last decade, measurements of the size of the GA as a marker for alterations in activity have been used in several rat studies examining functional changes in vestibular ganglion cells, acini of glands, neurons of the ventrolateral division of the hypothalamic ventromedial nucleus, and in hamster and rabbit studies in the parvocellular neurons of the parvocellular division of the paraventricular nucleus, spinal ganglion neurons, and satellite cell sheaths enveloping spinal ganglion neurons (32–36). Moreover, in human studies, activity changes were studied in vasopressinergic neurons of the supraoptic nucleus, neurons of the ventromedial and of the tuberomamillary nucleus, spinal anterior horn cells, neurons of the vertical limb of the diagonal band of Broca, neurons of the thalamus and the red nucleus, and neurons of the nucleus basalis of Meynert (17, 19, 37, 40, 41). In addition, morphologic changes in size and fragmentation of the GA have been demonstrated in human neurodegenerative diseases such as X-linked spinal and bulbar muscular atrophy, amyotrophic lateral sclerosis, and in AD (17, 19, 39, 42–45). Recent experimental studies showed the effect of AD pathology on GA morphology in a hepatoma cell line study, which showed fragmentation of the GA in response to proteasome inhibitors, whereas in rat hippocampal cultures and in mice expressing mutant tau, GA fragmentation and even GA duplication was observed (46–48).

**Quantitative Analysis of Golgi Size Within the Nucleus Basalis of Meynert**

The cholinergic neurons of the NBM form a diffuse cell system consisting of medial and lateral subdivisions of the anterior part, or Ch4am and Ch4al, as well as an intermediate (Ch4i) and a posterior (Ch4p) subfield (49). The selection of sections chosen for GA measurement was performed at a standard level of Ch4a based on the visualization of the fornix, the anterior commissure, the optic tract, and supraoptic nucleus. To confirm the cholinergic phenotype of the NBM neurons one section was immunostained using a polyclonal ChAT antibody (dilution 1:50, Chemicon, Temecula, CA), as previously described (17).

To visualize the GA, tissue was immunostained using the polyclonal antibody HG-130 raised against an 18 amino acids epitope of a dialglycoprotein MG-160 of the medial cisternae of the human GA. Dependent on the available tissue and to exclude any interassay variation, a once-only immunocytochemical staining was performed on one section for 18 subjects and on three sections for the other six subjects. The specificity and staining procedures of the HG-130 antibody were performed as described previously (17, 38), with the exception that the sections were exposed to microwave treatment for antigen retrieval in Tris/HCl buffer (pH 9.0) (50). Both immunostainings were visualized using 0.5 mg/mL TBS 3,3-diaminobenzidinetetrahydrochloride as the chromogen and enhanced with ammonium nickel sulphate 2.2 mg/mL TBS and 0.3% H2O2 for 30 minutes.

The immunocytochemical staining pattern is typically perinuclear. Immunoelectron microscopy studies with rat and human brain tissue and with rat myocardium showed prominent and exclusive staining of cis, medial, and occasionally trans cisternae of the GA (51), and is thus diffuse through the GA. In addition, GA morphology can be evaluated between different cells and cases, because the GA size measurements were constantly performed in only those cells that showed a clear nucleolus, ensuring a fixed measurement plane in each cell in each case. Measurements of the GA area per neuron were performed using an IBAS-KAT (Kontron, Eching/München, Germany) image analysis system as described previously (17–19). Briefly, using the 2.5× objective, an image covering the entire NBM in each section was loaded into the IBAS and displayed on the computer monitor. For sampling, this image was covered by a grid overlaying the area “seen by the camera” using a 40× objective. From this grid, a percentage of fields covering the outlined area of the NBM were selected in a systematic random fashion. For each patient, a total of 95 to 100 fields were selected. An automatic segmentation of the GA staining was done for each field resulting in a binary “Golgi mask” image. Subsequently, all neuron profiles containing a nucleolus were outlined manually regardless of GA size or visibility of the GA. From each of these neuron outlines, the area covered by the Golgi mask and the total profile area was determined. The GA and cell profile surface area were measured (expressed in μm²). From the neurons outlined, the GA area covered by the image analysis mask and the total profile area were automatically determined. Independent of the number of tissue sections, neurons were outlined for each subject in 95 to 100 randomly selected fields (covering the NBM), resulting in a mean number of 84.3 ± 1.4 (mean standard error of mean) neurons measured per patient. The investigator was blinded to the clinical diagnosis.

**Statistical Analysis**

Group differences in demographic characteristics were tested using the nonparametric Kruskal-Wallis test. The effect of these parameters on median GA size per subject was analyzed using analysis of covariance. Correlation between GA size and cell profile area was tested using the Spearman rank correlation test.

The variation of the GA sizes within patients was almost as large as the variations among patients, suggesting...
that the GA size measurements within patients can be considered to be independent. For this reason, we have pooled all GA sizes per group and depicted frequency distribution histograms to show the shape differences of the GA size distribution. We used the nonparametric Kolmogorov-Smirnov two-sample test to analyze differences in GA size distributions and evaluate more subtle differences. The same holds for the cell profile areas.

Differences in MMSE and GCS between the groups, based on their clinical diagnosis (NCI, MCI, or AD) or pathologic diagnosis (Braak I–II, III–IV, and V–VI), were tested using the nonparametric Mann-Whitney U test. Furthermore, to determine the relationship between the MMSE and GCS and the median GA size per subject, bivariate correlation (Pearson correlation) was performed. For all tests, the accepted level of significance is \( p < 0.05 \).

**RESULTS**

There were no significant differences in age (\( p = 0.179 \)), sex (\( p = 0.933 \)), postmortem interval (PMI; \( p = 0.86 \)), brain weight (\( p = 0.153 \)), education (\( p = 0.642 \)), or APOE genotype (\( p = 0.375 \)) between any of the clinically diagnosed groups (Table 1). Median GA size per subject was normally distributed (\( p = 0.200 \)); analysis of covariance revealed that age (\( p = 0.698 \)), sex (\( p = 0.903 \)), PMI (\( p = 0.801 \)), brain weight (\( p = 0.859 \)), education (\( p = 0.859 \)), and apolipoprotein E (APOE) genotype (\( p = 0.886 \)) had no significant effect on median GA size per subject in any of the groups. GA size and cell profile area of the NBM neurons were significantly correlated within each of the three groups examined (\( p < 0.001 \)).

**Cell Profile Area**

The patients with AD showed significantly more neurons with small cell profile areas compared with the MCI and NCI subjects (\( p < 0.001 \)). The NCI and MCI subjects showed no difference (\( p > 0.418 \)). The median cell profile areas were 650.5, 663.6, and 583.8 \( \mu\text{m}^2 \) for the NCI, MCI, and AD groups, respectively.

**Golgi Apparatus Size and Clinical Diagnosis**

In all sections that met our morphometric criteria, ChAT staining showed that the great majority of neurons appeared to be cholinergic, as reported before (52) (Fig. 1). GA immunoreactivity within the Ch4 NBM neurons displayed a granular cytoplasmic pattern with a preferential perinuclear distribution (Fig. 2). NBM neurons in the MCI subjects showed enlarged GA size, frequently with the GA extending into the dendrites (Fig. 2B).

The median GA size within NBM neurons of the NCI, MCI, and AD groups were 73.7, 90.4, and 42.7 \( \mu\text{m}^2 \), respectively. The GA size frequency distributions are depicted in Figure 3. The Kolmogorov-Smirnov test showed that MCI had significantly more neurons with a large GA size as compared with either the NCI or AD subjects (Fig. 3A). By contrast, the AD cases displayed significantly more neurons with extremely small GA size compared with NCI (Fig. 3B, C).

**Golgi Apparatus Size and Braak Stage**

Overall, the cases with Braak stage I–II, III–IV, and V–VI had median GA sizes of 62.4, 80.9, and 54.6 \( \mu\text{m}^2 \), respectively.
respectively. The frequency distributions of the GA sizes of the three neuropathologically defined groups are shown in Figure 3. Subjects with Braak stages III–VI revealed significantly more neurons with large GA sizes than cases with Braak stages I–II (Fig. 3D). Subjects with Braak stage V–VI had significantly more neurons with smaller GA sizes, and a percentage of neurons with larger GA sizes, compared with subjects with Braak III–IV and Braak I–II (Fig. 3E, F).

Correlation of Mini Mental State Examination and Global Cognitive Score and Median Golgi Apparatus Size

Figure 4 illustrates the MMSE score per patient and median GA size per patient for the NCI, MCI, and AD groups. There was a significant difference in median GCS and MMSE scores among the NCI, MCI, and AD groups, and significant correlations between the clinical diagnoses and GCS and MMSE score per patient.

DISCUSSION

Increased Nucleus Basalis of Meynert Metabolic Activity as a Neuronal Plasticity Response

The present study revealed an increase in GA size within Ch4a NBM neurons in subjects clinically diagnosed as MCI and those with a Braak III–IV stage. These findings suggest an increase in NBM metabolic activity during the early stages of AD, which may be a compensatory response to the disease process. After the increase in NBM metabolic activity in MCI, activity was dramatically reduced in subjects with AD who displayed neurons with extremely small GA sizes. These later findings are in agreement with the reduced metabolic activity previously reported in the tuberomamillary nucleus, the hippocampus, and the NBM in severe AD as measured by changes in GA size (17–19, 53, 54). The shift from increased to reduced metabolic activity within NBM neurons between MCI and AD suggests that decreased NBM metabolic activity may be a molecular marker for the transition from the early to the late stages of AD (15, 17, 18). This metabolic change is similar to the increase in ChAT activity seen in the hippocampus and superior frontal cortex of subjects with MCI followed by a reduction in AD (14, 20). Interestingly, the anterior Ch4 neurons provide a portion of the cholinergic innervation to the frontal cortex and are the least affected NBM subgroup in end-stage AD (55). Therefore, the observed increase in metabolic activity seen in these neurons may reflect the upregulation of cortical ChAT activity seen in MCI (14).

Nucleus Basalis of Meynert Metabolic Activity and Braak Pathology

The NBM is the only forebrain structure outside the medial temporal area that also displays NFT and tauopathy in aged subjects having a Braak I–II score supporting the selective vulnerability of these neurons to age-related and tau-based neurofibrillary pathology (56, 57). In the present investigation, when subjects were subdivided according to their clinical diagnosis or Braak stage, the differences in GA sizes overlapped significantly. Because NBM neurons display a wide spectrum of abnormal cytoskeletal pathology across Braak stages, these cytoskeletal changes might partly explain the difficulties in defining a case as MCI using Braak staging. In fact, other studies indicate that the progression of Braak pathology and cognitive impairment are not always inextricably bound (14, 20, 57–61).
FIGURE 3. (A-F) Frequency distribution histograms of nucleus basalis of Meynert Golgi apparatus (GA) sizes. Note the higher proportion of large GA sizes in mild cognitive impairment and in Braak stage III–IV. The Y-axis shows the percentage of the total number of neurons for that group. N, number of neurons measured.
In this regard, we previously reported an increase in NBM metabolic activity in cognitively intact subjects with Braak I–II scores compared with Braak 0 subjects (i.e. without any NFT neuropathology [17]). These findings suggest that NBM neurons might respond to NFT pathology by increasing their metabolic activity to compensate for neuronal dysfunction during the early phases of the disease process. Because Braak V–VI subjects show more neurons with extremely small GA sizes reflecting lower metabolic activity (17–19), this suggests a failure to maintain this compensatory metabolic response marking the transition from MCI and early AD to frank AD (14, 20). In fact, It has been suggested that the end-stage AD brain reaches a point of exhaustion for the maintenance of neural plasticity mechanisms (62).

The cell profile frequency distribution in the present study is in agreement with the study of Rinne et al, who showed that the population of large neurons with a diameter greater than 30 μm had almost disappeared in AD, and more neurons with small size classes were found in patients with AD than in NCI, suggesting that shrinkage may play a role (63).

**Interaction of Nucleus Basalis of Meynert Metabolic Activity With Other Cellular Impairments**

MCI subjects with a Braak III–IV score showed significantly increased NBM GA sizes compared with NCI and AD subjects with a similar Braak stage. This observation is in agreement with the findings showing a significant increase in hippocampal ChAT activity in MCI subjects with Braak III–IV compared with NCI and AD subjects with the same pathologic score (20). Because GA sizes increased in MCI compared with NCI with similar AD pathology, it is possible that NFT pathology is not the only factor initiating cholinergic plasticity responses in MCI (14). Indeed, although the number of NBM ChAT and vesicular acetylcholine transporter (VACHT)-immunoreactive neurons is preserved, and ChAT activity is increased in the hippocampus and frontal cortex in MCI and mild AD, many other neuronal changes occur that support the hypothesis that cholinergic neurons in MCI are not normally regulated (14, 24, 64). For example, the amount of mRNA of the NGF high-affinity signal transduction receptor TrkA is reduced in MCI and mild AD as well as the number of TrkA-immunoreactive NBM neurons (29, 65).

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In fact, these abnormalities are even more severe in advanced AD (58, 66, 67). In addition, the number of low-affinity neurotrophic receptor p75NTR-immunoreactive neurons in the NBM is also significantly reduced in MCI and early AD as well as in severe AD, although the expression of NBM p75NTR mRNA is not significantly different (60, 66, 67). Moreover, p75NTR protein is stable in cortical areas, whereas TrkA is reduced during the progression of AD (59). This preservation of p75NTR protein levels within the cortex may be related to the de novo expression of p75NTR-immunoreactive cortical neurons in AD (68). Recent findings indicate that p75NTR possesses complex autonomous signaling capabilities in response to NGF and proNGF that, paradoxically, regulate both cell survival (increased TrkA responsiveness to NGF) and cell death under different conditions (59, 69–71). Therefore, it is possible that changes in NGF receptor binding and/or production may be a factor triggering increased metabolic activity within NBF neurons in prodromal AD.

**FIGURE 4.** Histogram of the median Golgi apparatus size per patient and Mini Mental State Examination (MMSE) per patient per clinically diagnosed group. There was a significant difference in MMSE (p < 0.001) and Global Cognitive Score (GCS) (p = 0.001) between the no cognitive impairment (NCI), mild cognitive impairment (MCI), and Alzheimer disease (AD) groups. In the NCI and MCI subjects, the clinical diagnosis was significantly negatively correlated with GCS (p = 0.006, r = -0.632) and MMSE scores (p = 0.013, r = -0.588). In the MCI and AD subjects, clinical diagnosis was significantly positively correlated to the GCS (p = 0.004, r = 0.692) and MMSE scores (p = 0.002, r = 0.714).
CONCLUSIONS

Previous studies have shown that decreased metabolic activity, indicated by changes in GA size, is dependent on ApoE ε4 allele status in aged controls and in patients with AD (17, 18). The present study reveals that increased NBM neuron metabolic activity coincides with the development of NFT pathology in an inverted U-shaped manner. In this regard, Braak I–II compared with Braak 0 subjects and Braak III–IV compared with Braak I–II subjects showed increased GA sizes. However, Braak V–VI compared with Braak III–IV subjects showed reduced GA sizes. Because an increase in GA size was seen in MCI compared with NCI, both displaying similar AD pathology, NFT formation alone is not the only predisposing factor for the initiation of an increase in GA size. Another trigger may be the early dysfunction of the NGF receptor system within NBM neurons reported in people with MCI (72). We suggest that alterations in metabolic and neurotrophic activity within cholinergic NBM neurons mark the transition from MCI to AD. Perhaps further studies measuring the GA protein MG-160 gene expression or specific AD pathology proteins can be examined in relation to GA size changes during the progression of AD; or, possibly, visualization of the increased metabolic activity reported here can be evaluated in living brain.

Moreover, the changes discussed in the present report are examples of the ability of the aged diseased brain to display cellular plasticity in the face of the progression of AD. Finally, the present findings of an increase in NBM metabolic activity in MCI and the recent observation of NBM neuron fiber outgrowth after NGF delivery directly into this region (73) support a therapeutic strategy aimed at the reactivation of NBM neurons as a promising avenue for the treatment of AD.

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