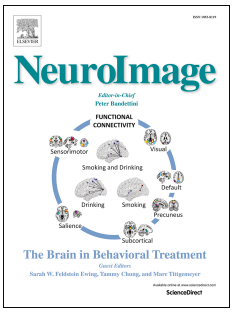
Accepted Manuscript



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PII: S1053-8119(17)30764-4

DOI: 10.1016/j.neuroimage.2017.09.016

Reference: YNIMG 14329

To appear in: *NeuroImage*

Received Date: 2 June 2017

Accepted Date: 8 September 2017

Please cite this article as: Kurth, F., Cherbuin, N., Luders, E., The impact of aging on subregions of the hippocampal complex in healthy adults, *NeuroImage* (2017), doi: 10.1016/j.neuroimage.2017.09.016.

Manuscript Title:

The Impact of Aging on Subregions of the Hippocampal Complex in Healthy Adults

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Number of Words in Abstract: **185**
Number of Figures: **1**
Number of Tables: **2**
Supplemental Material: **2 Tables**

Date of Submission: 07/24/2016

Date of Revision I: 06/01/2017

Date of Revision II: 08/08/2017

Keywords: aging, brain, gender, hippocampus, MRI, sex

Abstract

The hippocampal complex, an anatomical composite of several subregions, is known to decrease in size with increasing age. However, studies investigating which subregions are particularly prone to age-related tissue loss revealed conflicting findings. Possible reasons for such inconsistencies may reflect differences between studies in terms of the cohorts examined or techniques applied to define and measure hippocampal subregions. In the present study, we enhanced conventional MR-based information with microscopically defined cytoarchitectonic probabilities to investigate aging effects on the hippocampal complex in a carefully selected sample of 96 healthy subjects (48 males / 48 females) aged 18 – 69 years. We observed significant negative correlations between age and volumes of the cornu ammonis, fascia dentata, subiculum, and hippocampal-amygdaloid transition area, but not the entorhinal cortex. The estimated age-related annual atrophy rates were most pronounced in the left and right subiculum with -0.23% and -0.22%, respectively. These findings suggest age-related atrophy of the hippocampal complex overall, but with differential effects in its subregions. If confirmed in future studies, such region-specific information may prove useful for the assessment of diseases and disorders known to modulate age-related hippocampal volume loss.

1. Introduction

The hippocampal complex – an anatomical composite of several functionally and architectonically distinct subregions – is known to decrease in size with increasing age, even in healthy adults (for review see Fraser et al., 2015). Despite a wealth of literature describing the age-related hippocampal shrinkage, it is still unresolved whether some hippocampal subregions are more affected by the normal ageing process than others. Moreover, among studies that report such differential effects of aging, consensus is lacking. Some suggest the cornu ammonis and the dentate gyrus to be most prone to aging effects (Bender et al., 2013; Mueller et al., 2007; Mueller and Weiner, 2009; Raz et al., 2015; Shing et al., 2011), while others point to the subiculum (Jiang et al., 2014; La Joie et al., 2010; Thomann et al., 2013). On the one hand, discrepancies across studies may reflect differences in the investigated study sample. For example, the prevalence of cardiovascular problems, metabolic disorders, or risk factors for dementia – all of them impacting hippocampal anatomy (Cherbuin et al., 2015; de Flores et al., 2015a; Fotuhi et al., 2012; Korf et al., 2004; Small et al., 2011; Tabatabaei-Jafari et al., 2015) – is higher in elderly cohorts than in younger cohorts (Morris et al., 2013). On the other hand, conflicting findings across studies may arise from different methods applied to define and measure hippocampal subregions (Bender et al., 2013; Jiang et al., 2014; La Joie et al., 2010; Mueller et al., 2007; Mueller and Weiner, 2009; Raz et al., 2015; Shing et al., 2011; Thomann et al., 2013; Wisse et al., 2017; Yushkevich et al., 2015).

In the current study, we aimed to assess age-related atrophy of the hippocampus and its sub-regions in a very healthy population to provide a benchmark for hippocampal atrophy between 18 and 69 years uncontaminated by clinical pathology. For this purpose, we applied a state-of-the-art brain mapping technique combining MRI-based signal intensities and cytoarchitectonically defined maps (Kurth et al., 2015; Kurth et al., 2017a, b; Luders et al., 2013). This approach allows investigating

hippocampal morphology in a highly standardized way for three well-defined hippocampal subregions and two adjacent areas: the cornu ammonis (CA), the fascia dentata (FD), the subiculum (SUB), the entorhinal cortex (EC), and the hippocampal-amygdaloid transition area (HATA). While there is a significant body of literature on sex effects on hippocampal anatomy (Filipek et al., 1994; Goldstein et al., 2001; Han et al., 2013; Mouiha and Duchesne, 2011; Perlaki et al., 2014; Persson et al., 2014; Szabo et al., 2003) as well as on hippocampal pathology and age-related atrophy (Briellmann et al., 2000; Exner et al., 2008; Li et al., 2014; Murphy et al., 1996), a recent meta-analysis suggests that the human hippocampus is not sexually-dimorphic (Tan et al., 2016). Thus, in addition to computing the age-related correlations within the whole sample, we tested for age-by-sex interactions and also investigated whether volumetric differences in hippocampal / parahippocampal subregions between men (n=48) and women (n=48) are present independent of aging.

2. Methods

2.1 Subject Sample and Image Data

The study sample included 96 subjects (48 men, 48 women), ranging between 18 and 69 years of age (mean ± SD: 42.98 ± 13.89), whose brain scans were obtained from the International Consortium for Brain Mapping (ICBM) database of normal adults (http://www.loni.usc.edu/ICBM/Databases/). No significant sex difference in age was evident in the current sample, and an overview of the sex-specific distribution of subjects across the age range is given in **Supplementary Table 1**. Subjects with any potential medical disorders that could affect brain structure and/or function as well as subjects with brain-structural abnormalities in their MRI scans had been excluded from the ICBM database (Mazziotta et al., 2009). More specifically, any medical, neurological, neurosurgical, or psychiatric diseases, the use of prescription, over the counter, or illicit drugs except for the occasional use for

disease prevention, as well as elevated blood pressure or abnormal findings in a physical examination and history were considered exclusion criteria. This extensive set of exclusion criteria is detailed elsewhere (Mazziotta et al., 2009) and differs substantially from the usually applied less strict screening protocols for healthy controls. Importantly, out of the initial sample of volunteers who considered themselves "normal" and thus had signed up for the original ICBM project, only 10.7% were ultimately included. This highly selective (extremely healthy) sample constitutes the pool from which subjects were selected for the current study. All participants gave their informed consent in accordance with the policies and procedures of UCLA's Institutional Review Board. Structural brain data were acquired on a 1.5 Tesla Siemens Sonata scanner (Erlangen, Germany) using an 8-channel head coil and a T1-weighted magnetization-prepared rapid acquired gradient echo sequence with the following parameters: 1900 ms repetition time, 4.38 ms echo time, 15° flip angle, 160 contiguous sagittal slices, 256x256 mm^2 field-of-view, and 1x1x1 mm^3 voxel size.

2.2 Data Preprocessing

Data was analyzed using the SPM8 software (http://www.fil.ion.ucl.ac.uk/spm; version 4667) and the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm.html; version 435), as previously described (Kurth et al., 2015; Kurth et al., 2017a, b; Luders et al., 2013). All brain images were corrected for magnetic field inhomogeneities and tissue-classified into gray matter, white matter, and cerebrospinal fluid. The segmentation procedure was based on maximum *a posteriori* estimations (Rajapakse et al., 1997), used a partial volume estimation algorithm (Tohka et al., 2004), a spatially adapting non-linear means denoising filter (Manjon et al., 2010), as well as a hidden Markov Random Field model (Cuadra et al., 2005). The resulting gray matter partitions were spatially normalized to the DARTEL template provided by the VBM8 toolbox using 12-parameter affine transformations and high-dimensional

warping (Ashburner, 2007). The normalized gray matter segments were then divided by the non-linear components of the Jacobian derived from the normalization matrix. This latter modulation step served to preserve the actual voxel-wise gray matter content locally, while still accounting for the individual differences in brain size (via proportional scaling).

2.3 Combining Gray Matter Information with Cytoarchitectonic Tissue Probabilities

In order to investigate the impact of aging on the hippocampal complex we did not only look at the hippocampus overall but also on three hippocampal subregions (CA, FD and SUB) as well as two hippocampus-adjacent areas (EC and HATA) within the left and right hemisphere. The cytoarchitectonic probability maps of these five regions of interests (see **Figure 1)** were originally created using cell-body stained histological sections of 10 *post mortem* brains through cytoarchitectonic mapping, as detailed elsewhere (Amunts et al., 2005). Briefly, after removing the brains from the skull, each brain underwent structural MRI scanning and was embedded in paraffin, cut into 20 µm serial sections, and stained for cell bodies. Using the cell-body stained sections, the borders between the distinct hippocampal / parahippocamapal regions were established. Subsequently, these subregions were digitized and reconstructed in 3D space, warped into MNI single-subject space, and converted into region-specific probabilities. That is, each voxel within a cytoarchitectonic probability maps contains a count of how many brains (out of ten) have that voxel labeled as the respective hippocampal / parahippocampal subregion. The cytoarchitectonic probability maps are available for use in *in vivo* image analyses (Eickhoff et al., 2005) and can be accessed via the Anatomy Toolbox[1] (http://www.fz-juelich.de/inm/inm-1/EN/Forschung/_docs/SPMAnatomyToolbox/SPMAnatomyToolbox_node.html).

[1] For the current study, version 18 of the Anatomy Toolbox was used.

As detailed elsewhere (Kurth et al., 2015; Kurth et al., 2017a, b; Luders et al., 2013), the cytoarchitectonically derived probability maps were multiplied – voxel by voxel – with the normalized gray matter segments (see **Section 2.2**). Importantly, prior to this voxel-wise multiplication, all hippocampal / parahippocampal probability maps were spatially normalized to the DARTEL template to ensure an accurate spatial correspondence with the individual gray matter segments in DARTEL space. The resulting voxel-wise measures were then multiplied with the voxel volume, and summed up in order to reveal the gray matter volume (in mm^3) for each hippocampal / parahippocampal subregion. Note that these volumes are already corrected for inter-individual differences in brain size given the modulation of the gray matter segments (see **Section 2.2**).

– Figure 1 –

2.4 Statistical Analyses

Associations between age and the left and right hippocampal / parahippocampal subregions were investigated using a mass-univariate general linear model. Specifically, the measured volumes for CA, FD, SUB, EC, and HATA for each hemisphere were used as dependent variables, while age and sex were the independent variables. Age was centered on 50 years to facilitate interpretation of results, as prior research demonstrated acceleration in hippocampal atrophy at mid-life (Fraser et al., 2015). The beta estimates as well as the adjusted R^2 and F statistics for the model are given in **Supplementary Table 2**. Significance levels were Bonferroni-corrected for multiple comparisons and set at $p<0.01$. In addition, age-by-sex interactions as well as potential changes in the age-related decline as defined by quadratic effects of age were assessed but, as neither reached significance, these terms were not included in the final statistical model. Finally, the annual rates of volume

change as well as the volume differences between males and females were calculated using the beta estimates of the final model.

3. Results

All hippocampal / parahippocampal volumes were negatively associated with age, and significantly so for all subregions except for the EC. Annual atrophy rates (in %) are presented in **Table 1**, suggesting that, on average, every one year above age 50 years, is associated with a 0.09% (minimum) to a 0.23% (maximum) smaller volume. The minimum atrophy rate was evident for the left EC; the maximum atrophy rate for the left SUB. Across the age range investigated (18-69 years), these estimates equate to a volume loss of 6.8% (minimum) to 11.6% (maximum) in the different regions, and a volume loss of 8.5% for the entire hippocampus complex (HC). There were no significant quadratic effects of age, indicating that there are no differential rates of hippocampal decrease with increasing age. Mean volumes and standard deviations for each subregion stratified by sex are presented in **Table 2**. There was a significant main effect of sex, indicating that, on average, females had larger volumes (by 1%-10%) for every subregion, except for the right EC which was larger in males (by 1%) but not significantly so. There were no significant age-by-sex interactions, indicating that males and females tend to follow a similar trajectory with age.

– Table 1 –

– Table 2 –

4. Discussion

By integrating voxel-wise cytoarchitectonic probabilities with MR-based signal intensities in a sample of healthy subjects, we revealed significant negative correlations between age and four hippocampal / parahippocampal subregions, namely CA, FD, SUB, and HATA. In general, these findings are in good agreement with prior studies indicating significant age-related hippocampal atrophy (Bender et al., 2013; Jiang et al., 2014; La Joie et al., 2010; Mueller et al., 2007; Mueller and Weiner, 2009; Raz et al., 2015; Shing et al., 2011; Thomann et al., 2013; Wisse et al., 2014).

4.1 The Impact of the Methodology

Despite the overall good correspondence between current and previous results, there are still some discrepancies in terms of the hippocampal region(s) affected by age-related tissue loss. One possible reason for these inconsistencies across studies could be the different methods applied. For example, the hippocampal complex and its subregions are frequently investigated using traditional region-of-interest (ROI) analyses. There, the ROIs are established by either employing automated algorithms or manual tracings. However, either way, the creation of a ROI requires visible and/or detectable landmarks as well as a set of specific rules or protocols for the specification of the boundaries. Protocols for the definition of hippocampal ROIs often vary between studies, but even within studies may lead to variable ROIs due to variable (or entirely missing) macro-anatomic landmarks. Moreover, ROIs created manually may differ substantially from ROIs created automatically (de Flores et al., 2015b). Given that the present study used a methodology which does not rely on the identification of landmarks, it is not susceptible to the types of biases discussed above. Instead, it is based directly on the underlying microscopic anatomy as mapped *post mortem* (Amunts et al., 2005), thus maintaining a more systematic functional correspondence. Taken together, this may explain why some groups observed age-related volume loss in the CA/FD regions (Bender et al., 2013; Mueller et al., 2007;

Mueller and Weiner, 2009; Raz et al., 2015; Shing et al., 2011), while others detected effects within the SUB region (Jiang et al., 2014; La Joie et al., 2010; Thomann et al., 2013), and why the current study revealed effects within all of these subregions (CA, FD, SUB) in addition to HATA.

4.2 The Impact of the Study Sample

In addition to differences in methodology, variations between reported findings may be explained by differences in the study samples. As detailed above, the original pool our subjects had been recruited for the ICBM project with the explicit goal to avoid factors that may possibly impact brain anatomy or function (Mazziotta et al., 2001; Mazziotta et al., 2009). This led to an extensive set of exclusion criteria and, therefore, to an extremely healthy pool of subjects from which our sample was drawn. Although previous studies that investigated correlations between hippocampal subregions and age also included healthy subjects, exclusion criteria were often less strict compared to the ICBM cohort and, in addition, varied considerably among studies. This variation in exclusion criteria may have contributed to differences in reported results, as different health factors (diabetes mellitus, high blood pressure, etc.) not only have differential effects on the hippocampus overall, but have also been found to impact individual hippocampal subregions to varying degrees (den Heijer et al., 2005; Janowitz et al., 2014; Moran et al., 2013; Raz et al., 2005; Shing et al., 2011). For example, age-related atrophy within the hippocampus and particularly the CA has been reported to be modulated by hypertension, with enhanced atrophy in affected patients (Raz et al., 2005; Shing et al., 2011). Similarly, patients with major depressive disorders seem to be affected by an increased hippocampal atrophy and particularly so in the dentate gyrus (Samuels et al., 2015). Interestingly, regional effects also manifest when comparing hippocampal volume loss between patients with mild cognitive

impairment, Alzheimer's disease, and dementia with Lewy bodies (Delli Pizzi et al., 2016; Mak et al., 2016; Mueller et al., 2010; Mueller et al., 2007; Mueller and Weiner, 2009; Perrotin et al., 2015).

4.3 Estimated Annual Atrophy Rates

The most obvious discrepancy between previous reports and outcomes of the current study are the low atrophy rates of 0.17% per year for the hippocampal complex as a whole, as opposed to 0.85% per year according to a recent meta-analysis (Fraser et al., 2015). These discrepancies may be due to study-specific age ranges of the subjects examined, the inclusion / exclusion criteria applied, the nature of the image data processing, including tissue classification and spatial normalization, as well as the analysis design. More specifically, the previously reported hippocampal atrophy – as calculated from 28 studies (Fraser et al., 2015) – increased with increasing age, from 0.38% annual atrophy in subjects younger than 55 years to 1.12% in subjects older than 70 years. Given that the mean age of the current sample was around 43 years (with 75% of all subjects younger than 55 years), it is to be expected that the resulting atrophy rate would be lower than the estimate from the meta-analysis, which included a large proportion of older subjects. In addition, it has been demonstrated that cross-sectional studies usually yield a substantially lower estimate for annual atrophy rates than longitudinal studies (Fraser et al., 2015; Raz et al., 2005). Thus, the current (cross-sectional) estimates should be lower than the meta-analytic estimates that were derived exclusively from longitudinal data (Fraser et al., 2015). Finally, as described above, the cohort investigated in the present study had been meticulously screened for signs of diseases and disorders that may affect brain anatomy (Mazziotta et al., 2009), which may have further reduced the annual atrophy rates in the current sample. Therefore, while biased towards very healthy individuals, the present findings are important because they provide critical information on hippocampal atrophy associated with good health. This

does not only provide a benchmark for age-related hippocampal atrophy uncontaminated by clinical pathology, but may also be useful as a frame of reference when modeling hippocampal atrophy across the lifespan in various diseases, disabilities and disorders.

4.4 Summary and Implications for Future Studies

Our study significantly enhances this field of research by mapping age effects on the hippocampal complex, while discriminating between functionally relevant subregions as guided by micro-structure. Overall, the present findings in this extremely healthy sample are suggestive of annual atrophy rates of approximately one half of those expected in the broader population. In other words, while somewhat higher atrophy estimates were found for the SUB and somewhat lower for the EC, they tended to be low in all subregions. This is encouraging because it suggests that, in individuals with a profile indicative of better health than the average population, a lower level of age-related hippocampal shrinkage might be expected. However, future longitudinal studies are clearly necessary to confirm these findings, not only in individuals selected for their excellent health status but also in well-characterized normative cohorts as well as in carefully selected samples of individuals with specific chronic conditions, such as hypertension, diabetes, depression, etc. Estimates in these sub-groups will help quantify the level of regional hippocampal shrinkage that can be attributed to specific chronic diseases against an optimal benchmark obtained from healthy individuals. In addition, the current findings suggest that systematic reviews and meta-analyses aimed at summarizing atrophy rates in the hippocampus, its subregions, and other brain structures may want to consider producing separate estimates, not only discriminating between normative populations and samples affected by specific chronic conditions, but also focusing explicitly on very healthy cohorts.

Disclosure Statement

There are no actual or potential conflicts of interest.

Acknowledgments

NC is funded by Australian Research Council Future fellowship number 120100227. EL is funded by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health under award number R01HD081720 and further supported by the Cousins Center for Psychoneuroimmunology at the University of California, Los Angeles (UCLA).

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Figure Legends

Figure 1. Subregions of the Hippocampus. Top Row: Cytoarchitectonically derived probability maps of the cornu ammonis (CA), fascia dentata (FD), subiculum (SUB), entorhinal cortex (EC), and hippocampal-amygdaloid transition area (HATA), displayed on sagittal sections of the MNI single-subject template. Bottom Row: The same probability maps displayed on coronal sections of the MNI single-subject template, depicting hippocampal head (left), body (middle), and tail (right). The color bar encodes the region-specific probability.

Table 1. Age-related Hippocampal Atrophy

	Left		Right	
Region	Atrophy rate (%)	Significance (p, corrected)	Atrophy rate (%)	Significance (p, corrected)
CA	-0.18	<0.001	-0.17	0.003
FD	-0.19	0.001	-0.15	0.01
SUB	-0.23	<0.001	-0.22	<0.001
EC	-0.09	0.537	-0.13	0.081
HATA	-0.22	0.006	-0.17	0.008
HC	-0.17	0.001	-0.17	0.001

cornu ammonis (CA), fascia dentata (FD), subiculum (SUB), entorhinal cortex (EC), hippocampal-amygdaloid transition area (HATA), entire hippocampus complex (HC)

Table 2. Volumes and Sex differences

	Left			Right		
Region	Females (mm^3)	Males (mm^3)	Significance (p, corrected)	Females (mm^3)	Males (mm^3)	Significance (p, corrected)
CA	4,638 ± 285	4,296 ± 312	<0.001	4,748 ± 339	4,395 ± 305	<0.001
FD	2,399 ± 176	2,184 ± 161	<0.001	2,379 ± 176	2,174 ± 161	<0.001
SUB	3,100 ± 200	2,955 ± 204	0.004	3,277 ± 224	3,151 ± 201	0.028
EC	3,894 ± 348	3,855 ± 336	1	4,190 ± 360	4,218 ± 321	1
HATA	278 ± 24	269 ± 28	0.619	232 ± 19	224 ± 17	0.234
HC	14309 ± 849	13559 ± 891	<0.001	14827 ± 995	14161 ± 806	0.003

cornu ammonis (CA), fascia dentata (FD), subiculum (SUB), entorhinal cortex (EC), hippocampal-amygdaloid transition area (HATA), entire hippocampus complex (HC)