SUPPLEMENTAL METHODS

History of the Nun Study

The Nun Study began at the University of Minnesota (UMN) in 1986 as a pilot examination of physical functioning and aging in older members of the order of the School Sisters of Notre Dame (SSND). The project relocated to the University of Kentucky (UK) in 1992 with a broadening of scope to include cognitive decline, dementia, and brain autopsy. The neuropathology component was designed by Dr. William Markesbery (deceased, 2010), who personally carried out the gross and microscopic examinations. The histologic methods have been described in prior publications. The initial autopsy data collection and reading protocol were based on systems then in use at the UKY Alzheimer Disease Research Center. In 1994 the microscopic reading protocol and hard-copy form then in use for the HAAS were adopted for the Nun Study autopsies, thereby greatly enhancing subsequent comparisons. National Institute on Aging (NIA) funding for Nun Study data collection ended in 2009, following which the data and accrued tissue resources were transferred to the UMN School of Medicine. At present the study continues with sustaining support provided by the UMN with NIA grant funding for ongoing analyses.

History of the Honolulu-Asia Aging Study

The HAAS was initiated in 1991 and operated with funding from the NIA until 2012. The autopsy protocol and microscopic data collection methods were designed by Dr. Markesbery and the HAAS Principal Investigator, Lon White. Histologic methods have been described in prior publications. For the first decade of its operation, the HAAS autopsy microscopic reading component was overseen by Dr. Markesbery, who trained and shared responsibilities for readings with two other neuropathologists (Drs. D. Davis and J. Nelson). All of the gross brain autopsies were done by Dr. J. Hardman (deceased, 2002). After 2002 the gross brain dissections and examinations were done by Dr. J. Uyehara-Lock, who had been trained by Dr. Hardman. For the second decade (2002-2012), microscopic readings were overseen by Dr. Markesbery and Dr. T. Montine, training a team of neuropathologists comprised of Drs. J. Sonnen, C. Zarow, and J. Uyehara-Lock. This team met regularly during the remaining years to review difficult cases and to ensure inter-reader consistency.

For repeated neuropsychological assessments and for screening purposes, the HAAS employs the Cognitive Assessment and Screening Instrument (CASI), an instrument developed especially for use in the HAAS and cooperating studies in Seattle (the KAME project) and Hiroshima—collectively representing the Ni-Hon-Sea Project. While the CASI was developed as an improved and extended version of the MMSE and MMMSE, it also includes elements from the Hasegawa dementia assessment test. The full CASI was developed with finer scaling of several items in the MMSE and Hasegawa test, but allows extraction of scores corresponding very closely to a conventional MMSE and Hawagawa, modified only as necessary because of cultural and linguistic issues precluding use after direct translation.

Figure e-1: Distributions of final MMSE and MMSEc scores in autopsied NS and HAAS participants; the MMSEc is calculated from CASI for direct correspondence with conventional MMSE scores.

As expected, the NS scores are shifted toward better performance reflecting substantially greater educational attainment.

Figure e-1

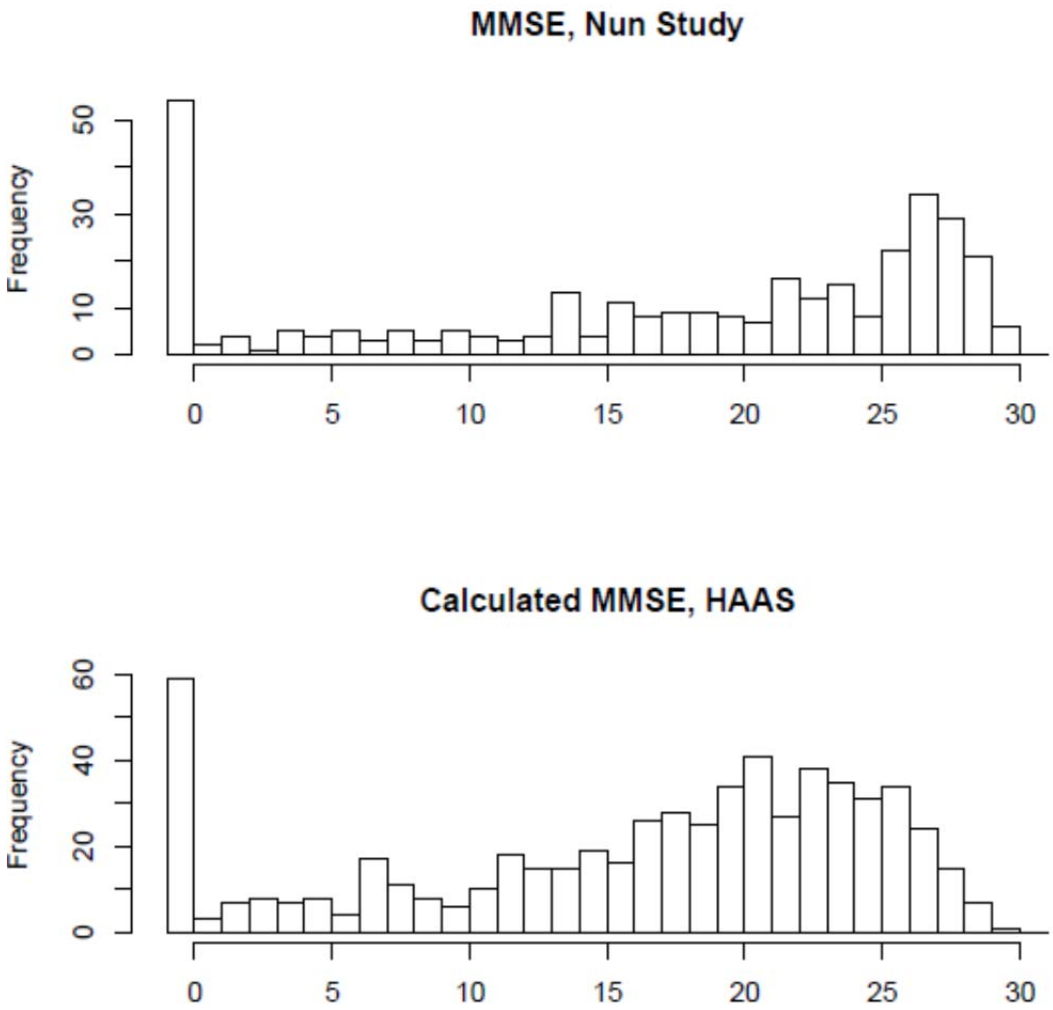

Table e-1: Correspondence of the MMSE, CASI, a CASI-derived MMSE (MMSEc), and total CERAD score.

Compared with the MMSE, the CASI involves more items (reflecting DSMIII-R criteria) and finer grading of responses to some items (as temporal orientation). The bolded and italicized levels indicate our cut-points separating moderate cognitive impairment from 'no or mild', and moderate from severe cognitive impairment. The substantially higher level of education in the NS undoubtedly influences these relationships, since the CERAD tests are weighted more toward verbal performance than either the MMSE or CASI. The NS data in this table were from the final testing prior to death. Data from the HAAS were collected during the 2002-2004 examination cycle.

TABLE e-1

Nun Study
final testing before death n=334

HAAS (based on surviving subjects)
testing at 2002-2004 exam n=917

Pearson correlation coefficients

CERAD x MMSE r = 0.885

-

CERAD x MMSEc r= 0.721
CERAD x CASI r= 0.806
MMSEc x CASI r= 0.949

MMSE	CERAD
28	78
26	71
24	59
22	***54***
19	47
17	***45***
14	31
10	26

CASI	MMSEc	CERAD
94	27	70
92	26	67
82	23	58
74	***22***	***51***
66	19	44
60	***17.5***	***43.6***
50	15	33
30	9	21.5

Table e-2: Linear regression analysis to identify predictors of the final MMSE (NS) or MMSEc (HAAS)
Data for this analysis were pooled for the two studies. Information was limited to 930 autopsies (334 from NS, 596 from HAAS, limited to autopsies within 3 years of final testing). A new variable was created (cohort; 0=HAAS, 1=NS) indicating cohort membership. Coefficients and p values are shown in Table e-2 in a full model, with MMSE (or MMSEc) score as the dependent variable. Coefficients show change in score for each unit of the predictor variable, estimated from the regression line. The analysis identifies as significant predictors all of the variables except the cohort. A major aim of this analysis is to examine possible differences in final cognitive test scores between HAAS and NS autopsied participants. In addition, this analysis is intended to complement logistic regression analyses addressing similar issues.

Predictors of the final MMSE (or MMSEc) for 930 autopsied Nun Study and HAAS participants

	coefficient	Std. Error		P
(Intercept)	25.8	4.38		<0.0001
age at final exam (yrs)	-0.127	0.048		0.0005
education (yrs)	0.414	0.08		<0.0001
interval test to death (yrs)	2.31	0.36		<0.0001
low brain wt index =0.4	-2.1	0.66		0.0005
low brain wt index= 1.0	-4.35	0.53		<0.0001
Alzheimer (Braak stage IV) index= 0.4	-2.39	0.61		<0,0001
Alzheimer (Braak stage V or VI) index= 1.0	-6.60	0.53		<0.0001
microinfarct index= 0.4	-1.41	0.59		0.0175
microinfarct index= 1.0	-3.42	0.61		<0.0001
Lewy body index= 0.4	-4.34	0.91		<0.0001
Lewy body index= 1.0	-6.83	1.07		<0.0001
Hippocampal sclerosis index= 0.4	-2.78	0.91		0.0023
Hippocampal sclerosis index= 1.0	-5.75	1.08		<0.0001
Cohort (HAAS=0; NS= 1) n= 774, 334	0.14	0.67		ns

A second analysis was done excluding those individuals who died after the first (and only) testing, thereby liming the subset to 898 decedents for whom at least two scores were available. For these analyses (data not shown) we included the baseline MMSE or MMSEc as an additional independent variable in the model. While most results differed only modestly from those presented in Table e-2, this redefinition of the dataset resulted in a reduction in the influence of education apparent in a fall in the coefficient from 0.414 to 0.13, with a corresponding increase in the the p value from <0.0001 to 0.069. A barely significant association (p=0.041) of cohort with the final MMSE score was also noted. These observations were likely the result of a highly significant association between the baseline and final MMSE, as well as related differences in the decline trajectory and in the interval between final testing and death for the two cohorts.

Table e-3: Results from five separate linear regression models for subsets stratified by Braak stage.

These analyses utilized the same dataset as employed for Table e-2, minus a small number for whom *APOE* genotypes had not been determined. Data were pooled from the two studies and the analyses were limited to 1108 participants who died within 3 years of final testing, and for whom all essential data (including *APOE* genotype) were available. The first subset includes Braak stages 0, I, and II. The results, given as coefficients and p values, identify predictors of the final MMSE or MMSEc. The cohort of membership is examined as one of the candidate predictors ("COHORT" 0=HAAS, 1=NS). Values in each cell indicate the change in MMSE score for each unit change in the predictor (independent) variable, based on the fitted regression line for that Braak stage subset.

These independent variables in the models included neocortical neurofibrillary tangles (NFTs), neuritic plaque (NP) counts, and *APOE* ε4 positivity. This was done to examine the possibility that simple Braak stage might fail to take account of the full range and severity of Alzheimer brain lesions. *APO*E ε4 positivity had no additional association with impairment beyond that implicit in its association with Braak stage. The results indicate significantly lower final MMSE scores with higher neocortical NFT counts in Braak stages VI and V subsets. The only apparent association with neocortical NP counts was slightly better scores with higher NP counts in the Braak stage VI subset. The substantial influence of neocortical NFT counts in the higher Braak stage subsets may indicate insufficiency of the Braak stages to represent the full range of Alzheimer pathology linked to cognitive decline.

The significant association of a higher cognitive test score with longer interval between final testing and death likely represents at least two factors: (1) worse cognitive function predicts a more imminent death, and (2) a longer interval allows further decline. Both of these phenomena are recognized and demonstrable with serial assessments in living persons with moderate levels of impairment. Since severely impaired older persons near the end of life rarely recover normal cognitive functioning, the bias imposed by an interval less than 3 years seems unlikely to result in the misclassification of truly unimpaired persons as demented or definitely impaired. The coefficients suggest declines in the MMSE score of about 2-3 for each year between final cognitive testing and death, at least in individuals with normal, marginal, or moderate impairment at the final examination.

Cohort membership was not significantly associated with the endpoint in any of these models. The concurrence of the findings shown, in two quite different cohorts, supports generalizability.

A summation of the 4 non-AD lesion indices ("4 lesion co-morbidity index") was a powerful predictor of poorer final cognitive test score at all Braak stages, least evident in the Braak stage VI subset. These findings emphasize the extreme importance of co-morbid neuropathologic abnormalities as drivers of cognitive decline in the final months and years of life. Results are presented in Table e-3.

Results of linear regression analyses of the merged NS and HAAS datasets among subsets stratified by Braak stage. The dependent variable is MMSE (MMSEc for HAAS). Each column presents results for a single model, one for each of these 5 Braak stage subsets. Numbers are coefficients, reflecting the change in MMSE score for each unit of the predictor variable. Negative values indicate an inverse relationship, i.e.declining scores with increases in the value of the independent variable. The variable "index4bwtmvl" is the 4-lesion co-morbidity index (sum of indices of Lewy bodies, microinfarcts, hippocampal sclerosis, and low brain weight)

Table e-3

	Braak <III n= 352	Braak III n= 182	Braak IV n= 155	Braak V n= 142	Braak VI n= 99
(Intercept)	28.02 ***	13.16 ns	39.17 ***	33.83 **	15.15 ns
e4pos	-0.64 ns	-1.87 ns	0.72 ns	0.81 ns	-1.18 ns
agelastexam	-0.16 *	0.01 ns	-0.27 *	-0.24 ns	-0.13 ns
edyrs	0.51 ***	0.32 ns	0.13 ns	0.28 ns	0.33 ns
testtodeathyrs	1.71 **	3.61 ***	2.19 *	2.91 **	2.39 *
tneo	0.08 ns	-0.71 ns	-0.49 ns	-0.27 *	-0.26 ***
npneo	-0.15 ns	0.16 ns	0.02 ns	-0.18 ns	0.4 *
index4bwtmvl	-5.24 ****	-4.91 ***	-4.94 ***	-5.67 ***	-2.01 *
Cohort (NS=1, HAAS=0)	2.24 *	1.43 ns	3.4 ns	3.44 ns	0.69 ns

*p<0.05 **p<0.01 ***p<0.001 ****p<0.0001

Since cognitive resilience is conventionally offered as a possible explanation for little or no impairment in individuals with substantial AD neuropathology, it can be most reasonably addressed in Braak strata in which individuals show a range of impairment, *i.e.*, some with impairment and others without. In Braak stage VI, the great majority were impaired providing little opportunity to examine resilience. In Braak stage III and below, and even in stage IV, the association with impairment in both HAAS and NS decedents is minimal (data not shown) or is attributable to non-Alzheimer abnormalities. Because of these findings we have viewed Braak stage V as most appropriate for analyses related to cognitive resilience.

Table e-4: Relationship of final MMSE to co-morbidity, and to the ADindex.

The dataset for this table was created by merging NS data (n=334) with HAAS data (n=774). For these tables the cognitive test score is the final MMSE (NS) or MMSEc (HAAS). The MMSE values shown are the mean for each cell. The co-morbidity index is the simple sum of the 5 neuropathologic indices (ADindex, LBindex, HSindex, MINFindex, and brain weight) – each with possible values of 0, 0.4, or 1.0. Because of special interest in AD brain lesions, this table shows scores and numbers for the total combined sample (far right column), plus scores and numbers for each of the ADindex values (3 columns to the left).

A co-morbidity index of 1.0 only exists if a single lesion type is present at that level of severity; *i.e.,* an index of 1.0 precludes more than one type of lesion, which must be present at a severe level. Among the 204 individuals with a co-morbidity index of 1.0, 67 had severe AD lesions (ADindex=1.0), while 137 had one of the other 4 lesion types at a severity index of 1.0. Among the 1108 decedents, 279 had a Braak stage of V or VI (requisite for AD index=1.0), 181 had a Braak stage if IV (ADindex=0.4), and the remaining 648 had Braak stages of III or less (ADindex=0). Among the 181 with and ADindex=0.4, all but 55 had at least one other lesion type. Among the 279 with severe AD lesions (ADindex=1.0), 212 had at least one other lesion type. This table demonstrates the dramatic impact of accumulated co-morbid pathology on severity of cognitive impairment as indicated by the mean MMSE score -- evident not just in the full sample, but also in the subsets stratified according to AD lesion severity. Note that AD lesions contribute to some but not all of the cases with high co-morbidity indices. The co-morbidity index is a major determinant of the final MMSE with or without AD lesion contribution.

TABLE e-4

	ADindex=0	**ADindex=0.4**	**ADindex=1**	**total**
	MMSE (n)	MMSE (n)	MMSE (n)	MMSE (n)
Co-morbidity index				
0	23.4 (n=208)	n=0	n=0	23.1 (n=208)
0.4	21.0 (n=154)	20.9 (n=51)	n=0	21.5 (n=205)
0.8	17.9 (n=39)	19.0 (n=35)	n=0	17.8 (n=74)
1.0	18.4 (n=**133**)	n=0	17.8 (n=**64**)	18.3 (n=197)
1.2	24.0 (n=2)	13.4 (n=9)	n=0	17.5 (n=11)
1.4	7.3 (n=58)	17.1 (n=39)	14.7 (n=55)	16.3(n=152)
1.6	n=0	4.6 (n=2)	n=0	9.0 (n=2)
1.8	14.0 (n=9)	13.5 (n=21)	12.3 (n=14)	13.4 (n=44)
2.0	13.5 (n=39)	n=0	10.0 (n=50)	12.1 (n=89)
2.2	n=0	5.8 (n=6)	20.0 (n=2)	14 (n=8)
2.4	8.8 (n=5)	10.5 (n=16)	5.0 (n 52)	7.1 (n=73)
2.8	n=0	19.5 (n=2)	6.3 (n=5)	6.6 (n=7)
3.0	11.5 (n=1)	n=0	7.4 (n=22)	7.7 (n=23)
3.4	n=0	n=0	6.7 (n=7)	6.7 (n=7)
3.8	n=0	n=0	6.0 (n=1)	6.0 (n=1)
4.0	n=0	n=0	0.0 (n=3)	0 (n=3)
4.4	n=0	n=0	6.0 (n=2)	6.0 (n=2)
5.0	n=0	n=0	6.0 (n=2	6.0 (n=2)
Total n=	**648**	**181**	**279**	**1108**

Table e-5: Associations among different neuropathologic abnormalities

The correlation matrices shown in Table e-5 demonstrate that the 5 neuropathologic abnormalities were largely mutually independent in their occurrence in both cohorts. Two substantial exceptions were evident: (1) the AD index was associated with low brain weight in the NS cohort, and (2) HS index was associated with the AD index in HAAS decedents. Among 102 NS brains with severe AD changes, 37% had a severe (low) brain weight index. In 27 NS brains with moderate AD changes, 36% had a severe low brain weight index. Among the 205 NS brains with negligible AD changes, 9 % had a severe low brain weight index. Among 83 HAAS decedents in whose brains either unilateral or bilateral hippocampal sclerosis was found, 44% had a severe AD lesion index. Among the 691 without HS, only 20% had a severe AD index. Weaker associations were also observed between AD and Lewy body indices in Nun Study brains, between microinfarct index and low brain weight in HAAS brains, and between HS index and low brain weight in the HAAS.

Correlation matrices showing associations among the 5 brain lesion indices and final MMSE score (Nun Study) and the final CASI score (HAAS) using results from brain autopsies

	Alzheimer's Disease	Lewy Body	Microinfarcts	Hippocampal Sclerosis	Low Brain Weight	
Nun Study N=334						**Last MMSE**
Alzheimer's Disease	1.0	0.14*	-0.0006 ns	0.09 ns	0.22***	-0.49***
Lewy Body		1.0	0.04 ns	0.001 ns	0.02 ns	-0.26***
Microinfarcts			1.0	0.01 ns	0.04 ns	-0.08 ns
Hippocampal Sclerosis				1.0	0.08 ns	-0.23***
Low Brain Weight					1.0	-0.23***
HAAS N=774						**Last CASI**
Alzheimer's Disease	1.0	0.05 n	0.06 ns	0.14***	0.06 ns	-0.27***
Lewy Body		1.0	-0.03 ns	-0.004 ns	0.04 ns	-0.17***
Microinfarcts			1.0	0.004 ns	0.08*	-0.19***
Hippocampal Sclerosis				1.0	0.08*	-0.22***
Low Brain Weight					1.0	-0.32***

Values are Spearman correlation coefficients. *p<0.05 **p<0.005 ***p<0.0005

Table e-6: Heterogeneity of brain lesions—single and co-morbid—in 138 NS and 269 HAAS decedents with severe cognitive impairment at final testing. These subsets of the two autopsy panels were considered separately to facilitate comparison with other autopsied dementia case series, such as the series of 22 autopsies recently reported from the ADNI project. Nearly all of the 130 sisters had been identified as demented. Among the 269 HAAS severely impaired decedents, dementia based on DSMIII-R criteria with CDR scores of 1.0 or greater was confirmed for more than 70% in those for whom full assessments had been completed.

Among the severely impaired sisters, 9.2 % had a co-morbidity lesion index of 0 (negligible lesions), while 59.2% had a co-morbidity index >1. In the HAAS men, 10 (3.7%) had a co-morbidity index of 0, 18 (10%) had a co-morbidity index =0.4 (one lesion type, moderate), 29 (19%) had a co-morbidity index = 0.8 (two types of lesion, both moderate), 39 (34%) had a co-morbidity index =1 (one type of lesion, severe), while 178 (66.2%) had a co-morbidity index greater than 1.

Among the 18 severely impaired HAAS decedents with a co-morbid index=0.4, 6 were AD lesions, 2 were Lewy bodies, 3 were microinfarcts, 2 were unilateral HS, and 5 were low brain weight. Among the 39 severely impaired HAAS decedents with a co-morbidity index=1.0, 9 had AD lesions, 1 had severe neocortical Lewy bodies, 11 had microinfarcts, 2 had bilateral HS, and 16 had severe low brain weight.

A similar heterogeneity of lesion types was observed in participants from both cohorts at all levels of the co-morbidity index.

These results confirm prior observations that cognitive impairment in late life is usually the result of at least two different types of brain abnormality. Although AD lesions are the single most common type, other types and other combinations are collectively dominant in their relevance to impairment. Any combination of the 5 major abnormalities that produces a substantial combined burden may be responsible for dementia or severe impairment. The type of abnormality seems to be less relevant than the total burden of brain pathology, whatever the lesion type.

Table e-6. **Brain lesions in Nun Study (138) and HAAS (269) participants with severe cognitive impairment**

		Alzheimer's Disease		Lewy Body		Infarcts		Hipp Sclerosis		Low Brain Weight	
		+	++	+	++	+	++	+	++	+	++
Nun Study											
Comorbidity											
Index 0	9 (6.5%)	0	0	0	0	0	0	0	0	0	0
Index 0.4 or 0.8	23 (16.7%)	8	0	6	0	6	0	3	0	7	0
Index 1.0	23 (16.7%)	0	.10	0	5	0	3	0	1	0	4
Index 1.2, 1.4, 1.6, or 1.8	28 (20.3%)	3	17	9	3	9	4	2	1	17	3
Index 2.0	20 (14.5%)	0	15	0	2	0	5	0	4	0	14

Index 2.4, 2.6, or 2.8	26 (18.8%)	1	24	14	4	7	4	4	5	3	15
Index ≥ 3	9 (6.5%)	0	9	0	7	3	1	0	4	0	9
TOTAL	**138 (100%)**	**12**	**75**	**29**	**21**	**25**	**17**	**9**	**15**	**27**	**45**
HAAS											
Comorbidity											
Index 0	10 (3.7%)	0	0	0	0	0	0	0	0	0	0
Index 0.4 or 0.8	48 (17.9%)	15	0	8	0	15	0	6	0	25	0
Index 1.0	43 (16.0%)	0	11	0	1	0	7	0	3	0	21
Index 1.2, 1.4, 1.6, or 1.8	76 (28.0%)	29	30	6	5	38	8	10	1	26	24
Index 2.0	32 (11.9%)	0	17	0	6	0	14	0	6	0	21
Index 2.4, 2.6, or 2.8	40 (14.9%)	15	22	4	4	10	19	14	2	11	28
Index ≥ 3	20 (7.5%)	0	19	1	7	3	9	2	10	1	19
TOTAL	**269 (100%)**	**59**	**99**	**19**	**23**	**66**	**57**	**32**	**22**	**63**	**113**

Values are the number of brains in which the comorbidity index or lesion type was observed. For each lesion type, + indicates moderate, and ++ severe.