Effects of Age and Amyloid Deposition on Aβ Dynamics in the Human Central Nervous System

Yafei Huang, PhD; Rachel Potter, BA; Wendy Sigurdson, MSN; Anna Santacruz, BSN; Shirley Shih, BA; Yo-El Ju, MD; Tom Kasten, PhD; John C. Morris, MD; Mark Mintun, MD; Stephen Duntley, MD; Randall J. Bateman, MD

Background: The amyloid hypothesis predicts that increased production or decreased clearance of β-amyloid (Aβ) leads to amyloidosis, which ultimately culminates in Alzheimer disease (AD).

Objective: To investigate whether dynamic changes in Aβ levels in the human central nervous system may be altered by aging or by the pathology of AD and thus contribute to the risk of AD.

Design: Repeated-measures case-control study.

Setting: Washington University School of Medicine in St Louis, Missouri.

Participants: Participants with amyloid deposition, participants without amyloid deposition, and younger normal control participants.

Main Outcome Measures: In this study, hourly cerebrospinal fluid (CSF) Aβ concentrations were compared with age, status of amyloid deposition, electroencephalography, and video recording data.

Results: Linear increases were observed over time in the Aβ levels in CSF samples obtained from the younger normal control participants and the older participants without amyloid deposition, but not from the older participants with amyloid deposition. Significant circadian patterns were observed in the Aβ levels in CSF samples obtained from the younger control participants. However, circadian amplitudes decreased in both older participants without amyloid deposition and older participants with amyloid deposition. Aβ diurnal concentrations were correlated with the amount of sleep but not with the various activities that the participants participated in while awake.

Conclusions: A reduction in the linear increase in the Aβ levels in CSF samples that is associated with amyloid deposition and a decreased CSF Aβ diurnal pattern associated with increasing age disrupt the normal physiology of Aβ dynamics and may contribute to AD.
One hypothesis suggests that neuronal activity is responsible for the dynamics of Aβ. Modulation of neuronal activity by electrical, pharmacological, and behavioral interventions has direct effects on the concentrations of Aβ in the central nervous system (CNS). For example, increased stress and decreased sleep have both been demonstrated to increase Aβ concentrations in animal models. Furthermore, a recent study demonstrated the effect of sleep on several synaptic markers, suggesting that sleep may modulate the metabolism of a number of CNS proteins.

Circadian rhythms have been described for a variety of biochemical, physiological, and behavioral processes that occur over a 24-hour cycle. Examples of circadian rhythms include body temperature, circulating levels of hormones such as cortisol, and blood levels of ions such as sodium. The level of Aβ in CSF demonstrates a circadian pattern in healthy younger participants; however, the dynamics of Aβ in aging and AD are less well understood. Although prior reports indicate that the level of Aβ42 in CSF is stable in individuals with AD, little is known about the effects of age and amyloid deposition on Aβ dynamics in the human CNS. Because age is the largest risk factor for AD, understanding changes in the dynamics of Aβ with aging may inform about the pathophysiological processes that lead to amyloidosis and ultimately AD. Furthermore, the effects of amyloidosis may reveal changes in CNS Aβ dynamics that are associated with the pathology of AD.

In our study, we investigated CSF Aβ dynamics and how they are affected by aging and amyloidosis. Samples of CSF were collected hourly from each participant, and electroencephalography (EEG) and video recordings were obtained continuously from a subset of study patients. The concentrations of CSF Aβ40 and Aβ42 were measured using an enzyme-linked immunosorbent assay and were analyzed over time for Aβ dynamics.

Figure 1. Diagram of a participant during the monitoring of Aβ levels in cerebrospinal fluid (CSF) samples. These samples were collected from a lumbar intrathecal catheter approximately every hour for 36 hours from each participant, and electroencephalograms (EEGs) and video recordings were obtained continuously from a subset of study patients. The concentrations of CSF Aβ40 and Aβ42 were measured using an enzyme-linked immunosorbent assay and were analyzed over time for Aβ dynamics.
SAMPLE COLLECTION
An intrathecal lumbar catheter was placed in all participants between 7:30 AM and 9:00 AM, and the collection of samples was started between 8:00 AM and 9:30 AM. For 36 hours, 6 mL of CSF were obtained every hour. Aliquots of CSF were frozen at −80°C immediately after being collected in 1-mL polypropylene tubes. Participants were encouraged to stay in bed and were allowed to choose when to sleep, read, watch television, or talk throughout the study period. Participants had meals served at 9:00 AM, 1:00 PM, and 6:00 PM.

ANALYSIS OF CSF SAMPLES
From each hour of collection, 1 mL of CSF was thawed, and Aβ40 and Aβ42 were measured by use of an enzyme-linked immunosorbent assay (ELISA). In brief, 2G3 (anti-Aβ40) and 21F12 (anti-Aβ42) antibodies were used as the capture antibodies, and biotinylated 3D6 antibody (anti-Aβ1-3) was used as the detection antibody. Each sample was assessed in duplicate. All samples from each participant were measured together on the same ELISA plate to avoid interplate variation. To measure the effect of ELISA variability, we ran separate ELISA plates for Aβ40 and Aβ42 with a single CSF sample for both assays. The mean percentage of the intrasample coefficient of variation for duplicates was 10.4% for a single CSF sample for both assays. The mean percentage of the intrasample coefficient of variation for duplicates at −80°C immediately after being collected in 1-mL polypropylene tubes. Participants were encouraged to stay in bed and were allowed to choose when to sleep, read, watch television, or talk throughout the study period. Participants had meals served at 9:00 AM, 1:00 PM, and 6:00 PM.

SLEEP STAGING AND EEG
Electroencephalographic data were collected for the YNC group. Ambulatory equipment (Tracklt; Lifelines Ltd, Southampton, England) was used to record signals from 6 EEG electrodes (F3, F4, C3, C4, O1, and O2), a chin electromyogram, and left and right electro-oculograms. This information was then imported and scored using Polysmith 6.0 (Nihon Kohden, Tokyo, Japan) using standard sleep scoring criteria.21 The sleep stage (awake, rapid-eye movement sleep, N1, N2, or N3) was scored for each 30-second epoch. Total sleep time (including the sleep (awake, rapid-eye movement sleep, N1, N2, or N3) was scored for each 30-second epoch. Total sleep time (including the sleep stages of rapid-eye movement sleep, N1, N2, and N3) was binned every hour and expressed as minutes of sleep per hour.

VIDEO RECORDING
A participant’s activity was video recorded using a Logitech Quickcam (Fremont, California) installed on a laptop computer for the duration of our study. Recording began shortly after the intrathecal catheter was inserted and continued for a 36- to 48-hour period. Videos were reviewed, and a participant’s activity was coded in 30-second intervals using Microsoft Excel 2007 (Microsoft Corp, Redmond, Washington). Activities were rated as sleeping, talking, eating, reading, watching television, defecation and/or urination, writing, computer use, sample collection, and catheter manipulation (eTable, http://www.archneurol.com). The position of the participant was coded as upright (≥60°), partially upright (15°-60°), or flat (<15°). After the videos were reviewed, they were quality-checked for accurate recording of the sample collection using patient charts. All video coders reviewed the same “test video,” and Cohen κ coefficients were used to measure inter-rater agreement of video coding. The weighted κ coefficients among the 3 different coders ranged from 0.77 to 0.79.

STATISTICAL ANALYSIS
All analyses were performed using SAS version 9.2 (SAS Institute, Cary, North Carolina). Graphs were plotted in GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego, California). Linear changes and circadian rhythms of Aβ fluctuation over time were explored in our study. Both individual time-course data for each patient and group-averaged data were used in circadian pattern recognition. Group-averaged data were calculated as follows: for each patient, (1) the mean Aβ40 and Aβ42 levels over a 36-hour period were calculated; (2) the mean-adjusted Aβ40 and Aβ42 levels were estimated for each time point and expressed as a percentage of the mean; and (3) hourly serial values of mean-adjusted Aβ40 and Aβ42 levels were grouped based on their amyloid plaque status, and an hourly average was calculated for each group.

COSINOR ANALYSIS
Single cosinor analysis was used to analyze the patterns of the Aβ40 and Aβ42 levels in each participant over the 36-hour period. A cosine transformation was applied to the time variable using 24 hours as the default circadian cycle, and the SAS PROC NLIN procedure (SAS Institute, Cary, North Carolina) was used to estimate the parameters of the circadian patterns for Aβ fluctuations. The mesor (midline of the Aβ oscillation), amplitude (distance between the peak and mesor), and acrophase (the time corresponding to the peak of the curve) were calculated for each patient and averaged within each group. Analysis of variance was used to assess the differences in mesor and amplitude among different groups. Similarly, group-averaged data were used to estimate the parameters of circadian rhythms in the 3 study groups.

DECREASED Aβ42 VARIABILITY IN AD
To compare the effects of age and amyloid deposition on hourly fluctuations, the YNC group was compared with the older amyloid⁺ and amyloid⁻ groups, as determined by 11C]PiB-PET. Variability of Aβ in each patient was calculated as the standard deviation of serial Aβ measurements over time, and the 36-hour mean Aβ concentration was averaged by group (the YNC, amyloid⁺, and amyloid⁻ groups), as shown in Tables 1 and 2.

RESULTS

Table 1. Data on the 36-Hour Mean Concentrations of Aβ42, by Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (SD)</th>
<th>Mean of SD</th>
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<tbody>
<tr>
<td></td>
<td>Aβ42 Concentrations Over 36 h, pM</td>
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<tr>
<td></td>
<td>SD</td>
<td>(SD)</td>
</tr>
<tr>
<td>YNC (n=20)</td>
<td>213.8 (80.6)</td>
<td>54.2 (25.5)</td>
</tr>
<tr>
<td>Amyloid⁻ (n=15)</td>
<td>226.2 (160.4)</td>
<td>42.5 (22.6)</td>
</tr>
<tr>
<td>Amyloid⁺ (n=11)</td>
<td>82.7 (53.8)</td>
<td>14.9 (9.9)</td>
</tr>
</tbody>
</table>

Abbreviations: Amyloid⁺, participants who tested negative for amyloid plaque; Amyloid⁻, participants who tested positive for amyloid plaque; YNC, younger normal control.

Comparisons were made between the amyloid⁺ and amyloid⁻ groups, using the YNC group as the reference.

 Differences were statistically significant at P<.01.

As expected, the CSF Aβ42 concentration was lower in the amyloid⁺ group than in the amyloid⁻ group (63%
lower; \( P < .01 \) (Table 1). In addition, there was also a 3-fold decrease in the variability (standard deviation) of A\( \beta \)42 hourly changes in the amyloid\(^+\) group compared with either the amyloid\(^-\) group or the YNC group (\( P < .01 \)). There was a nonsignificant trend toward decreased variability in the cognitively impaired amyloid\(^+\) group compared with the cognitively normal amyloid\(^-\) group: a mean A\( \beta \)40 standard deviation of 260pM for the 5 participants in the cognitively impaired amyloid\(^+\) group vs 562pM for the 6 participants in the cognitively normal amyloid\(^-\) group (\( P = .11 \)), and a mean A\( \beta \)42 standard deviation of 9.7pM for the 5 participants in the cognitively impaired amyloid\(^+\) group vs 19.2pM for the 6 participants in the cognitively normal amyloid\(^-\) group (\( P = .30 \)). However, there was no difference in variability between the amyloid\(^+\) and YNC groups, which indicates that the reduction in A\( \beta \)42 and A\( \beta \)44 variability was due to amyloid status, not age.

No difference was found in mean A\( \beta \)40 level or mean A\( \beta \)40 variability (\( P > .05 \); Table 2) between amyloid\(^-\) and amyloid\(^+\) groups; however, there was lower variability in the amyloid\(^-\) group than in the YNC group (\( P < .05 \)). Individual plots of A\( \beta \) concentration highlight the significant decrease in hourly variability between the amyloid\(^-\), amyloid\(^+\), and YNC groups (eFigure 1).

We explored CSF A\( \beta \) dynamics with respect to linear change over time and circadian rhythm. In the younger control group, a linear increase in the mean A\( \beta \) concentration was observed over time (Figure 2A). Circadian patterns remained after the linear trend was removed (Figure 2B).

### AMYLOID DEPOSITION EFFECTS ON THE LINEAR INCREASE IN A\( \beta \) LEVELS

To compare the associations between the linear increase in A\( \beta \) concentrations (the so-called A\( \beta \) linear rise), age, and amyloid deposition, calculations of the A\( \beta \) linear rise were expressed as the percent change over 24 hours for participants in the YNC, amyloid\(^-\), and amyloid\(^+\) groups. The mean percent change of the linear increase of A\( \beta \)42 concentrations over 24 hours was 17% for the YNC group, 24% for the amyloid\(^-\) group, and 7% for the amyloid\(^+\) group (Table 3). The amyloid\(^-\) group demonstrated a 66% lower A\( \beta \)42 linear rise compared with the combined results of the amyloid\(^+\) and YNC groups (\( P < .05 \)). Furthermore, with increased amyloid deposition, as measured by the mean cortical binding potential of [\( ^{11} \)C]PiB, there was less of a linear rise in A\( \beta \)42 level over 24 hours (Figure 3). Most participants with amyloid deposition (those in the amyloid\(^+\) group) had no significant A\( \beta \)42 linear rise. Conversely, most participants without amyloid deposition (those in the amyloid\(^-\) group) or those younger participants unlikely to have amyloid deposition by virtue of their age (those in the YNC group) had a significant A\( \beta \)42 linear rise.

The mean percent change in the A\( \beta \)40 linear rise per 24 hours was 19% for the YNC group, 24% for the amyloid\(^-\) group, and 15% for the amyloid\(^+\) group (Table 4). Although the amyloid\(^+\) group had a lower mean A\( \beta \)40 linear rise, the trend was not statistically different from the amyloid\(^-\) and YNC groups (\( P = .20 \)).

### DECREASING A\( \beta \) CIRCADIAN PATTERNS WITH AGE AND AMYLOID DEPOSITION

Cosinor analysis was used to assess the circadian patterns of A\( \beta \) dynamics in each individual participant. The mesor (midline of cosinor fit), amplitude (difference between me-
Table 3. Comparison of Linear Increases In and Cosinor Parameters for Aβ42 Among 3 Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Linear Increase in Aβ42 Level per 24 h, %</th>
<th>Aβ42 Circadian Pattern</th>
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<tbody>
<tr>
<td></td>
<td>Mesor, pM</td>
<td>Amplitude, pM</td>
</tr>
<tr>
<td>YNC (n=20)</td>
<td>17.4 (18.8)</td>
<td>215.4 (81.6)</td>
</tr>
<tr>
<td>Amyloid− (n=15)</td>
<td>24.4 (23.5)</td>
<td>226.7 (161.2)</td>
</tr>
<tr>
<td>Amyloid+ (n=11)</td>
<td>7.3 (18.4)</td>
<td>82.4 (54.1)</td>
</tr>
</tbody>
</table>

Abbreviations: Amyloid−, participants who tested negative for amyloid plaque; amyloid+, participants who tested positive for amyloid plaque; YNC, younger normative control.

a Comparisons were made between the amyloid− and amyloid+ groups, using the YNC group as the reference.

b Differences were statistically significant at P<.01.

c Demonstrated a statistical difference (P<.05) between the amyloid+ group and the participants who tested negative for amyloid plaque (by use of carbon 11–labeled Pittsburgh Compound B–positron emission tomography) and the YNC group combined.

To further explore the relationship between age and Aβ42 circadian rhythms, we plotted each participant’s Aβ42 circadian amplitude vs age (Figure 4). The amplitude of the Aβ42 circadian pattern was inversely correlated with age when the YNC and amyloid− groups were included (r=−0.49, P<.01) and when all 3 groups were included in the analysis (r=−0.61, P<.01). After controlling for age, there was no significant difference in amplitudes between the amyloid+, amyloid−, and YNC groups (P=.27). There was a nonsignificant trend toward a decreasing circadian pattern in the cognitively impaired amyloid+ group compared with the cognitively normal amyloid+ group: a mean Aβ40 circadian amplitude of 126 pM for the 5 participants in the cognitively impaired amyloid+ group vs 309 pM for the 6 participants in the cognitively normal amyloid+ group (P=.19), and a mean Aβ42 circadian amplitude of 3.2 pM for the 5 participants in the cognitively impaired amyloid+ group vs 8.8 pM for the 6 participants in the cognitively normal amyloid+ group (P=.08).

Cosinor analyses were also conducted on the group-averaged data for both Aβ40 and Aβ42 levels in the 3 groups (Figure 5). The range in mean Aβ levels over time before cosinor transformation was approximately 40% of the mean (Figure 5). Aβ40 circadian patterns were found by use of a cosinor curve fit (P<.01) in all 3 groups; however, an Aβ42 cosinor pattern was only found in the YNC group. Similar to individual cosinor analysis, the YNC group had higher circadian amplitudes in both Aβ40 and Aβ42. The peak CSF Aβ40 levels (acrophase) occurred at approximately 10 PM, and the lowest levels occurred at approximately 10 AM, for the YNC, amyloid−, and amyloid+

groups. Similarly, CSF Aβ42 amplitudes reached a maximum at 10 PM and a minimum at 10 AM for the YNC group.

**Relationship of Aβ Concentrations to Wakefulness and Sleep**

Previous animal studies have demonstrated a direct relationship between wakefulness and increases in Aβ level.

Herein, we assessed the correlation between Aβ levels and total sleep time in a subset of 12 participants who had EEG recordings in the YNC group (Figure 6). As expected, a circadian pattern was identified in the mean total sleep time (P<.01). Interestingly, the peak of wakefulness occurred at 4 AM, whereas the CSF Aβ peak occurred 6 hours later at 10 PM. The peak sleep time occurred at 4 AM, whereas the lowest CSF Aβ level occurred 6 hours later at 10 AM. A 6-hour delay from sleep to change in Aβ is expected because there is a 6-hour lag from the time of labeling to the time of detection of labeled Aβ in the lumbar CSF. Thus, both CSF Aβ40 and Aβ42 levels were inversely correlated with sleep after a 6-hour delay.

**Figure 3.** Mean percent change with respect to the linear increase in Aβ42 level per 24 hours, by mean cortical binding potential (MCBP) of carbon 11–labeled Pittsburgh Compound B. In general, individuals without amyloid deposition (younger normative controls [YNCs] and older cognitively normal controls who tested negative for amyloid plaque [amyloid−]) had significant increases in Aβ42 level, independent of age, whereas participants with amyloid deposition (older cognitively normal controls who tested positive for amyloid plaque [amyloid+]) had lower increases in Aβ42 level (P<.05).
Table 4. Comparison of Linear Increases In and Cosinor Parameters for Aβ40 Among 3 Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Linear Increase per 24 h, %</th>
<th>Mesor, pM</th>
<th>Amplitude, pM</th>
<th>Amplitude-to-Mesor Ratio, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>YNC (n=20)</td>
<td>18.9 (22.5)</td>
<td>2012.9 (917.8)</td>
<td>317.6 (187.2)</td>
<td>16.9 (7.8)</td>
</tr>
<tr>
<td>Amyloid− (n=15)</td>
<td>23.5 (24.5)</td>
<td>2599.7 (1682.6)</td>
<td>180.3 (115.5)b</td>
<td>9.8 (6.2)c</td>
</tr>
<tr>
<td>Amyloid+ (n=11)</td>
<td>14.5 (13.8)</td>
<td>2441.2 (1180.3)</td>
<td>226.3 (221.7)</td>
<td>11.3 (9.5)</td>
</tr>
</tbody>
</table>

Abbreviations: Amyloid−, participants who tested negative for amyloid plaque; Amyloid+, participants who tested positive for amyloid plaque; YNC, younger normal control.

a Differences were statistically significant at P < .05.

b Differences were statistically significant at P < .01.

c Differences were statistically significant at P < .05.

The relationship between total protein and Aβ40 among the YNC, amyloid−, and amyloid+ groups.

The mean levels of Aβ40 and total protein for each person over time were also calculated, and no correlation was found between them (r = 0.15, P = .44). As expected, mean total protein levels were significantly lower in young controls compared with the older participants (with a mean of 540.6 µg/mL for participants in the YNC group, 696.4 µg/mL for participants in the amyloid− group, and 656.1 µg/mL for participants in the amyloid+ group; P = .014). Total protein levels were averaged by group, and a cosinor fit was applied. There was no significant circadian pattern in total protein for any of the 3 groups (P > .05) (eFigure 2). Thus, CSF Aβ dynamics appear to be independent of CSF total protein changes.

INDIVIDUAL ACTIVITY AND Aβ LEVELS

Because individual behaviors may influence neuronal activity and therefore influence Aβ production, we assessed correlations between individual activities and hourly CSF Aβ levels. Video-rated activities included catheter manipulation, CSF sample collection, computer use, defecation and/or urination, eating, reading, sleeping, talking, watching television, and writing (eTable). A subset of 9 younger participants who were monitored with video analysis were included in the analysis. We assessed correlations between each activity and CSF Aβ40 and Aβ42 levels after a 6-hour delay due to an expected 6-hour lag time. However, there were no significant correlations between individual behaviors and CSF Aβ levels (11 of 22). Correlations remained low when assessed with no delay between activity and Aβ levels.

RELATIONSHIP OF Aβ AND TOTAL PROTEIN

For 30 participants, there was no hourly correlation between total protein and Aβ40 (r = 0.06 to 0.28; mean [SD] correlation, 0.06 [0.28]). Furthermore, there was no correlation between total protein and Aβ40 among the YNC, amyloid−, and amyloid+ groups.

The mean levels of Aβ40 and total protein for each person over time were also calculated, and no correlation was found between them (r = 0.15, P = .44). As expected, mean total protein levels were significantly lower in young controls compared with the older participants (with a mean of 540.6 µg/mL for participants in the YNC group, 696.4 µg/mL for participants in the amyloid− group, and 656.1 µg/mL for participants in the amyloid+ group; P = .014). Total protein levels were averaged by group, and a cosinor fit was applied. There was no significant circadian pattern in total protein for any of the 3 groups (P > .05) (eFigure 2). Thus, CSF Aβ dynamics appear to be independent of CSF total protein changes.
Amyloidosis may be associated with or may cause impairments in the clearance of Aβ to the CSF, thus blocking the normal Aβ linear rise.

The loss of dynamic patterns was more pronounced in Aβ42 than in Aβ40. More selective loss of dynamics in Aβ42 may be due to its greater propensity to aggregate and deposit in amyloid plaques. Studies of Aβ generation indicate brain Aβ is dynamic over minutes to hours\(^{11,12}\) and is circadian in animal models.\(^{16}\) Studies of single measures of CSF Aβ42 demonstrate low levels of CSF Aβ42 in the presence of amyloid deposition.\(^{23}\) In our study, we found decreased CSF Aβ42 dynamics in the presence of amyloid deposition. Taken together, these
results suggest that the dynamic changes in Aβ42 concentrations in the brain may be buffered by amyloid plaques that serve as a pool of Aβ42 species to both decrease CSF Aβ42 levels and buffer dynamic changes in CSF Aβ42 concentrations.

The level of CSF Aβ has been successfully used as a diagnostic,8 prognostic,9 and therapeutic biomarker.10 Our results are consistent with reports of decreased and stable levels of Aβ42 in AD,8 with higher variability in CSF Aβ40,9 and highly variable and dynamic Aβ changes in YNCs.10 The range of mean Aβ levels over time before cosinear transformation was approximately 20% to 40% of the mean (Figure 5), indicating that sampling time can significantly affect test results in both younger controls and older participants. Therefore, sampling at consistent times is helpful in making comparisons of CSF Aβ levels between patients and groups, especially for Aβ measurements in controls.

These findings provide insight into the normal dynamic changes of the Aβ protein in the human CNS, as well as the effects of aging and amyloidosis as they relate to AD. Further research into the mechanisms that contribute to the age- and amyloid-related changes in Aβ dynamics may offer novel therapeutic approaches for AD.

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Correspondence: Randall J. Bateman, MD, Department of Neurology, Washington University School of Medicine, 660 S Euclid, PO Box 8111, St Louis, MO 63110 (batemanr@wustl.edu).
Author Contributions: Study concept and design: Shi and Bateman. Acquisition of data: Sigurdson, Shi, Kasten, Morris, Duntley, and Bateman. Analysis and interpretation of data: Huang, Potter, Santacruz, Jü, Kasten, Mintun, Duntley, and Bateman. Drafting of the manuscript: Huang, Potter, Kasten, and Bateman. Critical revision of the manuscript for important intellectual content: Potter, Sigurdson, Santacruz, Shi, Jü, Kasten, Morris, Mintun, Duntley, and Bateman. Statistical analysis: Huang, Potter, and Bateman. Obtained funding: Morris and Bateman. Administrative, technical, and material support: Potter, Sigurdson, Santacruz, Kasten, Morris, Mintun, Duntley, and Bateman. Study supervision: Duntley and Bateman. Financial Disclosure: Eli Lilly provided antibodies for this research study.

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Online-Only Material: The eTable and eFigures are available at http://www.archneurol.com.

REFERENCES