

1 **Article**

2 **A next-generation, histological atlas of the human brain
3 and its application to automated brain MRI segmentation**

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5 Adrià Casamitjana^{1,2}, Matteo Mancini^{1,3,4}, Eleanor Robinson¹, Loïc Peter¹, Roberto Annunziata¹, Juri Althonayan¹,
6 Shauna Crampsie¹, Emily Blackburn¹, Benjamin Billot^{1,5}, Alessia Atzeni¹, Oula Puonti^{6,7}, Yaël Balbastre^{1,7}, Peter Schmidt⁸,
7 James Hughes⁸, Jean C Augustinack⁷, Brian L Edlow^{7,9}, Lilla Zöllei⁷, David L Thomas^{10,11}, Dorit Kliemann¹², Martina Bocchetta^{10,13},
8 Catherine Strand¹⁴, Janice L Holton¹⁴, Zane Jaunmuktane¹⁴, & Juan Eugenio Iglesias^{1,5,7,*}

9 1 Department of Medical Physics and Biomedical Engineering, University College London, London, United Kingdom
10 2 Research Institute of Computer Vision and Robotics, University of Girona, Girona, Spain
11 3 Department of Cardiovascular, Endocrine-Metabolic Diseases and Aging, Italian National Institute of Health, Rome, Italy
12 4 Cardiff University Brain Research Imaging Centre, Cardiff University, Cardiff, United Kingdom
13 5 Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge, MA, United States
14 6 Danish Research Centre for Magnetic Resonance, Centre for Functional and Diagnostic Imaging and Research, Copenhagen Uni-
15 versity Hospital - Amager and Hvidovre, Copenhagen, Denmark
16 7 Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital and Harvard Medical School, Boston, MA,
17 United States
18 8 Advanced Research Computing Centre, University College London, London, United Kingdom
19 9 Center for Neurotechnology and Neurorecovery, Department of Neurology, Massachusetts General Hospital and Harvard Medical
20 School, Boston, MA, United States
21 10 Dementia Research Centre, Department of Neurodegenerative Disease, UCL Queen Square Institute of Neurology, University Col-
22 lege London, London, United Kingdom
23 11 Neuroradiological Academic Unit, Department of Brain Repair and Rehabilitation, UCL Queen Square Institute of Neurology, Uni-
24 versity College London, London, United Kingdom
25 12 Department of Psychological and Brain Sciences, University of Iowa, Iowa City, IA, United States
26 13 Centre for Cognitive and Clinical Neuroscience, Division of Psychology, Department of Life Sciences, College of Health, Medicine
27 and Life Sciences, Brunel University London, United Kingdom
28 14 Queen Square Brain Bank for Neurological Disorders, Department of Clinical and Movement Neurosciences, UCL Queen Square
29 Institute of Neurology, University College London, London, United Kingdom
30
31 * Correspondence: jiglesiasgonzalez@mgh.harvard.edu

32 **Abstract:** Magnetic resonance imaging (MRI) is the standard tool to image the human brain *in vivo*. In this domain, digital
33 brain atlases are essential for subject-specific segmentation of anatomical regions of interest (ROIs) and spatial compar-
34 ison of neuroanatomy from different subjects in a common coordinate frame. High-resolution, digital atlases derived
35 from histology (e.g., Allen atlas [7], BigBrain [13], Julich [15]), are currently the state of the art and provide exquisite 3D
36 cytoarchitectural maps, but lack probabilistic labels throughout the whole brain. Here we present *NextBrain*, a next-
37 generation probabilistic atlas of human brain anatomy built from serial 3D histology and corresponding highly granular
38 delineations of five whole brain hemispheres. We developed AI techniques to align and reconstruct ~10,000 histological
39 sections into coherent 3D volumes with joint geometric constraints (no overlap or gaps between sections), as well as to
40 semi-automatically trace the boundaries of 333 distinct anatomical ROIs on all these sections. Comprehensive delineation
41 on multiple cases enabled us to build *the first probabilistic histological atlas of the whole human brain*. Further, we cre-
42 ated a companion Bayesian tool for automated segmentation of the 333 ROIs in any *in vivo* or *ex vivo* brain MRI scan
43 using the *NextBrain* atlas. We showcase two applications of the atlas: automated segmentation of ultra-high-resolution
44 *ex vivo* MRI and volumetric analysis of Alzheimer's disease and healthy brain ageing based on ~4,000 publicly available
45 *in vivo* MRI scans. We publicly release: the raw and aligned data (including an online visualisation tool); the probabilistic
46 atlas; the segmentation tool; and ground truth delineations for a 100 µm isotropic *ex vivo* hemisphere (that we use for
47 quantitative evaluation of our segmentation method in this paper). By enabling researchers worldwide to analyse brain
48 MRI scans at a superior level of granularity without manual effort or highly specific neuroanatomical knowledge, *Next-
49 Brain* holds promise to increase the specificity of MRI findings and ultimately accelerate our quest to understand the
50 human brain in health and disease.

51 Magnetic resonance imaging (MRI) is arguably the most
52 important tool to study the human brain *in vivo*. Its ex-
53 quisite contrast between different types of soft tissue
54 provides a window into the living brain without ionising
55 radiation, making it suitable to healthy volunteers. Ad-
56 vances in magnet strength, data acquisition and image
57 reconstruction methods [16-20] enable the acquisition
58 of millimetre-resolution MRI scans of the *whole* brain in
59 minutes. MRI can be acquired with different pulse se-
60 quences that image different tissue properties, includ-
61 ing: neuroanatomy with structural acquisitions [21];
62 brain activity with functional MRI based on blood oxy-
63 genation [23]; vasculature with perfusion imaging and
64 MR angiography [24-27]; or white matter fibres and mi-
65 crostructure with diffusion-weighted MRI [28,29].

66 Publicly available neuroimaging packages (Free-
67 Surfer [30], FSL [31], SPM [32], or AFNI [33]) enable re-
68 searchers to perform large-scale studies with thousands
69 of scans [34-37] to study of healthy ageing, as well as a
70 broad spectrum of brain diseases, such as Alzheimer's,
71 multiple sclerosis, or depression [38-41]. A core compo-
72 nent of these neuroimaging packages is digital 3D brain
73 atlases. These are reference 3D brain images that are
74 representative of a certain population and can comprise
75 image intensities, neuroanatomical labels, or both. We
76 note that, due to its highly convoluted structure, the
77 cerebral cortex is often modelled with specific atlases
78 defined on surface coordinate systems [42,43] – rather
79 than 3D images). We refer the reader to [44] for a com-
80 parative study.

81 Volumetric atlases are often computed by averaging
82 data from a large cohort of subjects [45], but they may
83 encompass as few as a single subject – particularly when
84 built from labour-intensive modalities like histol-
85 ogy [13]. Atlases enable aggregation of data from differ-
86 ent subjects into a common coordinate frame (CCF),
87 thus allowing analyses (e.g., group comparisons) as a
88 function of spatial location. Atlases that include neuro-
89 anatomical labels also provide prior spatial information
90 for analyses like automated image segmentation [46].

91 Most volumetric atlases, including those in neuroim-
92 aging packages, capitalise on the abundance of *in vivo*
93 MRI scans acquired at ~ 1 mm isotropic resolution. This
94 voxel size is sufficient to represent information at the
95 level of gyri, sulci, and subcortical nuclei. However, it is
96 insufficient to study the brain at the subregion level,
97 which is desirable as brain substructures (e.g., hippo-
98 campal subfields, nuclei of the thalamus) are known
99 from animal models and *postmortem* human studies to
100 have different function and connectivity [47]. This limi-
101 tation can be circumvented with higher resolution im-
102 ages acquired *ex vivo*, typically with MRI or histology.

103 *Ex vivo* MRI has no motion artifacts and enables long
104 acquisitions with voxels in the 100 μm range [3,48-50].
105 However, it fails to visualise cytoarchitecture and re-
106 solve many boundaries between brain areas. Histology,

107 on the other hand, is a microscopic 2D modality that can
108 visualise distinct aspects of cytoarchitecture using an ar-
109 ray of stains – thus revealing neuroanatomy with much
110 higher detail. Earlier versions of histological atlases
111 were printed, often not digitised, and comprised only a
112 small set of labelled sections. Representative examples
113 include the Morel atlas of the thalamus and basal gan-
114 glia [51] or the Mai atlas of the whole brain [1] (Fig. 1A).

115 While printed atlases are not useful for computational
116 analysis, serial histology can be combined with image
117 registration (alignment) methods to enable volumetric
118 reconstruction of 3D histology [52], thus opening the
119 door to creating 3D histological atlases. These have two
120 major advantages over MRI atlases: (i) providing a more
121 detailed CCF; and (ii) the ability to segment MRI scans at
122 finer resolution – with potentially higher sensitivity and
123 specificity to detect brain alterations caused by brain
124 diseases or to measure treatment effects.

125 Earlier 3D histological atlases were limited in terms of
126 anatomical coverage. Following the Morel atlas, two
127 digital atlases of the basal ganglia and thalamus were
128 presented [8,11] (Fig. 1B-C). To automatically obtain
129 segmentations for living subjects, one needs to register
130 their MRI scans with the histological atlases, which is dif-
131 ficult due to differences in image resolution and con-
132 trast between the two modalities. For this reason, the
133 authors mapped the atlases to 3D MRI templates (e.g.,
134 the MNI atlas [53]) that can be more easily registered to
135 *in vivo* images of other subjects. A similar atlas combin-
136 ing histological and MRI data was proposed for the hip-
137 pocampus [12] (Fig. 1D-F). Our group presented a histo-
138 logical atlas of the thalamus [14] (Fig. 1G), but instead of
139 using MNI as a stepping stone, we used Bayesian meth-
140 ods [54] to map our atlas to *in vivo* scans *directly*.

141 More recently, several efforts have aimed at the con-
142 siderably bigger endeavour of building histological at-
143 lases of the whole human brain:

144 - BigBrain [13] comprises over 7,000 histological sec-
145 tions of a single brain, which were accurately recon-
146 structed in 3D with an *ex vivo* MRI scan as reference
147 (Fig. 1H). BigBrain paved the road for its follow-up Ju-
148 lich-Brain [15], which aggregates data from 23 individu-
149 als. A subset of 10 cases have been provided to the com-
150 munity for labelling, which has led to the annotation of
151 248 cytoarchitectonic areas as part of 41 projects. The
152 maximum likelihood maps have been mapped to MNI
153 space for *in vivo* MRI analysis [55], but have two caveats
154 (Fig. 1I): they align poorly with the underlying MNI tem-
155 plate, and subcortical annotations are only partial.

156 - The Allen reference brain [7] (Fig. 1J) has comprehen-
157 sive anatomical annotations on high-resolution histol-
158 ogy and is integrated with the Allen gene expression at-
159 lases. However, it only has delineations for a sparse set
160 of histological sections of a single specimen (resembling
161 a printed atlas). For 3D analysis of *in vivo* MRI, the au-
162 thors have manually labelled the MNI template using a

163 protocol inspired by their own atlas (Fig. 1K), but with a
164 fraction of the labels and less accurately delineations –
165 since they are made on MRI and not histology.

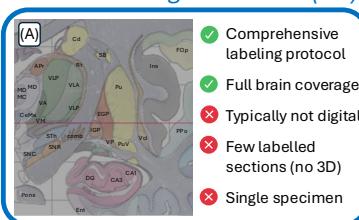
166 - The Ahead brains [22] (Fig. 1L-N) comprise quantitative
167 MRI and registered 3D histology for two separate
168 specimens. These have anatomical labels for a few
169 dozen structures, but almost exclusively of the basal
170 ganglia. Moreover, these labels were obtained from the
171 MRI with automated methods, rather than manually
172 traced on the high-resolution histology.

173 While these histological atlases of the whole brain
174 provide exquisite 3D cytoarchitectural maps, interoperability
175 with other datasets (e.g., gene expression), and
176 some degree of MRI-histology integration, there are
177 currently neither: (i) datasets with densely labelled 3D
178 histology of the whole brain; nor (ii) probabilistic atlases
179 built from such datasets, which would enable analyses

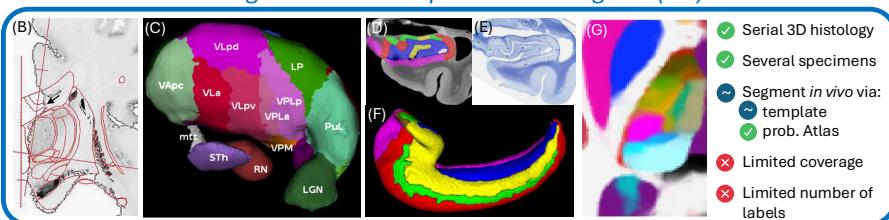
180 such as Bayesian segmentation or CCF mapping of the
181 whole brain at the subregion level.

182 In this article, we present *NextBrain*, a next-generation
183 probabilistic atlas of the human brain built from
184 comprehensively labelled, multi-modal 3D histology of
185 five half brains (Fig. 1O-P). The full dataset comprises
186 ~10,000 sections stained with Hematoxylin and Eosin
187 (H&E, which discerns cell nuclei vs cytoplasm) and Luxol
188 Fast Blue (LFB, which enhances myelin). These sections
189 were: (i) 3D-reconstructed with *ex vivo* MRI scans and
190 highly customised image registration methods powered
191 by artificial intelligence (AI); and (ii) densely segmented
192 into 333 regions of interest (ROIs) with AI-enabled,
193 semi-automated segmentation methods (Fig. 1Q). The
194 3D label maps are finally used to build a probabilistic atlas
195 (Fig. 1R), which is combined with a Bayesian tool for au-
196 tomated segmentation of MRI scans (Fig. 1S).

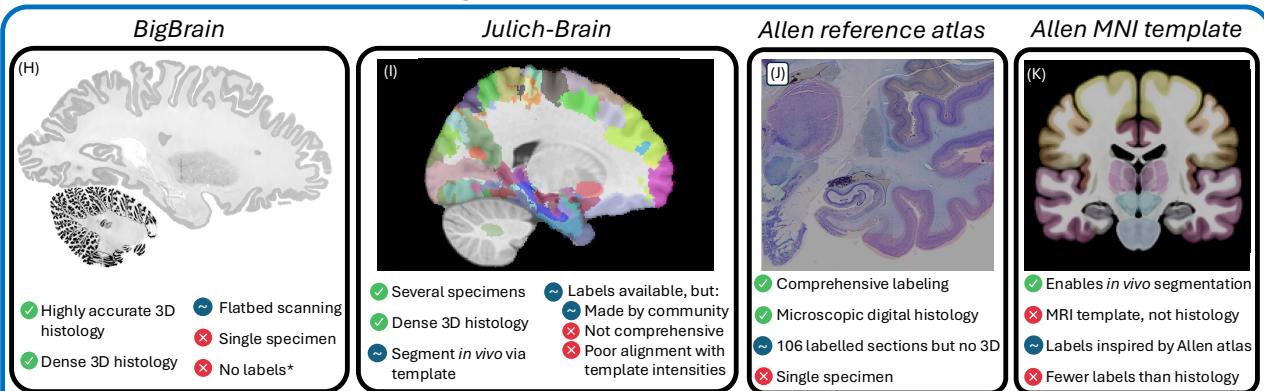
Printed histological atlases (2D)



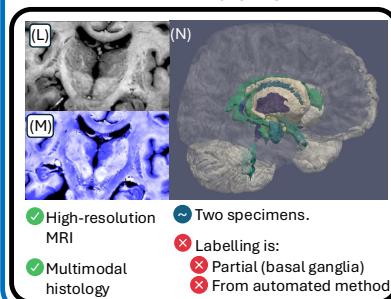
Histological atlases of specific brain regions (3D)



Histological atlases of the whole human brain



AHEAD brains



NextBrain (proposed)

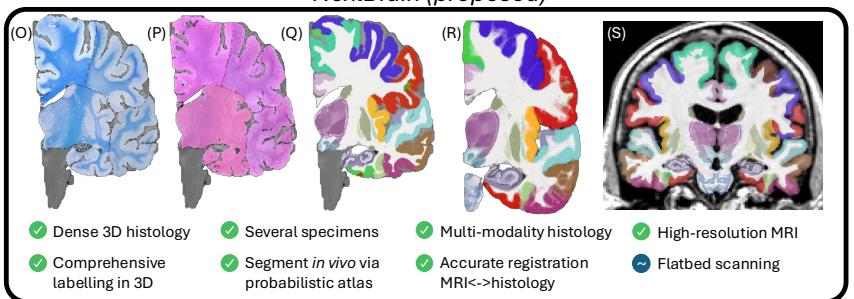


Fig. 1: NextBrain in the context of histological atlases, with advantages (✓), disadvantages (✗), and neutral points (⌚). (A) Printed atlas [1] with a sparse set of manually traced sections [1]. (B-G) Histological atlases of specific ROIs with limited coverage: (B) Manually traced section of basal ganglia [8]; (C) 3D rendering of deterministic thalamic atlas [11]; (D-F) Traced MRI slice, histological section, and 3D rendering of hippocampal atlas [12]; and (G) Slice of our probabilistic atlas of the thalamus [14]. (H-N) Histological atlases of the whole human brain: (H) 3D reconstructed slice of BigBrain [13]; (I) Slice of Julich-Brain labels on MNI template; (J) Labelled histological section of the Allen reference brain [7]; (K) Labelling of MNI template with protocol inspired by (J); and (L-N) MRI, histology, and 3D rendering of AHEAD brains [22]. (O-S) Our new atlas *NextBrain* includes dense 3D histology (O-P) and comprehensive manual labels (Q) of five specimens, enabling the construction of a probabilistic atlas (R) that can be combined with Bayesian techniques to automatically label 333 ROIs in *in vivo* MRI scans (S).

197 As the first densely labelled probabilistic atlas of the
198 human brain built from histology, *NextBrain* enables
199 brain MRI analysis at a level of detail that was previously
200 not possible. Our results showcase: the high accuracy of
201 our 3D histology reconstructions; *NextBrain*'s ability to
202 accurately segment MRI scans acquired *in vivo* or *ex*
203 *vivo*; its ability to separate diseased and control subjects
204 in an Alzheimer's group study; and a volumetric study of
205 healthy brain aging with unprecedented detail.

206 In addition to the atlas and companion segmentation
207 tool, our public release of *NextBrain* includes: (i) The
208 raw and registered images that were used to build the
209 atlas, which are an invaluable resource for MRI signal
210 modelling or histology registration studies; (ii) An online
211 visualisation tool for these data, for educational and
212 data inspection purposes; (iii) The source code and pipe-
213 lines, which do not require any highly specialised equip-
214 ment for intact coronally sliced full-brain procedures
215 used at select sites like Allen or Julich (e.g., full-brain mi-
216 crotome, custom glass slides), thus enabling wide ap-
217 plicability; and (iv) our manual 3D segmentation of a
218 publicly available 100 μm isotropic *ex vivo* scan [3] (used
219 here for quantitative evaluation), which is a valuable re-
220 source in its own right, e.g., for ROI analysis in the *ex*
221 *vivo* CCF of this scan, or for development and validation
222 of segmentations methods.

223 **Densely labelled 3D histology of five human
224 hemispheres**

225 The *NextBrain* workflow is summarised in Fig. 2 and de-
226 tailed in the Methods section. The first result of the
227 pipeline (panels A-G) is a multimodal dataset with hu-
228 man hemispheres from five donors (three right, two
229 left), including half cerebellum and brainstem. Each of
230 the five cases comprises accurately aligned high-res-
231 olution *ex vivo* MRI, serial histology (H&E and LFB stains),
232 and dense ground truth segmentations of 333 cortical and
233 subcortical brain ROIs.

234 Aligning the histology of a case is analogous to solving
235 a 2,000-piece jigsaw puzzle in 3D, with the *ex vivo* MRI
236 as reference (similar to the image on the box cover), and
237 with pieces that are deformed by sectioning and mount-
238 ing on glass slides – with occasional tissue folding or
239 tearing. This problem falls out of the scope of existing
240 inter-modality registration techniques [56], including
241 slice-to-volume [57] and 3D histology reconstruction
242 methods [52], which do not have to address the joint
243 constraints of thousands of sections, acquired in non-
244 parallel planes as part of different blocks.

245 Instead, we solve this challenging problem with a cus-
246 tom, state-of-the-art image registration framework (Fig.
247 3), which includes three components specifically devel-
248 oped for this project: (i) a differentiable regulariser that
249 minimises overlap of different blocks and gaps in be-

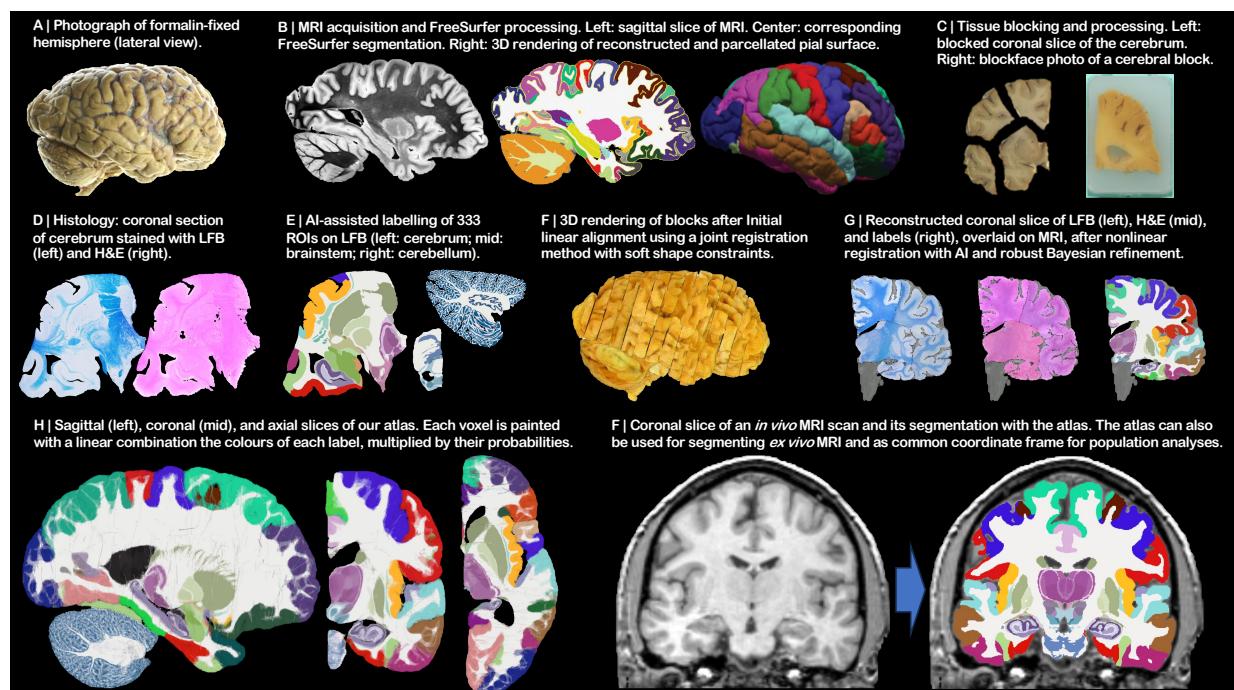


Fig. 2: *NextBrain* workflow. (A) Photograph of formalin-fixed hemisphere. (B) High-resolution (400 μm) *ex vivo* MRI scan, FreeSurfer seg-
mentation, and extracted pial surface (parcellated with FreeSurfer). (C) Tissue slabs and blocks, before and after paraffin embedding.
(D) Section stained with H&E and LFB. (E) Semi-automated labelling of 333 ROIs on sections using an AI method [5]. (F) Initialization of
affine alignment of tissue blocks using a custom registration algorithm that minimises overlap and gaps between blocks. (G) Refinement
of registration with histology and nonlinear transform, using a combination of AI and Bayesian techniques [9,10]. (H) Orthogonal slices of
3D probabilistic atlas. (I) Automated Bayesian segmentation of an *in vivo* scan into 333 ROIs using the atlas.

250 tween [58]; (ii) an AI registration method that uses con-
 251 trastive learning to provide highly accurate alignment of
 252 corresponding brain tissue across MRI and histol-
 253 ogy [10]; and (iii) a Bayesian refinement technique
 254 based on Lie algebra that guarantees the 3D smooth-
 255 ness of the reconstruction across modalities, even in the
 256 presence of outliers due to tissue folding and tear-
 257 ing [9]. We note that this is an evolution of our previ-
 258 ously presented pipeline [6], which incorporates the
 259 aforementioned contrastive AI method and jointly opti-
 260 mises the affine and nonlinear transforms to achieve a
 261 32% reduction in registration error (details below).

262 Qualitatively, it is apparent from Fig. 3 that a very high
 263 level of accuracy is achieved for the spatial alignment,
 264 despite the non-parallel sections and distortions in the
 265 raw data. The regulariser effectively aligns the block
 266 boundaries in 3D without gaps or overlap (Fig. 3A-C),
 267 with minor discontinuities across blocks (e.g., in the
 268 temporal lobe). When the segmentations of different
 269 blocks are combined (Fig. 3A, right), the result is a
 270 smooth mosaic of ROI labels.

271 The AI-enabled registration across MRI and histologi-
 272 cal stains is exemplified in Fig. 3B. Overlaying the main
 273 ROI contours on the different modalities reveals the
 274 highly accurate alignment of the three modalities (MRI,
 275 H&E, LFB), even in convoluted regions of the cortex and

276 the basal ganglia. The mosaic of modalities also high-
 277 lights the accurate alignment at the substructural level,
 278 e.g., subregions of the hippocampus.

279 Fig. 3C shows the 3D reconstruction in orientations
 280 orthogonal to the main plane of sectioning (coronal).
 281 This illustrates not only the lack of gaps and overlaps be-
 282 tween blocks, but also the smoothness that is achieved
 283 *within* blocks. This is thanks to the Bayesian refinement
 284 algorithm, which combines the best features of meth-
 285 ods that: (i) align each section independently (high fidel-
 286 ity to the reference, but jagged reconstructions) and
 287 (ii) those that align sections to their neighbours (smooth
 288 reconstructions, but with “banana effect”, i.e., straight-
 289 ening of curved structures).

290 Quantitative results are presented in Fig. 3D, as well
 291 as in Extended Data Figs. 1-4D. The registration error,
 292 evaluated with 250 manually placed pairs of landmarks
 293 (known to be a better proxy for the registration error
 294 than similarity of label overlap metrics [59]), is $0.99 \pm$
 295 0.51 mm – a considerable reduction with respect our
 296 previous pipeline [6], which yielded 1.45 ± 1.68 mm
 297 (Wilcoxon $p=2 \times 10^{-22}$). The spatial distribution of the er-
 298 rror is further visualised with kernel regression in Ex-
 299 tended Data Fig. 5, which shows that this distribution is
 300 fairly uniform, i.e., there is no obvious consistent pat-
 301 tern across cases.

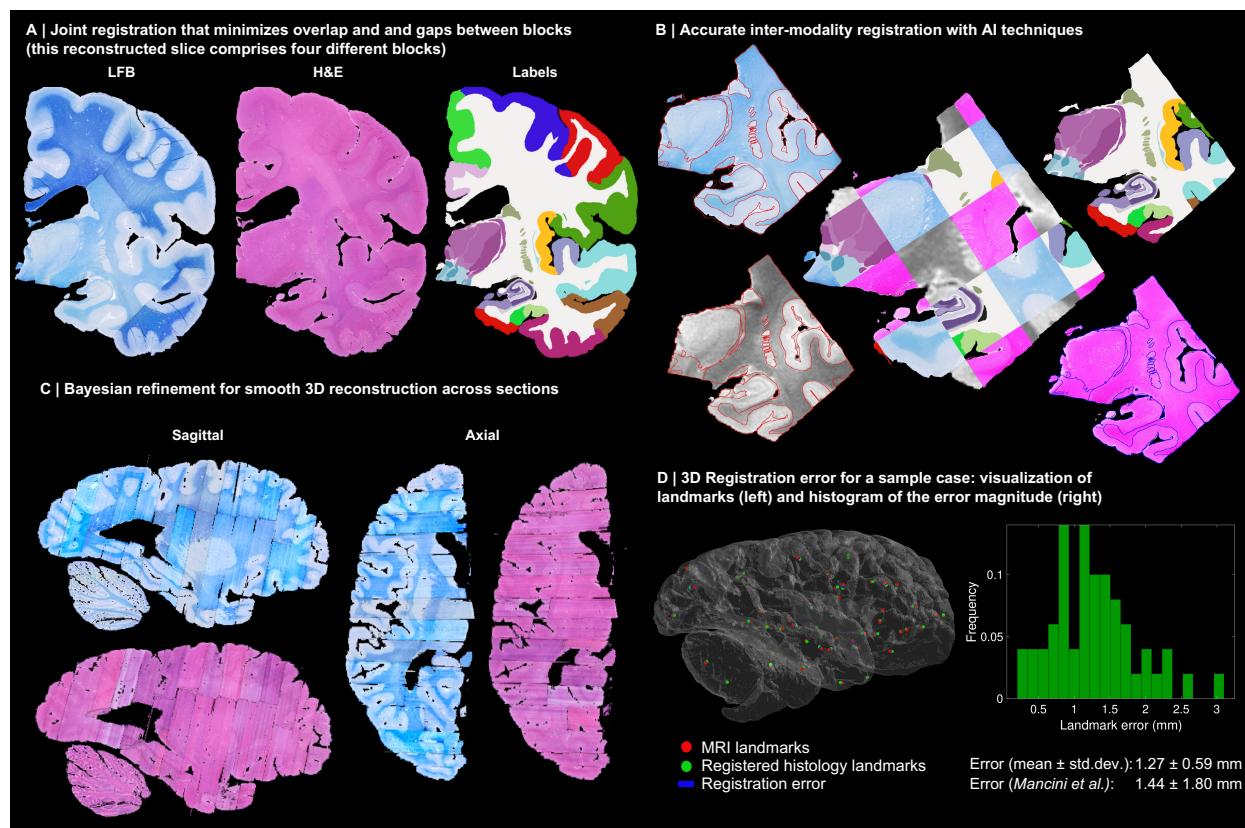


Fig. 3: 3D reconstruction of Case 1. (A) Coronal slice of 3D reconstruction; boundaries between blocks are noticeable from uneven staining. (B) Registered MRI, LFB, and H&E histology of a block, with tissue boundaries (traced on LFB) overlaid. (C) Orthogonal view of reconstruction, which is smooth thanks to the Bayesian refinement, and avoids gaps and overlaps thanks to the regulariser. (D) Visualization of 3D landmark registration error (left); histogram of its magnitude (right); and mean \pm standard deviation (bottom), compared with our previous pipeline [6]. See Extended Data for results on the other cases. The average landmark error across all cases is 0.99mm (vs 1.45 for [6]).

302 Our pipeline is widely applicable as it produces accurate 3D reconstructions from blocked tissue in standard-sized cassettes, sectioned with a standard microtome. 303 The computer code and aligned dataset is freely available in our public repository (see Data Availability). For 304 educational and data inspection purposes, we have built 305 an online visualisation tool for the multi-modality data, 306 which is available at: github-pages.ucl.ac.uk/NextBrain.

307 Supplementary Video 1 illustrates the aligned data, 308 which includes: (i) MRI at 400 μm isotropic resolution; 309 (ii) aligned H&E and LFB histology digitised at 4 μm resolution (with 250 or 500 μm spacing, depending on the 310 brain location); and (iii) ROI segmentations, obtained 311 with a semi-automated AI method [5]. The ROIs comprise 34 cortical labels (following the Desikan-Killiany 312 atlas [60]) and 299 subcortical labels (following different 313 atlas for different brain regions; see the Methods section 314 below and the supplement). This public dataset enables 315 researchers worldwide to conduct their own studies not 316 only in 3D histology reconstruction, but also other fields 317 like: high-resolution segmentation of MRI or histology 318 [61]; MRI-to-histology and histological stain-to-stain image 319 translation [62]; deriving MRI signal models from 320 histology [63]; and many others.

321 **A next-generation probabilistic atlas of the 322 human brain**

323 The labels from the five human hemispheres were 324 co-registered and merged into a probabilistic atlas. This 325 was achieved with a method that alternately registers 326 the volumes to the estimate of the template, and updates 327 the template via averaging [64]. The registration 328 method is diffeomorphic [65] to ensure preservation of 329 the neuroanatomic topology (e.g., ROIs do not split or 330 disappear in the deformation process). Crucially, we use 331

332 an initialization based on the MNI template, which 333 serves two important purposes: preventing biases towards 334 any of the cases (which would happen if we initialised 335 with one of them); and “centring” our atlas on a 336 well-established CCF computed from 305 subjects, 337 which largely mitigates our relatively low number of 338 cases. Since the MNI template is a greyscale volume, the 339 first iteration of atlas building uses registrations 340 computed with the *ex vivo* MRI scans. Subsequent iterations 341 register labels directly with a metric based on the 342 probability of the discrete labels according to the atlas [64].

343 Fig. 4 shows close-ups of orthogonal slices of the atlas, 344 which models voxel-wide probabilities for the 333 ROIs 345 on a 0.2mm isotropic grid. The resolution and detail of 346 the atlas represents a substantial advance with respect 347 to the SAMSEG atlas [2] currently in FreeSurfer (Fig. 4A). 348 SAMSEG models 13 brain ROIs at 1 mm resolution and 349 is, to the best of our knowledge, the most detailed 350 probabilistic atlas that covers all brain regions. The figure 351 also shows approximately corresponding slices of the 352 manual labelling of the MNI atlas with the simplified 353 Allen protocol [7]. Compared with *NextBrain*, this labelling 354 is not probabilistic and does not include many histological 355 boundaries that are invisible on the MNI template 356 (e.g., hippocampal subregions, in violet). For this reason, 357 it only has 138 ROIs – while *NextBrain* has 333.

358 A comprehensive comparison between and all digitised 359 sections of the printed atlas by Mai & Paxinos [1] 360 and approximately equivalent sections of the Allen reference 361 brain and *NextBrain* is included in the supplement. The 362 agreement between the three atlases is generally good, 363 especially for the outer boundaries of the 364 whole structures, e.g., the whole hippocampus, amygdala, 365 or thalamus. Mild differences can be found in the 366

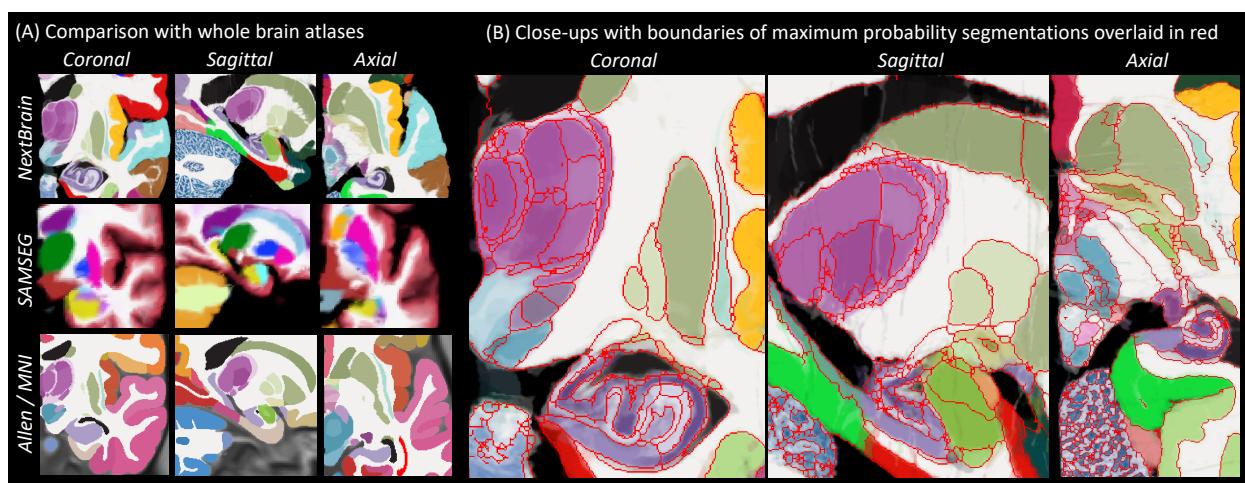


Fig. 4: *NextBrain* probabilistic atlas. (A) Portions of the *NextBrain* probabilistic atlas (which has 333 ROIs), the SAMSEG atlas in FreeSurfer [2] (13 ROIs), and the manual labels of MNI based on the Allen atlas [7] (138 ROIs). (B) Close-up of three orthogonal slices of *NextBrain*. The colour coding follows the convention of the Allen atlas [7], where the hue indicates the structure (e.g., purple is thalamus, violet is hippocampus, green is amygdala) and the saturation is proportional to neuronal density. The colour of each voxel is a weighted sum of the colour corresponding to the ROIs, weighted by the corresponding probabilities at that voxel. The red lines separate ROIs based on the most probable label at each voxel, thus highlighting boundaries between ROIs of similar colour; we note that the jagged boundaries are a common discretization artefact of probabilistic atlases in regions where two or more labels mix continuously, e.g., the two layers of the cerebellar cortex.

370 delineation of sub-structures, both cortical and subcor-
371 tical (e.g., subdivision of the accumbens), mainly due to:
372 (i) the forced choice of applying arbitrary anatomical cri-
373 teria in both atlases due to lack of contrast in smaller
374 regions; (ii) different anatomical definitions; and (iii) the
375 probabilistic nature of NextBrain. We emphasise that
376 these differences are not exclusive to NextBrain, as they
377 are also present between Mai-Paxinos and Allen.

378 Close-ups *NextBrain* slices centred on representative
379 brain regions are shown in Fig. 4B, with boundaries be-
380 tween the ROIs (computed from the maximum likeli-
381 hood segmentation) overlaid in red. These highlight the
382 anatomical granularity of the new atlas, with dozens of
383 subregions for areas such as the thalamus, hippocam-
384 pus, amygdala, midbrain, etc. An overview of the com-
385 plete atlas is shown in Supplementary Video 2, which il-
386 lustrates the atlas construction procedure and flies
387 through all the slices in axial, coronal, and sagittal view.

388 The probabilistic atlas is freely available as part of our
389 segmentation module distributed with FreeSurfer. The
390 maximum likelihood and colour-coded probabilistic
391 maps (as in Fig. 4) can also be downloaded separately
392 from our public repository, for quick inspection and ed-
393 ucational purposes (see Data Availability). Developers of
394 neuroimaging methods can freely capitalise on this re-
395 source, e.g., by extending the atlas via combination with
396 other atlases or manually tracing new labels; or by de-
397 signing their own segmentation methods using the at-
398 lass. Neuroimaging researchers can use the atlas for fine-
399 grained automated segmentation (as shown below), or
400 as a highly detailed CCF for population analyses.

401 **Automated segmentation of ultra-high reso- 402 lution *ex vivo* MRI**

403 One of the new analyses that *NextBrain* enables is the
404 automated fine-grained segmentation of ultra-high-res-
405 olution *ex vivo* MRI. Since motion is not a factor in *ex*
406 *vivo* imaging, very long MRI scanning times can be used
407 to acquire data at resolutions that are infeasible *in vivo*.
408 One example is the publicly available 100 μm isotropic
409 whole brain presented in [3], which was acquired in a
410 100-hour session on a 7T MRI scanner. Such datasets
411 have huge potential in mesoscopic studies connecting
412 microscopy with *in vivo* imaging [66].

413 Volumetric segmentation of ultra-high-resolution *ex*
414 *vivo* MRI can be highly advantageous in neuroimaging in
415 two different manners. First, by supplementing such
416 scans (like the 100-micron brain) with neuroanatomical
417 information that augments their value as atlases, e.g.,
418 as common coordinate frames or for segmentation pur-
419 poses [67]. And second, by enabling analyses of *ex vivo*
420 MRI datasets at scale (e.g., volumetry or shape analysis).

421 Dense manual segmentation of these datasets is prac-
422 tically infeasible, as it entails manually tracing ROIs on
423 over 1,000 slices. Moreover, one typically seeks to label

424 these images at a higher level of detail than *in vivo* (i.e.,
425 more ROIs of smaller sizes), which exacerbates the prob-
426 lem. One may utilise semi-automated methods like the
427 AI-assisted technique we used in to build *NextBrain* (see
428 previous section), which limits the manual segmenta-
429 tion to one every N slices [5] ($N=4$ in this work). How-
430 ever, such a strategy only ameliorates the problem to a
431 certain degree, as tedious manual segmentation is still
432 required for a significant fraction of slices.

433 A more appealing alternative is thus automated seg-
434 mentation. However, existing approaches have limita-
435 tions, as they either: (i) were designed for 1 mm *in vivo*
436 scans and do not capitalise on the increased resolution
437 of *ex vivo* MRI [2,54]; or (ii) utilise neural networks
438 trained with *ex vivo* scans but with a limited number of
439 ROIs, due to the immense labelling effort that is re-
440 quired to generate the training data [61].

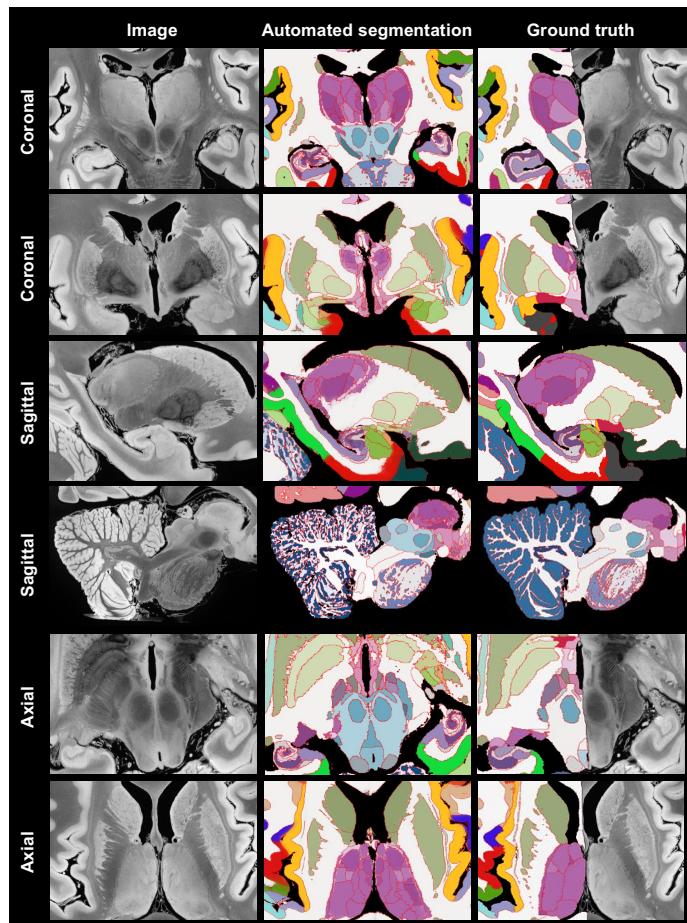
441 This limitation is circumvented by *NextBrain*: as a
442 probabilistic atlas of neuroanatomy, it can be combined
443 with well-established Bayesian segmentation methods
444 (which are adaptive to MRI contrast) to segment ultra-
445 high-resolution *ex vivo* MRI scans into 333 ROIs. We
446 have released in FreeSurfer an implementation that seg-
447 ments full brain scans in approximately 1h, using a desk-
448 top equipped with a graphics processing unit (GPU).

449 To quantitatively evaluate the segmentation method,
450 we have created a ground truth segmentation of the
451 public 100-micron brain [3], which we are publicly re-
452 leasing as part of *NextBrain*. To make this burdensome
453 task practical and feasible, we simplified it in five man-
454 ners: (i) downsampling the data to 200 μm resolution;
455 (ii) labelling only one hemisphere; (iii) using the same
456 semi-automated AI method as in *NextBrain* for faster
457 segmentation; (iv) using FreeSurfer to automatically
458 subdivide the cerebral cortex; and (v) labelling only a
459 subset of 98 visible ROIs (see Supplementary Videos 3
460 and 4). Even with these simplifications, labelling the
461 scan took over 100 hours of manual tracing effort.

462 We compared the ground truth labels with the auto-
463 mated segmentations produced by *NextBrain* using Dice
464 overlap scores. Since the ground truth has fewer ROIs
465 (particularly in the brainstem), we: (i) clustered the ROIs
466 in the automated segmentation that correspond with
467 the ROIs in the ground truth; and (ii) used a version of
468 *NextBrain* in which the brainstem ROIs are simplified to
469 better match those of the ground truth (with 264 labels
470 instead of 333). The results are shown in Extended Data
471 Table 1. As expected, there is a clear link between size
472 and Dice. Larger ROIs like the cerebral white matter or
473 cortex have Dice around 0.9. The smaller ROIs have
474 lower Dice, but very few are below 0.4 – which is enough
475 to *localize* ROIs. We note that the median Dice (0.667) is
476 comparable with that reported by other Bayesian seg-
477 mentation methods for brain subregions [68].

478 Sample slices and their corresponding automated
 479 and manual segmentations are shown in Fig. 5. The ex-
 480 quisite resolution and contrast of the dataset enables
 481 our atlas to accurately delineate a large number of ROIs
 482 with very different sizes, including small nuclei and sub-
 483 regions of the hippocampus, amygdala, thalamus, hypo-
 484 thalamus, midbrain, etc. Differences in label granularity
 485 aside, the consistency between the automated and
 486 ground truth segmentation is qualitatively very strong.

487 To the best of our knowledge, this is the most com-
 488 prehensive dense segmentation of a human brain MRI
 489 scan to date. As *ex vivo* datasets with tens of scans be-
 490 come available [61,69,70], our tool has great potential
 491 in augmenting mesoscopic studies of the human brain.
 492 Moreover, the labelled MRI that we are releasing has
 493 great potential in other neuroimaging studies, e.g., for
 494 training or evaluating segmentation algorithms; for ROI
 495 analysis in the high-resolution *ex vivo* space; or for vol-
 496 umetric analysis via registration-based segmentation.



546 **Fig. 5:** Automated Bayesian segmentation of publicly available ultra-high 546
 547 resolution *ex vivo* brain MRI [3] using the simplified version of *NextBrain*, 547
 548 and comparison with ground truth (only available for right hemisphere). We 548
 549 show two coronal, sagittal, and axial slices. The MRI was resampled to 549
 550 200 μm isotropic resolution for processing. As in previous figures, the seg- 550
 551 mentation uses the Allen colour map [7] with boundaries overlaid in red. 551
 We note that the manual segmentation uses a coarser labelling protocol.

497 **Fine-grained analysis of *in vivo* MRI**

498 *NextBrain* can also be used to automatically segment *in*
 499 *vivo* MRI scans at the resolution of the atlas (200 μm iso-
 500 tropic), yielding an unprecedented level of detail. Scans
 501 used in research typically have isotropic resolution with
 502 voxel sizes ranging from 0.7 to 1.2mm, and therefore do
 503 not reveal all ROI boundaries with as much detail as ultra-
 504 high-resolution *ex vivo* MRI. However, many bound-
 505 aries are still visible, including the external bound-
 506 aries of brain structures (hippocampus, thalamus, etc.) and
 507 some internal boundaries, e.g., between the anterome-
 508 dial and lateral posterior thalamus [14]. Bayesian seg-
 509 mentation capitalises on these visible boundaries and
 510 combines them with the prior knowledge encoded in
 511 the atlas to produce the full subdivision – albeit with
 512 lower reliability for the indistinct boundaries [49]. A
 513 sample segmentation is shown in Fig. 2F.

514 **Evaluation of segmentation accuracy:** We first eval-
 515 uated the *in vivo* segmentation quantitatively in two dif-
 516 ferent experiments. First, we downsampled the *ex vivo*
 517 MRI scan from the previous section to 1 mm isotropic
 518 resolution (i.e., the standard resolution of *in vivo* scans);
 519 segmented it at 200 μm resolution; and computed Dice
 520 scores with the high-resolution ground truth. The re-
 521 sults are displayed in Extended Data Table 1. The me-
 522 dian Dice is 0.590, which is 0.077 lower than at 200 μm ,
 523 but still fair for such small ROIs [68]. Moreover, most
 524 Dice scores remain 0.4, as for the ultra-high resolution,
 525 hinting that the priors can successfully provide a rough
 526 localization of internal boundaries, given the more visi-
 527 ble external boundaries.

528 In a second experiment, we analysed the Dice scores
 529 produced by *NextBrain* in OpenBHB [4], a public meta-
 530 dataset with \sim 1 mm isotropic T1-weighted scans of
 531 over 3,000 healthy individuals acquired at over 60 sites.
 532 Using FreeSurfer 7.0 as a silver standard, we computed
 533 Dice scores for our segmentations at the level of whole
 534 regions, i.e., the level of granularity provided by Free-
 535 Surfer. While these scores cannot assess segmentation
 536 accuracy at the subregion level, they do enable evalua-
 537 tion on a much larger multi-site cohort, as well as com-
 538 parison with the Allen MNI template – the only compet-
 539 ing histological (or rather, histology-inspired) atlas that
 540 can segment the whole brain *in vivo* (Fig. 1). The results
 541 (Extended Data Fig. 6) show that: (i) *NextBrain* con-
 542 stantly outperform the Allen MNI template, as expected
 543 from the fact that one atlas is probabilistic while the
 544 other is not; and (ii) *NextBrain* yields Dice scores in the
 545 range expected from Bayesian segmentation meth-
 546 ods [2] – despite using only five cases, thanks to the ex-
 547 cellent generalization ability of generative models [71].

548 **Application to Alzheimer's disease (AD) classification:**
 549 To further compare *NextBrain* with the Allen MNI tem-
 550 plate, we used an AD classification task based on linear
 551 discriminant analysis (LDA) of ROI volumes (corrected by

552 age and intracranial volume). Using a simple linear clas-
 553 sifier on a task where strong differences are expected
 554 allows us to use classification accuracy as a proxy for the
 555 quality of the input features, i.e., the ROI volumes de-
 556 rived from the automated segmentations. To enable di-
 557 rect comparison, we used a sample of 383 subjects from
 558 the ADNI dataset [72] (168 AD, 215 controls) that we
 559 used in previous publications [14,49,50].

560 Using the ROI volumes estimated by FreeSurfer 7.0
 561 (which do *not* include subregions) yields and area under
 562 the receiver operating characteristic curve (AUROC)
 563 equal to 0.911, which classification accuracy of 85.4% at
 564 its elbow. The Allen MNI template exploits subregion in-
 565 formation to achieve AUROC = 0.929 and 86.9% accu-
 566 racy. The increased segmentation accuracy and granu-
 567 larity of NextBrain enables it to achieve AUROC = 0.953
 568 and 90.3% accuracy – with a significant increase in
 569 AUROC with respect to the Allen MNI template ($p = 0.01$
 570 for a DeLong test). This AUROC is also superior to those
 571 of specific *ex vivo* atlases we have presented in the prior
 572 work [14,49,50] – which range from 0.830 to 0.931

573 **Application to fine-grained signature of aging:** We per-
 574 formed Bayesian segmentation with *NextBrain* on 705
 575 subjects (aged 36-90, mean 59.6 years) from the Ageing
 576 HCP dataset [73], which comprises high-quality *in vivo*
 577 scans at 0.8mm resolution. We computed the volumes
 578 of the ROIs for every subject, corrected them for total

579 intracranial volume (by division) and sex (by regression),
 580 and computed their Spearman correlation with age. We
 581 used the Spearman rather than Pearson correlation be-
 582 cause, being rank-based, it is a better model for ageing
 583 trajectories as they are known to be nonlinear for wide
 584 age ranges [74,75].

585 The result of this analysis is, to the best of our
 586 knowledge, the most comprehensive map of regional
 587 ageing of the human brain to date (Fig. 6A and Extended
 588 Data Fig. 7A; see also full trajectories for select ROIs in
 589 Extended Data Fig. 8). Cortically, we found significant
 590 negative correlations with age in the prefrontal cortex
 591 (marked with 'a' on the figure) and insula (b), whilst the
 592 temporal (c) and parahippocampal cortices (d) did not
 593 yield significant correlation; this is consistent with find-
 594 ings from studies of cortical thickness [38,76]. The white
 595 matter (e) is known to decline steadily after ~ 35
 596 years [74,75], and such negative correlation is also de-
 597 tected by *NextBrain*. Other general ageing patterns at
 598 the whole structure level [74,75] are also successfully
 599 captured, such as a steady volume decrease of the cau-
 600 date, thalamus, or putamen (f), or the volumetric reduc-
 601 tion of the hippocampus, amygdala, or globus pallidus.

602 Importantly, *NextBrain* also unveils more granular
 603 patterns of the relationship between volumes and age-
 604 ing within these regions. For example, the anterior cau-

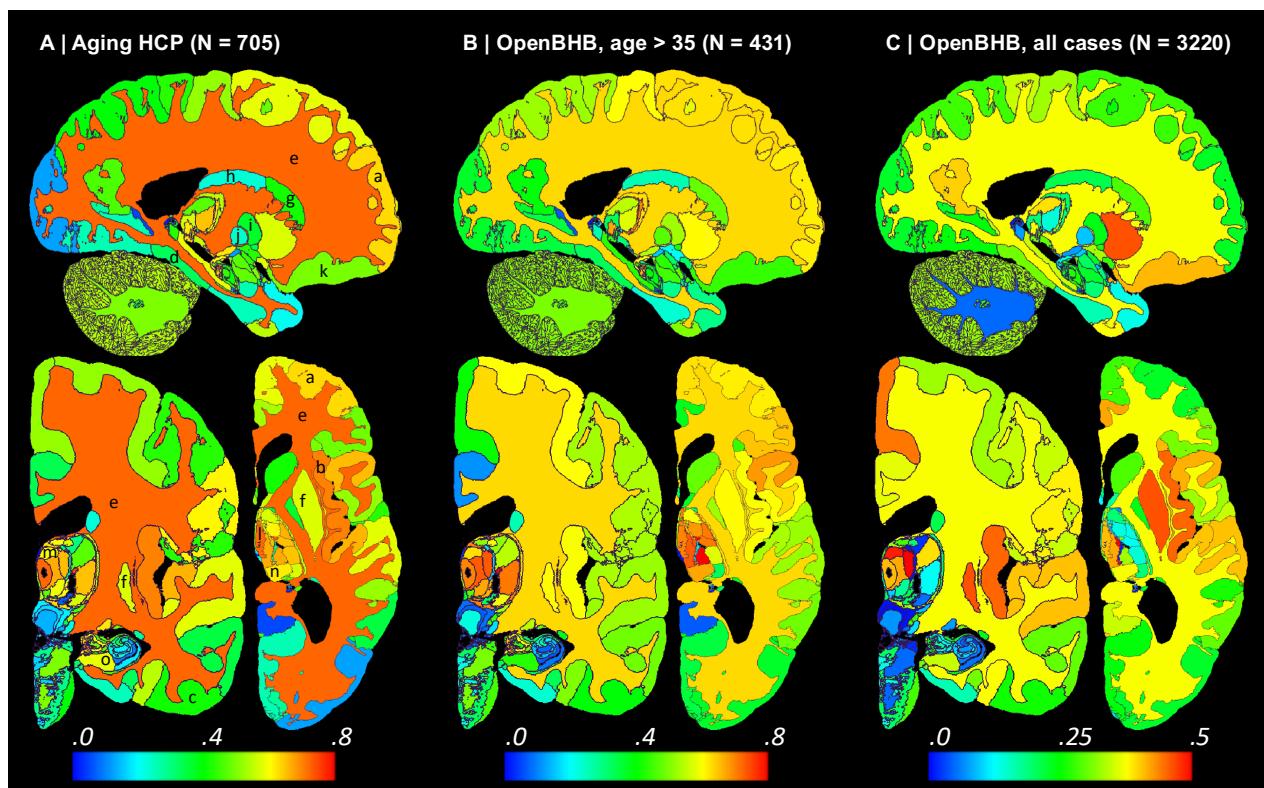


Fig. 6: Absolute value of Spearman correlation for ROI volumes vs age derived from *in vivo* MRI scans: (A) Ageing HCP dataset (image resolution: .8mm isotropic; age range: 36-90 years; mean age: 59.6 years); please see main text for meaning of markers (letters). (B) OpenBHB dataset [4], restricted to subjects with ages over 35 years to match Ageing HCP (resolution 1 mm isotropic; age range: 36-86 years; mean age: 57.9 years). (C) Full OpenBHB dataset (age range: 6-86 years, mean age: 25.2 years); please note the different scale of the colour bar. The ROI volumes are corrected by intracranial volume (by division) and sex (by regression). Further slices are shown in Extended Data Fig. 6.

605 date (g) showed a stronger negative correlation be-
606 tween age and volume than the posterior caudate (h).
607 Similarly, the external segment of the globus pallidus (i)
608 showed a stronger correlation than the internal seg-
609 ment (j) – an effect that was not observed in previous
610 work studying the whole pallidum [77]. The ability to in-
611 vestigate separate subregions highlights a differential
612 effect of ageing across brain networks, particularly a
613 stronger effect on the regions of the limbic and prefron-
614 tal networks, given the correlations we found in the cau-
615 date head (g), insula (b), orbitofrontal cortex (k), amyg-
616 dala, and thalamus [78]. Within the thalamus, the
617 correlation is more significant in the mediodorsal (l), an-
618 teroventral (m), and pulvinar subnuclei (n), key regions
619 in the limbic, lateral orbitofrontal and dorsolateral pre-
620 frontal circuits. In the hippocampus, subiculum regions
621 (o) correlate more strongly than the rest of the struc-
622 ture. The pattern of correlation strength is more homo-
623 geneous across subregions in the amygdala (key region
624 in the limbic system), hypothalamus, and cerebellum.

625 We then revisited the OpenBHB dataset and per-
626 formed the same regression analysis only for subjects
627 older than 35 years, to match the age range of the Age-
628 ing HCP dataset (N=431, aged 36-86 years, mean 57.9
629 years). The results are shown in Fig. 6B and Extended
630 Data Fig. 7B. Despite the differences in acquisition and
631 the huge heterogeneity of the OpenBHB dataset, the re-
632 sults are highly consistent with those from HCP – but
633 with slightly lower significance, possibly due to the in-
634 creased voxel size (twice as big, since $1/0.8^3 \approx 2$).

635 We also performed the same analysis with all 3,220
636 subjects in OpenBHB; see results in Fig. 6C and Extended
637 Data Fig. 7C. For many regions, widening the age range
638 to 6-86 years (mean age: 25.2) yields non-monotonic
639 ageing curves and therefore weaker Spearman corre-
640 lations. Therefore, these graphs highlight the regions
641 whose volumes start decreasing with age the earliest,
642 such as the putamen or medial thalamus. Many other
643 patterns of association between age and ROI volumes
644 remain very similar to those of the older populations
645 (e.g., basal ganglia or hippocampus).
646 The segmentation code is publicly available in Free-
647 Surfer: <https://surfer.nmr.mgh.harvard.edu/fswiki/His->
648 toAtlasSegmentation and can be run with a single line of
649 code. This enables researchers worldwide to analyse
650 their scans at a superior level of detail without manual
651 effort or highly specific neuroanatomical knowledge.

652 Discussion and Conclusion

653 *NextBrain* is a next-generation probabilistic human
654 brain atlas, which is publicly available and distributed
655 with a companion Bayesian segmentation tool and
656 multi-modal dataset. The dataset itself is already a
657 highly valuable resource: researchers have free access
658 to both the raw and registered data, which they can use
659 for their own research (e.g., in MRI signal modelling or

660 registration), or to augment the atlas with new ROIs,
661 e.g., by labelling them on the histology or MRI data and
662 rebuilding the atlas. The atlas itself is a novel, high-res-
663 olution common coordinate frame for population anal-
664 yses. The 3D segmentation of 100 μm *ex vivo* brain MRI
665 scan [3] is a valuable complement to this (already very
666 useful) resource. Finally, the Bayesian tool enables seg-
667 mentation of *ex vivo* and *in vivo* MRI at an unprece-
668 dented level of granularity.

669 Due to its volumetric and semantic nature, *NextBrain*
670 can be complemented by other segmentation methods
671 and atlases that describe other aspects of the brain. For
672 example, more accurate cortical segmentation and par-
673 cellation can be achieved with surface models [79]. We
674 are currently working on models that combine neural
675 networks with geometry processing to obtain laminar
676 segmentations from both *in vivo* and *ex vivo*
677 scans [80,81]. Surface placement will also warrant com-
678 patibility with cortical atlases obtained with multi-
679 modal data [43].

680 *NextBrain* is extensible: since all the data and code are
681 publicly available, it is possible to download the data,
682 modify (or extend) the manual annotations, and then
683 rerun all the scripts to build a custom atlas. However,
684 these tasks require domain expertise and compute
685 power. Automatising this process to make it more ac-
686 cessible is desirable, but also quite a large engineering
687 endeavour – and thus remains as future work.

688 The Bayesian segmentation tool in *NextBrain* is com-
689 patible with 1 mm isotropic scans, as illustrated by the
690 Alzheimer's and aging experiments. As with other prob-
691 abilistic atlases, Bayesian segmentation can be aug-
692 mented with models of pathology to automatically seg-
693 ment pathology, such as tumours [82] or white matter
694 hyperintensities [83]. Importantly, *NextBrain*'s high
695 level of detail enables us to fully take advantage of high-
696 resolution data, such as *ex vivo* MRI, ultra-high field MRI
697 (e.g., 7T), and exciting new modalities like HIP-CT [84].
698 As high-quality 3D brain images become increasingly
699 available, *NextBrain*'s ability to analyse them with su-
700 perior granularity holds great promise to advance
701 knowledge about the human brain in health and in dis-
702 ease.

703 Methods

704 Brain specimens

705 Hemispheres from five individuals (including half of the
706 cerebrum, cerebellum, and brainstem), were used in
707 this study, following informed consent to use the tissue
708 for research and the ethical approval for research by the
709 National Research Ethics Service (NRES) Committee Lon-
710 don-Central. All hemispheres were fixed in 10% neutral
711 buffered formalin (Fig. 2A). The laterality and de-
712 mographics are summarised in Table 1 below; the do-
713 nors were neurologically normal, but one case had an
714 undiagnosed, asymptomatic tumour (diameter:

715 ~10mm) in the white matter, adjacent to the pars opercularis. This tumour did not pose issues in any of the
716 processing steps described below.

718 **Table 1** Demographics of the five cases used in this study.

Case	Age at death	Sex	Laterality	Incidental findings
Case 1	94 years	Female	Right	None
Case 2	84 years	Female	Right	None
Case 3	80 years	Male	Right	None
Case 4	79 years	Female	Left	Tumour adjacent to pars opercularis
Case 5	78 years	Male	Left	None

719 **Data acquisition**

720 Our data acquisition pipeline largely leverages our previous work [6]. We summarise it here for completeness;
721 the reader is referred to the corresponding publication
722 for further details.

723 - *MRI scanning*. Prior to dissection, the hemispheres
724 were scanned on a 3T Siemens MAGNETOM Prisma
725 scanner. The specimens were placed in a container filled
726 with Fluorinert (perfluorocarbon), a proton-free fluid
727 with no MRI signal that yields excellent *ex vivo* MRI contrast
728 and does not affect downstream histological analysis
729 [85]. The MRI scans were acquired with a T2-
730 weighted sequence (optimised long echo train 3D fast
731 spin echo [86]) with parameters: TR = 500 ms, TEeff =
732 69ms, BW = 558 Hz/Px, echo spacing = 4.96ms, echo
733 train length = 58, 10 averages, with 400 μ m isotropic
734 resolution, acquisition time for each average = 547s, total
735 scanning time = 91 min. These scans were processed
736 with a combination of SAMSEG [2] and the FreeSurfer
737 7.0 cortical stream [79] to bias field correct the images,
738 generate rough subcortical segmentations, and obtain
739 white matter and pial surfaces with corresponding par-
740 cellations according to the Desikan-Killiany atlas [60]
741 (Fig. 2B).

742 - *Dissection*. After MRI scanning, each hemisphere is dis-
743 sected to fit into standard 74x52mm cassettes. First,
744 each hemisphere was split into cerebrum, cerebellum,
745 and brainstem. Using a metal frame as a guide, these
746 were subsequently cut into 10mm-thick slices in coro-
747 nal, sagittal, and axial orientation, respectively. These
748 slices were photographed inside a rectangular frame of
749 known dimensions for pixel size and perspective correc-
750 tion; we refer to these images as “whole slice photo-
751 graphs.” While the brainstem and cerebellum slices all
752 fit into the cassettes, the cerebrum slices were further
753 cut into as many blocks as needed. “Blocked slice photo-
754 graphs” were also taken for these blocks (Fig. 2C, left).

755 - *Tissue processing and sectioning*. After standard tissue
756 processing steps, each tissue block was embedded in
757 paraffin wax and sectioned with a sledge microtome at
758 25 μ m thickness. Before each cut, a photograph was

760 taken with a 24MPx Nikon D5100 camera (ISO = 100, ap-
761 erture = f/20, shutter speed = automatic) mounted right
762 above the microtome, pointed perpendicularly to the
763 sectioning plane. These photographs (henceforth
764 “blockface photographs”) were corrected for pixel size
765 and perspective using fiducial markers. The blockface
766 photographs have poor contrast between grey and
767 white matter (Fig. 2C, right) but also negligible nonlinear
768 geometric distortion, so they can be readily stacked into
769 3D volumes. A 2D convolutional neural network (CNN)
770 pretrained on the ImageNet dataset [87] and fine-tuned
771 on 50 manually labelled examples was used to automati-
772 cally produce binary tissue masks for the blockface im-
773 ages.

774 - *Staining and digitisation*. We mounted on glass slides
775 and stained two consecutive sections every N (see be-
776 low), one with Hematoxylin and Eosin (H&E) and one
777 with Luxol Fast Blue (LFB); see Fig. 2D. The sampling in-
778 terval was $N=10$ (i.e., 250 μ m) for blocks that included
779 subcortical structures in the cerebrum, medial struc-
780 tures of the cerebellum, or brainstem structures. The in-
781 terval was $N=20$ (500 μ m) for all other blocks. All stained
782 sections were digitised with a flatbed scanner at 6,400
783 DPI resolution (pixel size: 3.97 μ m). Tissue masks were
784 generated using a 2D CNN similar to the one used for
785 blockface photographs (pretrained on ImageNet and
786 finetuned on 100 manually labelled examples).

787 **Dense labelling of histology**

788 Segmentations of 333 ROIs (34 cortical, 299 subcortical)
789 were made by authors ER, JA, and EB (with guidance
790 from DK, MB, JZ, and JCA) for all the LFB sections, using
791 a combination of manual and automated techniques
792 (Fig. 2E). The general procedure to label each block was:
793 (i) produce an accurate segmentation for one of every
794 four sections; (ii) run SmartInterpol [5] to automatically
795 segment the sections in between; and (iii) manually cor-
796 rect these automatically segmented sections when
797 needed. SmartInterpol is a dedicated AI technique that
798 we have developed specifically to speed up segmenta-
799 tion of histological stacks in this project.

800 To obtain accurate segmentations on sparse sections,
801 we used two different strategies depending on the brain
802 region. For the blocks containing subcortical or brain-
803 stem structures, ROIs were manually traced from
804 scratch using a combination of ITK-SNAP [88] and Free-
805 Surfer’s viewer “Freeview”. For cerebellum blocks, we
806 first trained a 2D CNN (a U-Net [89]) on 20 sections on
807 which we had manually labelled the white matter and
808 the molecular and granular layers of the cortex. The
809 CNN was then run on the (sparse) sections, and the out-
810 puts manually corrected. This procedure saves a sub-
811 stantial amount of time, since manually tracing the con-
812 voluted shape of the arbor vitae is extremely time
813 consuming. For the cortical cerebrum blocks, we used a
814 similar strategy as for the cerebellum, labelling the tis-
815 sue as either white or grey matter. The subdivision of

816 the cortical grey matter into parcels was achieved by
817 taking the nearest neighbouring cortical label from the
818 aligned MRI scan (details on the alignment below).

819 The manual labelling followed neuroanatomical pro-
820 tocols based on different brain atlases, depending on
821 the brain region. Further details on the specific delineation
822 protocols are provided in the Supplementary Meth-
823 ods. The general ontology of the 333 ROIs is based on
824 the Allen reference brain [7], and is provide in a spread-
825 sheet as part of the Supplementary Data.

826 **3D histology reconstruction**

827 3D histology reconstruction is the inverse problem of re-
828 versing all the distortion that brain tissue undergoes
829 during acquisition, in order to reassemble a 3D shape
830 that accurately follows the original anatomy. For this
831 purpose, we used a framework with four modules.

832 - *Initial blockface alignment*. In order to roughly initialise
833 the 3D reconstruction, we relied on the stacks of block-
834 face photographs. Specifically, we used our previously
835 presented hierarchical joint registration framework [58]
836 that seeks to: (i) align each block to the MRI with a sim-
837 ilarity transform, by maximising the normalised cross-
838 correlation of their intensities; while (ii) discouraging
839 overlap between blocks or gaps in between, via a differ-
840 entiable regulariser. The similarity transforms allowed
841 for rigid deformation (rotation, translation), as well as
842 isotropic scaling to model the shrinking due to tissue
843 processing. The registration algorithm was initialised
844 with transforms derived from the whole slice, blocked
845 slice, and blockface photographs (see details in [6]). The
846 registration was hierarchical in the sense that groups of
847 transforms were forced to share the same parameters
848 in the earlier iterations of the optimisation, to reflect
849 our knowledge of the cutting procedure. In the first iter-
850 ations, we clustered the blocks into three groups: cere-
851 brum, cerebellum, and brainstem. In the following iter-
852 ations, we clustered the cerebral blocks that were cut
853 from the same slice, and allowed translations in all di-
854 rections, in-plane rotation, and global scaling. In the fi-
855 nal iterations, each block alignment was optimised inde-
856 pendently. The numerical optimisation used the LBFGS
857 algorithm [90]. The approximate average error after this
858 procedure was ~2mm [58]. A sample 3D reconstruction
859 is shown in Fig. 2F.

860 - *Refined alignment with preliminary nonlinear model*.
861 Once a good initial alignment is available, we can use the
862 LFB sections to refine the registration. These LFB images
863 have exquisite contrast (Fig. 2D) but suffer from nonlin-
864 ear distortion – rendering the good initialization from
865 the blockface images crucial. The registration procedure
866 was nearly identical to that of the blockface, with two
867 main differences. First, the similarity term used the local
868 (rather than global) normalised cross-correlation func-
869 tion [91] in order to handle uneven staining across sec-
870 tions. Second, the deformation model and optimisation

871 hierarchy were slightly different since nonlinear regis-
872 tration benefits from more robust methods. Specifically:
873 The first two levels of optimisation were the same, with
874 blocks grouped into cerebrum/cerebellum/brainstem
875 (first level) or cerebral slices (second level), and optimi-
876 sation of similarity transforms. The third level (i.e., each
877 block independently) was subdivided into four stages in
878 which we optimised transforms with increasing com-
879 plexity, such that the solution of every level of complex-
880 ity serves as initialisation to the next. In the first and
881 simplest level, we allowed for translations in all direc-
882 tions, in-plane rotation and global scaling (5 parameters
883 per block). In the second level, we added a different
884 scaling parameter in the normal direction of the block (6
885 parameters per block). In the third level, we allowed for
886 rotation in all directions (8 parameters per block). In the
887 fourth and final level, we added to every section in every
888 block a nonlinear field modelled with a grid of control
889 points (10mm spacing) and interpolating B-splines. This
890 final deformation model has approximately 100,000 pa-
891 rameters per case (~100 parameters per section, times
892 ~1,000 LFB sections).

893 - *Nonlinear AI registration*. We seek to produce final
894 nonlinear registrations that are accurate, consistent
895 with each other, and robust against tears and folds in
896 the sections. We capitalise on Synth-by-Reg (SbR [10]),
897 an AI tool for multimodal registration that we have re-
898 cently developed, to register histological sections to
899 MRI slices resampled to the plane of the histology (as
900 estimated by the linear alignment). SbR exploits the
901 facts that: (i) intra-modality registration is more accu-
902 rate than inter-modality registration [92]; and (ii) there
903 is a correspondence between histological sections and
904 MRI slices, i.e., they represent the same anatomy. In
905 short, SbR trains a CNN to make histological sections
906 look like MRI slices (a task known as style transfer [93]),
907 using a second CNN that has been previously trained to
908 register MRI slices to each other. The style transfer re-
909 lies on the fact that only good MRI synthesis will yield a
910 good match when used as input to the second CNN,
911 combined with a contrastive loss [94] that prevents
912 blurring and content shift due to overfitting. SbR pro-
913 duces highly accurate deformations parameterised as
914 stationary velocity fields (SVF [95]).

915 - *Bayesian refinement*. Running SbR for each stain and
916 section independently (i.e., LFB to resampled MRI, and
917 H&E to resampled MRI) yields a reconstruction that is
918 jagged and sensitive to folds and tears. One alternative
919 is to register each histological section to each neighbour
920 directly, which achieves smooth reconstructions but in-
921 curs the so-called “banana effect”, i.e., a straightening
922 of curved structures [52]. We have proposed a Bayesian
923 method that yields smooth reconstructions without ba-
924 nana effect [9]. This method follows an overconstrained
925 strategy, by computing registrations between: LFB and
926 MRI; H&E and MRI; H&E and LFB; each LFB section and

927 the two nearest neighbours in either direction across
928 the stack; each H&E section and its neighbours; and
929 each MRI slice and its neighbours. For a stack with S sec-
930 tions, this procedure yields $15 \times S - 18$ registrations, while
931 the underlying dimensionality of the spanning tree con-
932 necting all the images is just $3 \times S - 1$. We use a probabilis-
933 tic model of SVFs to infer the most likely spanning tree
934 given the computed registrations, which are seen as
935 noisy measurements of combinations of transforms in
936 the spanning tree. The probabilistic model uses a La-
937 place distribution, which relies on L1 norms and is thus
938 robust to outliers. Moreover, the properties of SVFs en-
939 able us to write the optimization problem as a linear
940 program, which we solve with a standard simplex algo-
941 rithm [96]. The result of this procedure was a 3D recon-
942 struction that is accurate (it is informed by many regis-
943 trations), robust, and smooth (Figures 2G and 3).

944 **Atlas construction**

945 The transforms for the LFB sections produced by the 3D
946 reconstructions were applied to the segmentations to
947 bring them into 3D space. Despite the regulariser
948 from [58], minor overlaps and gaps between blocks still
949 occur. The former were resolved by selecting the label
950 which is furthest inside the corresponding ROI. For the
951 latter, we used our previously developed smoothing ap-
952 proach [14].

953 Given the low number of available cases, we com-
954 bined the left (2) and right (3) hemispheres into a single
955 atlas. This was achieved by flipping the right hemi-
956 spheres and computing a probabilistic atlas of the left
957 hemisphere using an iterative technique [64]. To initial-
958 ise the procedure, we registered the MRI scans to the
959 MNI atlas [53] with the right hemisphere masked out,
960 and averaged the deformed segmentations to obtain an
961 initial estimate of the probabilistic atlas. This first regis-
962 tration was based on intensities, using a local normal-
963 ised cross-correlation loss. From that point on, the al-
964 gorithm operates exclusively on the segmentations.

965 Every iteration of the atlas construction process com-
966 prises two steps. First, the current estimate of the atlas
967 and the segmentations are co-registered one at the
968 time, using: (i) a diffeomorphic deformation model
969 based on SVFs parameterised by grids of control points
970 and B-splines (as implemented in NiftyReg [97]), which
971 preserves the topology of the segmentations; (ii) a data
972 term, which is the log-likelihood of the label at each
973 voxel according to the probabilities given by the de-
974 formed atlas (with a weak Dirichlet prior to prevent logs
975 of zero); and (iii) a regulariser based on the bending en-
976 ergy of the field, which encourages regularity in the de-
977 formations. The second step of each iteration updates
978 the atlas by averaging the segmentations. The proce-
979 dure converged (negligible change in the atlas) after five
980 iterations. Slices of the atlas are shown in Figs. 2H and 4.

981 **Bayesian segmentation**

982 Our Bayesian segmentation algorithm builds on well-es-
983 tablished methods in the neuroimaging litera-
984 ture [54, 98, 99]. In short, the algorithm jointly estimates
985 a set of parameters that best explain the observed im-
986 age in light of the probabilistic atlas, according to a gen-
987 erative model based on a Gaussian mixture model
988 (GMM) conditioned on the segmentation, combined
989 with a model of bias field. The parameters include the
990 deformation of the probabilistic atlas, a set of coeffi-
991 cients describing the bias field, and the means, vari-
992 ances, and weights of the GMM. The atlas deformation
993 is regularised in the same way as the atlas construction
994 (bending energy, in our case) and is estimated via nu-
995 merical optimisation with LBFGS. The bias field and
996 GMM parameters are estimated with the Expectation
997 Maximisation algorithm [100].

998 Compared with classical Bayesian segmentation
999 methods operating at 1 mm resolution with just a few
1000 classes (e.g., SAMSEG [2], SPM [54]), our proposed
1001 method has several distinct features:

- 1002 • Since the atlas only describes the left hemi-
1003 sphere, we use a fast deep learning registration
1004 method (EasyReg [101]) to register the input
1005 scan to MNI space, and use the resulting defor-
1006 mation to split the brain into two hemispheres
1007 that are processed independently.
- 1008 • Since the atlas only models brain tissue, we run
1009 SynthSeg [102] on the input scan to mask out
1010 the extracerebral tissue.
- 1011 • Clustering ROIs into tissue types (rather than let-
1012 ting each ROI have its own Gaussian) is particu-
1013 larly important, given the large number of ROIs
1014 (333). The user can specify the clustering via a
1015 configuration file; by default, our public imple-
1016 mentation uses a configuration with 15 tissue
1017 types, tailored to *in vivo* MRI segmentation.
- 1018 • The framework is implemented using the
1019 PyTorch package, which enables it to run on
1020 GPUs and cuts segmentation run times to
1021 about half an hour per hemisphere.

1022 Sample segmentations with this method can be found in
1023 Figures 2H (*in vivo*) and 5 (*ex vivo*).

1024 **Labelling of ultra-high resolution *ex vivo* brain MRI and** 1025 **simplified version of *NextBrain* atlas**

1026 In order to quantitatively assess the accuracy of our seg-
1027 mentation method on the ultra-high resolution *ex vivo*
1028 scan, we produced a gold standard segmentation of the
1029 publicly available 100 μm scan [3] as follows. First, we
1030 downsampled the data to 200 μm resolution and dis-
1031 carded the left hemisphere, to alleviate the manual la-
1032 belling requirements. Next, we used *Freeview* to man-
1033 ually label from scratch one coronal slice every 10; we
1034 labelled as many regions from the histological protocol
1035 as the MRI contrast allowed – without subdividing the
1036 cortex. Then, we used *SmartInterpol* [5] to complete the
1037 segmentation of the missing slices. Next, we manually

1038 corrected the SmartInterpol output as needed, until we
1039 were satisfied with the 200 μm isotropic segmentation.
1040 The cortex was subdivided using standard FreeSurfer
1041 routines. This labelling scheme led to a ground truth
1042 segmentation with 98 ROIs, which we have made pub-
1043 licly available (details under “Data Availability”). Supple-
1044 mentary Videos 3 and 4 fly over the coronal and axial
1045 slices of the labelled scan, respectively.

1046 As explained in the Results section, we used a simpli-
1047 fied version of the *NextBrain* atlas when segmenting the
1048 100 μm scan, in order to better match the ROIs of the
1049 automated segmentation and the ground truth (espe-
1050 cially in the brainstem). This version was created by re-
1051 placing the brainstem labels in the histological 3D recon-
1052 struction (Fig. 2G, right) by new segmentations made
1053 directly in the underlying MRI scan. These segmenta-
1054 tions were made with the same methods as for the
1055 100 μm isotropic scan. The new combined segmen-
1056 tions were used to rebuild the atlas.

1057 **Automated segmentation with Allen MNI template**

1058 Automated labelling with the Allen MNI template relied
1059 on registration-based segmentation with the NiftyReg
1060 package [65,97], which yields state-of-the art perfor-
1061 mance in brain MRI registration [103]. We used the
1062 same deformation model and parameters as the Nif-
1063 tityReg authors used in their own registration-based seg-
1064 mentation work [104]: (i) symmetric registration with a
1065 deformation model parameterised by a grid of control
1066 points (spacing: 2.5 mm = 5 voxels) and B-spline inter-
1067 polation; (ii) local normalised cross correlation as objec-
1068 tive function (standard deviation: 2.5mm); and
1069 (iii) bending energy regularisation (relative weight:
1070 0.001).

1071 **Linear discriminant analysis (LDA) for AD classification**

1072 Linear classification of AD vs controls based on ROI vol-
1073 umes was performed as follows. Leaving one subject out
1074 at the time, we used all other subjects to: (i) compute
1075 linear regression coefficients to correct for sex and age
1076 (intracranial volume was corrected by division); (ii) esti-
1077 mate mean vectors for the two classes ($\bar{\mu}_0, \bar{\mu}_1$), as well
1078 as a pooled covariance matrix (Σ); and (iii) use the
1079 means and covariance to compute an unbiased log-like-
1080 hood criterion L for the left-out subject:

$$1081 L(\bar{x}) = (\bar{\mu}_1 - \bar{\mu}_0)^t \Sigma^{-1} [\bar{x} - 0.5 (\bar{\mu}_1 + \bar{\mu}_0)],$$

1082 where \bar{x} is the vector with ICV-, sex-, and age-corrected
1083 volumes for the left-out subject. Once the criterion L
1084 has been computed for all subjects, we it can be globally
1085 thresholded for accuracy and ROC analysis. We note
1086 that, for *NextBrain*, the high number of ROIs renders the
1087 covariance matrix singular. We prevent this by using
1088 regularised LDA: we normalise all the ROIs to unit vari-
1089 ance and then compute the covariance as $\Sigma = S + \lambda I$,
1090 where S is the sample covariance, I is the identity ma-
1091 trix, and $\lambda = 1.0$ is a constant. We note that normalizing

1092 to unit variance enables us to use a fixed, unit λ – rather
1093 than having to estimate λ for every left-out subject.

1094 **B-spline fitting of aging trajectories**

1095 To compute the B-spline fits in Extended Data Fig. 8, we
1096 first corrected the ROI volumes by sex (using regression)
1097 and intracranial volume (by division). Next, we modelled
1098 the data with a Laplace distribution, which is robust
1099 against outliers which may be caused by potential seg-
1100 mentation mistakes. Specifically, we used an age-de-
1101 pendent Laplacian where the location μ and scale b are
1102 both B-splines with four evenly space control points at
1103 30, 51.6, 73.3, and 95 years. The fit is optimised with
1104 gradient ascent over the log-likelihood function:

$$1105 L(\theta_\mu, \theta_b) = \sum_{n=1}^N \log p[v_n; \mu(a_n; \theta_\mu), b(a_n; \theta_b)],$$

1106 where $p(x; \mu, b)$ is the Laplace distribution with loca-
1107 tion μ and scale b ; v_n is the volume of ROI for subject n ;
1108 a_n is the age of subject n ; $\mu(a_n; \theta_\mu)$ is a B-spline de-
1109 scribing the location, parameterised by θ_μ ; and
1110 $b(a_n; \theta_b)$ is a B-spline describing the scale, parameter-
1111 ised by θ_b . The 95% confidence interval of the Laplace
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1119 **Author contributions**

- 1120 - *Conceptualization*: JCA, BLE, JLH, ZJ, JEI
- 1121 - *Data curation*: AC, MM, ER, JA, SC, EB, BB, AA, LZ, DLT,
1122 DK, MB
- 1123 - *Formal analysis*: AC, LZ, JEI
- 1124 - *Funding acquisition*: JEI
- 1125 - *Investigation*: AC, MM, ER, LP, RA, JA, SC, EB, LZ, JEI
- 1126 - *Methodology*: AC, MM, OP, YB, JLH, ZJ, JEI
- 1127 - *Project administration*: ER, JLH, CS, ZJ, JEI
- 1128 - *Resources*: DLT, JLH, CS, ZJ
- 1129 - *Software*: AC, MM, BB, AA, OP, YB, PS, JH, JEI
- 1130 - *Supervision*: ER, LP, DK, MB, JLH, CS, ZJ, JEI
- 1131 - *Validation*: AC, JEI
- 1132 - *Visualisation*: AC, PS, JH, JEI
- 1133 - *Writing – original draft*: AC, ER, JEI
- 1134 - *Writing – review & editing*: all authors.

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1148	Data availability	1198	Yacoub, E. <i>et al.</i> Imaging brain function in humans at 7 Tesla. <i>Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine</i> 45 , 588–594 (2001).
1149	The raw data used in this article (MRI, histology, segmentations, etc.) can be downloaded from: https://doi.org/10.5522/04/24243835	1199	Griswold, M. A. <i>et al.</i> Generalized autocalibrating partially parallel acquisitions
1150		1200	
1151		1201	
1152	An online tool to interactively explore the 3D reconstructed data can be found here: https://github-pages.ucl.ac.uk/NextBrain	1202	
1153		1203	
1154		1204	
1155	This website also includes links to videos, publications, code, and other resources.	1205	
1156		1206	
1157	The segmentation of the <i>ex vivo</i> scan can be found at: https://openneuro.org/datasets/ds005422/versions/1.0.1	1207	
1158		1208	
1159		1209	
1160	Code availability	1210	
1161	The code used in this article for 3D histology reconstruction can be downloaded from: https://github.com/acasamitjana/ERC_reconstruction and used and distributed freely.	1211	
1162		1212	
1163		1213	
1164		1214	
1165	The segmentation tool is provided as Python code and is integrated in our neuroimaging toolkit “FreeSurfer”: https://surfer.nmr.mgh.harvard.edu/fswiki/HistoAtlas-Segmentation . The source code is available on GitHub: https://github.com/freesurfer/freesurfer/tree/dev/mri_histo_util	1215	
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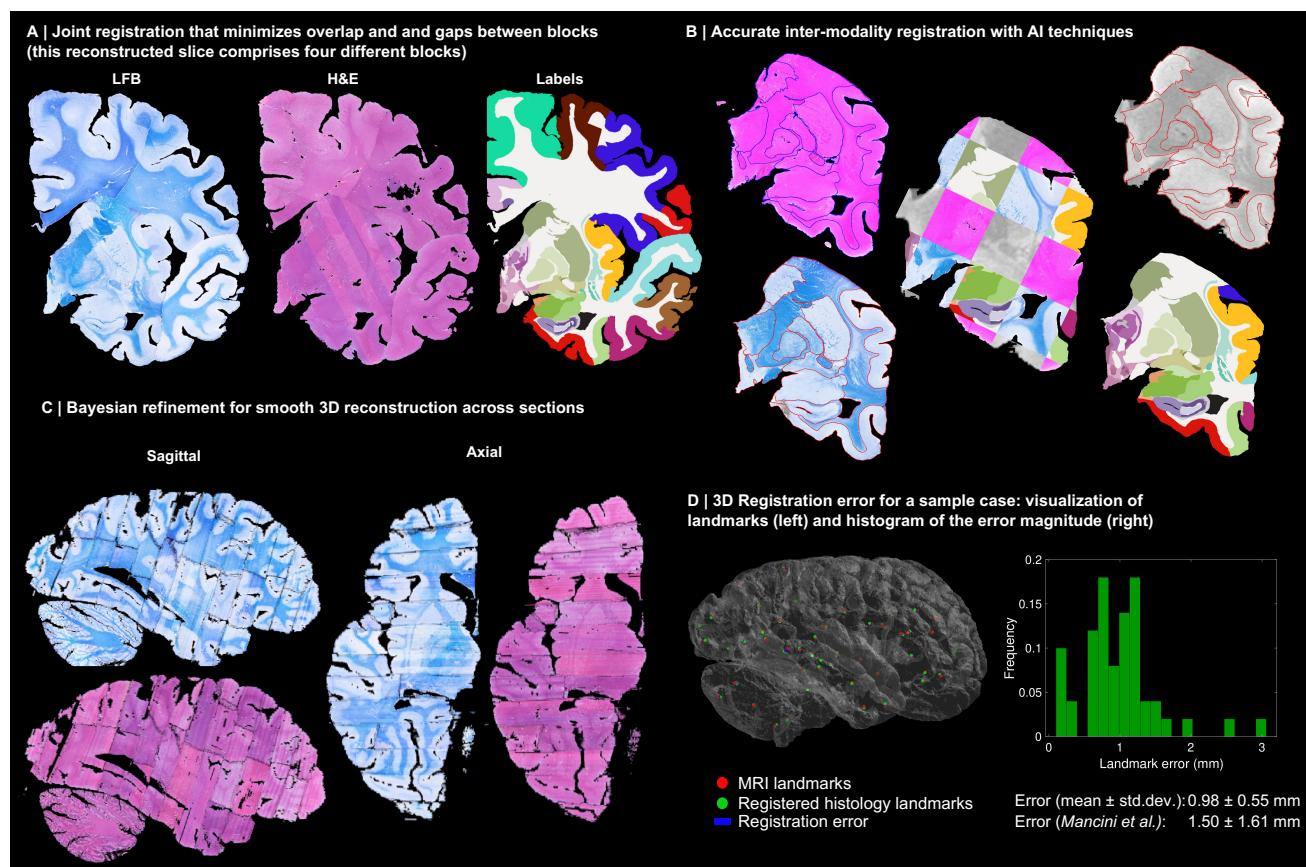
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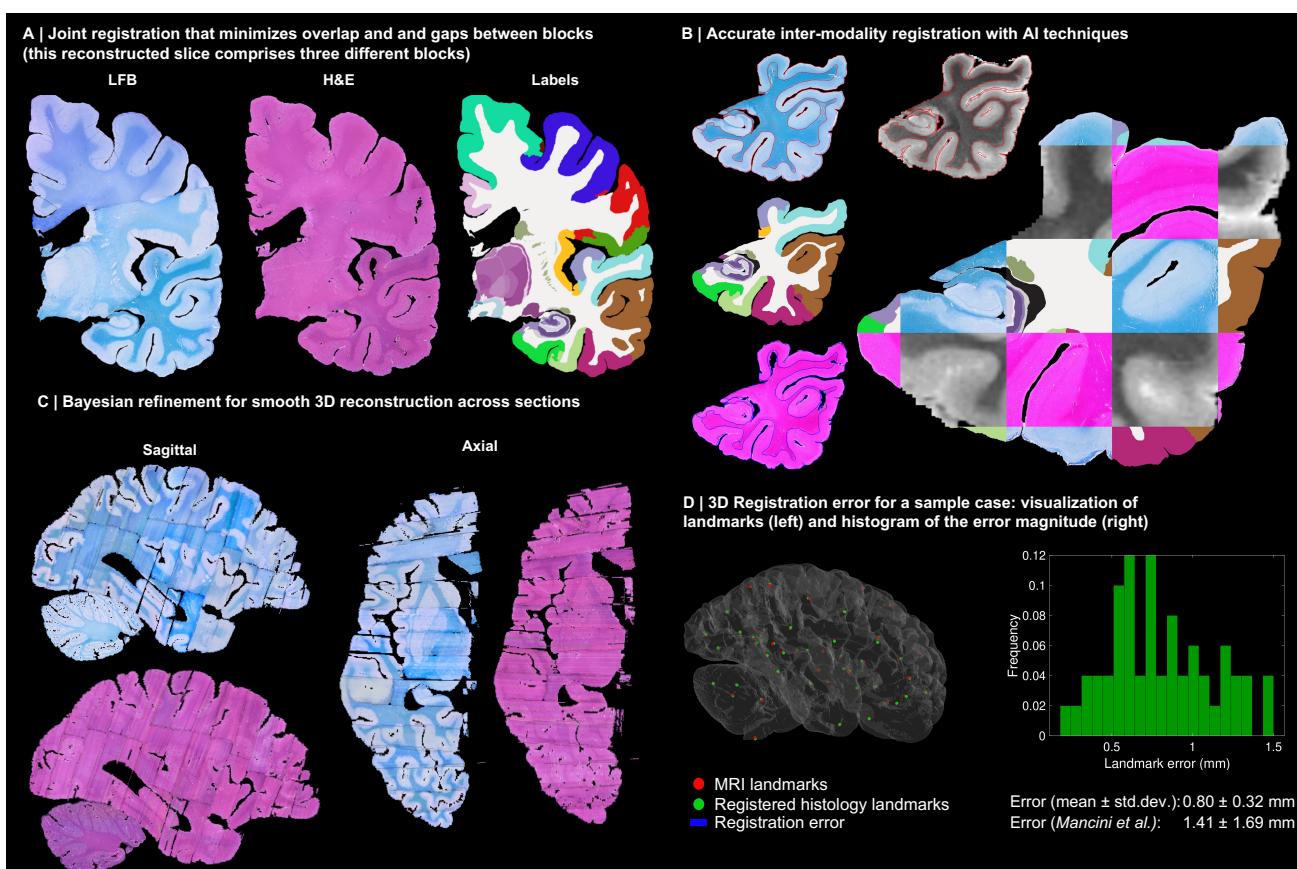
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Extended Data Fig. 1: 3D reconstruction of Case 2. The visualisation follows the same convention as in Figure 3: (A) Coronal slice of the 3D reconstruction. (B) Registered MRI, LFB, and H&E histology of a block, with tissue boundaries (traced on LFB) overlaid. (C) Orthogonal view of reconstruction, which is smooth and avoids gaps and overlaps. (D) Visualization of 3D landmark registration error (left); histogram of its error (right); and mean \pm standard deviation (bottom), compared with our previous pipeline (Mancini et al. [6]).



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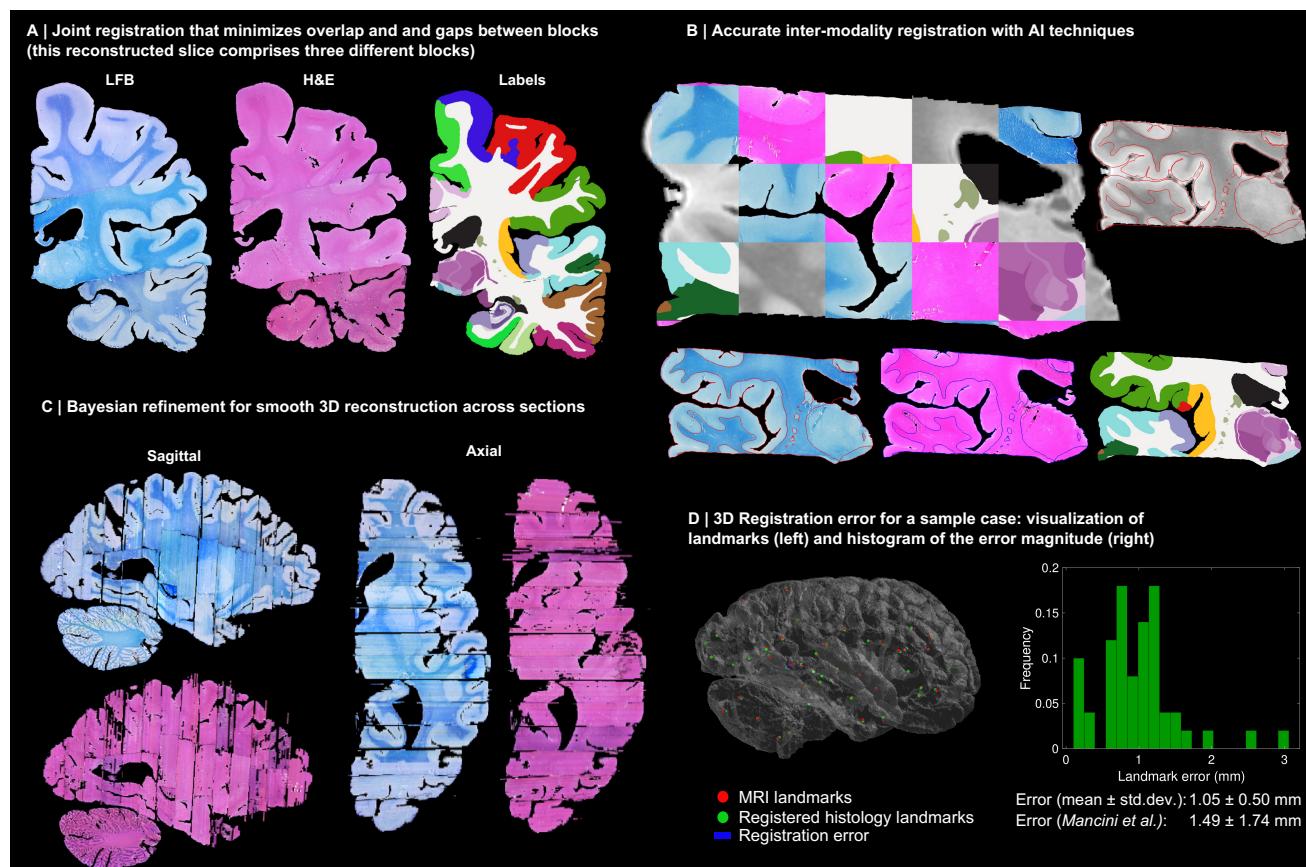
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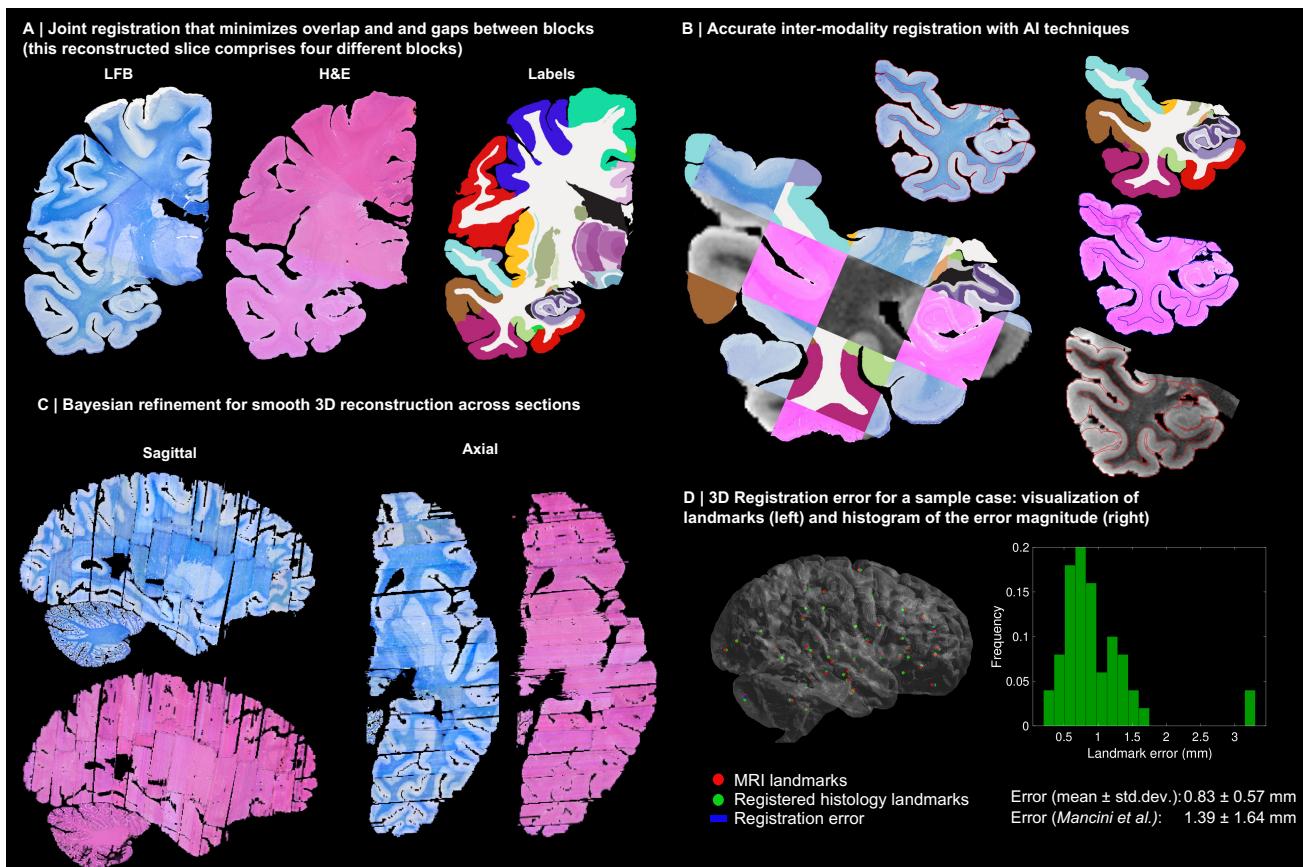
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Extended Data Fig. 2: 3D reconstruction of Case 3. The visualisation follows the same convention as in Figure 3: (A) Coronal slice of the 3D reconstruction. (B) Registered MRI, LFB, and H&E histology of a block, with tissue boundaries (traced on LFB) overlaid. (C) Orthogonal view of reconstruction, which is smooth and avoids gaps and overlaps. (D) Visualization of 3D landmark registration error (left); histogram of its error (right); and mean \pm standard deviation (bottom), compared with our previous pipeline (Mancini et al. [6]).



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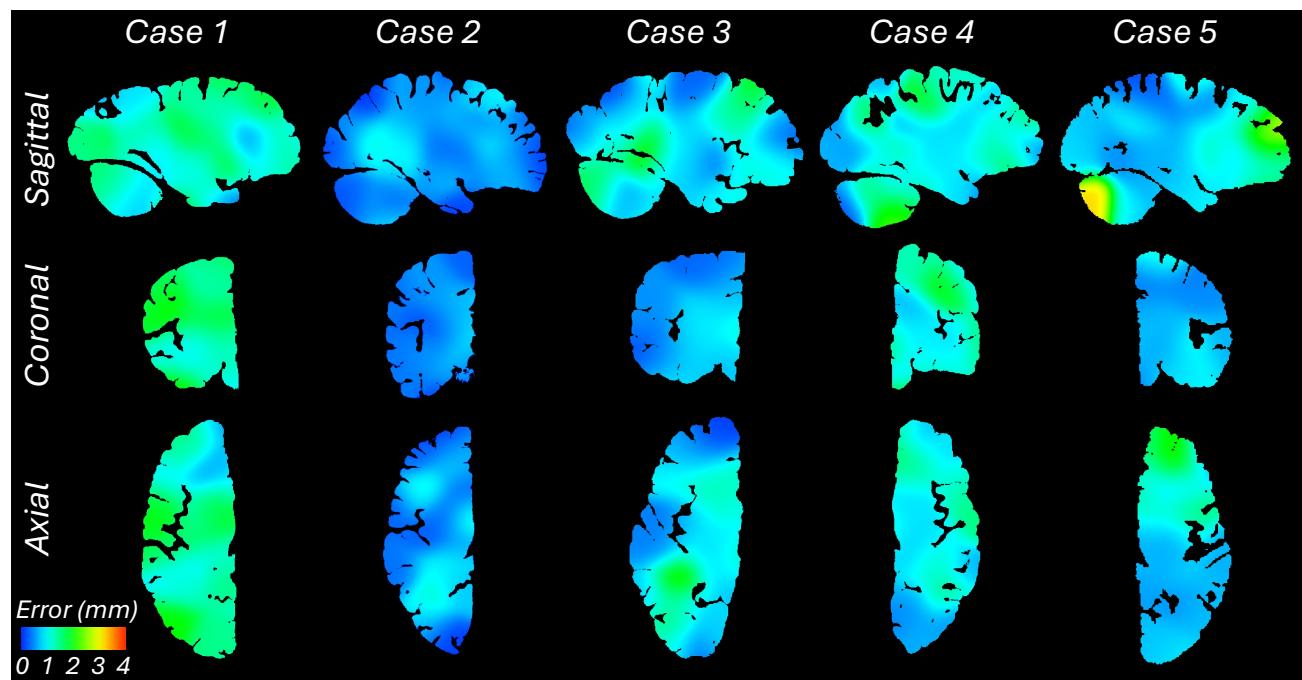
1608 **Extended Data Fig. 3:** 3D reconstruction of Case 4. The visualisation follows the same convention as in Figure 3: (A) Coronal slice of the 3D
1609 reconstruction. (B) Registered MRI, LFB, and H&E histology of a block, with tissue boundaries (traced on LFB) overlaid. (C) Orthogonal view of
1610 reconstruction, which is smooth and avoids gaps and overlaps. (D) Visualization of 3D landmark registration error (left); histogram of its error
1611 (right); and mean \pm standard deviation (bottom), compared with our previous pipeline (Mancini et al. [6]).



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1613 **Extended Data Fig. 4:** 3D reconstruction of Case 5. The visualisation follows the same convention as in Figure 3: (A) Coronal slice of the 3D
1614 reconstruction. (B) Registered MRI, LFB, and H&E histology of a block, with tissue boundaries (traced on LFB) overlaid. (C) Orthogonal view of
1615 reconstruction, which is smooth and avoids gaps and overlaps. (D) Visualization of 3D landmark registration error (left); histogram of its error
1616 (right); and mean \pm standard deviation (bottom), compared with our previous pipeline (Mancini et al. [6]).

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Extended Data Fig. 5: Sagittal, coronal, and axial slices of the continuous maps of the 3D landmark registration error. The maps are computed from the discrete landmarks (displayed in Fig. 3D and Extended Data Figs. 1-4D) using Gaussian kernel regression with $\sigma = 10$ mm. There is no clear spatial pattern for the anatomical distribution of the error across subjects.

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ROI	Dice (200 μm)	Dice (1 mm)
Cerebral-White-Matter	0.90871	0.81367
Ctx-whole	0.89455	0.81493
Cerebellum-Cortex	0.93205	0.85472
ctx-superiorfrontal	0.77579	0.67069
Cerebellum-White-Matter	0.84393	0.65269
ctx-inferiorparietal	0.77458	0.68367
ctx-precentral	0.73685	0.65937
ctx-rostralmiddlefrontal	0.72036	0.66448
ctx-superiortemporal	0.78506	0.69595
ctx-superiorparietal	0.63853	0.56005
ctx-middletemporal	0.71694	0.67675
ctx-inferiortemporal	0.64805	0.65658
ctx-lateraloccipital	0.61442	0.57929
ctx-postcentral	0.72245	0.60464
ctx-supramarginal	0.78025	0.66091
ctx-precuneus	0.72374	0.61229
ctx-fusiform	0.64278	0.5845
ctx-rh-lateralorbitofrontal	0.69867	0.66915
Brain-Stem	0.65342	0.64177
ctx-insula	0.80767	0.73846
ctx-caudalmiddlefrontal	0.65308	0.52521
ctx-medialorbitofrontal	0.73295	0.62687
ctx-lingual	0.66735	0.59501
ctx-parasubiculum	0.70901	0.63893
Left-Putamen	0.9199	0.86072
ctx-paracentral	0.66197	0.58969
ctx-parstriangularis	0.68932	0.65785
Left-Caudate	0.91102	0.86814
ctx-cuneus	0.56676	0.52349
Pons	0.73371	0.6236
ctx-caudalanteriorcingulate	0.6305	0.56515
ctx-bankssts	0.67939	0.4762
ctx-isthmuscingulate	0.70825	0.55557
ctx-parorbitalis	0.43236	0.42366
Pons-nuc	0.65229	0.5514
ctx-rh-posteriorcingulate	0.69731	0.56382
ctx-rostralanteriorcingulate	0.67935	0.41073
ctx-pericalcarine	0.5253	0.38703
ctx-entorhinal	0.72093	0.65273
ctx-temporalpole	0.54659	0.49421
ctx-parahippocampal	0.73123	0.66232
Left-PuL	0.76937	0.78917
Left-external-pallidum	0.78074	0.79407
Left-MDI	0.87316	0.86494
ctx-frontalpole	0.11589	0.3493
ctx-transversetemporal	0.63184	0.56123
Left-VA	0.6994	0.73112
CA1	0.78967	0.60729
Fornix	0.30751	0.29593
Clastrum	0.48364	0.3618

ROI	Dice (200 μm)	Dice (1 mm)
Reticular-of-thalamus	0.2386	0.3239
VPL	0.57387	0.62796
LP	0.73933	0.73133
molecular_layer_HP	0.50045	0.50313
Internal-pallidum	0.78939	0.83776
subiculum	0.69842	0.61734
Dentate-cerebellum	0.71523	0.54696
alveus	0.57837	0.3076
CA4_GC-DG	0.75655	0.7136
Accumbens-area	0.77455	0.67541
Thalamus	0.32506	0.32978
Lateral-nucleus	0.8586	0.75999
CeM	0.6668	0.70489
Substantia-Nigra	0.7045	0.63047
CA2_CA3	0.54381	0.4454
AV	0.59365	0.60739
Basal-nucleus	0.66909	0.61273
SCP	0.71921	0.67856
hypothalamus_posterior	0.58318	0.53634
hypothalamus_tubular_sup	0.51626	0.48914
Accessory-Basal-nucleus	0.74887	0.78098
hypothalamus_tubular_inf	0.65529	0.56586
PAG	0.76829	0.7809
Red-Nucleus	0.83407	0.83386
VTA	0.67032	0.4751
STN	0.71496	0.72738
Optic-Nerve	0.58216	0.44427
LGN	0.64318	0.62109
acomm	0.44371	0.42966
fimbria	0.264	0.157
MGN	0.37669	0.21371
VLa	0.32691	0.45109
LD	0.42601	0.46225
stria-terminals	0.50263	0.30588
Central-nucleus-inf-colliculus	0.78275	0.72433
Corticoamygdaloid-transitio	0.58151	0.48352
mammillary_body	0.56602	0.57353
DR	0.54393	0.49046
Inferior-olive	0.26264	0.046102
hypothalamus_anterior_sup	0.54939	0.45936
Medial-nucleus	0.32559	0.33181
Central-nucleus	0.52925	0.48942
Anterior-amygdaloid-area-AAA	0.21345	0.18373
zona-incerta	0.40755	0.45308
hypothalamus_anterior_inf	0.36912	0.32702
Paralaminar-nucleus	0.14105	0.13123
Cortical-nucleus	0.30083	0.23504
Rest of hippocampus	N/A	N/A
Rest of amygdala	N/A	N/A

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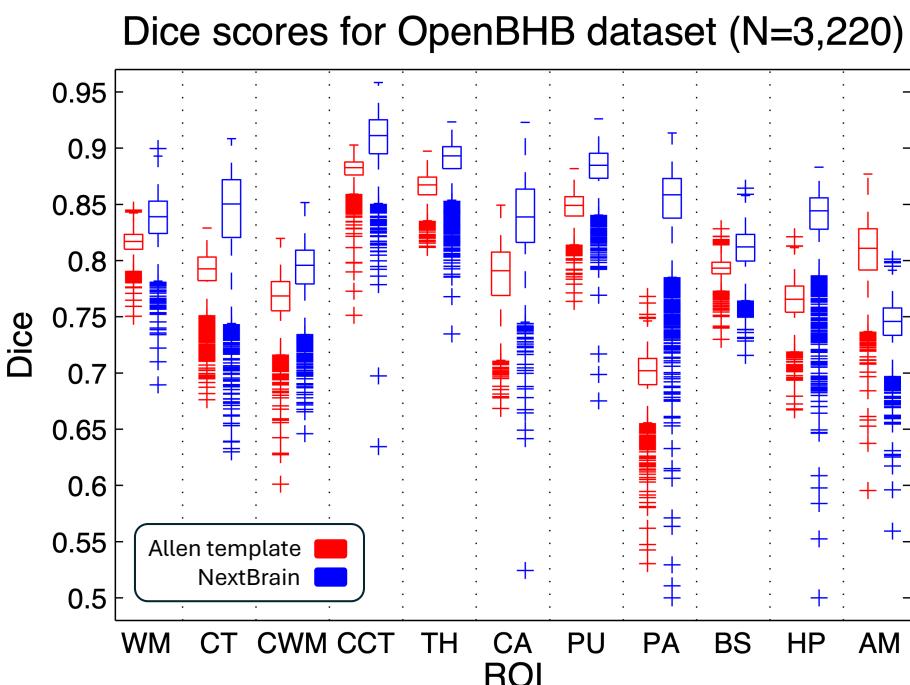
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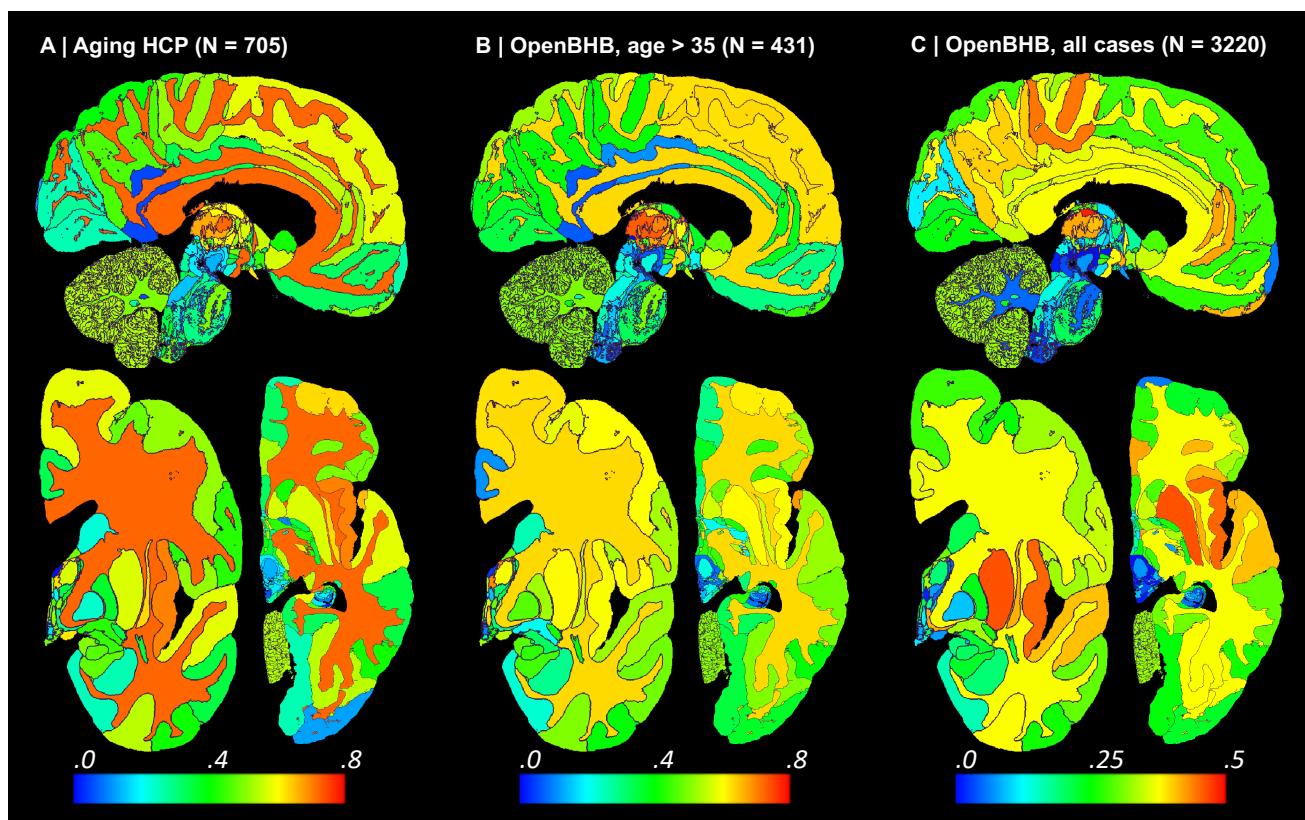
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Extended Data Tab. 1: Dice scores between the ground truth labels of the 100 μm *ex vivo* brain MRI scan presented in [3] and the automated segmentations obtained with *NextBrain*. ROIs are listed in decreasing order of size (volume). The Dice scores are shown for segmentations obtained at two different resolutions: 200 μm (the resolution at which we created the ground truth labels) and 1 mm (which is representative of *in vivo* data). We note that the Dice scores are computed from labels made on the right hemisphere (since we did not label the left side of the brain). We also note that the labels “rest of hippocampus” and “rest of amygdala” correspond to voxels that did not clearly belong to any of the manually labelled nuclei, and have therefore no direct correspondence with ROIs in *NextBrain*.



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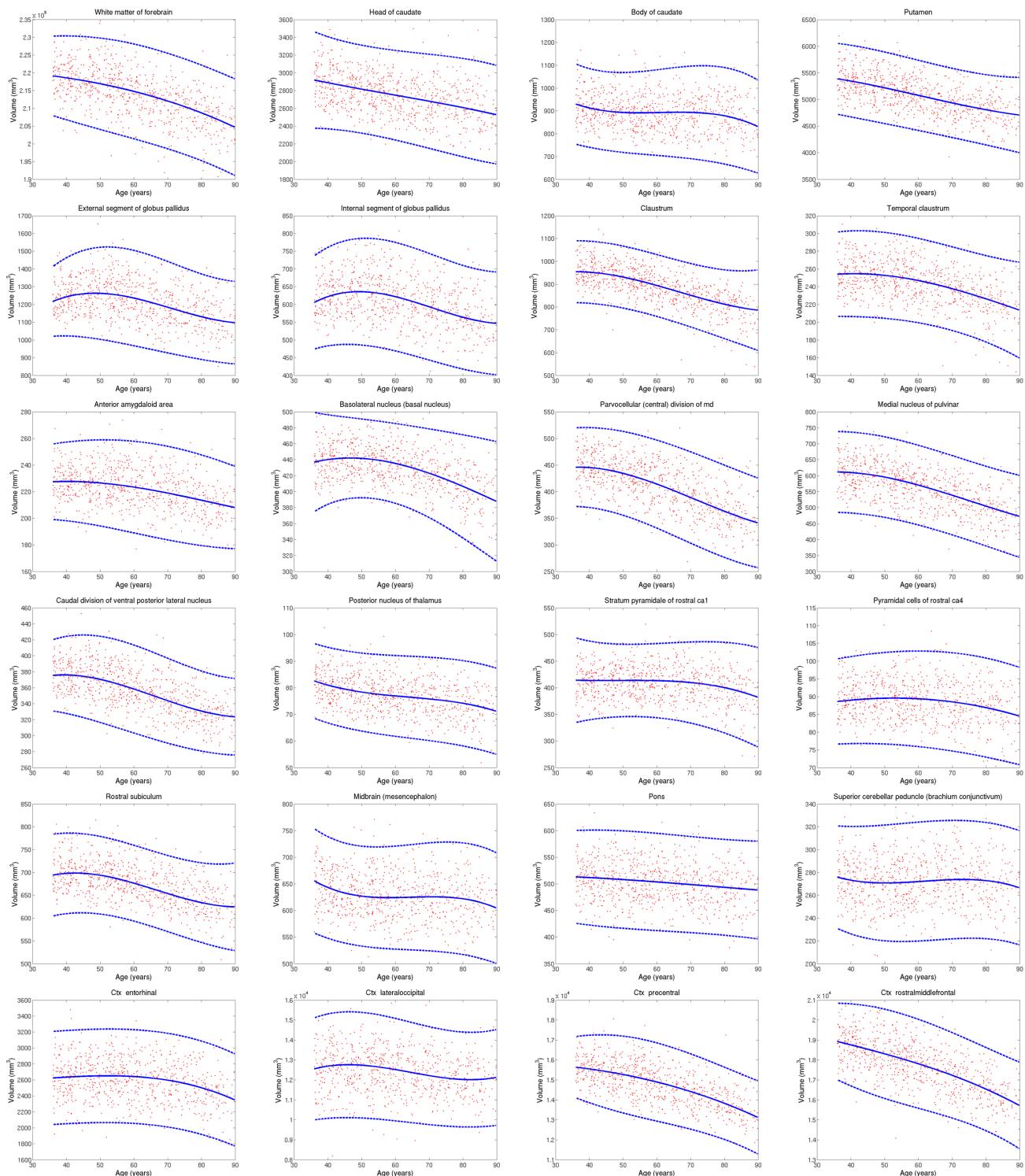
Extended Data Fig. 6: Box plots of the Dice scores for 11 representative ROIs computed on the OpenBHB dataset (3,330 subjects), using the Allen MNI template and NextBrain, with FreeSurfer segmentations as reference. The scores are computed at the whole regions level, i.e., the level of granularity at which FreeSurfer segments. On each box, the central mark indicates the median, the edges of the box indicate the 25th and 75th percentiles, the whiskers extend to the most extreme data points not considered outliers, and the outliers are plotted individually as '+'. The abbreviations for the regions are: WM = white matter of the cerebrum, CT = cortex of the cerebrum, CWM = cerebellar white matter, CCT = cerebellar cortex, TH = thalamus, CA = caudate, PU = putamen, PA = pallidum, BS = brainstem, HP = hippocampus, AM = amygdala.



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Extended Data Fig. 7: Absolute value of Spearman correlation for ROI volumes vs age derived from in vivo MRI scans (additional slices). The visualisation follows the same convention as in Figure 5: (A) Ageing HCP dataset. (B) OpenBHB dataset, restricted to ages over 35. (C) Full OpenBHB dataset.

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1643 **Extended Data Fig. 8:** Aging trajectories for select ROIs in HCP dataset, showing differential patterns in subregions of brain structures (thalamus, hippocampus, cortex, etc). The red dots correspond to the ROI volumes of individual subjects, corrected by intracranial volume (by division) and sex (by regression). The blue lines represent the maximum likelihood fit of a Laplace distribution with location and scale parameters parametrised by a B-spline with four control points (equally spaced between 30 and 95 years). The continuous blue line represents the location, whereas the dashed lines represent the 95% confidence interval (equal to three times the scale parameter in either direction). Volumes of contralateral structures are averaged across left and right.

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