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► **To cite this version:**

Mélissa Gentreau, Jerome Maller, Chantal Meslin, Fabienne Cyprien, Jorge Lopez-Castroman, et al.. Is Hippocampal Volume a Relevant Early Marker of Dementia?. *American Journal of Geriatric Psychiatry*, In press, 10.1016/j.jagp.2023.05.015 . hal-04153332

HAL Id: hal-04153332

<https://hal.science/hal-04153332>

Submitted on 6 Jul 2023

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1 **Is hippocampal volume a relevant early marker of dementia?**

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13

14

15 **ABSTRACT**

16 **Objective** Hippocampal volume (HV) is a key imaging marker to improve Alzheimer’s disease risk
17 prediction. However, longitudinal studies are rare, and hippocampus may also be implicated in the
18 subtle aging-related cognitive decline observed in dementia-free individuals. Our aim was to
19 determine whether HV, measured by manual or automatic segmentation, is associated with
20 dementia risk and cognitive decline in participants with and without incident dementia.

21 **Methods** At baseline, 510 dementia-free participants from the French longitudinal ESPRIT cohort
22 underwent magnetic resonance imaging. HV was measured by manual and by automatic segmentation
23 (FreeSurfer 6.0). The presence of dementia and cognitive functions were investigated at each follow-
24 up (2, 4, 7, 10, 12 and 15 years). Cox proportional hazards models and linear mixed models were used
25 to assess the association of HV with dementia risk and with cognitive decline, respectively.

26 **Results** During the 15-year follow-up, 42 participants developed dementia. Reduced HV (regardless of
27 the measurement method) was significantly associated with higher dementia risk and cognitive decline
28 in the whole sample. However, only the automatically measured HV was associated with cognitive
29 decline in dementia-free participants.

30 **Conclusions** These results suggest that HV can be used to predict the long-term risk of dementia but
31 also cognitive decline in a dementia-free population. This raises the question of the relevance of HV
32 measurement as an early marker of dementia in the general population.

33

34 Keywords: hippocampus, MRI, automatic segmentation, manual segmentation, dementia, cognition

35 1. INTRODUCTION

36 Hippocampus is a plastic brain structure with a central role in human cognition, memory,
37 learning, and spatial navigation [1,2]. Despite its plasticity, it can be damaged by a variety of exogenous
38 stimuli, including diet, air pollution, sleep alterations, inactivity and stress but also endogenous
39 conditions such as depression leading to a decreased volume [3]. Hippocampal volume (HV) is
40 correlated with local neuronal density [4], and its reduction is likely to be preceded by cellular changes,
41 such as synaptic loss and neuronal degeneration, that cannot be detected by conventional volumetric
42 measurements.

43 A smaller HV has been associated with the risk of dementia and Alzheimer’s disease (AD). It
44 has been shown that patients with AD or mild cognitive impairment have a smaller HV compared with
45 healthy aging controls [5]. Consequently, HV is of utmost interest in AD research, and has been
46 proposed as a possible surrogate biomarker to facilitate early diagnosis. However, most evidence
47 comes from epidemiological studies with short follow-up periods, and the role of HV reduction in
48 predicting the long-term AD risk remains unclear [6].

49 HV also decreases progressively with age in dementia-free older adults [7,8], but the functional
50 consequences of this age-related volumetric loss are not well characterized. Previous research has
51 produced controversial findings on hippocampus role in dementia-free older adults with cognitive
52 decline. While some studies found a significant association between HV and cognitive performance [9–
53 13], others did not [14–16]. Thus, the relationship between HV and cognitive decline in dementia-free
54 older adults remains poorly understood. Inconsistencies among studies are not surprising, because
55 most of them had a cross-sectional design or a short follow-up, and did not include information on the
56 participants’ cognitive outcome. In other words, it was not known whether the included participants
57 developed dementia during the follow-up.

58 HVs are generally investigated using brain magnetic resonance imaging (MRI) volumetric data.
59 The use of different measurement methods limits the comparisons between studies and explains, at
60 least partly, their discrepancies. Manual tracing is still considered the “gold standard” for volumetric

61 studies due to the hippocampus complex shape and ambiguous boundaries [17]. Unfortunately, this
62 approach is time-consuming when analyzing large MRI databases. To cope with these limitations,
63 automated segmentation methods based on software tools such as FreeSurfer have been developed.
64 It is yet unknown whether the association between HV and dementia risk or cognitive decline could
65 vary depending of the HV measurement method. No study to date has assessed this issue in a cohort
66 of older adults.

67 In summary, changes in cognitive performance during dementia screening and cognitive
68 assessment measures in patients with mild cognitive impairment and AD have been associated with
69 smaller HV. Conversely, it is unclear whether in the general population (i) HV predicts the long-term
70 risk of dementia; (ii) this marker is specific to the dementia stage or whether subtler variations in HV
71 might be observed also during dementia-free aging; and (iii) the association between HV and cognitive
72 performance is affected by the segmentation method used to measure HV.

73 The aim of this study was to determine whether lower HV at baseline (measured using manual
74 and automatic segmentation methods) is associated with dementia risk and cognitive performance
75 changes with and without incident dementia during a 15-year follow-up in a longitudinal population-
76 based cohort of French older adults.

77

78 **2. Materials and methods**

79 **2.1. Study sample**

80 The ESPRIT study is a longitudinal study of neuropsychiatric disorders in community-dwelling French
81 older adults and is part of the Three-City multicenter cohort study (Bordeaux, Dijon and Montpellier)
82 [18]. Eligible (≥ 65 -year-old and non-institutionalized) participants were recruited from the electoral
83 rolls between 1999 and 2001. The Bicêtre University Hospital Ethics Committee (France) approved
84 the Three-City protocol, and all participants signed an informed consent. Interviews were
85 administered by trained staff (nurses, psychologists, neurologists) at baseline and at 2, 4, 7, 10, 12

86 and 15 years of follow-up. From the 1863 participants recruited, only those younger than 80 years
87 were invited for an MRI at baseline. For the present analysis, 760 participants were randomly
88 selected, among whom 537 had complete data (including MRI). From this group, 28 participants were
89 excluded due to a diagnosis of dementia at baseline (n=11), of dementia with Lewy bodies (n=4) or
90 Parkinson's disease dementia (n=1) during the follow-up, and absence of follow-up (n=12). Thus, 510
91 participants were retained. Participants were not taking any prescribed medications for dementia.

92 2.2. Cognitive function evaluation

93 At each follow-up visit, trained neuropsychologists administered a battery of cognitive tests to
94 evaluate different cognitive domains. The Mini Mental State Examination (MMSE) was used as a
95 global measure of cognitive function [19]. The Benton's Visual Retention Test (BVRT) evaluates visual
96 memory and psychomotor speed [20]. The Trail Making Test, forms A and B (TMTA and TMTB),
97 assesses attention and visual motor processing speed (executive function) [21]. The score is the time
98 (in seconds) needed to execute the task: higher scores correspond to lower cognitive performance.
99 The TMTA and B were not filled in at the 2-year follow-up visit. The Isaacs Set Test (IST) measures
100 verbal fluency and semantic memory [22]. Fluency corresponds to the total score: the sum of the
101 number of words generated for each semantic category in 15 seconds.

102 2.3. Dementia diagnosis

103 At each follow-up visit, participants with suspected dementia (on the basis of their
104 neuropsychological test results) were examined by a neurologist. Then, an independent committee
105 of neurologists reviewed all potential cases of dementia to obtain a consensus on the diagnosis and
106 etiology based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition [23]. The
107 date of dementia onset was the middle of the interval between the last follow-up without the event
108 and the first follow-up with the event. The follow-up duration for each participant was calculated
109 from the baseline examination to death, diagnosis of dementia, or end of follow-up, whichever came
110 first.

111

112 2.4. MRI data analysis

113 At baseline, participants underwent MRI at the Gui De Chauliac Hospital (Montpellier, France). A 1.5T
114 GE Signa Imaging system (General Electric Medical Systems, Milwaukee, WI) was used to acquire a
115 contiguous AC-PC aligned axial IR-prepared SPGR T1-weighted sequence for volumetric estimations
116 (TR=12, TE=2.8, IT=6000, matrix, size=256x256, pixel spacing=0.9375x0.9375mm, NEX=1, slice
117 thickness=1.0mm).

118 2.4.1. *Manual segmentation of hippocampus*

119 Hippocampus regions of interest were manually outlined on consecutive coronal slices and the axial
120 and sagittal orientations were verified [24]. According to the protocol described by Watson et al [25],
121 the area from the anterior tip of the hippocampus to the slice before the opening of the crus of the
122 fornix corresponded to the hippocampus head and body and included the subiculum, CA1-4 areas, and
123 dentate gyrus [25]. The hippocampus tail was measured from the slice immediately posterior to the
124 last slice. The tail internal structure is the same as that of head and body. The CA areas and dentate
125 gyrus have a homogeneous structure. From the coronal plane perspective, measuring the
126 hippocampus until the crus of the fornix represents the part of the tail that coincides with the coronal
127 section of the pulvinar (which is situated in an upper-medial position). Voluminous choroidal plexuses
128 occupy portions of this region; hence, care was taken to exclude them laterally from the volumetric
129 estimates. The hippocampus was then followed posteriorly. On the initial slices, the tail appears bulgy
130 as an ovoid mass of gray matter on the lower-medial part of the lateral ventricle, and more posteriorly
131 it lies flattened on the superior surface of the parahippocampal gyrus. The tail was outlined up to the
132 point where the fasciolar gyrus becomes the subsplenium gyrus, curving around the splenium
133 posteroinferior margin. The upper border was easy to differentiate from the crus of the fornix. The
134 medial and inferior limits were also easily drawn because of the contrast between gray and white

135 matter. Images from standard atlases were used to ensure a consistent reference to the boundaries
136 and relevant landmarks.

137 *2.4.2. Automatic segmentation of the hippocampus*

138 Regional reconstruction and segmentation were performed with the FreeSurfer 6.0 image analysis
139 suite (<http://surfer.nmr.mgh.harvard.edu/>), as previously described [26]. The standard FreeSurfer
140 automated subcortical segmentation protocol for the hippocampus is based on a probabilistic atlas
141 [27].

142 *2.4.3. Intracranial volume measurement*

143 Using the segment m-file in SPM5 (Wellcome Department of Cognitive Neurology, London, United
144 Kingdom), the intracranial volume (gray + white matter + cerebrospinal fluid) was calculated and used
145 as covariate in the models to minimize the effect due to global brain-size differences.

146 2.5. Covariates

147 The standardized interview included information on sociodemographic data, education level (no
148 school, primary school, high school, or graduate level), medical history, and medication intake. History
149 of cardiovascular disease included myocardial infarction, coronary surgery, coronary angioplasty, and
150 arterial surgery of the legs for arteritis. Daily activity impairment was assessed with the Instrumental
151 Activities of Daily Living (IADL) scale. Impairment was defined as increased difficulty in at least one
152 IADL item [28]. Self-reported information was also obtained on health and sleep quality, appetite loss,
153 social isolation, alcohol consumption (categorized as no drinking, drinking), and smoking status (never,
154 past, or current). Apolipoprotein E genotyping was described elsewhere [29]. APOE4 carriers had at
155 least one ϵ 4 allele.

156 2.6. Statistical analyses

157 The participants' socio-demographic and clinical characteristics were analyzed with Cox proportional
158 hazards models (adjusted for sex, education, age). Variables with $P < 0.20$ were included in the
159 multivariate models. Because the left hippocampus is generally more impacted than the right

160 hippocampus in the dementia process [5,30], we assessed the asymmetric association between
161 hippocampal volume and risk of dementia or cognitive decline by analyzing left and right hippocampal
162 volume separately.

163 Cox proportional hazards models with delayed entry were used for the longitudinal analysis of
164 dementia onset during the 15-year follow-up, taking age as the timescale [31]. HVs were normally
165 distributed and the assumption of hazard log-linearity was checked for each HV measurement.

166 Linear random-effect models, with random intercept and slope (follow-up time), were used to evaluate
167 the association between baseline HVs and cognitive performance (each test) over time. To normalize
168 the distributions, the MMSE and TMT scores were transformed using $(30\text{-MMSE})^{1/2}$ and the natural
169 logarithm of TMT. For each cognitive test, the HVs x time interaction was tested. This represents the
170 difference in the cognitive change slope for one-unit increase in the baseline HV. As no interaction was
171 significant, this interaction was not included in the final model. In this model, the β coefficient for HV
172 represents the association of HV with the mean cognitive performance during the 15-year follow-up.
173 For BVRT and IST, a positive β coefficient indicates a reduced mean cognitive performance during the
174 15-year follow-up for 1 cm³ decrease in the baseline HV. For MMSE and TMT, a negative β coefficient
175 indicates a reduced mean cognitive performance during the 15-year follow-up for 1 cm³ decrease in
176 the baseline HV. Finally, for each confounding factor, the interaction with time was tested and was
177 added to the models when significant (i.e. age x time, sex x time, intracranial volume x time). These
178 analyses were repeated after exclusion of the participants who developed dementia during the 15-
179 year follow-up (n = 42).

180 The degree of agreement between manual and automatic methods was assessed with Bland-Altman
181 plots [32]. The difference between volumes for all participants were plotted against the mean of both
182 measurements on a graph.

183 All statistical analyses were performed in SAS 9.4 and the graphs were plotted in R 3.4.3.

184

185 **3. Results**

186 In the selected ESPRIT study sample (n=510), the participants' mean age at baseline was 71.3 (\pm 4)
 187 years and 52.8% were women. Among all participants, 42 developed dementias during the 15-year
 188 follow-up. They were more likely to be older and were more often APOE4 carriers (Table 1).

189 Table 1. Baseline demographic and clinical characteristics of the study participants.

Baseline characteristics	Total sample n = 510	Incident dementia n = 42	Dementia-free n = 478	P value*
Age, mean (SD)	71.3 (4.0)	73.2 (4.5)	71.1 (3.9)	0.012
Women, % (n)	52.8 (269)	52.4 (22)	52.5 (251)	0.64
Education level, % (n)				0.10
No formal education	25.3 (129)	35.7 (15)	24.4 (114)	
Primary	28.4 (145)	16.7 (7)	29.5 (138)	
Secondary	19.8 (101)	21.4 (9)	19.7 (92)	
Higher	26.5 (135)	26.2 (11)	26.5 (124)	
Hypertension†, % (n)	48.0 (245)	50.0 (21)	47.9 (224)	0.80
Hypercholesterolemia‡, % (n)	52.0 (265)	59.5 (25)	51.4 (240)	0.30
Diabetes§, % (n)	9.9 (50)	9.8 (4)	9.9 (46)	0.71
History of head trauma, % (n)	10.1 (51)	7.1 (3)	10.4 (48)	0.70
Cardiovascular history, % (n)	6.7 (34)	7.1 (3)	6.6 (31)	0.33
History of stroke, % (n)	3.5 (18)	2.4 (1)	3.6 (17)	0.94
Depressive symptomatology¶, % (n)	15.4 (78)	14.3 (6)	15.5 (72)	0.92
Antidepressants, % (n)	3.9 (20)	7.1 (3)	3.6 (17)	0.17
APOE4 carriers, % (n)	21.6 (109)	36.6 (15)	20.3 (94)	<0.001
Anticholinergic drugs, % (n)	4.3 (22)	2.4 (1)	4.5 (21)	0.64
Alcohol use, % (n)	85.0 (431)	90.5 (38)	84.5 (393)	0.21
Tobacco use (current or past), % (n)	44.3 (226)	40.5 (17)	44.6 (209)	0.83
Insomnia, % (n)	18.5 (91)	20.0 (8)	18.3 (83)	0.52
Good subjective health, % (n)	95.9 (488)	97.6 (41)	95.7 (447)	0.75
Appetite loss, % (n)	7.1 (36)	7.3 (3)	7.1 (33)	0.75
Living alone, % (n)	19.5 (99)	19.1 (8)	19.5 (91)	0.65
Difficulty with at least 1 IADL, % (n)	1.4 (7)	2.4 (1)	1.3 (6)	0.40
Hippocampal volume (cm ³): manual method				
Right, mean (SD)	2.76 (0.37)	2.77 (0.37)	2.64 (0.42)	-
Left, mean (SD)	3.01 (0.42)	3.02 (0.40)	2.81 (0.54)	-
Hippocampal volume(cm ³): automatic method				
Right, mean (SD)	3.12 (0.36)	3.13 (0.35)	2.99 (0.43)	-
Left, mean (SD)	3.14 (0.36)	3.15 (0.36)	2.98 (0.34)	-

190 *Cox models with delayed entry were performed with age as the basic timescale and birth as the time origin,
 191 and adjusted for sex and education level, (except for age, sex, and education level). P values were determined
 192 using the Wald Chi-square test with 1 degree of freedom (df) expect for education level (df = 3).

193 †Blood pressure $\geq 140/90$ mmHg or treated

194 ‡Cholesterol level >6.2 mmol/L. or treated

195 §Fasting blood glucose ≥ 7.0 mmol/L or treated

196 ¶¶Depressive symptomatology: CESD-S ≥ 17 for men and ≥ 23 for women

197 Abbreviations: SD, standard deviation; APOE4, Apolipoprotein $\epsilon 4$; MMSE, Mini Mental State Examination; IADL,
 198 Instrumental Activities of Daily Living; CESD-S, Centre for Epidemiologic Studies of Depression scale.

199

200 3.1. Reduced HV is associated with higher risk of dementia

201 Reduction of right and left HV was associated with higher risk of dementia over the 15-year follow-up
 202 (Table 2). Results were similar regardless of the HV measurement method, although a larger effect
 203 size was observed with the automatic method (Fig. 1).

204

205 Table 2. Association between baseline hippocampal volumes and risk of dementia during the 15-year
 206 follow-up.

Hippocampus (cm ³)	Model 1 ^a n = 510		Model 2 ^b n = 501	
	HR* (CI)	P value	HR* (CI)	P value
Manual method				
Right	1.93 (1.24-2.99)	0.004	1.69 (1.09-2.61)	0.019
Left	2.09 (1.49-3.01)	<0.0001	1.88 (1.36-2.61)	0.0001
Automatic method: FreeSurfer 6.0				
Right	2.45 (1.45-4.34)	0.009	2.18 (1.19-3.99)	0.012
Left	2.92 (1.75-4.55)	<0.0001	2.70 (1.56-4.70)	0.0004

207 Cox proportional hazard models with delayed entry and age as the time scale were adjusted for:

208 ^a sex, education level, and intracranial volume

209 ^b Model 1 + antidepressant intake, alcohol use, APOE4 carriers

210 P values were obtained using the Wald Chi-square test with 1 degree of freedom

211 * HR, hazard ratio for one standard deviation decrease in hippocampal volume

212

213 3.2. Reduced HV, manually and automatically measured, is associated with lower cognitive
214 performance in the sample including participants with incident dementia

215 Using the manual method, a reduced right HV was significantly associated with reduced BVRT (by 0.36
216 points) and IST (by 1.51 points) performance over the 15-year follow-up. Similar results were obtained
217 for the left HV (Table 3).

218 Using the automatic method, reduced HV (right and left sides) was associated with lower performance
219 in all cognitive tests. The effect size was larger for the right hippocampus (Table 3). Indeed, a reduced
220 right HV was associated with lower MMSE (by 0.50 points), BVRT (by 0.67 points), TMTA (by 7.55
221 seconds), TMTB (by 12.8 seconds), and IST (by 3.17 points) scores during the 15-year follow-up.

222 Table 3. Association between baseline hippocampal volumes and cognitive performance during the 15-year follow-up.

Hippocampus (cm ³)	MMSE		BVRT		TMT A		TMT B		IST	
	$\sqrt{30 - MMSE}$ n = 482		n = 482		$\log(TMTA)$ n = 437		$\log(TMTB)$ n = 428		n = 483	
	β (SE)	P value	β (SE)	P value	β (SE)	P value	β (SE)	P value	β (SE)	P value
Manual segmentation										
Right	-0.089 (0.063)	0.15	0.361 (0.173)	0.037	-0.056 (0.039)	0.15	-0.060 (0.040)	0.14	1.513 (0.630)	0.017
Left	-0.053 (0.056)	0.34	0.300 (0.154)	0.052	-0.059 (0.034)	0.086	-0.043 (0.036)	0.23	1.301 (0.564)	0.02
Automatic segmentation: FreeSurfer 6.0										
Right	-0.190 (0.071)	0.007	0.665 (0.195)	0.0007	-0.149 (0.044)	0.0007	-0.133 (0.046)	0.004	3.174 (0.709)	<0.0001
Left	-0.129 (0.071)	0.069	0.556 (0.837)	0.004	-0.115 (0.044)	0.009	-0.117 (0.045)	0.01	2.929 (0.704)	<0.0001

223 Linear mixed models adjusted for time, age, sex, education level, intracranial volume, antidepressant intake, alcohol use, APOE4 carrier, and interaction terms (age x time,
 224 sex x time, intracranial volume x time). P values were obtained using the Student's t-test with respectively 1305, 1288, 734, 785, and 1267 degrees of freedom for MMSE,
 225 BVRT, TMTA, TMTB, and IST.

226 To transform back to the original scale:

227 MMSE: $-\beta^2 - (2\beta\sqrt{30 - m_{MMSE}})$ where $m_{MMSE} = 28$

228 TMTA et TMTB: $\exp(\beta) m_{TMT} - m_{TMT}$ where $m_{TMTA} = 47 \text{ sec}$ and $m_{TMTB} = 90 \text{ sec}$

229 m is the median of the cognitive score in the sample.

230 Abbreviations: BVRT, Benton Visual Retention Test; IST, Isaacs Set Test; MMSE, Mini Mental State Examination; SE, standard error; TMT, Trail Making Test.

231

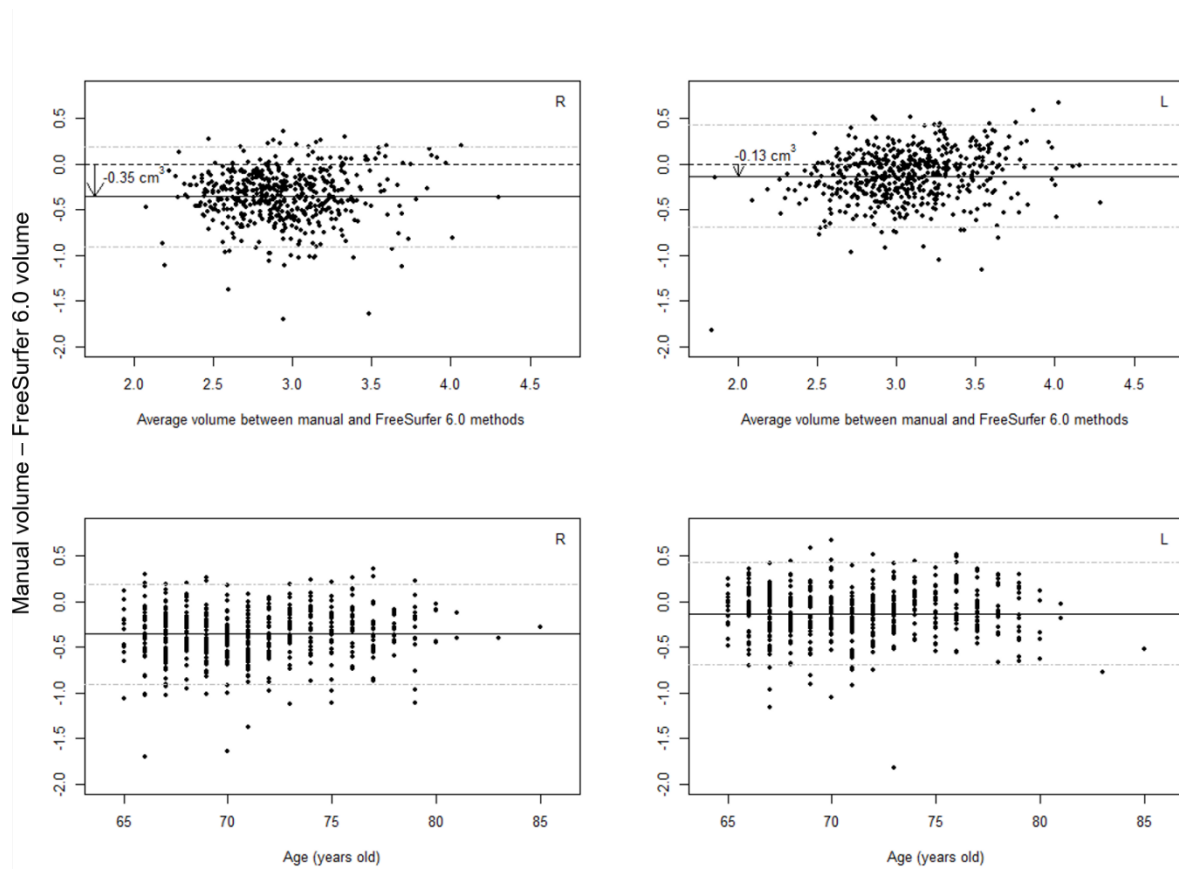
232 3.3. Reduced HV, automatically measured, is associated with lower cognitive performance in
233 dementia-free participants

234 Then, the association between HVs and cognitive performance in each test over time was investigated
235 after exclusion of participants with incident dementia (n = 42). Using the manual method, HV was no
236 longer associated with the BVRT and IST scores. Conversely, using the automatic method, the results
237 for the right hippocampus were similar to those obtained in the analysis including participants with
238 incident dementia, although the effect size was slightly weaker (Table 4). Specifically, reduced right HV
239 was associated with a decrease in the MMSE (by 0.45 points), BVRT (by 0.49 points), TMTA (by 5.32
240 seconds), TMTB (by 8.58 seconds), and IST (by 2.92 points) performance during the 15-year follow-up.

241

242 3.4. HVs are larger with the automatic method than with the manual method

243 The HVs obtained with the automatic method were larger than those obtained by manual
244 measurement (Fig. 1). The right and left HVs were, on average, 0.35 cm³ and 0.13 cm³ larger with the
245 automatic method compared to the manual method. This difference increased slightly (but not
246 significantly) with the participants' age.



248 Fig. 1. Bland-Altman plots showing the differences in the mean hippocampal volumes obtained with
 249 the two segmentation methods (top). Differences between manual and automatic (FreeSurfer 6.0)
 250 tracing according to the participants' baseline age (bottom). Left panels: differences between right
 251 (R) hippocampal volumes. Right panels: differences between left (L) hippocampal volumes. Solid lines
 252 represent the mean difference between manual and automatic (FreeSurfer 6.0) volumes. Dashed
 253 lines represent the upper and lower 95% limits of agreement.

254

255 Table 4. Association between baseline hippocampal volumes and cognitive performance during the 15-year follow-up in the sample without participants with
 256 incident dementia (n = 42).

Hippocampus (cm ³)	MMSE $\sqrt{30 - MMSE}$ n = 442		BVRT n = 442		TMT A $\log(TMTA)$ n = 400		TMT B $\log(TMTB)$ n = 392		IST n = 443	
	β (SE)	P value	β (SE)	P value	β (SE)	P value	β (SE)	P value	β (SE)	P value
Manual segmentation										
Right	-0.061 (0.065)	0.35	0.221 (0.175)	0.21	-0.034 (0.040)	0.40	-0.022 (0.041)	0.59	1.180 (0.662)	0.07
Left	0.010 (0.059)	0.87	0.124 (0.179)	0.45	-0.038 (0.037)	0.31	-0.005 (0.038)	0.90	1.049 (0.609)	0.09
Automatic segmentation: FreeSurfer 6.0										
Right	-0.170 (0.074)	0.021	0.488 (0.199)	0.014	-0.123 (0.046)	0.008	-0.092 (0.048)	0.054	2.915 (0.748)	0.0001
Left	-0.092 (0.072)	0.203	0.390 (0.194)	0.047	-0.083 (0.045)	0.066	-0.069 (0.046)	0.14	2.587 (0.732)	0.0004

257 Linear mixed models adjusted for time, age, sex, education level, intracranial volume, antidepressant intake, alcohol use, APOE4 carrier, and interaction terms (age x time,
 258 sex x time, intracranial volume x time). P values were obtained using the Student's t-test with respectively 1200, 1189, 677, 731, and 1170 degrees of freedom for MMSE,
 259 BVRT, TMTA, TMTB, and IST.

260 To transform back to the original scale:

261 MMSE: $-\beta^2 - (2\beta\sqrt{30 - m_{MMSE}})$ where $m_{MMSE} = 28$

262 TMTA et TMTB: $\exp(\beta) m_{TMT} - m_{TMT}$ where $m_{TMTA} = 46 \text{ sec}$ and $m_{TMTB} = 89 \text{ sec}$

263 m is the median of cognitive score in the sample.

264 Abbreviations: BVRT, Benton Visual Retention Test; IST, Isaacs Set Test; MMSE, Mini Mental State Examination; SE, standard error; TMT, Trail Making Test.

265 4. Discussion

266 In this longitudinal analysis of a population-based cohort, we found that reduced baseline HVs
267 were associated with higher risk of dementia and decreased cognitive performance during the 15-year
268 follow-up. However, the association between HV and cognitive performance remained only for the
269 automatically measured HVs when participants with incident dementia were excluded. These results
270 suggest that 1) HV is a predictor of cognitive decline also in dementia-free older adults; 2) the
271 segmentation method influences the results.

272 Manual segmentation, a laborious time-consuming task, is usually considered the gold
273 standard, but many manual tracing protocols are now available. A recent article showed that
274 FreeSurfer and manual tracing provide similar estimations of hippocampal atrophy in terms of
275 biological interpretation, but, in agreement with our observations, volumes were larger with
276 FreeSurfer. The proportion of overlapping voxels between manual tracing and FreeSurfer was 78%
277 [33]. Comparison between manual tracing and FreeSurfer (version 5.3) suggests that FreeSurfer
278 delineation differs by the border location between the hippocampus and the amygdala, and by
279 including boundary voxels along the lateral and dorsal surfaces that contains mixture tissue (grey,
280 white, or cerebrospinal fluid) [34]. Moreover, FreeSurfer might also tend to over-segment larger
281 hippocampi than smaller ones [35]. However, the routine use of FreeSurfer has considerable
282 advantages. As FreeSurfer is an algorithm-based software, it cannot be influenced by the brain general
283 shape, unlike clinicians and investigators [36]. FreeSurfer is less time-consuming than manual tracing
284 and facilitates comparisons between studies. Our results also suggest that FreeSurfer can detect subtle
285 HV changes during normal cognitive aging, compared with the manual method.

286 Many studies have shown a reduction in HV in AD and dementia. A meta-analysis of 23 cross-
287 sectional studies found a decrease of the right and left HVs by 23.2% and 24.2%, respectively, in
288 patients with AD compared with healthy controls [5]. Hippocampus is generally considered as the main
289 target of AD hallmarks: neurofibrillary tangles, amyloid plaques, and neuronal loss. Thus, it has been
290 incorporated in AD diagnostic criteria [37,38]. However, few studies have reported smaller HVs as a

291 risk factor of dementia in longitudinal analyses. Our results are in line with the prospective analysis by
292 the Rotterdam Scan Study showing that smaller HVs are associated with higher risk of dementia during
293 a 10-year follow-up [30].

294 We also found that reduced baseline HVs were associated with lower performances in all
295 cognitive domains during the 15-year follow-up. However, when we excluded participants with
296 incident dementia, only the right HV was associated with lower cognitive performance in global
297 cognition, visual memory and psychomotor speed, attention and visual-motor processing speed, and
298 semantic memory. This suggests that the right HV could be a better predictor of cognitive decline in
299 dementia-free older adults. The strongest and most robust association was between HV and semantic
300 memory. Therefore, semantic memory may be the first cognitive function altered upon HV reduction
301 during normal cognitive aging.

302 Although several studies assessed the association between HV and cognitive decline in
303 dementia-free individuals, they were limited by a short follow-up or small sample size. A cross-
304 sectional study suggested the possible role of hippocampus in executive function and processing speed
305 [9]. The authors also showed a role of aging-related HV changes in episodic memory and verbal
306 learning. In another study, total HV (measured with FreeSurfer 6.0) was associated with all cognitive
307 tests, except verbal learning, during a mean follow-up of 5.5 years [39]. Unlike our study, another
308 population-based study did not find any significant association between HV and cognitive performance
309 during the follow-up [40]. This discrepancy can be explained by the shorter follow-up (5 years versus
310 15 years) and the use of data collected only at one follow-up visit, whereas we considered the changes
311 in cognitive performance over several assessments. Another study also found that in a sample of 40
312 women, reduced HV was associated with a decline in executive functions, but not episodic memory
313 [41].

314 According to our results, the HV alone does not identify individuals who will develop dementia,
315 but might help to detect individuals at risk of cognitive decline, particularly when using FreeSurfer as
316 a segmentation method. The fact that HV also predicts cognitive decline during dementia-free aging is

317 not a surprising result. Indeed, because of the high neuroplasticity of the hippocampus, HV correlates
318 with many environmental conditions and pathologies that can also affect cognition. HV changes
319 combined with other biomarkers, such as amyloid A β ₄₂ and tau concentration in cerebrospinal fluid
320 and in brain (PET imaging) [38], might allow differentiating individuals at risk of dementia and AD from
321 those with stable cognitive decline.

322 The major strengths of our study are the prospective population-based design, the important
323 number of participants for an MRI-based study and the long follow-up period with regular diagnosis of
324 incident dementia and cognitive testing during the follow-up. Our study also presents some limitations.
325 We did not perform a longitudinal brain MRI assessment to monitor hippocampal atrophy. HVs,
326 adjusted only to intracranial volume, may not provide an entirely valid index of hippocampal atrophy
327 because of the wide range of such volumes [42]. Another measurement of the HV later in the follow-
328 up could have refined our results. Then, some HV subfields (e.g. the subiculum) have been associated
329 with the risk of dementia and executive function impairment [39]. However, we did not assess the
330 association between HV subfields and the risk of dementia or cognitive decline because manual
331 measurements could not be compared with FreeSurfer automatic measurements. Finally, we did not
332 adjust for multiple testing which increase the type I error but prevent the increase of type II error [43–
333 45].

334 Reduced HV was significantly associated with the risk of dementia but also cognitive decline during
335 aging in our study. The use of the FreeSurfer automatic method to measure HV could help to better
336 identify older adults at risk of cognitive decline. Thus, one single measure of HV is not a relevant early
337 marker of dementia in general population, but individuals with a reduced hippocampus also
338 constitutes a high-risk population for age-related cognitive decline, which can lead to disabilities.
339 Thus, all individuals with a reduced hippocampus should benefit from targeted preventive measures
340 to slow down or prevent cognitive decline.

341

342 **Funding** The 3C Study is carried out under a partnership agreement between Inserm, the Victor
343 Segalen–Bordeaux II University, and Sanofi-Synthélabo. The Fondation pour la Recherche Médicale
344 funded the preparation and first phase of the study. The 3C Study is also supported by the Caisse
345 Nationale Maladie des Travailleurs Salariés, Direction Générale de la Santé, MGEN, the Institut de la
346 Longévité, Agence Française de Sécurité Sanitaire des Produits de Santé, the Regional Governments
347 of Aquitaine, Bourgogne and Languedoc-Roussillon, Fondation de France, and the Ministry of
348 Research-Inserm Programme ‘Cohorts and collection of biological material’. This study was also
349 supported by the Agence Nationale pour la Recherche (ANR) Dentalcog ANR-22-CE36-0003-01 and
350 by Union France Alzheimer .

351 **Credit author contribution statement** Conceptualization, SA; methodology, MG, JJM, CM; software,
352 MG, JJM, CM; validation, SA; formal analysis, MG; investigation, MG; resources, SA ; data curation,
353 SA; writing—original draft preparation, MG, SA; writing—review and editing, MG, SA, FB, CM, JJM,
354 JLC; visualization, MG; supervision, SA; project administration, SA; funding acquisition, SA.

355 **Declaration of competing of interest** The authors declare no conflict of interest.

356 **Acknowledgments** The authors thank Elisabetta Andermarcher for English editing.

357 **Data statement.** The data have been presented as a poster at a scientific meeting (AAIC, virtual
358 conference, July 2020)

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