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INCOMPLETE HIPPOCAMPAL INVERSION AND ITS RELATIONSHIP TO HIPPOCAMPAL SUBFIELD VOLUMES AND AGING

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ABSTRACT

Background and Purpose: Incomplete hippocampal inversion (IHI) is an atypical anatomical pattern presented by the hippocampus. It is associated with several neuropathological conditions and is thought to be a factor of susceptibility to hippocampal sclerosis and loss of volume. The volume loss of hippocampus is an inevitable consequence of aging, and when accelerated it is commonly considered an imaging biomarker of Alzheimer's disease dementia.

Methods: We have studied the relationship between IHI and hippocampal subfield volumes in a cohort of 60 healthy participants of 49 to 87 years of age. The presence and severity of IHI and hippocampal subfield volumes were quantified from T2 MR images acquired at 3T.

Results: It was found that IHI presented in 23.3% of participants. Right unilateral IHI was rare (2 cases, 3.3%) in comparison to left unilateral IHI (9 cases, 15%), with 3 (5%) of participants showing bilateral IHI. No significant relationships between the whole hippocampal volumes and IHI was observed. Instead, significant relationships between the volumes of the left and right Cornu Ammonis subfield-1 (CA1) and IHI scores were evident.

Conclusions: The rates of IHI prevalence in the current cohort are similar to those previously reported in healthy cohorts. The IHI severity is related to hippocampal subfield volumes, most notably the CA1, which is a novel finding with potential implications in research on aging and dementia.

INTRODUCTION

In normal foetal development the hippocampus inverts within the medial temporal lobe characterised by progressive unfolding of the dentate gyrus (DG), cornu ammonis (CA), subiculum and parahippocampal gyrus around the progressively decreasing hippocampal sulcus[1] to form a distinct oval configuration in the coronal plane.[2] Incomplete hippocampal inversion (IHI) describes an atypical anatomical pattern whereby the hippocampus retains a rounded shape and is medially orientated to a deep collateral sulcus, a phenomenon which occurs predominantly, but not exclusively, in the left hippocampus.[3] IHI has been associated with several pathological conditions and has a high prevalence in epilepsy.[3] Baulac et al.[4] reported that 13 out of 19 patients with temporal lobe epilepsy displayed IHI as characterised by medial positioning of the hippocampus, an abnormally rounded hippocampal body or an unidentifiable indentation of the hippocampal fissure. Furthermore, it has been suggested that IHI may be a factor of susceptibility to neuropathological processes which leads to neuronal loss and hippocampal sclerosis.[5] However, IHI is not specific to pathological conditions and also presents in healthy subjects, albeit with a lower frequency.[2] Cury et al. reported a prevalence of 23% in 2089 adolescents.[3]

Abnormal alterations in hippocampal volume and morphology in healthy aging have received considerable interest as early markers of neurodegeneration preceding imminent cognitive impairment[6] particularly in Alzheimer's disease dementia (ADD).[7] Total hippocampal volume is used commonly as an MR biomarker of ADD[8] although more recently efforts to measure hippocampal subfields have gained significant momentum.[9] Hippocampal subfields have been reported to be differentially vulnerable to both the effects of age[9-12] and ADD,[13] for example the CA1 demonstrates volume loss in normal aging;[9] and also shows significant volume loss in Mild Cognitive Impairment (MCI) along with the subiculum.[11, 14] Moreover, CA1 volume loss has been suggested to be superior to total hippocampal volume loss for separating healthy participants from those predestined to MCI.[11] Accordingly, the examination of hippocampal subfields is potentially informative for both aging and dementia research. Furthermore, abnormal morphological alterations, such as those which may display in incidents of IHI, have also been associated with cognitive impairment,[10, 15, 16] particularly in the anterior hippocampus[15, 16] and the inferred location of the CA1 subfield.[17] Therefore, the investigation of both hippocampal subfield volumes and IHI

is of interest in aging and dementia research, providing an insight into macroscopic alterations occurring within the hippocampus. However, the relationship between IHI and subfield volumes is yet to be established.

The current study was undertaken (a) to examine incidence of IHI in normal aging and (b) to determine whether IHI and hippocampal subfield volumes, in particular CA1, are associated with each other in normal aging.

METHODS

Participants and MRI Acquisitions

Ethical approval was obtained from the University of Bristol Faculty of Science Research Ethics Committee and Research Ethics Committee South West, Frenchay. Written informed consent was received from all participants prior to data collection. A sample of 60 healthy participants (23 male, 37 female), aged 49-87 years (mean = 67.8), were enrolled. Participants were self-reported to be free of any neurological or psychiatric disorder. In addition, participants had to score above 26 on the Montreal Cognitive Assessment.[18]

MRI data was collected using a 3T Siemens Magnetom Skyra scanner and a 32-channel head coil. T1 weighted images were acquired for anatomical reference, total brain volume (TBV) and positioning of T2 slices using a 3D MPRAGE sequence (TR= 2200 ms, TI= 900 ms, TE= 2.42 ms, alpha flip angle= 9^{o}, FOV= 220 x 184 x 230 mm, resolution= 0.34 x 0.34 x 1.6 mm^2 after 2-fold interpolation in-plane, GRAPPA factor= 2, acquisition time= 5.25 minutes). T2-weighted images were acquired using a 2D multi-echo spin echo sequence, based on a vendor-supplied pulse sequence adapted to phase cycle the refocusing pulse, with a scan time of ~12 minutes (TR= 5500 ms, echo spacing= 12 ms, 12 echoes, slice thickness= 1.72 mm, FOV= 184 x 218 x 58 mm^3, in-plane resolution= 0.34 x 0.34 after 2-fold interpolation). The T2 images corresponding to an entire echo train were summed. All T2 MR images were acquired in an oblique plane in which the long axis of the hippocampus was perpendicular to the coronal plane in the resulting images.

Hippocampal subfield masks

Hippocampal subfield masks were manually applied using FSL software following the segmentation procedure described in Wood et al.[19] This protocol defines boundaries using image contrast, external anatomical boundaries and geometric rules and allows the identification of six subfields: CA1, CA2, CA3, DG, subiculum and lumped stratum radiatum, stratum lacunosum, stratum moleculare (SR/SL/SM). Example hippocampal masks are shown in Figure 1. The absolute volume of each hippocampal subfield mask for the left and the right hemisphere was calculated using FSLstats, a function of FSL software.[20] Each of the six subfield volumes were also calculated relative to the participant's total brain volume. To calculate normalised volumes, the given volume was divided by the participant's total brain volume (determined by FSLstats in mm^3) and multiplied by 1000. The six subfield volumes were summed to give a total hippocampal volume in cubic millimetres.[3] To obtain measures of reliability for the segmentation protocol, three randomly selected participants were re-segmented and the Dice Kappa metric analysis applied as recently described. [19]

Criteria for IHI quantification

The protocol used for determination of IHI followed the five criteria described by Cury et al. in a study validated on a sample of 2,089 young, healthy participants.[3] The five criteria are summarised below.

(a) Roundness and verticality of hippocampal body. In order to quantify roundness and verticality, two distances were observed: the width of the hippocampal body (H1) from the medial part of the DG to the lateral part of CA1, and the depth of the hippocampal body (H2) from the dorsal part of CA1 to the ventral part of CA3 (Figure 1).

The roundness was evaluated on the basis of three categories: flat (width > depth), round (width = depth) or oval (width < depth). The verticality was also evaluated on the basis of three categories: horizontal (width = horizontal, with a tolerance of roughly ten degrees), oblique (width = neither horizontal nor vertical, at roughly 45 degrees) or vertical (width = vertical, with a tolerance of roughly ten degrees).[3] For example, a flat, horizontal hippocampal body scored 0.

(b) Verticality and depth of collateral sulcus. The collateral sulcus separates the fourth and fifth convolution of the temporal lobe. The verticality and depth of the collateral sulcus was observed relative to the depth of the hippocampal body, with the lateral limit of the hippocampal body being used to define the variable. The depth of the collateral sulcus (CS) and depth of the hippocampal body (H2) (Figure 1). If the CS did not overlap with the lateral limit, it was scored between 0 and 1, depending on its distance from the lateral limit. If the CS did overlap with the lateral limit, it was scored between 1 and 2, depending on the extent of overlap.

(c) Medial positioning of the hippocampus. To evaluate medial positioning, the length of the subiculum inferior of the DG (S1) was observed, relative to the length of CA1 also inferior of the DG (C1) (Figure 1). Even when the hippocampus was vertical or oblique in shape, the length of the subiculum and CA1 were defined orthogonally to the brain midline. Medial positioning was evaluated on the basis of five categories on a continuum from very lateral positioning to very medial positioning.[3]

(d) Thickness of the subiculum. We followed the methodology from Bernasconi et al. to quantify this property.[5] Thickness of the subiculum was considered by an abnormal bulge upwards (Figure 1), causing the subiculum to look thickened. More specifically, thickness of the subiculum was quantified by its protrusion into the usually empty choroidal fissure. Protrusion into the choroidal fissure was given a score of 2; otherwise, the subiculum was considered normal and scored 0.

(e) Depth of the sulci of the fusiform gyrus. This criterion took into account the occipitotemporal sulcus, as well as the collateral sulcus that was previously evaluated in the second criterion. The occipitotemporal sulcus separates the third and fourth temporal convolutions. If the superior part of either the collateral sulcus or occipitotemporal sulcus exceeded the lateral limit of the subiculum with a vertical orientation, 2 points were scored. If either exceeded the lateral limit with an oblique orientation, 1 point was scored. If neither sulci exceeded the lateral limit, the score was 0. The CS, the occipitotemporal sulcus (OTS) and lateral limit of the subiculum (S2) are shown in Figure 1.

Determination of IHI score and intra-rater reliability

A trained rater scored the five criterion for IHI on each coronal slice throughout the hippocampal body in both hemispheres. Scores from all coronal slices were averaged for each criterion in each hemisphere to give a measure of IHI. Each criterion was scored between 0 and 2, allowing for a cumulative IHI score out of maximal 10 for each hemisphere. To appraise the intra-rater reliability of evaluating IHI using visual inspection, the trained rater blindly re-evaluated IHI scores of 10 participants.

Statistical analyses

A two-way mixed intraclass correlation coefficient (ICC) model assessing absolute agreement was used to estimate intra-rater reliability of IHI score. The proportion of participants with left and right IHI was determined using Cury et al.'s optimal threshold of 3.75.[3] This was shown to be a reliable and valid threshold of IHI in a cohort of 2,089 participants. Pearson's bivariate correlations were used to assess the relationship between age and IHI scores, total hippocampal volume, hippocampal subfield volumes. Pearson's bivariate and partial correlations controlling for age and gender were used to assess the relationship between IHI scores, total hippocampal volumes and hippocampal subfield volumes. To justify use of the parametric tests, we performed Shapiro-Wilk test to assess whether the values were normally distributed. The Shapiro-Wilk test confirmed that the data were normally distributed. Further correlations were used to explore bilateral asymmetry between total hippocampal volume as well as hippocampal subfield volumes. All statistics were performed using SPSS statistics 23.

RESULTS

Whole hippocampal and hippocampal subfield volumes are shown both in absolute and normalised terms in Table 1. No significant differences in any of the volumes, either in absolute terms or normalised to total brain volume, were observed between left and right hemispheres. Absolute volumes for the whole hippocampi are shown as a function of age (Figure 2). It is evident that volumes of both hippocampi decrease with age. The correlation coefficient, controlled for gender, was on the left r= -0.425 ($p< 0.05$) and r= -0.585, ($p<0.05$) on the right (no significance between hemispheres). Hippocampal subfield volumes are plotted as function of age in Figure 3. Mean Dice-kappa values ranged from 0.72 to 0.78 for all other subfields except CA2 which showed Dice-kappa range from 0.51 to 0.54. Dice-kappa data were consistent with those reported in literature confirming good reliability of subfield segmentation.[19, 21] Table 2 summarise correlation coefficients for subfield volumes both in absolute and normalised terms with age, as controlled for gender. Significant age-dependent negative correlations (i.e. volume loss) were observed in CA1 on the left and in CA1, DG and SR/SL/SM on the right (Table 2).

IHI scores are shown as a function of age (Figure 4). Fourteen (23.3%) of the 60 participants met optimal threshold for either unilateral or bilateral IHI.[3] Right unilateral IHI was rare (2 cases, 3.3%) in comparison to left unilateral IHI (9 cases, 15%), with 3 (5%) of participants showing bilateral IHI. Pearson's bivariate correlations revealed no significant relationship between age and left (r = 0.174, p = 0.19) or right (r = 0.232, p = 0.08) IHI score. The single measures ICC revealed high intra-rater reliability for both left (f = 0.970, p = <0.01) and right (f = 0.934, p = <0.01) IHI scores, conveying that visual inspection using the described criteria was a reproducible measure of IHI.

Partial correlations, controlling for age and gender, between IHI score and volumes of hippocampal structures are shown in Table 3. The partial correlations showed no significant relationships between the whole hippocampal volumes. Instead, significant

relationships between the left and right CA1 and IHI scores were evident. On the right, also CA3 volume and IHI scores correlated significantly.

DISCUSSION

This study investigated the prevalence of IHI in healthy, aged individuals as well as the relationship between IHI and hippocampal subfield volumes. The results are in agreement with those of Cury et al.[3] with respect to prevalence of IHI in a healthy population, reporting a 23.3% prevalence in an aged cohort. Furthermore, right and left unilateral IHI mimicked the frequencies previously reported.[3] Our results also suggest that IHI is correlated with certain hippocampal subfield volumes, most notably that of the CA1 bilaterally. These are novel findings providing insight into the intra-hippocampal macroscopic underpinnings for IHI in aging.

Studies of hippocampal volumes in later-life populations have reported loss of volume in certain subfields, particularly in the CA1 bilaterally, but also in the left SR/SL/SM and the right CA3, DG and subiculum. For example, Mueller et al. [9] reported marked age related of the CA1 bilaterally, which accelerated in the 7th decade. Likewise Mueller et al.[11] found a decline in volume of the CA1 with age, as well as in the CA3/DG combined subfield and Raz et al.[12] found that advanced age was differentially associated with the combined CA1/CA2 subfield. In contrast, Voineskos et al.[10] reported a linear relationship between age and all subfield volumes, apart from the CA1. However, this discrepancy could be due to the age of the cohort studied, with Voineskos et al. examining subfield volumes across a wide adult lifespan (18-86 years).[10] It is worth pointing out that age-dependent decline was evident in our study both in absolute and normalised total hippocampal and subfield volumes.

This study found a relationship between IHI score and subfield volumes including the CA1 bilaterally and the right CA3 which was independent of age. This suggests that IHI severity may be related to a decline in certain hippocampal subfield volumes. To the best of our knowledge, this is the first study to have investigated the relationship between IHI and subfield volumes and therefore this is a novel finding. Given that both CA1 volume and IHI have been proposed as factors which indicate susceptibility to neuronal loss in neuropathological conditions, this relationship warrants further

investigation and could hold significance for such diseases as ADD. Previous studies have reported an association between hippocampal morphology and subfield volumes using global surface-based descriptions of global hippocampal morphology and vertex-wise analysis to assess local regions of inward and outward displacement.[10] Voineskos et al. reported that both hippocampal morphology and subfield volumes showed associations to cognitive performance, proposing that both could be used as markers of cognitive decline.[10] Certain hippocampal subfield volumes have, in particular been associated with working memory performance.[10] The relationship between cognitive performance and IHI severity is yet to be established but may be an insightful addition to the body of knowledge surrounding macroscopic alterations in the hippocampus and cognitive decline, particularly in light of the finding that it is related to subfield volumes.

The relationship between hippocampal subfields and hippocampal morphology has further been investigated using surface based statistics to infer subfield locations on 3D surface maps of the hippocampus. Costafreda et al. exploited pattern recognition algorithms to create 3D mesh models of hippocampi from T1-weighted MRI data to infer subfield locations and link morphological displacements to cognitive decline.[15] They reported that severe inward displacement in the anterior CA1 subfield was the most strongly associated with cognitive decline. Similarly, Yang et al. reported inward displacement in the CA1 subfield with age, again implicating alterations in the CA1 associated with age, in keeping with the findings of this study.[17] Costafreda et al. also demonstrated that hippocampal morphology was an accurate predictor of conversion of MCI to ADD, implicating morphology as a useful conversion prediction tool.[15] It is yet to be seen whether IHI would also serve as a useful predictor of neuropathological aging but since it is a means of quantifying abnormal shape characteristics in the hippocampus this would be an insightful investigation. Furthermore, it has been suggested that combining volumetric measurements of the hippocampus with shape analyses, for which IHI could be a surrogate marker, offers a superior method of distinguishing healthy and pathological aging[22, 23] indicating that IHI scores used in combination with subfield volumes has potential as a useful clinical tool.

The study has some limitations which should be acknowledged. Firstly, it employs a cross-sectional design which although offers an insight into the relationship between IHI, hippocampal subfield volumes and age, cannot demonstrate changes over time.

Furthermore, the sample size is somewhat small and larger samples are required to clarify findings. It also adopts a method of quantifying IHI which only samples from the hippocampal body.[3] Significant differences in hippocampal shape have frequently been reported with age in the head region of the hippocampus[10, 16, 17, 23] meaning that the inclusion of this region could offer further insight into the relationship between IHI and age.

In conclusion, this study has investigated how IHI relates to hippocampal subfields in healthy aging. It has reported rates of IHI prevalence similar to those previously reported in healthy cohorts. It has also established that IHI severity is related to several hippocampal subfield volumes, notably the CA1, which to the best of our knowledge is a novel observation. Given that studies investigating the morphology of the hippocampus have strongly implicated morphological abnormalities with cognitive decline and CA1 volume has been suggested as a precursor to dementia pathology, this relationship may hold particular relevance to dementia research. Overall this study is the first to provide a novel insight into the macroscopic underpinnings of hippocampal IHI in a healthy, aging cohort.

Table 1. Volumes of given brain structures in the entire cohort.

Structure	**Left (absolute in mm^3)**	**Left (normalised)**	**Right (absolute in mm^3)**	**Right (normalised)**
Whole hippocampus	2600 (400)	1.97 (0.26)	2700 (410)	2.03 (0.27)
CA1	820 (180)	0.67 (0.13)	880 (160)	0.67 (0.10)
CA2	40 (12)	0.03 (0.01)	45 (10)	0.03 (0.01)
CA3	180 (53)	0.14 (0.04)	190 (50)	0.14 (0.03)
DG	550 (100)	0.41 (0.07)	600 (110)	0.45 (0.08)
SR/SL/SM	700 (120)	0.52 (0.08)	720 (130)	0.53 (0.09)
Subiculum	290 (62)	0.22 (0.04)	290 (67)	0.22 (0.05)

Values (mean and standard deviation in brackets) are given in absolute terms ('absolute' indicated in mm^3) and normalised to total brain volume ('normalised). Abbreviations: CA = Cornu Ammonis; DG = Dentate Gyrus; SR/SL/SM = Stratum Radiatum/ Stratum Lacunosum/Stratum Moleculare**.**

Table 2. Correlations between age, controlling for gender, and volume for hippocampal subfields.

Hippocampal subfield	**Left (absolute)**	**Left (normalised)**	**Right (absolute)**	**Right (normalised)**
CA1	-0.425***** *(p=0.001)*	-0.538***** *(p= 0.000013)*	-0.585***** *(p= 0.00001)*	-0.35 *(p= 0.007)*
CA2	-0.154 *(p= 0.25)*	-0.09 *(p= 0.50)*	-0.261 *(p= 0.048)*	-0.188 *(p= 0.16)*
CA3	-0.210 *(p= 0.114)*	-0.153 *(p= 0.25)*	-0.341 *(p = 0.017)*	-0.26 *(p= 0.048)*
DG	-0.276 *(p= 0.036)*	-0.185 *(p= 0.165)*	-0.461***** *(p= 0.00027)*	-0.387 *(p= 0.003)*
SR/SL/SM	-0.367 *(p= 0.005)*	-0.275 *(p= 0.037)*	-0.439***** *(p= 0.001)*	-0.357 *(p= 0.006)*
Subiculum	-0.241 *(p= 0.069)*	-0.18 *(p= 0.176)*	-0.263 *(p= 0.046)*	-0.132 *(p= 0.324)*

Table 2 gives correlation coefficients and (p) values for both absolute ('absolute') and normalised ('normalised') hippocampal subfield volumes with age. Abbreviations: CA = Cornu Ammonis; DG = Dentate Gyrus; SR/SL/SM = Stratum Radiatum/ Stratum Lacunosum/Stratum Moleculare**.** Benjamini-Hochberg procedure was used to control the false detection rate. Asterisk (*****) indicate significance at $p < 0.05$.

Table 3. Partial correlation results, controlling for age and gender, between incomplete hippocampal inversion score and volume for hippocampal subfields and the whole hippocampi.

Structure	**Left**	**Right**
CA1	-0.46***** *(p= 0.000032)*	-0.78***** *(p= 0.000086)*
CA2	-0.05 *(p= 0.70)*	-0.238 *(p= 0.074)*
CA3	-0.164 *(p= 0.22)*	-0.309* *(p= 0.019)*
DG	-0.099 *(p= 0.47)*	-0.258 *(p= 0.053)*
SR/SL/SM	-0.092 *(p= 0.49)*	-0.253 *(p= 0.055)*
Subiculum	-0.052 *(p= 0.70)*	-0.10 *(p= 0.46)*
Whole hippocampus	-0.034 *(p= 0.80)*	-0.207 *(p= 0.123)*

Table 3 gives correlation coefficients and (p) values. Abbreviations: CA = Cornu Ammonis; DG = Dentate Gyrus; SR/SL/SM = Stratum Radiatum/ Stratum Lacunosum/Stratum Moleculare**.** Benjamini-Hochberg procedure was used to control the false detection rate. Asterisk (*****) indicate significance at $p < 0.05$.

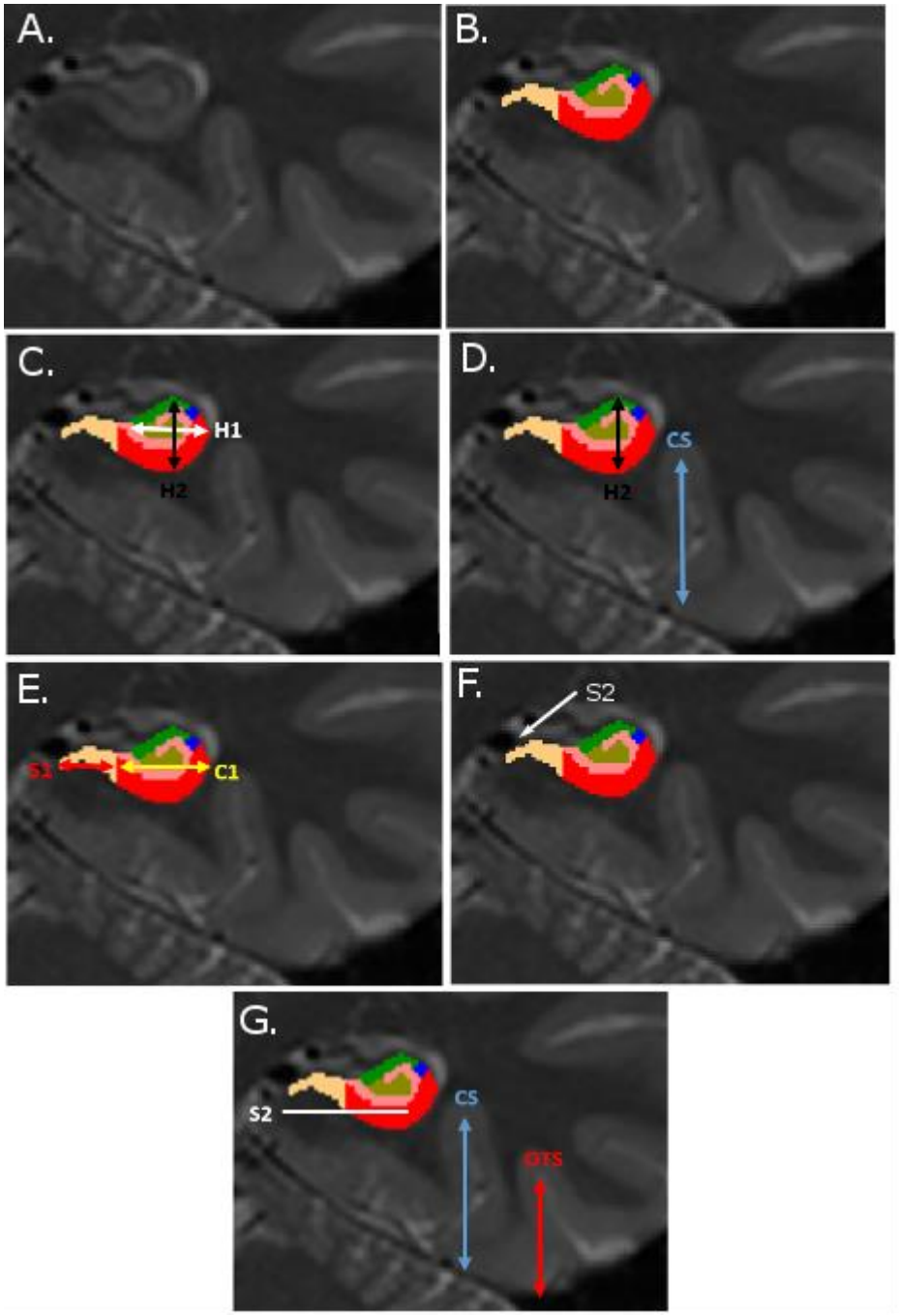

Figure 1. Hippocampal subfields are represented in the following colors: CA1 = red, CA2 = blue, CA3 = bright green, DG = khaki green, SLSMSR = pink, subiculum = yellow.

A) A coronal slice taken from the medial temporal lobe of a healthy participant. B) The six hippocampal subfields are labelled with colored masks on the same coronal slice that was given in image A. C) Distances for the width of the hippocampal body (H1, white arrow) and the depth of the hippocampal body (H2, black arrow) used to evaluate the roundness and verticality of the hippocampal body. D) Depths of the collateral sulcus (CS, blue arrow) and H2 (black arrow) used to evaluate the verticality and depth of the collateral sulcus. E) The length of the subiculum inferior of the dentate gyrus (S1, red arrow) and the length of CA1 inferior of the DG (C1, yellow arrow) were used to evaluate the medial positioning of

the hippocampus. F) An example of a thickened subiculum labelled S2 (white arrow). G) The CS (blue arrow), occipitotemporal sulcus (OTS, red arrow) and lateral limit of the subiculum labelled S2 (white line).

Abbreviations: CA = Cornu Ammonis; DG = Dentate Gyrus; SR/SL/SM = Stratum Radiatum/ Stratum Lacunosum/Stratum Moleculare; CS = collateral sulcus; OTS = occipitotemporal sulcus; H1 = the width of the hippocampal body; H2 = the depth of the hippocampal body; C1 = the length of CA1 inferior to the dentage gyrus; S1 = the length of subiculum inferior to dentate gyrus.

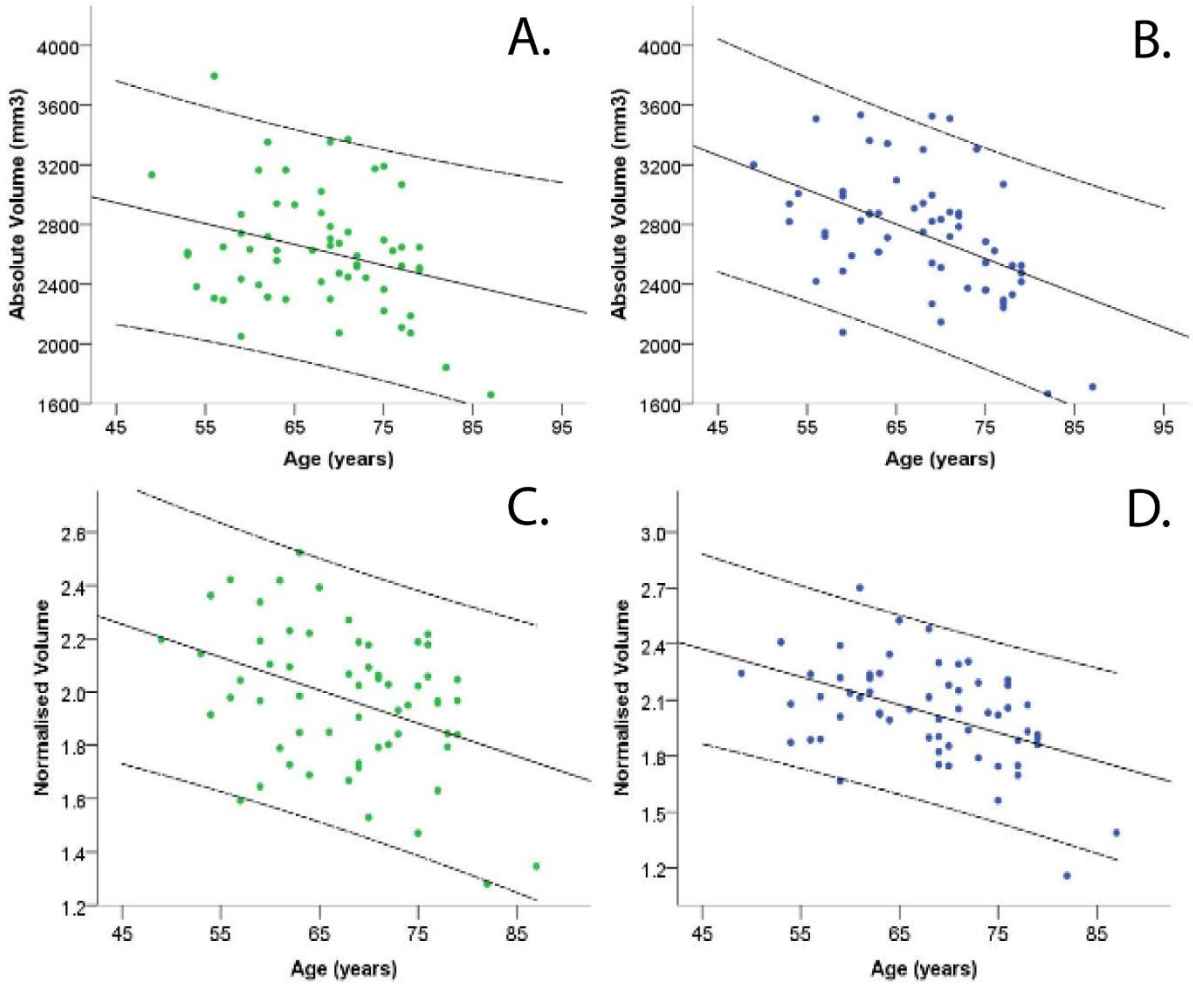

Figure 2: Absolute (A, B) and normalised (C, D) volumes for the left (A,C) and right (B, D) whole hippocampus as a function of age.

In panels A and B each symbol represents a volume of given subfield in mm^3. In panels C and D the normalised hippocampal volumes as described in the Methods section. Fit to a linear regression is given by a solid line, 95% confidence intervals with dotted lines.

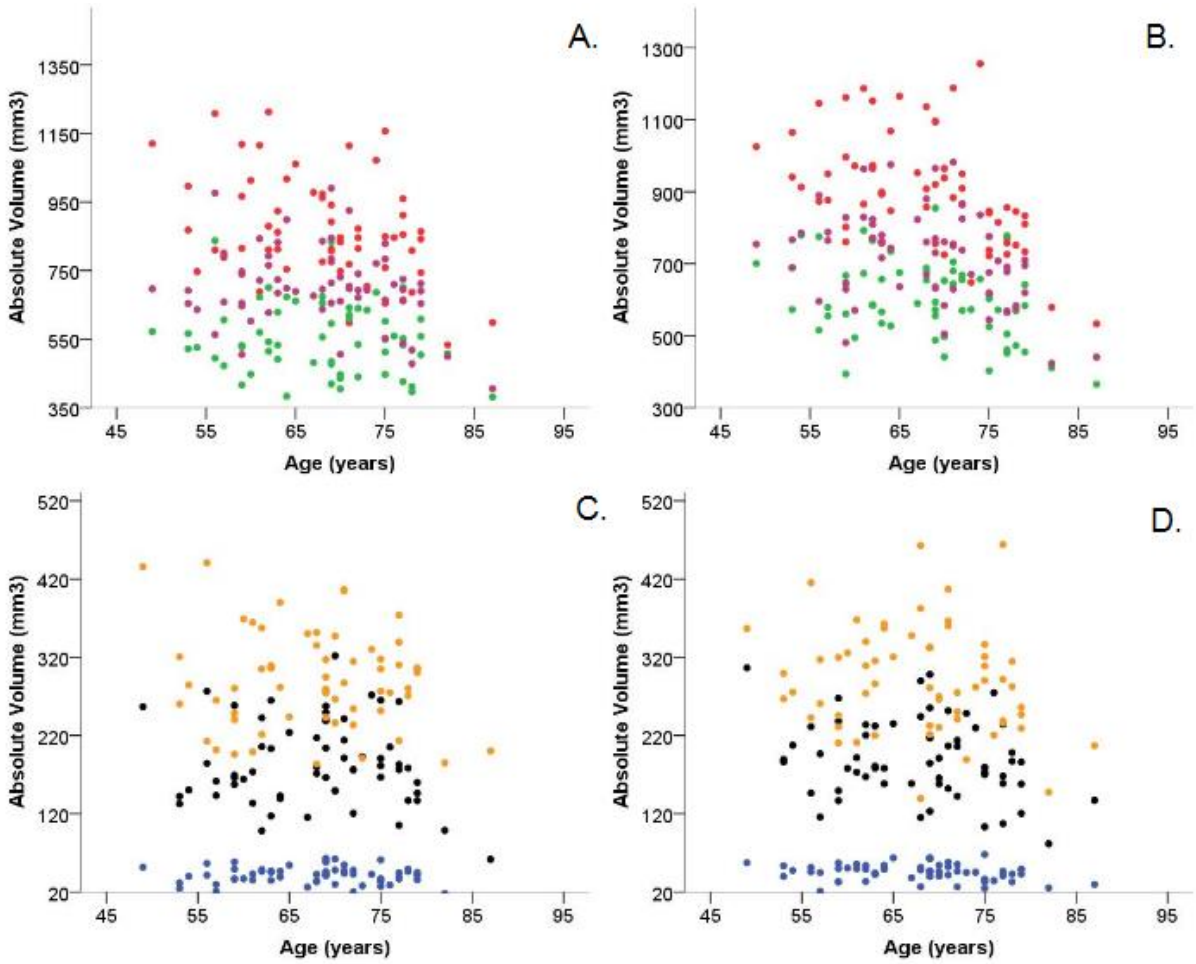

Figure 3: Absolute volumes for the left (A,C) and the right (B,D) hippocampal subfields as a function of age.

Symbols are as follows: Red = CA1, Blue = CA2, Black = CA3, Purple = SR/SL/SM, Green = DG and Orange = Subiculum. Each symbol represents a volume of a given subfield in mm^3. Abbreviations: CA = Cornu Ammonis; DG = Dentate Gyrus; SR/SL/SM = Stratum Radiatum/ Stratum Lacunosum/Stratum Moleculare

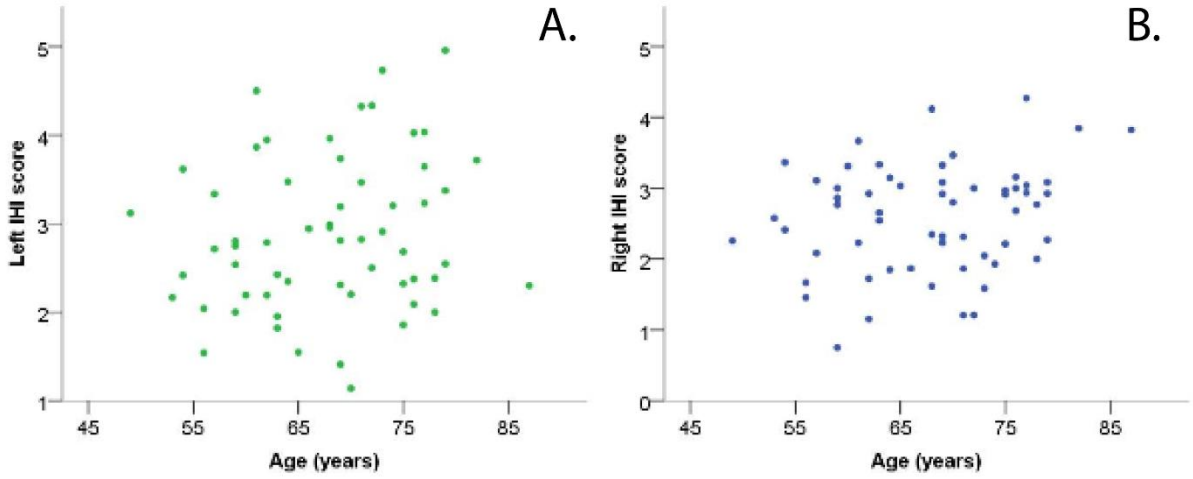

Figure 4. Incomplete hippocampal inversion scores for left (A) and right (B) hippocampus as a function of age.

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