Supplemental Information

Inclusion and diagnostic criteria of the study sample

In the present study only those subjects from the OASIS-database (1) aged 20 years or older were included. Given that subjects in their twenties are overrepresented in the OASIS-database, we only included the first 36 subjects of the 20 – 29 age range under the constraint of gender homogeneity, in order to achieve a more uniform age-distribution of the studied sample.

Healthy controls in the present study had to pass a Mini-Mental State Exam (MMSE)-threshold of ≥ 26 points, leading to the exclusion of one male control subject (90 years old). Given the absence of clinical follow-up data, this threshold was chosen in order to reduce the risk of including subjects harboring undetected AD pathology.

All preprocessed MRI scans were inspected visually for processing artifacts. As a result of this visual inspection, one very mildly demented female subject (clinical dementia rating (CDR) = 0.5; MMSE = 28; 75 years old) had to be excluded due to inaccurate tissue segmentation results.

Scanner/acquisition settings

T1-weighted images of the OASIS-database were acquired sagittally and MP-RAGE parameters were empirically optimized for gray–white contrast (TR = 9.7 ms, TE = 4.0 ms, flip angle 10°, TI = 20 ms, TD = 200 ms, spatial resolution = 1 x 1 x 1.25 mm^3, number of slices = 128). Furthermore, “head movement was minimized by cushioning and a thermoplastic face mask. Headphones were provided for communication. A vitamin E capsule was placed over the left forehead to provide a reference marker of anatomical side. Positioning was low in the head coil (toward the feet) to optimize imaging of the cerebral cortex” (1).

Basal forebrain cytoarchitectonic map and study-specific basal forebrain and hippocampus masks

The cytoarchitectonic map of basal forebrain (BF) cholinergic nuclei in Montreal Neurological Institute (MNI) space (2) was generated by combined histology and MRI of one post-

mortem brain of a 76-year-old woman who had died of myocardial infarction with a relapsing course and was relatively free of AD-pathology. The cholinergic nuclei of the BF were identified on digitalized cryo-gallocyanin stained sections of the left hemisphere. The location of the nuclei was then manually transferred to the corresponding slices of the post-mortem MRI sequence in native space using manual drawing of the region-of-interest (ROI). An affine 12-parameter transformation matrix from the post-mortem MRI to the ICBM152 MNI standard template was then applied to the BF ROI in post-mortem native space, resulting in an approximate distribution of BF cholinergic nuclei in MNI space.

Manual segmentation of the hippocampus was performed on structural (T1-weighted) versions of the lifespan template and the elderly-AD template. These were generated by warping all individual T1-weighted structural scans to the respective template in MNI space using the flow-fields of the DARTEL registration and the affine transform from the study-specific templates to MNI space. The arithmetic means of the warped structural scans represent the study-specific structural templates in MNI space. The final hippocampus masks cover left and right hemispheric volumes of 3975 mm^3 and 4139 mm^3, respectively, for the lifespan template and 3216 mm^3 and 3453 mm^3, respectively, for the elderly-AD template.

For the study of age-effects on basal forebrain cholinergic system (BFCS) gray matter volume we used the original BFCS mask (2), which is aligned to the ICBM152 standard template and hence matches the characteristics of the lifespan template of healthy adults in MNI space. A study-specific BFCS mask that matches the characteristics of the study group of healthy elderly and patients with AD was generated by warping the original BFCS mask to the elderly-AD template. The ICBM152 standard template was registered to the elderly-AD template in MNI space using DARTEL and the resulting warp was applied to the BFCS mask. To account for interpolation effects of the warp, the warped BFCS mask was binarized again by applying a threshold of 0.6. Based on this threshold the warped BFCS mask covers a volume of 942 mm^3 in each hemisphere, being similar to the original size of 958 mm^3 in each hemisphere.

Table S1. Subject demographics

	Healthy Subjects	**Healthy Elderly**	**Very Mild AD**	**AD**
Number of Subjects	211	95	69	28
Age, years	55.8 ± 21.1 [20-94]	75.6 ± 8.9 [60-94]	76.2 ± 7.2 [62-92]	77.8 ± 7.0 [65-96]
Gender (F / M)	136/75	70/25	38/31^{+}	19/9
MMSE	-	29.0 ± 1.2	25.6 ± 3.5**	21.7 ± 3.8$^{**, \#}$
CDR	0	0	0.5	1

AD, Alzheimer's disease; F, female; M, male.
MMSE: Mini-Mental State Exam, where the range from best to worst performance is 30-0.
CDR: Clinical Dementia Rating, where CDR = 0 no dementia, CDR = 0.5 questionable dementia/very mild AD, CDR = 1 clinically manifest AD.
$^{+}$ distribution differs significantly from distribution of healthy elderly (chi^2-test).
** value differs significantly from the healthy elderly group (at $p < 0.001$, 2-sample *t*-test).
$^{\#}$ value differs significantly from the very mild AD group (at $p < 0.001$, 2-sample *t*-test).

Table S2. Negative effects of age on basal forebrain gray matter volume

Basal forebrain region	**Side**	**CS**	**Coordinates (mm)**			**T_{207}**	**r_{part}**
			x	***y***	***z***		
Anterior lateral nucleus basalis (Ch4al)	**R**	**942**	**18**	**10**	**-12**	**15.48**	**0.73**
Anterior to intermediate nucleus basalis (Ch4a-Ch4i)	R		11	2	-10	14.85	0.72
Vertical limb of the diagonal band (Ch2)	R		1	6	-8	13.36	0.68
Anterior lateral nucleus basalis (Ch4al)	**L**	**872**	**-17**	**7**	**-11**	**13.76**	**0.69**
Intermediate to posterior nucleus basalis (Ch4i-Ch4p)	L		-17	-3	-11	13.42	0.68
Anterior to intermediate nucleus basalis (Ch4a-Ch4i)	L		-9	2	-10	13.34	0.68

CS, cluster size; df, degrees of freedom; L, left; MNI, Montreal Neurological Institute; R, right; sqrt, square root.

The height threshold was set at $p < 0.01$, corrected. The cluster extension, representing the number of contiguous voxels passing the height threshold was set at ≥ 5. Coordinates (MNI-space) in bold delineate a cluster and the peak T-value (207 degrees of freedom) within the cluster. Subsequent non-bold coordinates identify further peaks within the same cluster that meet the significance level. r_{part} is the corresponding (partial) correlation coefficient of the regression analysis ($r = T/\mathrm{sqrt}(T^2 + df)$).

CS = number of contiguous voxels passing the height threshold.

Table S3. Reduced basal forebrain gray matter volume in very mild AD as compared to healthy elderly controls (corrected for head size)

Basal forebrain region	**Side**	**CS**	**Coordinates (mm)**			**T_{186}**
			x	***y***	***z***	
Posterior nucleus basalis (Ch4p)	**R**	**239**	**22**	**-4**	**-15**	**6.04**
Posterior nucleus basalis (Ch4p)	**L**	**280**	**-25**	**-9**	**-16**	**5.51**
Intermediate nucleus basalis (Ch4i)	L		-24	-1	-14	4.28
Anterior to intermediate nucleus basalis (Ch4a-Ch4i)	L		-17	3	-11	3.32
Anterior lateral nucleus basalis (Ch4al)	**L**	**12**	**-20**	**12**	**-14**	**3.96**
Horizontal limb of the diagonal band (Ch3)	**R**	**72**	**10**	**5**	**-10**	**3.73**
Anterior lateral nucleus basalis (Ch4al)	**L**	**8**	**-24**	**9**	**-12**	**3.52**
Anterior lateral nucleus basalis (Ch4al)	**R**	**8**	**20**	**12**	**-15**	**3.22**

AD, Alzheimer's disease; CS, cluster size; L, left; MNI, Montreal Neurological Institute; R, right.
Results are corrected for total intracranial volume. The height threshold was set at $p < 0.01$, corrected. The cluster extension, representing the number of contiguous voxels passing the height threshold was set at ≥ 5. Coordinates (in MNI-space) in bold delineate a cluster and the peak T-value (186 degrees of freedom) within the cluster. Subsequent non-bold coordinates identify further peaks within the same cluster that meet the significance level.
CS = number of contiguous voxels passing the height threshold.

Table S4. Reduced basal forebrain gray matter volume in AD as compared to healthy elderly controls (corrected for head size)

Basal forebrain region	**Side**	**CS**	**Coordinates (mm)**			**T_{186}**
			x	***y***	***z***	
Posterior nucleus basalis (Ch4p)	**R**	**836**	**23**	**-4**	**-15**	**5.62**
Horizontal limb of the diagonal band (Ch2) / anterior medial nucleus basalis (Ch4am)	R		12	5	-10	4.69
Anterior lateral nucleus basalis (Ch4al)	R		23	9	-14	4.33
Posterior nucleus basalis (Ch4p)	**L**	**767**	**-26**	**-9**	**-17**	**5.33**
Anterior lateral nucleus basalis (Ch4al)	L		-21	8	-13	5.21
Horizontal limb of the diagonal band (Ch2)	L		-13	7	-12	5.06

AD, Alzheimer's disease; CS, cluster size; L, left; MNI, Montreal Neurological Institute; R, right.
Results are corrected for total intracranial volume. The height threshold was set at $p < 0.01$, corrected. The cluster extension, representing the number of contiguous voxels passing the height threshold was set at ≥ 5. Coordinates (in MNI-space) in bold delineate a cluster and the peak T-value (186 degrees of freedom) within the cluster. Subsequent non-bold coordinates identify further peaks within the same cluster that meet the significance level.
CS = number of contiguous voxels passing the height threshold.

Table S5. Reduced basal forebrain gray matter volume in very mild AD as compared to healthy elderly controls (corrected for global gray matter)

Basal forebrain region	**Side**	**CS**	**Coordinates (mm)**			**T_{186}**
			x	***y***	***z***	
Posterior nucleus basalis (Ch4p)	**R**	**206**	**22**	**-4**	**-15**	**4.97**
Posterior nucleus basalis (Ch4p)	**L**	**148**	**-25**	**-9**	**-16**	**4.24**
Posterior nucleus basalis (Ch4p)	L		-19	-4	-11	3.34

AD, Alzheimer's disease; CS, cluster size; L, left; MNI, Montreal Neurological Institute; R, right.
Results are corrected for total gray matter volume. The height threshold was set at $p < 0.01$, corrected. The cluster extension, representing the number of contiguous voxels passing the height threshold was set at ≥ 5. Coordinates (in MNI-space) in bold delineate a cluster and the peak T-value (186 degrees of freedom) within the cluster. Subsequent non-bold coordinates identify further peaks within the same cluster that meet the significance level.
CS = number of contiguous voxels passing the height threshold.

Table S6. Reduced basal forebrain gray matter volume in AD as compared to healthy elderly controls (corrected for global gray matter)

Basal forebrain region	**Side**	**CS**	**Coordinates (mm)**			**T_{186}**
			x	***y***	***z***	
Posterior nucleus basalis (Ch4p)	**R**	**499**	**22**	**-4**	**-12**	**4.63**
Horizontal limb of the diagonal band (Ch3)	R		13	8	-11	3.57
Anterior lateral nucleus basalis (Ch4al)	R		23	9	-14	3.54
Anterior lateral nucleus basalis (Ch4al)	**L**	**549**	**-21**	**8**	**-13**	**4.44**
Posterior nucleus basalis (Ch4p)	L		-26	-9	-17	4.09
Horizontal limb of the diagonal band (Ch3) / anterior medial nucleus basalis (Ch4am)	L		-14	5	-10	3.89

AD, Alzheimer's disease; CS, cluster size; L, left; MNI, Montreal Neurological Institute; R, right.
Results are corrected for total gray matter volume. The height threshold was set at $p < 0.01$, corrected. The cluster extension, representing the number of contiguous voxels passing the height threshold was set at ≥ 5. Coordinates (in MNI-space) in bold delineate a cluster and the peak T-value (186 degrees of freedom) within the cluster. Subsequent non-bold coordinates identify further peaks within the same cluster that meet the significance level.
CS = number of contiguous voxels passing the height threshold.

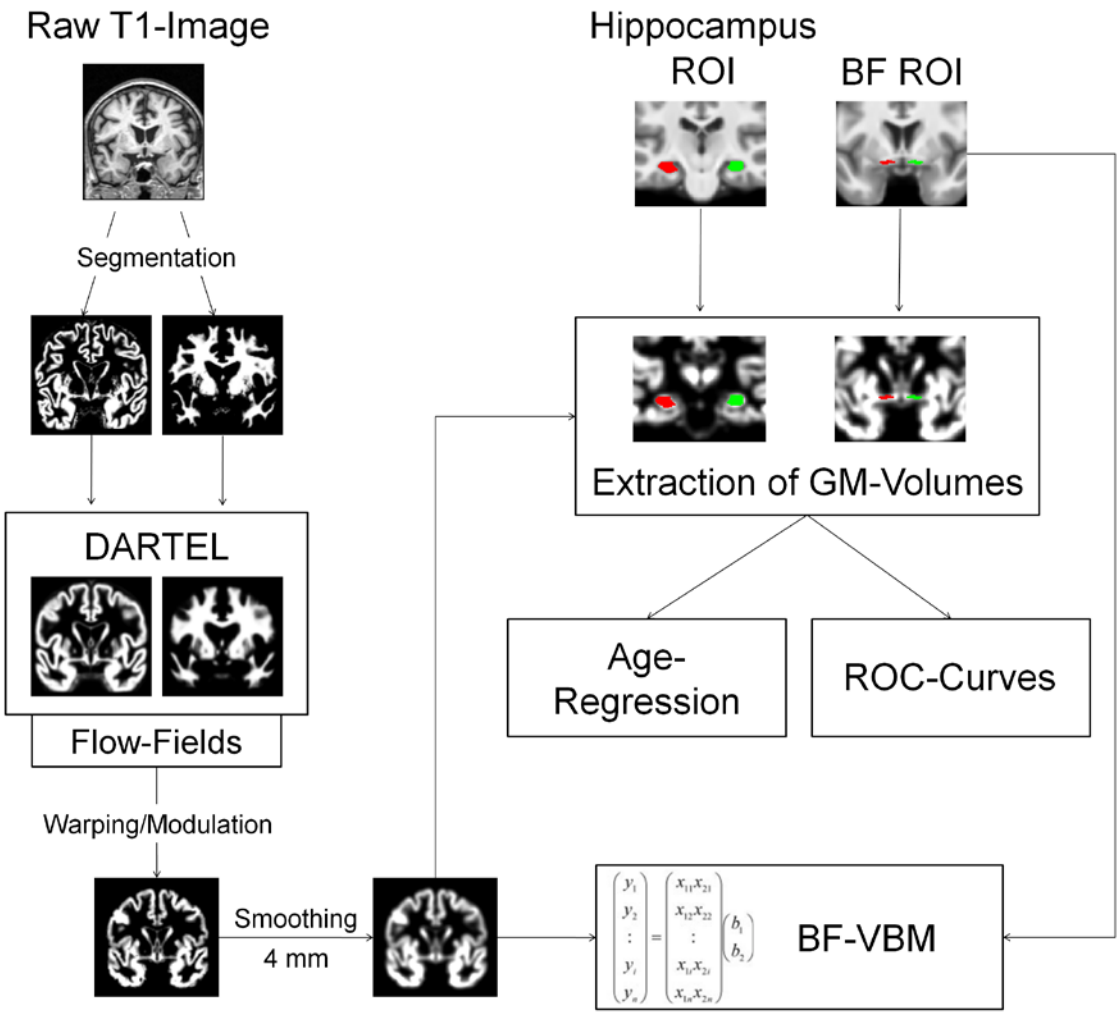

Figure S1. Workflow of the main processing steps and statistical analyses. Structural MRI scans were segmented into different tissue-types and high-dimensionally registered to study-specific templates of group average anatomy. Warping parameters were applied to individual GM maps and GM-voxel values were modulated to preserve the absolute amount of GM-volume. Modulated GM maps were smoothed by an isotropic smoothing kernel of 4 mm and subjected to voxel-based analyses restricted to the BFCS. In addition, hippocampus ROI maps were manually drawn in the two population-specific reference spaces and BFCS-ROIs were derived from a cytoarchitectonic map of the BFCS in MNI space. Individual hippocampus and BFCS GM values were automatically extracted by summing up the modulated GM voxel values within the hippocampus- and BFCS-ROIs, respectively. These individual hippocampus and BFCS GM values were plotted against age for the healthy cohort and also subjected to ROC analyses to estimate the diagnostic potential for separation of diagnostic groups. BFCS, basal forebrain cholinergic system; GM, gray matter; MNI, Montreal Neurological Institute; ROC, receiver operating characteristic; ROI, region of interest; VBM, voxel-based morphometry.

References

1. Marcus DS, Wang TH, Parker J, Csernansky JG, Morris JC, Buckner RL (2007): Open Access Series of Imaging Studies (OASIS): cross-sectional MRI data in young, middle aged, nondemented, and demented older adults. *J Cogn Neurosci* 19(9): 1498-507.

2. Teipel SJ, Flatz WH, Heinsen H, Bokde AL, Schoenberg SO, Stockel S, *et al.* (2005): Measurement of basal forebrain atrophy in Alzheimer's disease using MRI. *Brain* 128(Pt 11): 2626-44.