

# 1 Lifelong musical activity is associated with multi-domain cognitive and brain 2 benefits in older adults

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58 **1 ABSTRACT**

59 Regular musical activity as a highly-stimulating lifestyle activity is proposed to be protective  
60 against age-related cognitive decline and Alzheimer's disease (AD). This study investigated  
61 associations between lifelong regular musical instrument playing, late-life cognitive abilities and brain  
62 morphology in older adults. We show that musical activity over the life course is associated with better  
63 global cognition, working memory, executive functions, language, and visuospatial abilities accounting  
64 for reserve proxies. Playing music is not significantly associated with gray matter volume in regions  
65 most affected by aging and AD. Selectively in the musically active participants, multi-domain cognitive  
66 abilities were enhanced with preserved gray matter volume in frontal and temporal regions. Our  
67 correlational findings suggest that playing a musical instrument may improve the recruitment of  
68 existing brain resources to facilitate late-life cognitive capacities. We propose that engaging in regular  
69 musical activity could serve as a low-threshold multimodal enrichment strategy that may promote  
70 cognitive resilience in advanced age.

71 **Keywords:** cognitive reserve, resilience, prevention, brain plasticity, instrument playing

## 72 2 INTRODUCTION

73 Healthy lifestyle activities are proposed to enhance brain and cognitive resilience in older adults <sup>1</sup>  
74 through multiple neuroprotective pathways <sup>2-4</sup> and may thereby offer protection against age-related  
75 neurodegenerative diseases, such as Alzheimer's disease (AD). Among others, regular musical  
76 activity, such as playing an instrument, has been associated with reduced risk of developing dementia  
77 <sup>5,6</sup>. To advance targeted intervention strategies, it is important to delineate cognitive benefits and  
78 underlying brain mechanisms associated with musical activity in advanced age.

79 Musical activity is suggested to share communalities with the concept of environmental  
80 enrichment <sup>7,8</sup>, shown to promote far-reaching neurobiological and behavioral benefits in animal  
81 models <sup>9</sup>. Playing a musical instrument entails complex skills involving the simultaneous perception  
82 and integration of motor, sensory, cognitive, emotional, and social stimulations, thought to facilitate  
83 beneficial brain plasticity <sup>10</sup>. Consistently, there is evidence indicating that playing music could  
84 preserve higher-order cognitive abilities in older adults <sup>11,12</sup>. In this population, benefits of regular  
85 musical activity have been shown to transfer to multiple cognitive domains that typically decline with  
86 higher age, including executive functions, attention, language, visuospatial as well as memory abilities  
87 <sup>13-17</sup>. Together these findings imply that complex multimodal stimulation, as inherent to playing music,  
88 might help retain cognitive capacities in late life.

89 Comparatively little is, however, known about the neurobiological underpinnings of regular  
90 musical activity in older adults <sup>18</sup>. Studies that have investigated brain correlates of playing music in  
91 young and middle-aged cohorts suggest that this activity leads to plasticity, as reflected in measurable  
92 volume increases in distributed brain areas <sup>19,20</sup>. Those areas comprise multisensory frontal, parietal,  
93 and temporal regions <sup>21-23</sup>, which are strongly affected by healthy and pathological aging <sup>24-26</sup>.  
94 Regularly participating in musical activity may also be protective for the hippocampus. In young  
95 musicians compared to controls, musical activity is associated with enhanced volume and functions of  
96 the hippocampus <sup>27-29</sup>. In older adults, there appears to be a volume increase in frontal and temporal  
97 areas associated with playing music <sup>30</sup>. Such benefits in brain resources may contribute to better late-  
98 life cognitive abilities associated with this lifestyle activity.

99 Overall, the existing findings propose that regular musical activity may protect brain and  
100 cognitive health via multiple pathways. There might be a boost in functional brain capacities and/or an  
101 increase in structural brain resources, both of which may help counteract neuropathological burden in

102 advanced age<sup>31</sup>. To shed light onto these mechanisms, this cross-sectional study investigated  
103 potential cognitive and brain benefits of playing music during the entire life in the older population.  
104 Taking into account reserve proxies of educational attainment, crystallized intelligence, socioeconomic  
105 status (SES), and physical activity, we hypothesized that lifelong regular musical activity is associated  
106 with better late-life cognitive abilities in multiple domains as well as larger gray matter volume (GMV),  
107 particularly in regions affected by healthy and pathological aging. We further investigated, whether  
108 playing an instrument has a positive influence on the association between regional brain structure and  
109 cognitive performance in older adults, proposed to convey cognitive resilience in advanced age.

## 110 **3 MATERIAL AND METHODS**

### 111 **3.1 Overall design of the DELCODE study**

112 The data used in this study were obtained from the DZNE-Longitudinal Cognitive Impairment  
113 and Dementia cohort. The detailed study protocol can be found in a previous report <sup>32</sup>. In brief, the  
114 DELCODE cohort was set up to recruit 1000 participants at baseline with five groups of participants.  
115 Specifically, these groups are healthy controls (HC), first-degree relatives of AD patients (family  
116 history, FH) as well as participants with subjective cognitive decline (SCD), mild cognitive impairment  
117 (MCI), and mild AD dementia. At baseline assessment, all participants received extensive clinical,  
118 neuropsychological, and behavioral assessments. To minimize site-effects and ensure high data  
119 quality, assessment protocols were standardized across sites using Standard Operating Procedures  
120 (SOP). Post-scanning MRI image quality assessments were conducted by the DZNE Magdeburg. The  
121 DELCODE study protocol agreed with ethical principles for human experimentation in accordance with  
122 the Declaration of Helsinki. At each participating study sites, the protocol was approved by the local  
123 ethical committees. All participants gave their written informed consent. DELCODE was registered at  
124 the German Clinical Trials Register (DRKS00007966; April 5, 2015).

### 125 **3.2 Participants**

126 In the present study, cognitively healthy participants (HC, FH, and SCD) were included and  
127 merged across the three groups to increase the final sample size. Recruitment procedures including  
128 inclusion and exclusion criteria are described in detail elsewhere <sup>32</sup>. In brief, all participants were aged  
129  $\geq 60$  years, German speaking, able to provide informed consent and had a study partner serving as an  
130 informant. Normal cognitive function was defined as a test performance within -1.5 standard deviations  
131 of age-, sex- and education-adjusted norms on all subtests of the Consortium to Establish a Registry  
132 of Alzheimer's Disease (CERAD) test battery <sup>49</sup>. Exclusion criteria for HC, FH, and SCD were  
133 comprised of medical conditions including current or past major medical, neurological, or psychiatric  
134 disorders. Presence of SCD was defined by subjectively reported decline in cognitive functioning with  
135 concerns <sup>50</sup>. Diagnostic criteria for MCI and mild AD dementia are provided in Jessen et al. (2018).

136 The DELCODE baseline dataset (total:  $n = 1079$ ) was used to select a subset of participants  
137 into the present study as follows (see Figure 1): At the time of our analysis, data from 943 participants  
138 with a structural cranial magnetic resonance imaging (MRI) assessment at baseline were available. Of

139 these participants, cognitively healthy participants were selected (i.e., HC, SCD, FH, total:  $n = 678$ ).  
140 Afterwards, participants who reported regular musical activity across the life span (i.e., group of  
141 interest) and participants with no musical activity during life (i.e., control group) were identified (total:  $n$   
142 = 429; for methodological details see below). Finally, we matched the control group and included only  
143 participants with complete datasets regarding variables of interest, resulting in a final sample of  $n =$   
144 140 (for methodological details see below).

### 145 **3.3 Measurements**

#### 146 **3.3.1 Measurement of musical activity**

147 Musical activity across lifespan was assessed using the Lifetime of Experiences Questionnaire  
148 <sup>47</sup>, adapted for the German population <sup>51</sup>. Details on the LEQ and the coding scheme used to assess  
149 lifelong regular musical activity are provided in the supplementary material. In brief, the self-reported  
150 questionnaire measures educational, occupational, and leisure activities across three life periods  
151 (young adulthood: 13 – 30 years, mid-life: 30 – 65 years, and late-life: 65 years onwards). One self-  
152 reported lifestyle activity inquired by the LEQ was the frequency of playing a musical instrument and  
153 this information was used to operationalize musical activity across the lifespan. Similar to a previous  
154 study <sup>33</sup>, we constructed a variable that was comprised of two groups: (1) The musical activity group  
155 (group of interest) included those participants that were musically active in all life periods and reported  
156 regular musical activity (2 times per month or more) in at least one life period. (2) The no musical  
157 activity or control group included participants that reported to never have played a musical instrument  
158 in any of the life periods.

#### 159 **3.3.2 Measures of cognitive abilities**

160 Cognitive functioning was assessed using latent factors over five cognitive domains, namely  
161 (1) learning and memory, (2) working memory, (3) executive functions and mental processing speed,  
162 (4) language, and (5) visuospatial abilities, created by summarizing cognitive tests from the extensive  
163 neuropsychological test battery in the overall DELCODE cohort as described previously <sup>52</sup>. In brief,  
164 Wolfsgruber et al. (2020) used confirmatory factor analyses (CFA) to extract the factor structure using  
165 data from the extensive neuropsychological test battery applied during baseline assessment.  
166 Additionally, a global cognitive performance score was calculated by taking the mean of the five  
167 cognitive scores <sup>52</sup>. For the present analysis, we used performance measures for global cognition and

168 the five cognitive domain scores. Each cognitive score was z-transformed using the selected  
169 DELCODE neuroimaging sample including HC, FH, and SCD participants.

### 170 **3.3.3 MRI acquisition and processing**

171 MRI data were acquired using Siemens MRI scanners (Siemens, Erlangen, Germany),  
172 including three TIM Trio systems, four Verio systems, one Skyra system, and one Prisma system. The  
173 extensive MRI protocol of the DELCODE study is described elsewhere <sup>32</sup>. For the present analysis, we  
174 used T1-weighted images (i.e., 3D GRAPPA PAT 2, 1 mm<sup>3</sup> isotropic, 256 × 256 px, 192 slices,  
175 sagittal, ~ 5min, TR 2500 ms, TE 4.33 ms, TI 110 ms, FA 7°) and T2-weighted images (i.e., 0.5 × 0.5 ×  
176 1.5 mm<sup>3</sup>, 384 × 384 px, 64 slices, orthogonal to hippocampal long axis, ~12 min, TR 3500 ms, TE 353  
177 ms, optimized for volumetric assessment of the medial temporal lobe). All scans underwent quality  
178 assessment provided by the DZNE imaging network (iNET, Magdeburg).

179 Regional GMV analysis was conducted in pre-selected regions-of-interest (ROI), robustly  
180 affected by healthy and pathological aging due to AD <sup>24-26</sup>. Based on these findings, we chose two  
181 regions, that is, the frontal lobe and the hippocampus. For each of these ROIs, we used regional  
182 volume measures provided in the DELCODE database, as described previously <sup>53</sup>. In brief, structural  
183 MRI images were segmented in native space using an automated cortical parcellation pipeline <sup>54</sup>  
184 implemented in FreeSurfer (version 6.0, <http://surfer.nmr.mgh.harvard.edu/>) and an advanced  
185 segmentation tool <sup>55</sup> to derive ROI-based GMV. To enhance reliability, image segmentation was based  
186 on T1-weighted and high-resolution T2-weighted images. Left and right hippocampal volume were  
187 summed for a measure of the overall hippocampal volume. Frontal volume was calculated as the sum  
188 over left and right frontal ROI of the Desikan atlas following the procedure described elsewhere <sup>56</sup>. In  
189 addition, cortical GMV was evaluated as a global measure of brain integrity. Regional GMV measures  
190 were adjusted for total intracranial volume (TIV), as estimated using FreeSurfer <sup>57</sup>, using a ratio.

191 We also assessed GMV at the voxel level. Structural MRI images were segmented to extract  
192 GM, WM, and CSF tissues using the unified segmentation algorithm in CAT12 (version 12.6,  
193 <http://dbm.neuro.uni-jena.de/vbm>) with default parameters. Warping to the Montreal Neurological  
194 Institute (MNI) template space was performed using Diffeomorphic Anatomical Registration Through  
195 Exponentiated Lie Algebra (DARTEL) with default parameters and registration to existing templates <sup>58</sup>.  
196 Total intracranial volume (TIV) was computed as the sum of volumes of GM, WM, and CSF using the  
197 SPM “Estimate TIV and global tissue volumes” routine. Voxel-based statistical analyses were

198 performed on the warped and modulated GMV maps, which were smoothed by a three-dimensional  
199 Gaussian kernel with full width at half maximum of 8 mm<sup>3</sup>.

### 200 **3.3.4 Additional measures**

201 Age, sex, education, intelligence, SES, self-reported participation in physical activity and  
202 diagnostic group were considered as potential confounders. Educational attainment was measured in  
203 years of education. Crystallized intelligence was estimated using the Multiple-Choice Vocabulary  
204 Intelligence Test (MWT, min. score: 0, max. score: 37), with scores proportional to crystallized  
205 intelligence <sup>59</sup>.

206 Participation in long-term physical activity was estimated using respective information from the  
207 LEQ. A mean score was calculated over responses on the frequency of physical activity over two or  
208 three life stages (i.e., < 65 years and ≥ 65 years, respectively). In addition, current physical activity  
209 was assessed through the Physical Activity Scale for the Elderly PASE, <sup>60</sup>. The PASE includes leisure,  
210 household and occupational activities assessed over the previous week. Based on frequency,  
211 duration, and intensity of these activities, a total score is calculated with higher scores indicating  
212 greater levels of physical activity. Long-term physical activity was significantly correlated with current  
213 physical activity in the matched sample ( $n = 140$ ,  $r = 0.35$ ,  $p < 0.001$ ), supporting the validity of the  
214 measure. Long-term physical activity was used as a covariate in statistical analyses, since the  
215 measure was available from all participants.

216 The SES was calculated for each participant using information on occupational activity  
217 assessed by the LEQ. Details are provided in the supplementary material. In brief, details on  
218 occupational activities of each respondent were obtained using 10 five-year intervals across middle- to  
219 late-life adulthood (i.e., 30 to 79 years of age). The information was used to calculate the international  
220 socio-economic index of occupational information (ISEI, min. score: 16, max. score: 90) <sup>61</sup> using a  
221 fully-automated procedure. The ISEI scores were averaged across time intervals to obtain one mean  
222 SES measure per participant. The SES measure was positively and significantly associated with the  
223 LEQ sum scores measuring educational as well as occupational activity for young ( $n = 140$ ,  $r = 0.54$ ,  $p$   
224  $< 0.001$ ) and middle ( $n = 140$ ,  $r = 0.69$ ,  $p < 0.001$ ) adulthood, indicating the validity of the estimated  
225 ISEI scores.

## 226 **3.4 Statistical analyses**



227 Statistical analyses were conducted using *R* (version 3.5.1.) and Statistical Parametric Mapping  
228 (SPM, version 12, Wellcome Trust Centre for Neuroimaging, London, UK). Figures were generated  
229 using the package *ggplot2*<sup>62</sup>. Before conducting statistical models, statistical assumptions were  
230 assessed visually using diagnostic plots.

### 231 **3.4.1 Sample characteristics and matching procedure**

232 Participants with lifelong musical activities and controls with no musical activity were matched  
233 using a one-to-one matching procedure taking into account age, sex, diagnostic group, education,  
234 SES, intelligence, and physical activity. Details are provided in the supplementary material. The  
235 procedure was carried out using propensity score matching with the *R* package *MatchIt* (version  
236 4.1.0.)<sup>63</sup>. Observations were matched based on the nearest-neighbor method, as a simple and  
237 effective procedure for selecting well-matched groups<sup>64</sup>. Musical activity groups were compared in  
238 baseline demographic, behavioral, neuropsychological, and neuroimaging variables. Independent  
239 Student's *t*-tests were used for all continuous variable and chi-squared ( $\chi^2$ ) tests were applied for all  
240 categorical variables.

### 241 **3.4.2 ROI-based analyses**

242 To assess our main hypotheses, multiple linear regression models were used. In these statistical  
243 analyses, an alpha value of 0.05 was considered statistically significant. In addition, correction for  
244 multiple comparisons was performed using a false discovery rate (FDR)-adjusted p-value threshold  
245 (alpha) of 0.05<sup>65</sup>. Uncorrected p-values were reported, when results survived FDR correction, this is  
246 specifically indicated.

247 Firstly, the association of musical activity (modelled as a main effect) with global cognition  
248 followed by the domain-specific abilities were assessed. Multiple linear regression models were  
249 performed including with musical activity (binary group variable) as an independent variable and each  
250 cognitive measure (z-transformed composite score) as a dependent variable, respectively. Next, the  
251 association between musical activity and brain structure was examined using similar multiple linear  
252 regressions. Models included musical activity (binary group) as independent variable and ROI-based  
253 GMV (frontal region and hippocampal region, both TIV adjusted) as dependent variable along with  
254 scanner site as covariate (dummy coded). Selected relationships were visualized to facilitate the  
255 interpretation of findings using box plots of unadjusted data.

256 Secondly, we assessed the moderating effect of musical activity on the relationship between ROI-  
257 based GMV and cognitive abilities. To do this, musical activity, ROI-based GMV (frontal region and  
258 hippocampal region, both mean-centered), the interaction term (musical activity  $\times$  GMV), and scanner  
259 site as covariate (dummy coded) were entered into regression models with each cognitive factor score  
260 as dependent variable. To specify the directionality of the interactions, simple slope analyses were  
261 conducted<sup>66,67</sup>. Interaction effects were visualized using unadjusted data as follows: General and  
262 domain-specific cognitive scores (z-transformed) were graphed as a function of musical activity and  
263 ROI-based GMV, respectively. In addition, we examined whether or not the respective relationships  
264 differed significantly from zero within each group.

### 265 **3.4.3 Voxel-based analysis**

266 To further evaluate the spatial distribution of musical activity-associated effects on brain  
267 structure at the voxel level, exploratory voxel-wise general linear models (GLM) were conducted in  
268 SPM12. For the present purpose, voxel-wise results were presented at  $p < 0.001$  uncorrected at peak  
269 level in combination with the estimated expected voxels per cluster ( $k$ ) as automatically calculated by  
270 SPM.

271 Firstly, a GLM was computed with musical activity as independent variable and the modulated,  
272 warped, and smoothed GMV maps as dependent variable. Secondly, a moderating effect of musical  
273 activity was evaluated at the voxel level. This GLM included musical activity, the respective cognitive  
274 measure (z-transformed composite score), and the interaction term (musical activity  $\times$  cognitive  
275 measure) as independent variables with GMV maps as dependent variable. The later analysis was  
276 carried out for global cognition and all cognitive domains. For reasons of simplicity, results of this  
277 analysis were displayed for one cognitive domain, selected by the strongest interaction effect in the  
278 ROI-based analysis.

279 All voxel-based analyses were adjusted for TIV as well as scanner site (dummy coded) and  
280 restricted to cerebral GM using an explicit binary GM mask derived from the present sample (i.e.,  
281 average GM maps thresholded at a level of  $> 0.3$ , excluding cerebellum and brain stem). Cluster  
282 peaks are specified by their anatomical site, labelled using the Hammersmith atlas<sup>68</sup> provided by the  
283 CAT12 toolbox. Finally, mean values were extracted in significant clusters for each participant from the  
284 warped, modulated, and non-smoothed GMV images using the Marsbar toolbox (release: 0.44;  
285 <http://marsbar.sourceforge.net/>)<sup>69</sup>, to provide complementary visualizations of the associations.

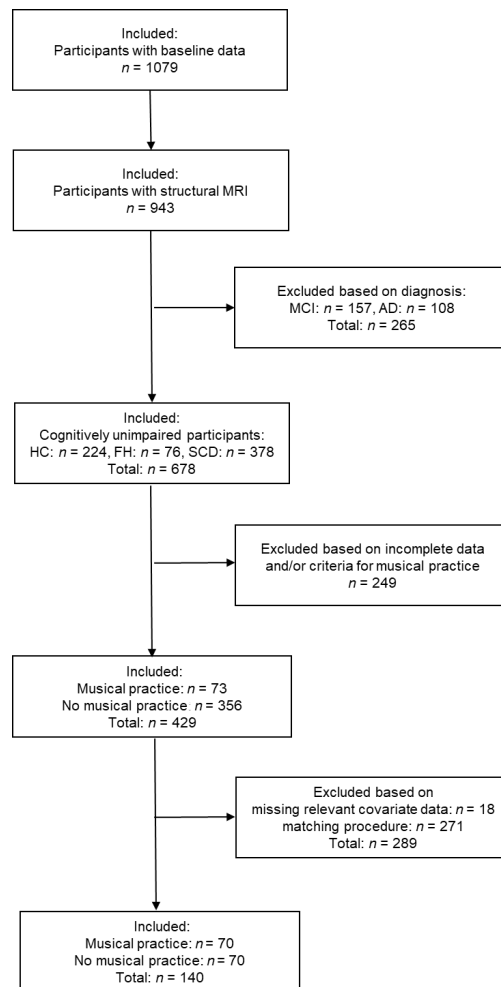
286 **4 RESULTS**

287 **4.1 Sample characteristics**

288 This study included a total sample of 140 older participants (aged  $\geq 60$  years) selected from the  
289 ongoing, multi-center, observational DELCODE cohorts<sup>32</sup>. The present sample comprised 70  
290 individuals with lifelong regular musical activity and 70 controls with no musical activity over the life  
291 course (see Figure 1). The two groups (musical activity, no musical activity) were comparable in age,  
292 sex, distribution of diagnostic groups as well as reserve proxies of higher education, crystallized  
293 intelligence, SES, and participation in both long-term and current physical activity (all  $p$ 's  $> 0.05$ , Table  
294 1). Slight group differences in frontal and total GMV (unadjusted raw values) were found, with larger  
295 volumes in the older participants with lifelong musical activity compared to controls.

296 =====

297 **Figure 1**



298

299 **Figure 1: Participant selection flowchart.** The graph displays the selection procedure from the  
300 DELCODE database. **Key:** HC, healthy controls; FH, family history of AD; AD, Alzheimer's disease;  
301 MCI, mild cognitive impairment; Magnetic resonance imaging (MRI).

302 =====

303 **Table 1**

| <b>Table 1: Descriptive characteristics of the matched sample (n = 140)</b> |                       |                       |         |
|---|-----------------------|-----------------------|---------|
|   | Musical activity      | No musical activity   | P value |
| Number (n)  | 70                    | 70                    | -       |
| Age (years)   | 68.23 (6.62)          | 69.01 (5.44)          | 0.445   |
| Gender female/male (n)  | 31/39                 | 35/35                 | 0.498   |
| Education (years)   | 16.20 (2.71)          | 15.96 (2.74)          | 0.598   |
| Diagnostic group HC/ FH/SCD (n)   | 19/7/44               | 24/6/40               | 0.654   |
| SES <sup>a</sup>  | 66.27 (16.32)         | 65.21 (16.04)         | 0.699   |
| Crystallized intelligence <sup>b</sup>                                      | 33.31 (2.14)          | 33.04 (2.22)          | 0.463   |
| Physical activity, long-term <sup>c</sup>                                   | 4.25 (0.78)           | 4.32 (0.71)           | 0.611   |
| Physical activity, current <sup>d</sup>                                     | 33.86 (11.80), n = 66 | 32.45 (12.85), n = 69 | 0.507   |
| Total hippocampal GMV (sum, ml)   | 6.26 (0.71)           | 6.21 (0.66)           | 0.692   |
| Total frontal GMV (ml)  | 138.86 (12.44)        | 134.69 (11.88)        | 0.044*  |
| Total cortical GMV (ml)   | 453.83 (37.56)        | 441.69 (37.13)        | 0.049*  |

Descriptive data are given if applicable as mean and standard deviation (in parenthesis). The actual sample size is provided, if different from sample size specified in first row. P values correspond to independent t-tests for unequal variance with participant group as independent variable. Chi-square statistic was used to compare the distribution of categorical variables.  
\*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ .  
**Key:** HC, healthy control participants; FH, participants with family history of AD; GMV, gray matter volume; SCD, participants with subjective cognitive decline; SES, socioeconomic status.  
<sup>a</sup> International socio-economic index (ISEI); <sup>b</sup> Multiple-Choice Vocabulary Intelligence Test (MWT); <sup>c</sup> Lifetime of Experiences Questionnaire (LEQ); <sup>d</sup> Physical Activity Scale for the Elderly (PASE).

304 =====

## 305 **4.2 Musical activity and cognition**

306 Applying multiple linear regression models to assess the associations between musical activity  
307 and cognitive performance, we found significant group differences for global cognition, working  
308 memory, executive function, language and visuospatial abilities (Table 2). Performance in these  
309 cognitive domains was significantly better in the older participants with lifelong regular musical activity  
310 compared to controls. In contrast, no association of musical activity was found with the domain of  
311 learning and memory ( $p = 0.209$ ).

312 =====

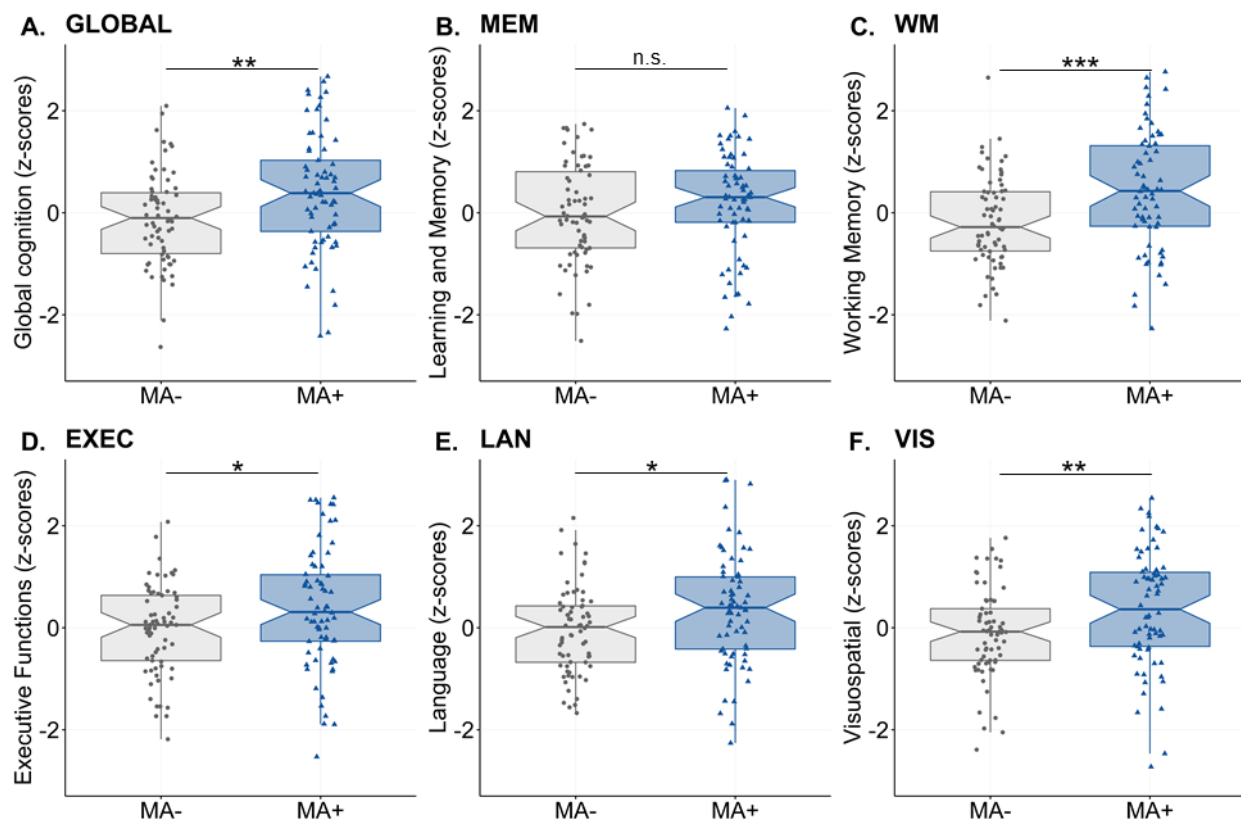
313 **Table 2**

| Table 2: Results of linear regression analyses between musical activity and cognition |                     |                      |       |       |       |            |                             |
|---|---------------------|----------------------|-------|-------|-------|------------|-----------------------------|
|   | Dependent variable  | Independent variable | B     | SE B  | Beta  | P value    | Total R <sup>2</sup> (adj.) |
| 1   | Global cognition    | Musical Activity     | 0.540 | 0.178 | 0.250 | 0.003***   | 0.062 (0.056)               |
| 2   | Learning and Memory | Musical Activity     | 0.209 | 0.165 | 0.107 | 0.209      | 0.011 (0.004)               |
| 3   | Working Memory      | Musical Activity     | 0.669 | 0.178 | 0.304 | < 0.001*** | 0.092 (0.086)               |
| 4   | Executive Functions | Musical Activity     | 0.465 | 0.180 | 0.214 | 0.011*†    | 0.046 (0.039)               |
| 5   | Language            | Musical Activity     | 0.443 | 0.170 | 0.216 | 0.010*†    | 0.047 (0.040)               |
| 6   | Visuospatial        | Musical Activity     | 0.522 | 0.176 | 0.245 | 0.003***   | 0.060 (0.053)               |

Musical activity was included as binary predictor, dummy coded with musical activity = 1, no musical activity = 0.  
 \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ .  
 †  $p < 0.05$  false discovery rate (FDR)-adjusted for statistical tests performed across cognitive domains.  
**Key:** B, unstandardized coefficient; SE, standard error; Beta, standardized coefficient; R<sup>2</sup>, explained variance

314 =====

315 **Figure 2**



316

317 **Figure 2: Main effect of lifelong musical activity on cognitive abilities.** Significant group differences were  
 318 found for global cognition (A, GLOBAL), working memory (C, WM), executive function (D, EXEC), language (E,  
 319 LAN) and visuospatial abilities (F, VIS). These multi-domain cognitive abilities were enhanced for participants with  
 320 lifelong musical activity (MA+, blue) compared to controls (no musical activity across lifespan, MA-, gray). The  
 321 association was not significant for the learning and memory composite (B, MEM). Boxplots display unadjusted  
 322 data with individual data points. The “notch” shows the median with 95% confidence intervals and interquartile  
 323 range with lower (25th) and upper percentiles (75th). Significance levels (uncorrected): \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p$   
 324  $< 0.05$ . **Key:** MA+, musical activity; MA-, no musical activity.

325 =====

### 326 4.3 Musical activity and brain structure in regions-of-interest

327 Results for the associations between musical activity and GMV (TIV-adjusted values) in the  
328 selected ROIs are shown in Table 3. There were no significant differences between participants with  
329 lifelong musical practice compared to controls in frontal and hippocampal volume (all  $p$ 's > 0.5). Also,  
330 the groups did not differ significantly in total cortical GMV ( $p = 0.722$ ).

331 =====

332 **Table 3**

|   | <b>Dependent variable</b> | <b>Independent variable</b> | <b>B</b> | <b>SE B</b> | <b>Beta</b> | <b>P value</b> | <b>Total R<sup>2</sup> (adj.)</b> |
|---|---------------------------|-----------------------------|----------|-------------|-------------|----------------|-----------------------------------|
| 1 | Frontal GMV               | Musical Activity            | -0.046   | 0.204       | -0.020      | 0.822          | 0.120 (0.052)                     |
| 1 | Hpc GMV                   | Musical Activity            | -0.007   | 0.012       | -0.052      | 0.551          | 0.102 (0.032)                     |
| 3 | Cortical GMV              | Musical Activity            | -0.303   | 0.850       | -0.031      | 0.722          | 0.132 (0.065)                     |

Models adjusted for scanner site.  
Musical activity was included as binary predictor, dummy coded with musical activity = 1, no musical activity = 0.  
Regional GMV was adjusted by total intracranial volume (TIV).  
\*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ .  
†  $p < 0.05$  false discovery rate (FDR)-adjusted for statistical tests performed across ROIs.  
**Key:** B, unstandardized coefficient; Hpc, Hippocampus; SE, standard error; Beta, standardized coefficient; R<sup>2</sup>, explained variance; GMV, gray matter volume

333 =====

### 334 4.4 Moderations of musical activity in regions-of-interest

335 Moderation analyses were applied to assess the influence of musical activity on the  
336 associations between late-life regional GMV and cognitive performance (Table 4 and 5). Frontal GMV  
337 was positively associated with global cognition and domain-specific cognitive abilities (all  $p$ 's ≤ 0.001,  
338 data not shown). Importantly though, significant interactions were observed between musical activity  
339 and frontal GMV for global cognition, working memory, and language abilities (all  $p$ 's < 0.05; Table 4).  
340 Visualization of these relationships (Figure 3) indicated that these cognitive abilities were selectively  
341 enhanced in participants with musical activity and preserved GMV in the frontal regions (i.e., above the  
342 90% percentile of the GMV distribution in AD patients). No similar effect was detected for the learning  
343 and memory domain. Hippocampal GMV was also positively associated with global cognition and  
344 domain-specific cognitive abilities (all  $p$ 's < 0.01, data not shown). There were, however, no significant  
345 interactions between musical activity and hippocampal volume in the multi-domain cognitive abilities  
346 (all  $p$ 's > 0.1, Table 5 and supplementary Figure 4).

347 =====

348 **Table 4**

|   | <b>Dependent variable</b> | <b>Independent variable</b>  | <b>B</b> | <b>SE B</b> | <b>Beta</b> | <b>P value</b> | <b>Total R<sup>2</sup> (adj.)</b> |
|---|---------------------------|------------------------------|----------|-------------|-------------|----------------|-----------------------------------|
| 1 | Global cognition          | Music Activity × Frontal GMV | 0.318    | 0.139       | 0.261       | 0.024*†        | 0.332 (0.269)                     |
| 2 | Learning and Memory       | Music Activity × Frontal GMV | 0.102    | 0.132       | 0.092       | 0.441          | 0.263 (0.193)                     |
| 3 | Working Memory            | Music Activity × Frontal GMV | 0.432    | 0.141       | 0.348       | 0.003**†       | 0.335 (0.273)                     |
| 4 | Executive Functions       | Music Activity × Frontal GMV | 0.278    | 0.145       | 0.228       | 0.058          | 0.273 (0.204)                     |
| 5 | Language                  | Music Activity × Frontal GMV | 0.316    | 0.133       | 0.274       | 0.019*†        | 0.320 (0.256)                     |
| 6 | Visuospatial              | Music Activity × Frontal GMV | 0.227    | 0.145       | 0.189       | 0.119          | 0.251 (0.180)                     |

Models adjusted for scanner site.  
 Musical activity was included as binary predictor, dummy coded with musical activity = 1, no musical activity = 0.  
 Frontal GMV was adjusted for total intracranial volume and mean centered.  
 \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ .  
 †  $p < 0.05$  false discovery rate (FDR)-adjusted for statistical tests performed across cognitive domains.  
**Key:** B, unstandardized coefficient; SE, standard error; Beta, standardized coefficient; R<sup>2</sup>, explained variance

349 =====

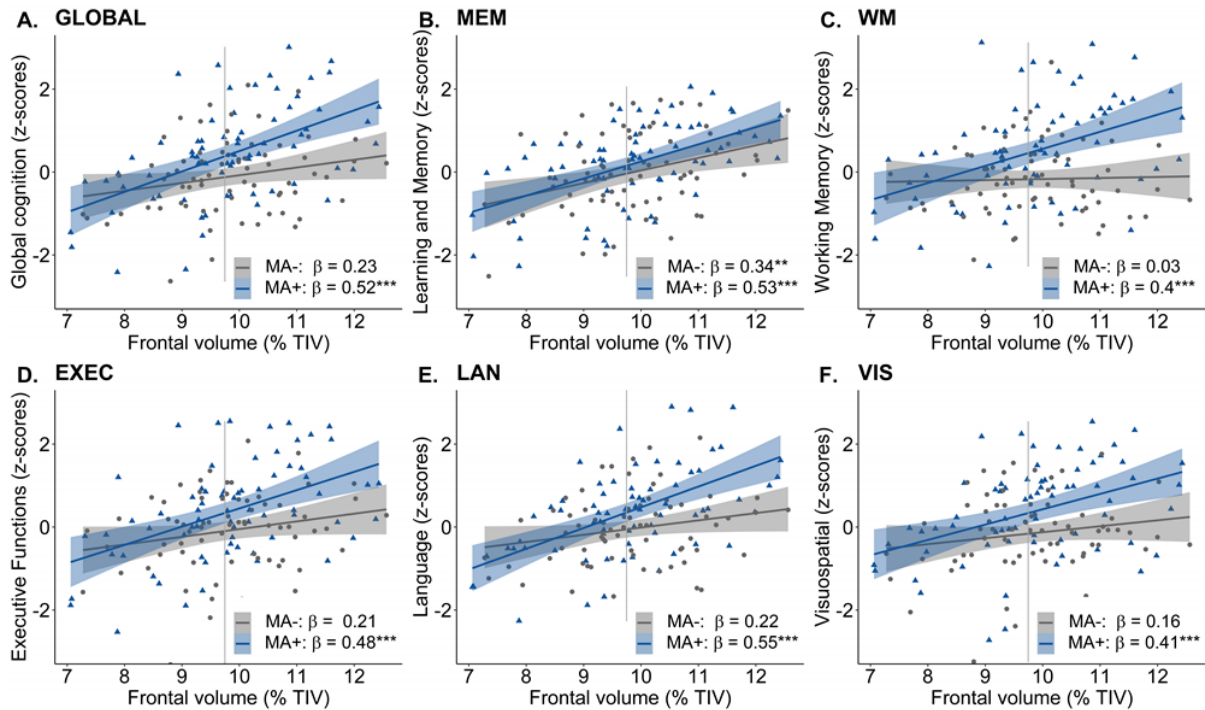
350 **Table 5**

|   | <b>Dependent variable</b> | <b>Independent variable</b> | <b>B</b> | <b>SE B</b> | <b>Beta</b> | <b>P value</b> | <b>Total R<sup>2</sup> (adj.)</b> |
|---|---------------------------|-----------------------------|----------|-------------|-------------|----------------|-----------------------------------|
| 1 | Global cognition          | Music Activity × Hpc GMV    | 2.248    | 2.515       | 0.118       | 0.373          | 0.330 (0.266)                     |
| 2 | Learning and Memory       | Music Activity × Hpc GMV    | -0.727   | 2.350       | -0.042      | 0.757          | 0.284 (0.216)                     |
| 3 | Working Memory            | Music Activity × Hpc GMV    | 4.109    | 2.620       | 0.211       | 0.119          | 0.299 (0.233)                     |
| 4 | Executive Functions       | Music Activity × Hpc GMV    | 2.268    | 2.640       | 0.118       | 0.392          | 0.268 (0.199)                     |
| 5 | Language                  | Music Activity × Hpc GMV    | 2.782    | 2.435       | 0.154       | 0.255          | 0.300 (0.234)                     |
| 6 | Visuospatial              | Music Activity × Hpc GMV    | 0.897    | 2.547       | 0.047       | 0.725          | 0.290 (0.223)                     |

Models adjusted for scanner site.  
 Musical activity was included as binary predictor, dummy coded with musical activity = 1, no musical activity = 0.  
 Hippocampal GMV was adjusted for total intracranial volume and mean centered.  
 \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ .  
 †  $p < 0.05$  false discovery rate (FDR)-adjusted for statistical tests performed across cognitive domains.  
**Key:** B, unstandardized coefficient; Hpc, hippocampus; SE, standard error; Beta, standardized coefficient; R<sup>2</sup>, explained variance

351 =====

352 **Figure 3**



353

354 **Figure 3: Moderation effect of lifelong musical activity in the frontal region.** A significant moderation effect of  
355 musical activity was observed for global cognition (A, GLOBAL), working memory (C, WM), and language abilities  
356 (E, LAN), such that larger frontal volume (above the 90th percentile of the frontal volume distribution in AD  
357 patients) was associated with better global in participants with lifelong musical activity (MA+, blue) compared to  
358 controls (MA-, gray). This interaction was not significant for learning and memory (B, MEM), executive functions  
359 (D, EXEC), and visuospatial abilities (F, VIS). Individual data points (dots and triangles), linear trends (solid lines),  
360 95% confidence intervals (shaded areas), and standardized regression coefficients ( $\beta$ ) within each group are  
361 provided. Gray vertical lines display the 90th percentile of the frontal GMV distribution in AD patients of the  
362 DELCODE study. Significance levels (uncorrected): \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . **Key:** GMV, gray matter  
363 volume; MA+, musical activity; MA-, no musical activity; TIV, total intracranial volume.

364 =====

365 **4.5 Voxel-based analysis**

366 Results of the exploratory analyses at the voxel level are presented in Table 6 and Figure 4.  
367 There was a subtle positive association between lifelong regular musical activity and GMV within a  
368 smaller cluster in the left postcentral gyrus ( $p < 0.001$  uncorrected). No other significant clusters were  
369 found. The interaction analysis corroborated a significant moderation of musical activity on the  
370 association between working memory and regional GMV ( $p < 0.001$  uncorrected) in frontal (lateral and  
371 medial), inferior temporal, and precentral regions. Results of the moderation analyses across all



372 cognitive measures were essentially similar, with some variations in the number of significant clusters  
 373 (supplementary Figure 6). No significant interaction effect was found for the domain of learning and  
 374 memory at the voxel level.

375 =====

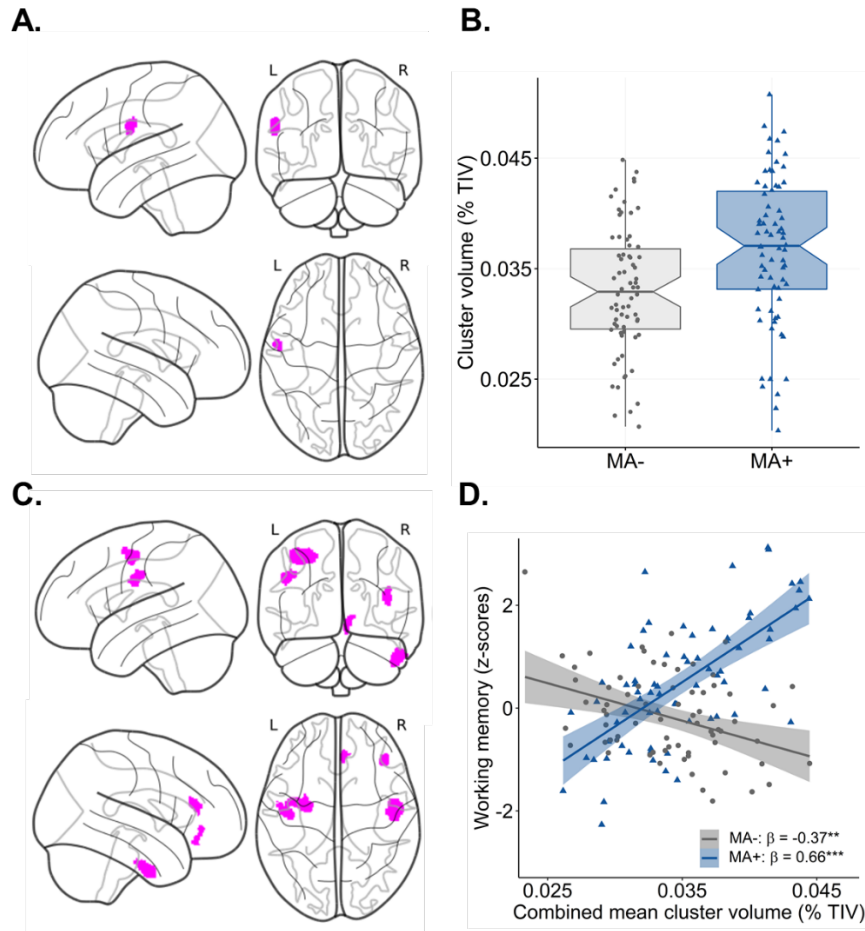
376 **Table 6**

| Table 6: Results of analyses between musical activity and GMV at the voxel level |             |                                |                |         |      |                 |                         |     |     |
|--|-------------|--------------------------------|----------------|---------|------|-----------------|-------------------------|-----|-----|
| Model / contrast   | No. cluster | Label                          | Hemisp here    | Cluster |      | Peak of cluster |                         |     |     |
|  |             |                                |                | p       | size | Z value         | MNI coordinates (x y z) |     |     |
| <b>Main effect <sup>a</sup></b>  |             |                                |                |         |      |                 |                         |     |     |
| Positive   | 1           | Postcentral gyrus              | left           | 0.176   | 239  | 3.66            | -57                     | -6  | 26  |
| <b>Interaction effect <sup>b</sup></b>   |             |                                |                |         |      |                 |                         |     |     |
|  | 1           | Precentral gyrus               | left           | 0.019   | 646  | 4.83            | -28                     | -10 | 48  |
|  | 2           | Precentral gyrus               | left           | 0.123   | 251  | 4.46            | -38                     | -8  | 52  |
|  | 3           | Inferior middle temporal gyrus | right          | 0.048   | 437  | 4.42            | 51                      | -12 | -42 |
|  | 4           | Inferior frontal gyrus         | right/ lateral | 0.171   | 194  | 3.70            | 40                      | 33  | 12  |
|  | 5           | Superior frontal gyrus         | right/ medial  | 0.273   | 122  | 3.47            | 6                       | 36  | -12 |

Models adjusted for scanner site and TIV.  
 Musical activity was included as binary predictor, dummy coded with musical activity = 1, no musical activity = 0.  
<sup>a</sup> Results from the main effect model with musical activity and GMV ( $p < 0.001$  uncorrected, expected voxels per cluster  $k = 139$ ).  
<sup>b</sup> Results from the interaction effect model with musical activity, working memory, and GMV ( $p < 0.001$  uncorrected, expected voxels per cluster  $k = 110$ ).  
 Cluster peaks are specified by their anatomical site, labelled using the Hammersmith atlas provided by the CAT12 toolbox.  
**Key:** GMV, gray matter volume; MNI x y z [mm], coordinates MNI space in millimeters; TIV, total intracranial volume

377 =====

378 **Figure 5**



379

380 **Figure 5: Associations between lifelong musical activity and regional volume distribution. A-B. Results of**

381 **the main effect analysis.** Statistical map (A) shows significant clusters ( $p < 0.001$  uncorrected, color-coded in

382 magenta) with larger GMV in participants with musical activity compared to controls. The corresponding graph (B)

383 displays the association using mean GMV values extracted from the corresponding cluster in the postcentral

384 gyrus. The box plot displays the median with 95% confidence intervals, interquartile range with lower (25th) and

385 upper percentiles (75th), and individual data points. **C-D. Results of the interaction analysis.** The statistical

386 map (C) displays clusters ( $p < 0.001$  uncorrected, color-coded in magenta) with a significant moderation effect of

387 musical activity. The corresponding scatter plot (D) shows the association using mean values extracted from the

388 GMV maps in the combined cluster. Larger GMV in the combined cluster was associated with better working

389 memory ability selectively in the musically active participants (MA+, blue) compared to controls (MA-, gray).

390 Individual data points, linear trends (solid lines), 95% confidence intervals (shaded areas), and standardized

391 regression coefficients ( $\beta$ ) within each musical activity group are provided. The statistical maps are depicted on a

392 glass brain. Significance levels (uncorrected): \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ . **Key:** MA+, musical activity; MA-,

393 no musical activity; GMV, gray matter volume; TIV, total intracranial volume.

394 =====

## 395 **5 DISCUSSION**

### 396 **5.1 Summary**

397 The current study examined late-life cognitive abilities, brain morphology, and their interplay in  
398 cognitively healthy older adults as a function of regularly playing a musical instrument over the life  
399 course. Participants with a self-reported history of lifelong musical activity were compared to matched  
400 controls without musical activity across the lifespan. Results of this study highlight that regularly  
401 playing a musical instrument is associated with global and multi-domain cognitive benefits in older  
402 adults, with no significant benefit in gray matter structure in regions affected by aging and AD. In the  
403 musically active participants, cognitive abilities were enhanced with preserved regional GMV for some  
404 cognitive domains, pointing towards a facilitated recruitment of existing brain resources in this group.  
405 Overall, our findings may imply that a history of regular musical activity could promote cognitive and  
406 brain benefits in older adults and thereby strengthen resilience against cognitive decline.

### 407 **5.2 Musical activity and cognition**

408 We demonstrate that participants, who reported a lifelong regular engagement in musical  
409 activity, outperformed matched controls in cognitive performance. More precisely, superior cognitive  
410 abilities were found in global cognition and multiple cognitive domains including working memory,  
411 executive functions, language and visuospatial abilities in the musically active older people, with the  
412 largest effect size seen for working memory. These findings directly support and expand previous  
413 studies, showing that playing a musical instrument may preserve higher-order cognitive skills that  
414 typically decline in older adults<sup>14,15,33,34</sup>. By contrast, we did not identify benefits of musical activity on  
415 learning and memory, although these must be perceived as essential cognitive skill involved in playing  
416 music. In older adults, some studies report that regular musical activity is associated with better  
417 episodic memory<sup>13,14,33</sup>, while others do not<sup>8,15,16</sup>. It might, however, be argued that specific  
418 hippocampus-related processes are enhanced by musical activity, such as long-term musical memory  
419 or navigation in acoustic space<sup>27,35</sup>, but cannot be captured by the memory composite used in the  
420 current study. While sensitive experimental and neuroimaging markers are needed to gain insights into  
421 presumed memory benefits, current findings appear to confirm that regular musical activity may  
422 particularly favor late-life cognitive abilities involving the frontal lobe.

### 423 **5.3 Musical activity and brain structure**

424 Our data demonstrate that playing music during life was not significantly associated with larger  
425 GMV in age-sensitive brain regions. Specifically, we did not detect volumetric differences between  
426 musically active people and controls in frontal or hippocampal regions. The voxel-based analysis  
427 confirmed this observation. A slight volume increase was found in somatosensory areas of the  
428 musically active group, presumably reflecting brain plasticity in response to tactile stimulations induced  
429 by playing an instrument<sup>21,22</sup>. However, we failed to identify respective GMV modulations in higher-  
430 order brain regions. Earlier studies have shown positive associations between musical activity and  
431 brain volume in frontal, temporal, and parietal regions mainly in younger cohorts<sup>21,22,27</sup>, with limited  
432 indication in older adults<sup>30</sup>. Similar to previous studies<sup>15,16,33</sup>, we accounted for several reserve  
433 proxies that may help maintain late-life brain structure<sup>3,36</sup>. Given this effort, it seems plausible to  
434 assume that there is little benefit of regular musical activity on structural brain resources in older age.  
435 Alternatively, subtle effects could be unnoticed due to increased variability in GMV through differential  
436 brain aging and/or brain pathology<sup>37</sup>.

#### 437 **5.4 Moderations of musical activity**

438 Importantly though, our current results revealed that lifelong regular musical activity could act as  
439 a protective factor in the associations between late-life brain resources and cognitive performance. We  
440 found an interaction between playing music and GMV, such that performance in some cognitive  
441 domains was enhanced with preserved frontal volume selectively in the musically active participants.  
442 This specific moderation effect was significant for global cognition, language, as well as working  
443 memory and extended to inferior temporal as well as motor-sensory regions at the voxel level. In other  
444 words, although gray matter structure was not substantially associated with musical activity, it  
445 facilitated late-life cognitive performance in synergy with playing music. This may reflect a more  
446 efficient use of an overall younger brain age, as previously reported in amateur musicians compared to  
447 controls<sup>38</sup>. Our observation further parallels existing findings<sup>29</sup>. In their study, a larger hippocampal  
448 volume was associated with better general cognitive abilities in younger musicians, but not in non-  
449 musicians, implying that musically active people may be able to use existing brain resources more  
450 efficiently<sup>29</sup>. The current findings essentially indicate that this functional advantage of playing music is  
451 detectable in older adults, where it is linked to distributed brain regions and multiple cognitive domains.  
452 Frontal and temporal brain regions, in particular, are part of wide-spread brain networks shown to  
453 support cognitive reserve processes<sup>36,39-42</sup>. It therefore appears that playing music over the life course

454 could facilitate the recruitment of structural brain resources, as a key benefit to support late-life  
455 cognitive functioning.

## 456 **5.5 Synopsis**

457 Taken together, the present study adds considerable insight to the picture that musical activity  
458 over the life course, even at a moderate frequency, could act as protective factor in late life stages.  
459 Given that playing a musical instrument requires the simultaneous integration of intense multimodal  
460 motor, sensory, cognitive, emotional, and social sensations, this lifestyle activity may induce lasting  
461 functional plasticity in higher-order neural networks supporting multi-domain cognitive functions <sup>43,44</sup>.  
462 Even passive listening to music was previously shown to modulate functional connectivity in  
463 distributed brain networks <sup>45</sup>, a mechanism suggested to convey therapeutic benefits of music-based  
464 interventions <sup>7</sup>. Enhanced functional connectivity in higher-order brain networks is an essential  
465 mechanism shown to be protective against neuropathological burden in older adults <sup>39,41</sup>. In light of our  
466 findings, it may be proposed that musical activity could act as a resilience factor through functional  
467 brain resources, which need to be examined in future studies.

468 Nevertheless, the observed health benefits associated with lifelong regular musical activity  
469 could be encouraged by a general engagement in advantageous lifestyles. Similar to previous studies  
470 <sup>33</sup>, participants with regular musical activity during the life course were characterized by a high reserve  
471 profile including higher education, SES, intelligence, and more frequent physical activity. Notably  
472 though, we observed better cognitive abilities in the musically active group with these factors  
473 accounted for, suggesting an added benefit of musical activity on late-life brain and cognitive functions  
474 beyond known reserve proxies. Overall, our results propose that playing a musical instrument could  
475 serve as a low-threshold multimodal enrichment strategy that may help preserve cognitive and brain  
476 health. Targeted intervention studies are required to evaluate the impact of playing music on cognitive  
477 performance and underlying brain mechanisms in older people <sup>46</sup>.

## 478 **5.6 Strengths and limitations**

479 Our study has several strengths and limitations. We assembled data from the longitudinal  
480 observational DELCODE cohort to assess a well-characterized sample of cognitively unimpaired older  
481 adults with measures of demographics, cognition, lifestyle behaviour, and brain structure. This detailed  
482 phenotyping provided new evidence on potential health benefits of regular musical activity in older

483 adults. This study identified older people with a history of musical activity over three life stages and  
484 statistical analyses were based on multi-domain cognitive abilities and morphological brain measures  
485 all measured in the same participants.

486 Limitations of our cohort-based approach include the assessment of musical activity. While we  
487 obtained the frequency of musical activity using the LEQ<sup>47</sup>, more detailed information on musical  
488 instrument type, age of acquisition, and intensity would be desirable given that these features may  
489 differentially impact brain plasticity and cognitive skills<sup>14,48</sup>. Furthermore, the present cross-sectional  
490 study design does not permit causal interpretations of the investigated associations. It might be  
491 possible that certain factors that were not accounted for, facilitate playing an instrument over the life  
492 course. Such factors may include genetic predispositions or advantageous early-life exposures, which  
493 could play a role in the observed relationships, warranting further investigations. Finally, potential  
494 benefits of musical activity call for a longitudinal study design, to evaluate if musically active older  
495 people are indeed more protected against cognitive decline, which will have important implications on  
496 public health strategies.

## 497 **5.7 Conclusion**

498 Results of the present study are promising and suggest that lifelong regular musical activity, as  
499 an accessible and multimodal leisure activity, could help mitigating age-related cognitive decline  
500 through benefits in functional brain resources. Further research is needed to assess detailed  
501 information about the nature of playing music and functional brain mechanisms associated with a  
502 history of regular musical activity over the life course. Given that world populations are aging and that  
503 age-related diseases pose healthcare challenges of utmost importance, interventional studies  
504 examining the protective effects of musical activity on the brain and cognitive functioning in older  
505 adults are greatly needed.

## 506 **6 AVAILABILITY STATEMENTS**

### 507 **6.1 Data availability**

508 The data that support findings of the present study are available on reasonable request.

### 509 **6.2 Code availability**

510 For this study, existing data analysis packages for statistical analyses were used. Scripts for the use of  
511 these packages are available online from the authors on reasonable request.

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## 538 **8 DECLARATIONS**

### 539 **8.1 Ethics approval and consent to participate**

540 The DELCODE study protocol was approved by the ethical committees of the medical faculties  
541 of participating sites. All participants gave written informed consent prior to study inclusion.

### 542 **8.2 Availability of data and materials**

543 The data that support findings of this study are available on reasonable request.

### 544 **8.3 Disclosures**

545 O. Peters received fees for consultation from Abbvie, Biogen, Eisai, Grifols, MSD Roche, and  
546 Schwabe. J. Priller received fees for consultation, lectures, and patents from Neurimmune, Axon,  
547 Desitin, and Epomedics. J. Wiltfang is an advisory board member of Abbott, Biogen, Boehringer  
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