ELSEVIER

Neurobiology of Aging xx (2010) xxx

www.elsevier.com/locate/neuaging

An MRI substudy of a donepezil clinical trial in mild cognitive impairment

Norbert Schuff[a,b,*], Joyce Suhy[c], Robert Goldman[d], Yikang Xu[d], Yijun Sun[e], Diana Truran-Sacrey[a], Anita Murthy[e]

[a] *Veterans Affairs Medical Center, San Francisco, CA, United States*
[b] *University of California San Francisco, San Francisco, CA, United States*
[c] *Synarc Inc., San Francisco, CA, United States*
[d] *Pfizer Inc, New York, NY, United States*
[e] *Eisai Inc., Woodcliff Lake, NJ, United States*

Received 15 October 2009; received in revised form 24 March 2010; accepted 5 April 2010

Abstract

A magnetic resonance imaging (MRI) study was conducted as part of an intervention study in subjects with amnestic mild cognitive impairment (aMCI) to assess donepezil's treatment effect on brain atrophy. Adults with aMCI were randomly assigned to double-blind treatment with 10 mg/day donepezil hydrochloride or placebo for 48 weeks. Brain MRI scans were acquired at baseline and endpoint. The primary outcome measure was annualized percentage change (APC) in hippocampal volume; the main secondary outcome measure was APC in whole brain volumes. An analysis of variance (ANOVA) model including terms for treatment, site, and age was used to compare the treatment groups. APCs for hippocampal volumes were not significantly different between treatment groups. There were significant differences favoring the donepezil group for total ($p = 0.001$), ventricular region ($p = 0.0002$), and cortical region ($p = 0.003$) whole brain volumes. Although the primary MRI outcome measure was negative, the main secondary MRI outcome measure showed a positive result. These findings suggest a treatment effect of donepezil on brain atrophy in aMCI.

Keywords: Amnestic MCI; Serial MRI volumetric imaging; Cognitive decline; Donepezil; Atrophy; Whole brain analysis; Hippocampus; Randomized clinical trial; Surrogate marker

1. Introduction

The concept of mild cognitive impairment (MCI) describes a heterogeneous clinical condition with several subtypes and multiple etiologies (Petersen et al., 2001). The amnestic subtype (aMCI) is defined as a significant decline in memory with either slight or no impairment in activities of daily living (Petersen et al., 2001). As many as 80% of patients with aMCI have been reported to progress to Alzheimer's disease (AD) within 6 years of diagnosis (Petersen et al., 2001), which is consistent with the finding that aMCI is often of a degenerative etiology (Morris et al., 2001). Together these data suggest that aMCI may represent the prodromal state of AD (Morris et al., 2001). As a result, cholinesterase inhibitors (ChEIs), the mainstay of treatment for AD, have been evaluated for the treatment of MCI.

Donepezil, an acetylcholinesterase inhibitor, has been shown to delay progression to AD over a period of 1 year, though the rate of progression to AD after 3 years was not lower among patients treated with donepezil (Petersen et al., 2005). Galantamine and donepezil have shown benefit in subjects with MCI on secondary outcome measures (Salloway et al., 2004; Winblad et al., 2008). Nonetheless, no agent tested in a randomized clinical trial in MCI has met its primary efficacy objectives, all of which have been defined by clinical rating instruments (Doody et al., 2009; Feldman et al., 2007; Petersen et al., 2001; Salloway et al., 2004;

* Corresponding author at: Center for Imaging of Neurodegenerative Disease, Veterans Affairs Medical Center, 114M, 4150 Clement St., San Francisco, CA 94121, United States. Tel.: +1 415 221 4810 × 4904.
E-mail address: Norbert.Schuff@ucsf.edu (N. Schuff).

0197-4580/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2010.04.005

Thal et al., 2005; Winblad et al., 2008). Therefore, an objective marker of disease progression in MCI has been sought to supplement the clinical rating scales and cognitive tests (Jelic et al., 2006).

Identifying how donepezil might slow neurodegenerative progression in aMCI in a manner that could be detected by structural magnetic resonance imaging (MRI) is complicated by the fact that the mechanisms responsible for neuronal loss in AD are largely unknown. However, evidence from clinical trials of donepezil in the treatment of AD provides support for the possibility that, compared with placebo, donepezil slows neurodegenerative progression in treated patients with AD (Hashimoto et al., 2005; Krishnan et al., 2003). These prospective, placebo-controlled studies demonstrated significantly reduced hippocampal brain atrophy in the donepezil-treated group compared with the control group. In addition, evidence from animal studies suggests that donepezil at clinically relevant concentrations can attenuate amyloid ($A\beta_{25\text{-}35}$)-induced toxicity (Meunier et al., 2006; Svensson and Nordberg, 1998), which may slow the neurodegenerative process and thus stabilize brain atrophy. Therefore, if MCI is an early form of AD in a substantial proportion of patients, these preclinical and clinical data indicate that rates of hippocampal brain atrophy may be reduced with donepezil treatment in comparison with rates in a placebo-treated control group.

Serial MRI, which has been utilized extensively in studies of MCI, has shown significant differences between MCI patients and controls in rates of hippocampal and whole brain volume loss (Fox et al., 1996, 2000; Jack et al., 2004, 2008). In particular, higher rates of hippocampal atrophy in MCI were associated with generally higher cognitive decline, suggestive of accumulative AD structural changes (van de Pol et al., 2007). Moreover, several MRI studies demonstrated an association between increased rates of brain atrophy, including shrinkage of the hippocampus, and higher rates of clinical progression from MCI to AD (Misra et al., 2009; Spulber et al., 2008). Serial MRI scans (1–5 years apart) in 160 adults have shown that ventricular expansion and atrophy rates of the whole brain, hippocampus, and entorhinal cortex (ERC) were greater in cognitively normal subjects who progressed to MCI or AD than among those who remained stable, and greater among MCI subjects who converted to AD than among those who did not (Jack et al., 2004). In 131 subjects who had serial MRI scans during the vitamin E and donepezil study (Petersen et al., 2005), significant correlations between cognitive test performance and regional and whole brain atrophy rates were demonstrated (Jack et al., 2008), indicating that the neuronal correlate of clinical symptoms in MCI is progressive brain atrophy.

These data suggest that measurements of brain atrophy progression are associated with clinical symptoms of cognitive decline, may distinguish between MCI and normal aging, and may be used to evaluate the effects of therapeutic interventions in MCI. Therefore, an MRI substudy was included as part of an intervention study of donepezil in patients with aMCI (NCT00293176) (Doody et al., 2009). The primary hypothesis was that donepezil treatment would slow the rate of brain atrophy. A secondary hypothesis was that the rate of brain atrophy would correlate with clinical measures of cognitive decline.

2. Methods

2.1. Patient population

Participants comprised a subset from the primary clinical trial of donepezil for the treatment of aMCI (Clinicaltrials.gov NCT00293176). The details of the parent study have been previously described (Doody et al., 2009). Briefly, subjects (45–90 years of age) met criteria for aMCI (Petersen et al., 2001), with a recent ($<$1 year) brain scan showing no evidence of focal lesions. At screening evaluation, participants had a global Clinical Dementia Rating (CDR) score of 0.5, memory box score between 0.5 and 1.0 (Berg, 1988), Mini-Mental Status Examination (MMSE) score between 24 and 28 inclusive (Folstein et al., 1975), and Rosen-modified Hachinski ischemia scale score $\leq$4. Exclusionary criteria included current diagnosis of any major neurological, psychiatric, or substance use disorder; uncontrolled diabetes or hypertension; a sleep disorder that could affect cognitive performance; or treatment with a ChEI or memantine for $>$1 month or within 3 months of screening.

2.2. Study design

Subjects participated in a 3-week single-blind, placebo run-in period followed by a 48-week double-blind period during which they were randomly assigned to treatment with 10 mg/day donepezil hydrochloride or placebo (Fig. 1). The modified Alzheimer Disease Assessment Scale-cognitive subscale (ADAS-cog) (Doody et al., 2009; Rozzini et al., 2008) was administered at each visit except week 3, the CDR sum of boxes (SB) (O'Bryant et al., 2008) at each visit. The study protocol was approved by the institutional review board of each site, and informed consent was obtained from all subjects. Brain MRI scans were acquired at 36 qualified investigative sites at baseline and after 50 weeks of treatment, or after at least 6 months in subjects who terminated the study prematurely.

2.3. MRI methodology

The sponsor mandated CCBR-Synarc (San Francisco, California), a provider of central radiology services, to oversee the image acquisition, quality control, and off-site independent blinded central review of images performed at the Center for Imaging of Neurodegenerative Diseases. Procedures were put in place to ensure the standardization and quality of MRI acquisition and image assessment.

For each scan, fast scout scans were initially obtained to determine head position in the magnet, followed by coronal

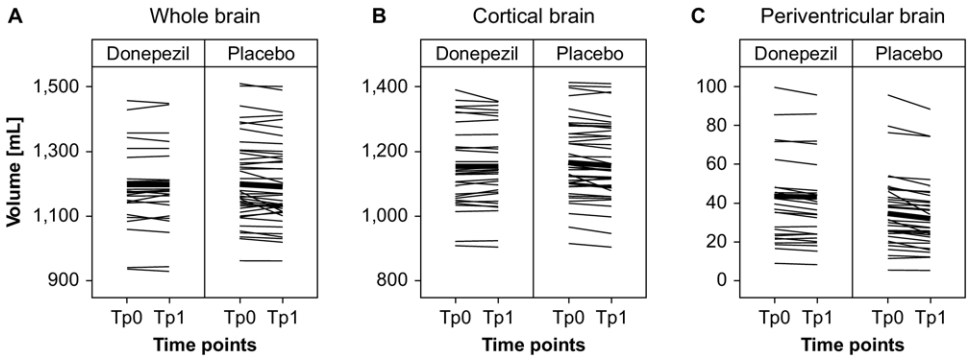

Fig. 1. Individual trajectories of brain volume change. Graphs display change over time in the donepezil and placebo groups based on boundary shift integral (BSI) measurements from baseline (Tp0) to follow-up scan (Tp1). Randomly selected subjects comprising 50% of each group are shown for better visualization of individual changes. The thick solid lines represent the mean change in each group for each of the BSI measures (whole, cortical, and periventricular brain). Note, the vertical scales differ between BSI measures.

In total, a decrease in entorhinal cortex (ERC), hippocampus, and whole brain volume was observed for 60.0%, 58.3%, and 55.4% of donepezil-treated patients and for 62.5%, 75.4% and 81.1% of placebo-treated patients, respectively. All other patients showed an increase in brain volumes, except for 1 patient, in the donepezil group, who had unchanged ERC volume.

3-dimensional T1-weighted images performed at 1 × 1.5-mm^2 resolution and angulated perpendicular to the long axis of the hippocampus. An axial T2-weighted fast spin echo sequence was obtained to determine intracranial volume (ICV) and to conduct clinical readings at the clinical site. An axial fluid-attenuated inversion recovery (FLAIR) sequence was collected to assess white matter lesions and lacunar infarcts at the clinical site.

MRI scans collected for the study were sent to Synarc for quality control and archiving, and then transferred to the lead author and his group for evaluation. Readers were blinded to treatment assignment, chronological order of images, and relevant clinical information. The main criteria for judging MRI quality included appearance of gray/white matter contrast, severity of image artifacts (e.g., ringing, ghosting, and head movements), and the ability to co-register each subject's serial MRI scans. In the few cases that had discrepant readings, the lead author (NS) made the final decision for pass or fail.

Quantitative volumes of the ERC, hippocampus, and whole brain atrophy were obtained from the T1-weighted MRI data using established and previously published procedures. The ERC was traced manually (Du et al., 2001) following an established protocol (Insausti et al., 1998) (figs e–1A). Tracing of the left and right hippocampus was performed using a semiautomated brain mapping method based on a high-dimensional fluid transformation algorithm (figs e–1B) (Christensen et al., 1997). A commercially available version of the algorithm was used (Medtronic Surgical Navigation Technologies, Louisville, Colorado).

Whole brain atrophy was measured using the boundary shift integral (BSI) (Ezekiel et al., 2004) generally following established procedures (figs e–1C) (Freeborough and Fox, 1997). Measurements of whole brain atrophy were further classified into atrophy of periventricular regions and cortical regions based on tissue classifications using image segmentation software (Tanabe et al., 1997). The final markings of ERC, hippocampus, and BSI were checked visually by 2 experienced readers independently and either accepted or rejected by consensus.

2.4. Statistical analysis

Subjects included in the MRI substudy intent-to-treat (ITT) population were those who were randomized, received at least 1 dose of double-blind study medication, had baseline and at least 1 postbaseline assessment of at least 1 efficacy variable, and had a baseline and follow-up MRI scan. The MRI variables analyzed were rates of hippocampal, ERC, and whole brain volume change; hippocampal volume change was the primary outcome measure. Intracranial volume (ICV) was used as the denominator to derive head size-adjusted volumes.

Rate measurements were expressed as annualized percentage change (APC) from baseline volumes, according to:

$$[(V_{ij} - V_{i0})/V_{i0}] \times (365/\Delta t_{ij}) \times 100$$

Here, V_{i0} and V_{ij} are the volumes of i-th subject measured at study baseline ($t = 0$) and end date ($t = j$), respectively; and Δt_{ij} is the scan interval of the i-th subject. Rate measurements were further classified by apolipoprotein E (APOE) genotype (APOE e4 allele carriers versus noncarriers).

An analysis of variance (ANOVA) model with terms for treatment, site, age, and baseline MMSE category (≤ 28, ≥ 29) was used to compare the treatment groups, as well as their corresponding APOE-defined subgroups, for each of these variables. Differences in least squares means, 95% confidence interval, and p values for each variable were determined. The ANOVA tests of a treatment effect on brain volume changes were supplemented post hoc by linear

Table 1
Demographic and patient characteristics

	MRI substudy-BSI qualified		MRI substudy-ITT		MRI substudy		MCI total population	
	Placebo (n = 90)	Donepezil (n = 74)	Placebo (n = 125)	Donepezil (n = 109)	Placebo (n = 199)	Donepezil (n = 193)	Placebo (n = 387)	Donepezil (n = 391)
Age, years, mean ± SD	67.5 ± 10.1	70.0 ± 9.8	68.4 ± 9.9	70.6 ± 9.8	69.2 ± 10.4	69.3 ± 10.4	69.8 ± 10.3	70.2 ± 9.7
Years since onset, mean ± SD	4.4 ± 2.5	4.3 ± 2.0	4.6 ± 2.9	4.2 ± 2.0	4.5 ± 2.7	4.4 ± 3.4	4.2 ± 2.8	4.1 ± 2.4
Sex, % male	53.3	58.1	56.8	56.0	53.8	50.3	57.4	51.7
Race, white, n (%)	80 (88.9)	72 (97.3)	110 (88.0)	104 (95.4)	177 (88.9)	178 (92.2)	330 (85.3)	346 (88.5)
APOE genotype, negative for APOE e4, n (%)	35 (53.0)	28 (54.9)	49 (53.3)	41 (55.4)	66 (54.1)	60 (55.0)	162 (58.1)[a]	154 (58.8)[a]
Education, n (%)								
0–7 years	1 (1.1)	0 (0.0)	2 (1.6)	0 (0.0)	2 (1.0)	0 (0.0)	3 (0.8)	2 (0.5)
8–15 years	43 (47.8)	35 (47.3)	58 (46.4)	52 (47.7)	106 (53.3)	105 (54.4)	210 (54.3)	201 (51.4)
> 15 years	48 (51.1)	39 (52.7)	65 (52.0)	57 (52.3)	91 (45.7)	88 (45.6)	174 (45.0)	188 (48.1)
Baseline MMSE ≤ 28, n (%)	67 (74.4)	56 (75.7)	95 (76.0)	81 (74.3)	155 (77.9)	151 (78.2)	322 (83.2)	328 (83.9)
Baseline scores, mean ± SD								
Modified ADAS-cog	17.6 ± 6.8	18.1 ± 6.1	17.8 ± 7.0	17.9 ± 6.4	17.7 ± 7.3	18.3 ± 6.7	18.3 ± 6.6	18.2 ± 7.0
CDR-SB	1.1 ± 0.7	1.2 ± 0.8	1.2 ± 0.9	1.1 ± 0.7	1.2 ± 0.8	1.2 ± 0.8	1.5 ± 0.9	1.5 ± 0.9
MMSE	27.7 ± 2.0	27.9 ± 1.6	27.5 ± 2.1	27.8 ± 1.8	27.6 ± 1.8	27.5 ± 2.0	27.5 ± 1.9	27.4 ± 1.9
Delayed recall score	3.9 ± 2.4	4.1 ± 2.4	3.9 ± 2.3	4.2 ± 2.3	4.0 ± 2.3	3.9 ± 2.3	3.9 ± 2.3	4.0 ± 2.3

APOE genotyping was introduced as a protocol amendment after the start of the study. The test, although scheduled for week 3, was also allowed at any other visit or at an unscheduled visit if necessary.

Key: ADAS-cog, Alzheimer disease Assessment Scale-cognitive subscale; CDR-SB, Clinical Dementia Rating-sum of boxes; APOE, apolipoprotein E; BSI, boundary shift integral; ITT, intent-to-treat; MCI, mild cognitive impairment; MMSE, Mini Mental State Examination; MRI, magnetic resonance imaging.

[a] $n_{placebo}$ = 279; $n_{donepezil}$ = 262.

mixed effects analyses, in which the response variable (e.g., hippocampal volume) was regressed against an explanatory variable (e.g., time) as fixed effect, and variation in baseline volumes was included as random effect to account for a potential bias from smaller volumes.

To determine whether there was a relationship between cognitive measures and brain volumes, correlations between MRI assessments and clinical assessments (modified ADAS-cog and CDR-SB) at baseline and at endpoint, as well as for annualized percentage change (APC), as defined above, were also investigated. Spearman's coefficients were used to determine the correlation between these MRI and clinical variables, and p values (null hypothesis: $\rho = 0$) were determined.

A post hoc analysis of the APC for hippocampal and ERC volumes was performed, restricted to those scan pairs that qualified for whole brain analysis, based on the criteria set forth for BSI quality control, because high variability and artifacts compatible with drifts in gradient calibration (which can mimic brain atrophy) were observed in the ITT dataset. The rationale for this analysis was to reduce these variations and potential systematic errors in the MRI data. Selection of these scan pairs was done completely blinded to subjects' treatment status and baseline demographic and clinical characteristics.

3. Results

3.1. Subjects

Of the 2037 subjects screened, 821 subjects (donepezil, n = 409; placebo, n = 412), were recruited from 74 sites and randomized; and 788 subjects were included in the ITT population (donepezil, n = 397; placebo, n = 391) (Fig. 1). A total of 392 subjects participated in the MRI substudy, of whom 234 were included in the ITT population (donepezil, n = 109; placebo, n = 125). APOE data were available for 74 patients (67.9%) in the donepezil group and 92 (73.6%) in the placebo group; of these patients, 33 (44.6%) in the donepezil group and 43 (46.7%) in the placebo group were APOE e4 carriers. Demographic and baseline illness characteristics were similar for the MRI study subpopulation and the MCI total study population (Table 1).

3.2. MRI data

The mean interval between baseline and endpoint scans was 356 days (range, 205–479) for the donepezil group and 361 days (range, 252–424) for the placebo group. The number of scan pairs that passed the protocol-specified quality assessments was: ERC, 182 of 212; hippocampus, 215 of 234; and BSI, 164 of 234. Baseline volumes are shown in Table 2; differences between the placebo and treatment groups were not significant.

3.3. Hippocampal volume change

For the protocol-defined sample of scans, the APCs for total, left, and right hippocampal volumes were not significantly different between treatment groups (Table 3). There were also no significant treatment group differences when stratified by ApoE status (Table e–1).

For the post hoc sample of scans restricted to those that passed the BSI quality check, the between-group difference of the APC for right hippocampal volume was significant (p = 0.044) and the between-group difference of the APC for

Table 2
Baseline volumetric measures (cubic centimeters)

Brain measure	Placebo	*n*	Donepezil	*n*	Placebo	*n*	Donepezil	*n*
Total hippocampal	4.044 ± 0.849	125	3.938 ± 0.757	105	4.113 ± 879	90	4.036 ± 0.832	72
Left hippocampal	1.991 ± 0.438	125	1.947 ± 0.375	105				
Right hippocampal	2.053 ± 0.450	125	1.992 ± 0.410	105				
Total entorhinal cortex (ERC)	1.076 ± 0.461	112	1.125 ± 0.440	100	1.118 ± 484	80	1.085 ± 0.358	70
Left ERC	0.531 ± 0.224	112	0.571 ± 0.242					
Right ERC	0.545 ± 0.265	112	0.555 ± 0.230					
Total whole brain					1199 ± 127	74	1193 ± 135	74
Ventricular region					35.00 ± 21.83	74	43.79 ± 29.93	74
Cortical region					1164 ± 122	74	1149 ± 129	74

Data are given as mean ± standard error of the mean. All differences between placebo and donepezil were nonsignificant.

total hippocampal volume demonstrated a trend ($p = 0.076$) (Table 3). The linear mixed effects analyses were supportive of the original ANOVA (Table e–2).

3.4. ERC volume change

For the protocol-defined and the post hoc sample of scans, the APCs for total, left, and right ERC volumes were not significantly different between treatment groups (Table 3).

There were also no significant treatment group differences when stratified for ApoE status (Table e–1). The linear mixed effects analyses were supportive of the original analyses (Table e–2).

3.5. Whole brain volume change

A significant difference was noted between treatment groups in the APC of total whole brain volume ($p = 0.001$), ventricular region whole brain volume atrophy ($p = 0.0002$), and cortical region whole brain volume ($p = 0.003$) (Table 3). For each of these measures, the donepezil group exhibited a slower rate of atrophy than the placebo group (Fig. 1). To exclude the possibility that slight differences in MRI scan intervals between the groups resulted in bias in rate measurements, we added variations in scan intervals to the model and obtained virtually identical results.

3.6. Correlational analysis

Most cross-sectional MRI measures correlated significantly with the ADAS-cog scores (Table 4). Specifically, the correlations between ADAS-cog scores and hippocampal, ERC, and total, cortical and ventricular whole brain measures were significant at baseline and endpoint (except for total whole brain volume at baseline). Fewer significant correlations were noted between CDR-SB scores and brain volume measures at baseline or endpoint (6 of 18, compared with 17 of 18 for ADAS-cog; Table 4).

The APC for the whole brain volumes correlated significantly with the APC for ADAS-cog scores, but not for CDR-SB scores. In contrast, the only significant correlations for the regional brain volume changes were between the APCs for total and right ERC volumes and the APC of CDR-SB, and between the APC for right hippocampal volume and the APC of ADAS-cog. These results were not appreciably altered in the post hoc sample of scans restricted to those that passed the BSI quality check (4 cor-

Table 3
Effect of donepezil versus placebo on percent change in brain volumes per year

Volume	Percent rate of change per year[a]				Treatment difference (95% CI)	*p*-value
	n	Placebo	*n*	Donepezil		
Total hippocampal[b]	125	−2.23 ± 0.64	105	−1.64 ± 0.66	0.59 (-0.94 to 2.12)	0.446
Left hippocampal[b]	125	−2.03 ± 0.76	105	−1.96 ± 0.79	0.06 (−1.76 to 1.88)	0.945
Right hippocampal[b]	125	−2.39 ± 0.74	105	−1.19 ± 0.77	1.21 (−0.56 to 2.97)	0.180
Total hippocampal[c]	90	−2.02 ± 0.66	72	−0.65 ± 0.68	1.37 (−0.14 to 2.88)	0.076
Left hippocampal[c]	90	−2.19 ± 0.82	72	−1.25 ± 0.85	0.94 (−0.94 to 2.83)	0.325
Right hippocampal[c]	90	−1.83 ± 0.81	72	−0.08 ± 0.84	1.91 (0.05–3.76)	0.044
Total ERC	112	−3.00 ± 1.88	100	−0.85 ± 1.93	2.14 (−2.22 to 6.50)	0.334
Left ERC	112	−2.09 ± 2.18	100	0.09 ± 2.24	2.18 (−2.89 to 7.24)	0.398
Right ERC	112	−3.43 ± 2.20	100	−0.79 ± 2.26	2.64 (−2.47 to 7.76)	0.309
Total whole brain atrophy	90	−0.54 ± 0.14	74	0.01 ± 0.14	0.55 (−0.88 to −0.23)	0.0010
Ventricular region	90	−5.63 ± 0.81	74	−2.02 ± 0.83	3.61 (−5.49 to −1.72)	0.0002
Cortical region	90	−0.41 ± 0.13	74	0.07 ± 0.14	0.48 (−0.78 to −0.17)	0.0025

Key: CI, confidence interval.
[a] 100 × ([end value-baseline value]/baseline value) × 365.25/(end date-baseline date); least squares mean ± standard error of the mean.
[b] Protocol-defined intent-to-treat (ITT) population.
[c] Boundary shift integral (BSI)-qualified scans.

Table 4
Correlation between brain volumes and clinical assessments (SC)

Volume	Baseline		Study endpoint[a]		% Rate of change per year	
	ADAS-cog	CDR-SB	ADAS-cog	CDR-SB	ADAS-cog	CDR-SB
Total hippocampal	−0.375[b]	−0.040	−0.408[b]	−0.173	−0.127	−0.107
Left hippocampal	−0.404[b]	−0.061	−0.441[b]	−0.180	−0.063	−0.074
Right hippocampal	−0.328[b]	−0.025	−0.348[b]	−0.154	−0.147	−0.109
Total ERC	−0.217[c]	−0.072	−0.259[d]	−0.158[e]	−0.063	−0.166[e]
Left ERC	−0.203[c]	−0.101	−0.284[b]	−0.110	−0.055	−0.133
Right ERC	−0.203[c]	−0.047	−0.215[c]	−0.166[e]	−0.088	−0.151[e]
Whole brain atrophy	−0.115	0.010	0.233[c]	0.086	0.218[c]	0.105
Ventricular region	0.261[d]	0.136[e]	0.291[d]	0.140	0.222[c]	0.099
Cortical region	−0.155[e]	−0.003	0.187[e]	0.056	0.176[e]	0.079

Number of subjects: hippocampal, $n = 230$; ERC, $n = 213$ (baseline), $n = 212$ (endpoint and % rate of change/year); whole brain atrophy, ventricular, and cortical regions, $n = 214$ (baseline), $n = 164$ (endpoint and % rate of change/year).
Key: ERC, entorhinal cortex; CDR-SB, Clinical Dementia Rating-sum of boxes; ADAS-cog, modified Alzheimer Disease Assessment Scale-cognitive subscale (Doody et al., 2009; Rozzini et al., 2008); SC, Spearman correlation coefficient.
[a] For whole brain atrophy and ventricular and cortical regions, change from baseline to study endpoint.
[b] $p<0.0001$.
[c] $p<0.001$.
[d] $p<0.01$.
[e] $p<0.05$.

relations that had been significant for hippocampal measures were no longer significant).

4. Discussion

This study did not demonstrate a treatment effect of donepezil on hippocampal volume change over 1 year, the primary outcome measure, but did show a potential treatment effect of pharmacotherapy on other brain volume changes. Specifically, donepezil 10 mg/day was associated with slowed progression of whole brain atrophy over the course of 1 year. In this study, the difference between the donepezil and placebo groups on the primary outcome measure, APC in hippocampal volume, was not significant. However, the donepezil group did differ significantly from placebo on the secondary outcome measure, APC in whole brain volumes. This study is therefore the first clinical trial to demonstrate a treatment effect of donepezil on brain volume changes in aMCI. Moreover, the statistical significance of this effect was roughly an order of magnitude greater than that of the change in ADAS-cog ($p < 0.001$ versus $p = 0.01$) (Doody et al., 2009). Although donepezil treatment did not significantly slow the rate of hippocampal or ERC atrophy, a trend was noted toward slowing of the progression of total hippocampal atrophy and a significant slowing of right hippocampal atrophy in the post hoc analysis restricted to BSI-qualified scan pairs. Because selection of this subgroup was carried out blinded to treatment and chronological scan order, it could not have been driven by either of these factors. However, because this result was only detected by post hoc analysis and was not corrected for multiple comparisons, it must be considered tentative. Our study also showed that cognitive function, as measured by the modified ADAS-cog, correlated with whole brain volume measures at baseline and—most importantly—with respect to APC. The result is consistent with previous findings (Petersen et al., 2005) and further supports the view that atrophy rates measured with MRI are surrogate markers of disease progression. Taken together, these results imply a possible disease-modifying effect of donepezil in aMCI.

Several mechanisms have been identified by which cholinesterase inhibition might slow the progression of neuronal loss in aMCI. In vitro studies have found that muscarinic receptor stimulation decreases beta-amyloid production (Wolf et al., 1995) and that nicotinic receptor stimulation partially protects neurons from beta-amyloid-induced neurotoxicity (Svensson and Nordberg, 1998). Perhaps more clinically relevant is the hypothesis that ChEI treatment, by facilitating cholinergic neurotransmission, may promote maintenance of synaptic integrity and thereby resistance to neurodegeneration (Cummings, 2005). Recent functional MRI studies in subjects with MCI, showing that recruitment of the hippocampus and of other brain regions in response to a range of experimental tasks is significantly increased by ChEI treatment, are supportive of this hypothesis (Goekoop et al., 2004; Gron et al., 2006).

The greater impact of donepezil treatment on whole brain atrophy than on hippocampal or ERC atrophy is noteworthy, given that aMCI is considered to be, in most cases, a transitional stage to AD, and AD structural changes are thought to start in the ERC and hippocampus before spreading to cortical regions (Morris et al., 2001; Petersen et al., 2001). Interestingly, similar 1-year results were reported for rivastigmine in a large clinical trial in MCI, in which ventricular atrophy, but not ERC or hippocampal atrophy, was significantly less in the rivastigmine group compared with the placebo group ($p = 0.009$, uncorrected), though this difference was not present at 3 or 4 years (Feldman et al., 2007). It is possible that pathological factors are responsible

for this observation. For example, it is well documented that beta-amyloid is more widespread in cortical regions than in the hippocampus, especially at an early stage of the disease (Braak and Braak, 1996). Based on the hypothesis that donepezil may attenuate amyloid-induced neuronal toxicity (Svensson and Nordberg, 1998; Wolf et al., 1995), it is conceivable that the treatment has a greater effect on the cortex and whole brain in general than on the hippocampus. This would also explain the dominance of the effect on the whole brain despite observations that both treated and untreated patients showed greater atrophy progression in the hippocampus. However, it cannot be completely ruled out that methodological differences between measurements of whole brain and hippocampal atrophy rates are responsible for the observation. For example, power to detect a treatment effect is more limited for the hippocampus than for cortical regions because tracing the anatomical boundary of the hippocampus (and ERC) reliably is notoriously difficult. Furthermore, hippocampal and ERC tracings are based on hard segmentation of image intensities, whereas BSI uses soft segmentation that can exploit partial volume effects and may therefore capture subtle changes more effectively (Ezekiel et al., 2004). In fact, prior work in this field has shown the greater sensitivity of BSI for detecting whole brain atrophy in MCI as compared with focal measurements of hippocampal or ERC atrophy (Ezekiel et al., 2004; Misra et al., 2009; Spulber et al., 2008).

In contrast to the results of the present trial, a prior donepezil aMCI clinical study employing similar MRI volumetric analysis (Jack et al., 2008) did not find a treatment effect, even though MRI scan intervals in that study were more than twice as long. Several reasons may account for the discrepant findings. First, the sample size was larger in the present study (placebo, $n = 90$ versus $n = 54$; donepezil, $n = 74$ versus $n = 37$). Second, a high degree of heterogeneity exists among patients diagnosed with aMCI, which may lead to important differences in the patient populations examined (Fleisher et al., 2007). Third, this MRI substudy was included in the initial protocol of the main study, whereas the prior MRI substudy was grafted on at a later date. Consequently, the methodological rigor employed in this study with respect to site qualification, scan acquisition, image quality control, and reader training and oversight was greater than in the previous study.

Stratifying subjects by APOE status did not result in the demonstration of a treatment effect, although APOE e4-positive subjects did demonstrate a higher rate of atrophy overall than the APOE e4-negative group, as has been observed previously (Hashimoto et al., 2005; Jack et al., 2008).

Modified ADAS-cog scores correlated significantly, but not very strongly, with all but one of the volumetric measures at baseline and endpoint, and with the APC for total, cortical, and ventricular whole brain atrophy, consistent with findings from a previous study of donepezil in aMCI (Jack et al., 2008). The CDR-SB, however, did not correlate well with volumetric measures at baseline, endpoint, or with respect to change. Moreover, in this trial and 2 preceding trials of donepezil in MCI, a significant treatment benefit was observed on the modified ADAS-cog, but not the CDR-SB (Doody et al., 2009; Petersen et al., 2005; Rozzini et al., 2008; Salloway et al., 2004). These findings suggest that donepezil treatment may have a greater effect on cognitive function than on global rating in aMCI.

This study has several limitations. First, the subjects have not been followed long enough to determine the incidence of incipient AD in each group. Thus, the difference in the rates of brain atrophy and cognitive decline between the study groups may partly reflect a difference in the proportion of subjects with preclinical AD within each group. Second, the cognitive tests and rating scales used in this study may not have been sufficiently sensitive to the subtle and slower changes characteristic of aMCI to provide a highly accurate assessment of change. Third, white matter lesions, an indication of cerebrovascular disease, have been shown to modulate hippocampal atrophy (Fein et al., 2000). It is therefore possible that the slowing of brain atrophy from treatment with donepezil may differ as a function of white matter lesions.

Overall, these findings suggest that donepezil has a treatment effect on whole brain volume changes in aMCI. The hippocampal volumetric results are more encouraging than those previously reported and suggest their possible utility as well. These results provide support for the use of whole brain MRI volumetric analysis in studying both the natural history of disease progression and therapeutic interventions in aMCI. The combined use of MRI brain volumetric measures with clinical rating scales and cognitive tests will allow for greater statistical power to detect change in studies of patients with aMCI.

Disclosure statement

Dr. Schuff received financial support from Synarc Inc. for research, and is funded by several grants from the National Institutes of Health, the Department of Defense, and the Michael J. Fox Foundation for Parkinson's disease. Drs. Xu and Goldman are Pfizer employees and hold stock options in Pfizer. Drs. Sun and Murthy are Eisai employees. Ms. Truran-Sacrey reports funding by Synarc, Inc. and multiple National Institutes of Health sources.

The study protocol was approved by the institutional review board of each site, and informed consent was obtained from all subjects.

Acknowledgements

This study was sponsored by Eisai Inc. and Pfizer Inc. Editorial support was provided by B. Kadish, MD of PAREXEL and funded by Eisai Inc. and Pfizer Inc. Study

design, data collection, and manuscript content were conceived by the authors.

References

Berg, L., 1988. Clinical Dementia Rating (CDR). Psychopharmacol. Bull. 24, 637–639.

Braak, H., Braak, E., 1996. Evolution of the neuropathology of Alzheimer's disease. Acta Neurol. Scand. Suppl. 165, 3–12.

Christensen, G.E., Joshi, S.C., Miller, M.I., 1997. Volumetric transformation of brain anatomy. IEEE Trans. Med. Imaging 16, 864–877.

Cummings, J.L., 2005. Searching for methods to detect, prevent, and treat Alzheimer's disease. Am. J. Psychiatry 162, 645–647.

Doody, R.S., Ferris, S.H., Salloway, S., Sun, Y., Goldman, R., Watkins, W.E., Xu, Y., Murthy, A.K., 2009. Donepezil treatment of patients with MCI: a 48-week randomized, placebo-controlled trial. Neurology 72, 1555–1561.

Du, A.T., Schuff, N., Amend, D., Laakso, M.P., Hsu, Y.Y., Jagust, W.J., Yaffe, K., Kramer, J.H., Reed, B., Norman, D., Chui, H.C., Weiner, M.W., 2001. Magnetic resonance imaging of the entorhinal cortex and hippocampus in mild cognitive impairment and Alzheimer's disease. J. Neurol. Neurosurg. Psychiatry 71, 441–447.

Ezekiel, F., Chao, L., Kornak, J., Du, A.T., Cardenas, V., Truran, D., Jagust, W., Chui, H., Miller, B., Yaffe, K., Schuff, N., Weiner, M., 2004. Comparisons between global and focal brain atrophy rates in normal aging and Alzheimer disease: Boundary Shift Integral versus tracing of the entorhinal cortex and hippocampus. Alzheimer Dis. Assoc. Disord. 18, 196–201.

Fein, G., Di Sclafani, V., Tanabe, J., Cardenas, V., Weiner, M.W., Jagust, W.J., Reed, B.R., Norman, D., Schuff, N., Kusdra, L., Greenfield, T., Chui, H., 2000. Hippocampal and cortical atrophy predict dementia in subcortical ischemic vascular disease. Neurology 55, 1626–1635.

Feldman, H.H., Ferris, S., Winblad, B., Sfikas, N., Mancione, L., He, Y., Tekin, S., Burns, A., Cummings, J., Del Ser, T., Inzitari, D., Orgogozo, J.M., Sauer, H., Scheltens, P., Scarpini, E., Herrmann, N., Farlow, M., Potkin, S., Charles, H.C., Fox, N.C., Lane, R., 2007. Effect of rivastigmine on delay to diagnosis of Alzheimer's disease from mild cognitive impairment: the InDDEx study [Erratum in: 2007. 6, 849]. Lancet Neurol. 6, 501–512.

Fleisher, A.S., Sowell, B.B., Taylor, C., Gamst, A.C., Petersen, R.C., Thal, L.J., 2007. Clinical predictors of progression to Alzheimer disease in amnestic mild cognitive impairment. Neurology 68, 1588–1595.

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J. Psychiatry Res. 12, 189–198.

Fox, N.C., Cousens, S., Scahill, R., Harvey, R., Rossor, M., 2000. Using serial registered brain magnetic resonance imaging to measure disease progression in Alzheimer's disease. Arch. Neurol. 57, 339–344.

Fox, N.C., Warrington, E.K., Freeborough, P.A., Hartikainen, P., Kennedy, A.M., Stevens, J.M., Rossor, M.N., 1996. Presymptomatic hippocampal atrophy in Alzheimer's disease. A longitudinal MRI study. Brain 119, 2001–2007.

Freeborough, P.A., Fox, N.C., 1997. The boundary shift integral: an accurate and robust measure of cerebral volume changes from registered repeat MRI. IEEE Trans. Med. Imaging. 16, 623–629.

Goekoop, R., Rombouts, S.A., Jonker, C., Hibbel, A., Knol, D.L., Truyen, L., Barkhof, F., Scheltens, P., 2004. Challenging the cholinergic system in mild cognitive impairment: a pharmacological fMRI study. NeuroImage 23, 1450–1459.

Gron, G., Brandenburg, I., Wunderlich, A., Riepe, M.W., 2006. Inhibition of hippocampal function in mild cognitive impairment: targeting the cholinergic hypothesis. Neurobiol. Aging 27, 78–87.

Hashimoto, M., Kazui, H., Matsumoto, K., Nakano, Y., Yasuda, M., Mori, E., 2005. Does donepezil treatment slow the progression of hippocampal atrophy in patients with Alzheimer's disease? Am. J. Psychiatry 162, 676–682.

Insausti, R., Juottonen, K., Soininen, H., Insausti, A.M., Partanen, K., Vainio, P., Laakso, M.P., Pitkanen, A., 1998. MR volumetric analysis of the human entorhinal, perirhinal, and temporopolar cortices. AJNR Am. J. Neuroradiol. 19, 659–671.

Jack, C.R., Jr, Petersen, R.C., Grundman, M., Jin, S., Gamst, A., Ward, C.P., Sencakova, D., Doody, R.S., Thal, L.J., 2008. Longitudinal MRI findings from the vitamin E and donepezil treatment study for MCI. Neurobiol. Aging 29, 1285–1295.

Jack, C.R., Jr, Shiung, M.M., Gunter, J.L., O'Brien, P.C., Weigand, S.D., Knopman, D.S., Boeve, B.F., Ivnik, R.J., Smith, G.E., Cha, R.H., Tangalos, E.G., Petersen, R.C., 2004. Comparison of different MRI brain atrophy rate measures with clinical disease progression in AD. Neurology 62, 591–600.

Jelic, V., Kivepelto, M., Winblad, B., 2006. Clinical trials in mild cognitive impairment: lessons for the future. J. Neurol. Neurosurg. Psychiatry 77, 429–438.

Krishnan, K.R., Charles, H.C., Doraiswamy, P.M., Mintzer, J., Weisler, R., Yu, X., Perdomo, C., Ieni, J.R., Rogers, S., 2003. Randomized, placebo-controlled trial of the effects of donepezil on neuronal markers and hippocampal volumes in Alzheimer's disease. Am. J. Psychiatry 160, 2003–2011.

Meunier, J., Ieni, J., Maurice, T., 2006. The anti-amnesic and neuroprotective effects of donepezil against amyloid beta(25–35) peptide-induced toxicity in mice involve an interaction with the sigma(one) receptor. Br. J. Pharmacol. 149, 998–1012.

Misra, C., Fan, Y., Davatzikos, C., 2009. Baseline and longitudinal patterns of brain atrophy in MCI patients, and their use in prediction of short-term conversion to AD: results from ADNI. Neuroimage 44, 1415–1422.

Morris, J.C., Storandt, M., Miller, J.P., McKeel, D.W., Jr, Price, J.L., Rubin, E.H., Berg, L., 2001. Mild cognitive impairment represents early-stage Alzheimer's disease. Arch. Neurol. 58, 397–405.

O'Bryant, S.E., Waring, S.C., Cullum, C.M., Hall, J., Lacritz, L., Massman, P.J., Lupo, P.J., Reisch, J.S., Doody, R., 2008. Staging dementia using Clinical Dementia Rating Scale Sum of Boxes scores: a Texas Alzheimer's research consortium study. Arch. Neurol. 65, 1091–1095.

Petersen, R.C., Doody, R., Kurz, A., Mohs, R.C., Morris, J.C., Rabins, P.V., Ritchie, K., Rossor, M., Thal, L., Winblad, B., 2001. Current concepts in mild cognitive impairment. Arch. Neurol. 58, 1985–1992.

Petersen, R.C., Thomas, R.G., Grundman, M., Bennett, D., Doody, R., Ferris, S., Galasko, D., Jin, S., Kaye, J., Levey, A., Pfeiffer, E., Sano, M., van Dyck, C.H., Thal, L.J., 2005. Vitamin E and donepezil for the treatment of mild cognitive impairment. Alzheimer's Disease Cooperative Study Group. N. Engl. J. Med. 352, 2379–2388.

Rozzini, L., Vicini, C.B., Bertoletti, E., Conti, M., delRio, I., Trabucchi, M., Padovani, A., 2008. The importance of Alzheimer disease assessment scale-cognitive part in predicting progress for amnestic mild cognitive impairment to Alzheimer disease. J. Geriatr. Psychiatry Neurol. 21, 261–267.

Salloway, S., Ferris, S., Kluger, A., Goldman, R., Griesing, T., Kumar, D., Richardson, S., 2004. Efficacy of donepezil in mild cognitive impairment: a randomized placebo-controlled trial. Neurology 63, 651–657.

Spulber, G., Niskanen, E., MacDonald, S., Smilovici, O., Chen, K., Reiman, E.M., Jauhiainen, A.M., Hallikainen, M., Tervo, S., Wahlund, L.O., Vanninen, R., Kivipelto, M., Soininen, H., 2008. Whole brain atrophy rate predicts progression from MCI to Alzheimer's disease. Neurobiol. Aging, doi:10.1016/j.neurobiolaging.2008.08.018.

Svensson, A.L., Nordberg, A., 1998. Tacrine and donepezil attenuate the neurotoxic effect of A beta(25–35) in rat PC12 cells. Neuroreport 9, 1519–1522.

Tanabe, J.L., Ezekiel, F., Jagust, W.J., Schuff, N., Fein, G., 1997. Volumetric method for evaluating magnetization transfer ratio of tissue categories: application to areas of white matter signal hyperintensity in the elderly. Radiology 204, 570–575.

Thal, L.J., Ferris, S.H., Kirby, L., Block, G.A., Lines, C.R., Yuen, E., Assaid, C., Nessly, M.L., Norman, B.A., Baranak, C.C., Reines, S.A., 2005. A randomized, double-blind, study of rofecoxib in patients with mild cognitive impairment. Rofecoxib Protocol 078 Study Group. Neuropsychopharmacology 30, 1204–1215.

van de Pol, L.A., Korf, E.S., Van Der Flier, W.M., Brashear, H.R., Fox, N.C., Barkhof, F., Scheltens, P., 2007. Magnetic resonance imaging predictors of cognition in mild cognitive impairment. Arch. Neurol. 64, 1023–1028.

Winblad, B., Gauthier, S., Scinto, L., Feldman, H., Wilcock, G.K., Truyen, L., Mayorga, A.J., Wang, D., Brashear, H.R., Nye, J.S., 2008. Safety and efficacy of galantamine in subjects with mild cognitive impairment. Neurology 70, 2024–2035.

Wolf, B.A., Wertkin, A.M., Jolly, Y.C., Yasuda, R.P., Wolfe, B.B., Konrad, R.J., Manning, D., Ravi, S., Williamson, J.R., Lee, V.M., 1995. Muscarinic regulation of Alzheimer's disease amyloid precursor protein secretion and amyloid beta-protein production in human neuronal NT2N cells. J. Biol. Chem. 270, 4916–4922.

Online Supplement

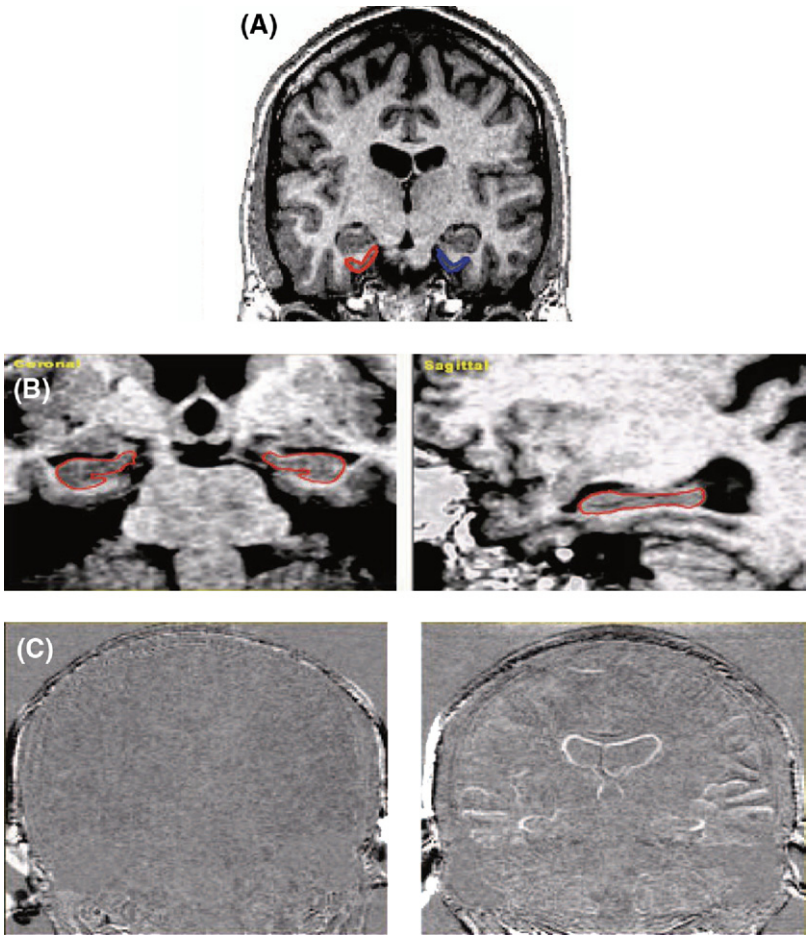

Figure e-1. Magnetic resonance images from the training manual. (A) Entorhinal cortex (ERC) on coronal slice: representative T1-weighted magnetic resonance imaging (MRI) scan showing a coronal section through the ERC. The boundaries of this small brain structure, derived manually using anatomical landmarks, are highlighted in red (right ERC) and blue (left ERC). (B) Hippocampus on coronal and sagittal slices: representative T1-weighted MRI showing a coronal and sagittal section through the mesial temporal lobe including the hippocampus. The boundaries of the hippocampus, derived automatically by warping an atlas brain onto this individual brain image, are highlighted in red. (C) Pairs of whole brain scans with boundary shifts highlighed: results of boundary shift integral (BSI) analyses of 2 pairs of images acquired about 1 year apart. The BSI results on the left, lacking any observable feature, suggest that there has been no major volume change of the brain. The BSI results on the right, highlighting boundary shifts, indicate enlargements of ventricles and sulcal spaces, consistent with brain volume loss.

Table e-1
Percent rate of change per year in total hippocampal volume by ApoE genotype[a]

Treatment group (*n*)	ApoE positive		ApoE negative	
	Donepezil (23)	Placebo (31)	Donepezil (28)	Placebo (35)
Mean (SD)	−2.06 (3.935)	−1.66 (5.103)	−0.91 (4.404)	−2.97 (4.865)
Range	−7.9 to 4.6	−8.4 to 19.7	−8.5 to 10.6	−21.5 to 3.3
LS mean[2]	−2.63	−3.70	−0.42	−2.64
SE[2]	1.427	1.348	1.154	1.106
Treatment difference (95% CI)	1.07 (−1.66 to 3.81)		2.22 (−0.45 to 4.89)	
p Value	0.4319		0.1008	

Key: ApoE, apolipoprotein E; BSI, boundary shift integral; CI, confidence interval; LS, least-squares estimated mean; SD, standard deviation; SE, standard error; (2): from a linear mixed-effects analysis.

[a] The data presented in the table are limited to BSI-qualified scans. Thus, the number of samples is lower (placebo, 66; donepezil, 51) than the total number of samples with ApoE data (placebo, 92; donepezil, 74). The results in the full data set are no different (ApoE negative: 0.91 [−1.31 to 3.13], $p = 0.4164$; ApoE positive 1.65 [−1.17 to 4.46] $p = 0.2473$).

Table e-2
Linear mixed effects analysis[a] of total hippocampal APC (restricted to BSI-qualified scans)

	Value [mm^3]	SE [mm^3]	*df*	*t* value	*p* value
Intercept	4024.594	65.71662	160	61.24164	< 0.0001
Scan interval	−65.784	15.79048	160	−4.16602	0.0001
Treatment group	86.558	65.71662	160	1.31713	0.1897
Scan interval by treatment group interaction	**−28.811**	**15.79048**	**160**	**−1.82456**	**0.0699**

Random effects, intercept SD = 820.2 mm^3, residuals = 138.7; fixed effects formula, Volume = Intercept + a1*Scan interval + a2*Treatment group + a3*Scan interval *Treatment group; here: Volume is total hippocampal volume; a1, a2, a3 are coefficients of the regression number of observations = 324; number of groups = 162. Bold text indicates treatment effect (the corresponding ANOVA *p* value for total hippocampal APC restricted to BSI-qualified scans was $p = 0.076$ [Table 2, main text]).

Key: ANOVA, analysis of variance; APC, annualized percentage change; ApoE4, apolipoprotein E4; BSI, boundary shift integral; *df*, degrees of freedom; SD, standard deviation; SE, standard error.

[a] The ANOVA tests of a treatment effect on brain volume changes were supplemented post hoc by linear mixed effects analyses, in which volumes were regressed against time as fixed effects separately from variations in baseline volumes as random effects. Other explanatory variables, e.g., treatment, age, or ApoE4 status, were added into the model as appropriate. To determine if the addition of explanatory variables, especially the inclusion of treatment, improved explanatory power, paired models were designed with and without the additional explanatory variable.

e-References

American College of Radiology, 2002. Site scanning instructions for use of the MR phantom for the ACR MRI Accreditation Program. Available at: www.acr.org/accreditation/mri/mri_qc_forms/site_scanning_instructions_phantom.aspx.

American College of Radiology, 2004. MRI Quality Control Manual. American College of Radiology: Reston, VA.

American College of Radiology, 2005. Phantom test guidance for the ACR MRI Accreditation, available at: www.acr.org/accreditation/mri/mri_qc_forms/phantom_test_guidance.aspx.