3 Tesla Magnetic Resonance Imaging of Hippocampal Asymmetry: Results from the Dallas Heart Study

by

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ABSTRACT

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Background: Asymmetry of the hippocampus is regarded as an important clinical finding but limited data on hippocampal asymmetry is available for the general population. Here we present hippocampal asymmetry data from the Dallas Heart Study determined by automated methods and its relationship to age, sex, and ethnicity.

Methods: 3D-MPRAGE MRI were obtained in 2082 DHS-2 participants. The MR images were analyzed using two standard automated brain segmentation programs, FSL-FIRST and Freesurfer. Individuals with imaging error, self-reported stroke, or major structural abnormalities were excluded. Statistical analyses were performed to determine significance of the findings across age, sex, and ethnicity.

Results: At the 90th percentile FSL-FIRST demonstrated hippocampal asymmetry of 9.8% (95% CI 9.3 to 10.5%). The 90th percentile of hippocampal asymmetry measured by the difference between hippocampii over the larger hippocampus was 17.9% (95% CI 17.0 to 19.1%). Hippocampal asymmetry increases with age (P=0.0216) and men have greater asymmetry than women as shown by FSL-FIRST (P=0.0036), but ethnicity is not significantly correlated with asymmetry.

To confirm these findings Freesurfer was used. Freesurfer showed asymmetry of 4.4% (95% CI 4.3 to 4.7%) normalized to total volume, and 8.5% (95% CI 8.3 to 9.0%) when normalized by difference/larger hippocampus. Freesurfer also showed that hippocampal asymmetry increases with age (P=0.0024), and that men had greater asymmetry than women (P=0.03).

Conclusion: There is a significant degree of hippocampal asymmetry in the population. The data provided will aid in the research, diagnosis, and treatment of temporal lobe epilepsy and other neurological disease.

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- **Lucarelli RT,** Khera A, Peshock RM, McColl R, Ayers C, King KS. CRP, IL-18, and BNP are Associated with Regional Brain Atrophy: Results from the Dallas Heart Study. Abstract: Submitted August 14, 2012: *Honolulu HI, International Stroke Conference,* 2013.
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- **Lucarelli RT,** Peshock RM, Hulsey K, McColl R, Whittemore A, King KS. Abstract: Asymmetry of the Hippocampus at 3 T: From the Dallas Heart Study. Abstract: *Chicago, Ill: American Society Neuroradiology*. April 2012.
- **Lucarelli RT,** McColl R, King KS, Hulsey K, Ayers C, Peshock RM. Automated Analysis of Regional Brain Atrophy in the Dallas Heart Study. Abstract: *UT Southwestern: 50th Annual Medical Student Research Forum*, 2012.
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CHAPTER 1

Asymmetry of the hippocampus is relevant in neurological disease and is an important clinical finding. However, we lack hippocampal asymmetry data representative of the general population to guide the interpretation of hippocampal asymmetry. Additionally, the reliability of prior work analyzing asymmetry using user guided segmentation has recently been called into question.[1] At present, there are no large studies with broad ethnic representation, utilizing high resolution MRI, and with consistent application of automated measures of the hippocampus to define the range of values of hippocampal asymmetry in the general population.

Hippocampal asymmetry is one of the most important findings suggestive of mesial temporal sclerosis, the most commonly diagnosed structural cause of temporal lobe epilepsy,[2] and is used to lateralize the seizure focus for epilepsy surgery.[3-7] Other imaging findings in the hippocampus of mesial temporal sclerosis include T2 prolongation on MRI, and loss of internal architecture.[8] Hippocampal asymmetry is associated with Mild Cognitive Impairment and Alzheimer's disease, providing a potential bio-marker for early diagnosis that has been reported to be more accurate than bilateral hippocampal atrophy.[9] Additionally, several studies have suggested a link between asymmetry of the hippocampus in depression and schizophrenia .[10-12]

We report a study of a large population-based probability sample, with broad ethnic and age representation to ascertain the distribution of hippocampal asymmetry and its variance in relation to age, ethnicity, and sex.

CHAPTER 2

Study Population

The Dallas Heart Study (DHS) is a large, multi-ethnic, population-based cohort study of Dallas County residents which began in 1999. The DHS study was designed to produce unbiased population estimates of biologic and social variables, as has been previously described.[13] Briefly, population sampling was based on US Postal Service delivery sequence file with selection probabilities increased for strata with larger concentrations of African Americans so that they would constitute approximately half the study sample. The study was approved by the Institutional Review Board at the University of Texas Southwestern Medical Center and all participants provided written informed consent.

Between September 2007 and December 2009, original DHS subjects were asked to participate in a continuation of the original study termed the Dallas Heart Study-2. Family members and spouses of the original participants were able to participate in the DHS-2. Participants underwent magnetic resonance imaging at University of Texas Southwestern Medical Center. Individuals with previous surgery for an aneurysm in the brain, metal fragments in the eyes, brain, or spinal canal, cardiac pacemaker, implantable cardiodefibrillators, cochlear implant, spinal cord stimulators, or other internal electrical device, pregnancy, and occupations associated with exposure to metal fragments were excluded from MR imaging.

A total of 2,082 participants underwent brain MRI. Thirty seven were excluded for self-reported stroke. Images of outliers as found by Robust Minimum Covariance Distance analysis of brain segments,[18, 19] individuals flagged for exclusion in previous DHS2 MRI brain studies, and individuals who had error flags generated during automated analysis were reviewed by a neuroradiologist (KSK). Upon MRI image review, 70 individuals with major structural defects (such as corpus collosum agenesis, imaging evidence of stroke, and hydrocephalus), or image acquisition errors (such as metal and motion

artifact and other noise), were excluded. In total, 107 individuals were excluded from subsequent analysis.

MRI protocol

Brain MR images were obtained on a 3T MRI scanner (Achieva; Philips Medical Systems, Best, The Netherlands) using 3D magnetization-prepared rapid acquisition gradient (3D-MPRAGE). Images were obtained from the vertex of the skull to the foramen magnum in true axial orientation. Specifications for 3D MPRAGE were the following: axial sections reconstructed at 1.0-mm slice thickness; TR, 9.6 ms; TE, 5.8 ms; flip angle, 12°; FOV 260mmx260,mm with a voxel size of 1.0 x 0.9 x 0.9mm.

Image analysis

MRI quantification was performed using the freely available FMRIB software library, FSL-FIRST.[14, 15] Volumes of the left and right hippocampus were derived from 3D-MPRAGE sequences. In brief, the skull was removed from the 3D-MPRAGE images and the remaining images were segmented into three classes: CSF, WM, and GM. A mask for the hippocampus was created using the FSL-toolkit. Volumetric data was collected using the FSLstats routine. The present study was focused on the hippocampus and therefore the cerebellum and brainstem were excluded from analysis.

Further MRI quantification was performed using the Freesurfer image analysis suite, version 4.4, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/). The fully-automated analysis was run at the Texas Advanced Computing Center at The University of Texas at Austin, Austin, Texas. Volumes of the left and right hippocampus, along with other cortical and subcortical structures not reported here, were derived from MPRAGE sequences. Individuals who had Talairach

atlas registration error (n=11) had the atlas manually aligned following the procedures in the Freesurfer documentation, and the images were re-analyzed. Individuals with minor error on analysis (n=2) or timeout errors (n=9) were re-analyzed and the masks generated by Freesurfer were verified by a neuroradiologist (KSK).

Hippocampal asymmetry was calculated by taking the absolute value of the difference of left and right hippocampal volume and dividing by the total hippocampal volume. Another measure which has practical significance when interpreting MRI was calculated by taking the value of the difference between the left and right volume and dividing by the larger of the right or left hippocampus.

Statistical Analysis

Statistical analyses were performed using SAS Version 9.2.0 (Cary, NC, USA). Ninety-five percent distribution-free confidence intervals were generated with the Hahn and Meeker method.[16] Differences in hippocampal asymmetry as measured by FSL-FIRST for gender were evaluated with two-sided Mann-Whitney U-test at a significance level of <0.05. Differences in hippocampal asymmetry as measured by Freesurfer, to confirm FSL-FIRST results, were evaluated with one-sided Mann-Whitney U-test at significance level of <0.05. The correlation between age and hippocampal asymmetry was evaluated using Spearman Rank-Order correlation at a significance level of <0.05. Comparison of mean age among men and women was compared using the student T-test and among whites, Hispanics, and African Americans using ANOVA with Tukey's multiple comparison procedure. The correlation between ethnicity and hippocampal asymmetry was evaluated adjusting for age as a co-variate using the general nonparametric approach developed by Schacht, et al.[17]

CHAPTER 3

After exclusion analysis, 1975 individuals were evaluated with a gender makeup of 58.3% (n=1151) female, and 41.7% (n=824) male and an ethnic makeup of 46.3% (n=914) African Americans, 37.2% (n=735) white, 14.1% (n=279) Hispanic and 2.4% (n=47) other or not reported. The mean age was 49.8 ± 10.5 years.

The degree of hippocampal asymmetry and its relationship to gender is shown in Table 1 and Table 2. FSL-FIRST demonstrated significant asymmetry in our population (Table 1). After normalizing the difference in left and right hippocampal volume to the total hippocampal volume, the magnitude of hippocampal asymmetry at the 90^{th} percentile for FSL-FIRST was 9.8% (95% CI 9.3% to 10.5%). When clinically evaluating images for asymmetry, the magnitude is typically assessed by comparing the size of the smaller hippocampus with that of the larger, and this measure is shown in Table 3. FSL-FIRST also showed that men had significantly more asymmetry than women (P=0.0036). The median degree of hippocampal asymmetry for men was 3.8% (95% CI 3.5% to 4.2%) and it was 3.4% (95% CI 3.1% to 3.7%) for women. The magnitude of asymmetry was also shown to increase with age (P=0.0216) when evaluated with FSL-FIRST and increased from 2.7% in the 2^{nd} decade to 4.5% in the 7^{th} decade (Figure 1). This increase in hippocampal asymmetry with age was more pronounced in the top 90^{th} percentile of individuals and increased from 9.9% to 11.3% as measured by FSL-FIRST (Figure 1). When evaluating for ethnic differences in hippocampal asymmetry we found that there was a significant difference in age amongst Hispanics, whites, and African Americans (P<0.0001). After controlling for differences in age, there was no significant correlation between ethnicity and hippocampal asymmetry.

To confirm the findings found with FSL-FIRST, another automated program, Freesurfer, was used to evaluate asymmetry as shown in Table 1 and 2. Freesurfer also showed a significant degree of

asymmetry in our population when normalizing asymmetry by taking the difference between the left and right hippocampus over the total hippocampal volume (Table 1). This difference in asymmetry was also apparent when normalizing the difference between the left and right hippocampus to the larger of the two hippocampii (Table 2). Freesurfer further demonstrated an increase in hippocampal asymmetry with increasing age (P=0.0024) as shown in Figure 1. The difference between men and women seen with FSL-FIRST was confirmed with Freesurfer (P=0.03), but ethnicity was not associated with asymmetry when controlled for age.

CHAPTER 4

Our study reports a detailed description of the magnitude of hippocampal asymmetry analyzed with fully-automated segmentation methods using high magnetic field strength MRI among participants in the Dallas Heart Study. To our knowledge, this is the first description of the magnitude of hippocampal asymmetry with evaluation of distributions by age, gender, and ethnicity in a multi-ethnic population-based probability sample of community dwelling individuals. We initially performed our analysis using FSL-FIRST and noted a significant degree of hippocampal asymmetry in the population. To confirm these findings, we used a second commonly used software package, Freesurfer, which confirmed the finding of hippocampal asymmetry in our population. We took a conservative approach by reporting quartiles and the top 90^{th} percentile, yet by definition 10% of individuals in our study have an even greater degree of asymmetry than reported here (more detailed results are shown in supplemental Tables 1 and 2). We chose to report asymmetry in two different ways. The majority of our analysis utilizes a traditional approach reporting hippocampal asymmetry normalized by total hippocampal volume (Table 1 and Figure 1). However, we also report hippocampal asymmetry normalized by the larger of the two hippocampii as this reflects the evaluation when attempting to visually determine symmetry between hippocampi in a clinical setting (Table 2).

This work expands our knowledge of hippocampal asymmetry in several ways. Our current conceptions regarding hippocampal asymmetry are based largely on studies with user guided segmentation performed by experts in neuroanatomy. However, a recent analysis of user guided segmentation has shown the technique to be flawed. By presenting randomly inserted mirror images for analysis, investigators were able to determine a consistent left-right bias in subcortical segmentation which was most significant for the hippocampus, in some cases as high as 11%. The authors proposed 'laterality of

visual perception' as an inherent limitation in any study employing user interaction in hippocampal segmentation and further suggested a reappraisal of prior work in light of this finding.[1] Thus, data previously reported using manual segmentation has the potential for material bias.

Previous studies of hippocampal volumes and asymmetry were limited by the time intensive nature of user guided segmentation, and as a result no large population based studies have been conducted. Pedraza et al. sought to address the small size of these previous studies by performing a meta-analysis on 82 studies for a total of 3,564 participants from the control groups.[20] This approach primarily addresses type II errors, or low power, among prior studies. This approach does not, however, address selection bias, as control groups are not designed to reflect the general population.[21, 22] Using automated techniques we were able to examine data from 1975 individuals using the same segmentation methods, imaging technique, and MRI hardware.

Because signal strength may influence image quality, and thus the accuracy of segmentation, our analysis was conducted using 3 Tesla 3D-MPRAGE images reconstructed at 1mm slices, providing high resolution volumetric images compared to previous 1.5 Tesla studies. The amount of hippocampal asymmetry demonstrated is higher than reported in previous studies and while the reason for this is presently unknown and may be due to technical improvements in our study, it could also be due to other existing pathology present in a population-based sample which may affect the hippocampal asymmetry seen in our study participants.

Prior studies have validated the automated methods used for the segmentation of deep brain nuclei using manual segmentation and post-mortem autopsy as the gold standard references.[23-25] While the validity of manual segmentation itself has recently been called into question,[1] one would not expect these automated measures to suffer from the same systematic bias of manual tracing. Recently there has been

considerable effort to compare the sensitivity of these automated methods in determining hippocampal volume. Pardoe and Morey in separate studies both conclude that Freesurfer is more sensitive at detecting hippocampal atrophy than FSL-FIRST.[26, 27] Pardoe further concludes that Freesurfer is less likely than FSL-FIRST to fail to detect atrophy of the hippocampus when compared to manual segmentation.[27] Notably, FSL-FIRST was found to have greater variation than Freesurfer when determining hippocampal volume compared to manual tracing.[26] However, FSL-FIRST has several advantages compared to Freesurfer including much faster processing time and requiring considerably less computing resources. It needs to be clearly stated that such prior validation efforts and our own analysis are designed for accurate assessment of hippocampal asymmetry in the population and targeted groups. This work does not advocate replacing qualitative analysis of MR images for individual clinical diagnosis. Additionally, while our data shows a significant degree of hippocampal asymmetry in the general population as measured by automated methods, it is not clear at what magnitude this asymmetry would be detected clinically. It would be fruitful to investigate what percent difference in hippocampal asymmetry is detectable clinically by expert reviewers to further elucidate the impact of the findings reported herein.

It should be noted that the sample we have evaluated in this study was designed to be representative of the adult population of Dallas County, with oversampling to ensure approximately half our population was from African Americans. We evaluated hippocampal asymmetry with respect to ethnicity and did not see a significant difference in our sample. We did not evaluate or exclude any persons based on their neurological or psychiatric histories. Our study is designed to be reflective of the general population without history of stroke. Diseases which have a high prevalence in the general population that do not preclude independent living in the community are likely included in this sample. In the case of epilepsy, prior studies of prevalence would suggest we would have approximately 5

individuals per 1000 with a history of epilepsy.[28] By focusing on median values and percentile ranges, and using non-parametric analysis the effects of specific outliers is limited. However, early changes that may lead to Mild Cognitive Impairment or eventually Alzheimer's disease may be observed in our study to the same extent that they would be observed among the general population.

Our data suggests a large degree of asymmetry in the general population and questions how much weight should be placed on mild hippocampal asymmetry as a solitary imaging finding. For the example of mesial temporal sclerosis, we would suggest that future quantitative studies evaluate the degree of hippocampal asymmetry rather than presence or absence. Further studies should also include other abnormalities such as increased signal on FLAIR or loss of the normal internal architecture into a comprehensive predictive model. In the example of suspected mesial temporal sclerosis this may lead to improved diagnostic accuracy of pre-surgical imaging and more precise patient selection for epilepsy surgery.

The data presented herein on the magnitude of hippocampal asymmetry provides the first large, population based study with broad ethnic representation using high resolution MRI and fully-automated methods. Our findings suggest that age and gender should be considered when evaluating hippocampal asymmetry and that caution is warranted in the interpretation of hippocampal asymmetry as an indicator of pathology.

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Hippocampal Asymmetry (with Respect to Total Hippocampal Volume) (95% CI)				
FSL-FIRST				
	25%ile	50%ile	75%ile	90%ile
Total	1.6% (1.5 - 1.7%)	3.6% (3.4 - 3.8%)	6.2% (5.9 - 6.6%)	9.8% (9.3 - 10.54%)
Men	1.8% (1.5 - 1.9%)	3.8% (3.5 - 4.2%)	6.8% (6.2 - 7.3%)	11.2% (9.9 - 11.8%)
Women	1.5% (1.4 - 1.7%)	3.4% (3.1 - 3.7%)	5.9% (5.7 - 6.3%)	9.2% (8.7 - 9.9%)
Freesurfer				
Total	0.8% (0.7 - 0.8%)	1.7% (1.6 - 1.8%)	2.9% (2.8 - 3.1%)	4.5% (4.3 - 4.7%)
Men	0.8% (0.7 - 0.9%)	1.8% (1.6 - 1.9%)	3.2% (2.9 - 3.3%)	4.7% (4.4 – 5.0%)
Women	0.8% (0.7 - 0.8%)	1.7% (1.6 - 1.8%)	2.9% (2.8 - 3.1%)	4.5% (4.3 - 4.7%)

Table 1: FSL-FIRST and Freesurfer measures of hippocampal asymmetry in the total population and grouped by gender. Asymmetry is defined by absolute difference in left to right hippocampal volumes with respect to total hippocampal volume.

Hippocampal Asymmetry (with Respect to Larger Hippocampus) (95% CI)				
	25%ile	50%ile	75%ile	90%ile
FSL-FIRST	3.1% (2.9 - 3.4%)	6.9% (6.5 - 7.2%)	11.7% (11.3 - 12.4%)	17.9% (17.0 - 19.1%)
Freesurfer	1.5% (1.4 - 1.6%)	3.3% (3.1 - 3.5%)	5.7% (5.4 - 5.9%)	8.5% (8.3 -9%)

Table 2: Hippocampal asymmetry, defined by the absolute difference in left to right hippocampal volumes with respect to the volume of the larger side.

LIST OF SUPPLEMENTAL TABLES

Hippocampal Asymmetry (with Respect to Total Hippocampal Volume)						
FSL-FIRST						
	25%ile	50%ile	75%ile	90%ile	98%ile	99%ile
Total	1.6%	3.6%	6.2%	9.8%	16.7%	19.9%
Men	1.8%	3.8%	6.8%	11.2%	18.0%	20.7%
Women	1.5%	3.4%	5.9%	9.2%	14.5%	18.4%
Freesurfer						
Total	0.8%	1.7%	2.9%	4.5%	6.9%	8.5%
Men	0.8%	1.8%	3.2%	4.7%	7.5%	8.5%
Women	0.8%	1.7%	2.9%	4.5%	6.4%	8.5%

Supplemental Table 1: Extended table of FSL-FIRST and Freesurfer measures of hippocampal asymmetry in the total population and grouped by gender. Asymmetry is defined by absolute difference in left to right hippocampal volumes with respect to total hippocampal volume.

Hippocampal Asymmetry (with Respect to Total Hippocampal Volume)						
FSL-FIRST						
Decade	25%ile	50%ile	75%ile	90%ile	98%ile	99%ile
2nd	1.7%	2.7%	6.1%	8.9%	16.1%	16.1%
3rd	1.5%	3.1%	5.4%	8.6%	12.4%	17.3%
4th	1.7%	3.7%	6.5%	9.8%	16.5%	20.7%
5th	1.5%	3.5%	6.4%	11.0%	17.0%	19.0%
6th	1.5%	3.7%	6.6%	9.5%	18.5%	27.5%
7th	3.1%	4.5%	7.5%	11.3%	15.6%	16.7%
Freesurfer						
2nd	0.7%	1.7%	2.5%	3.7%	4.3%	4.3%
3rd	0.7%	1.6%	2.5%	4.0%	6.8%	7.8%
4th	0.8%	1.6%	2.8%	4.4%	6.8%	8.9%
5th	0.8%	1.7%	3.0%	4.5%	7.0%	8.5%
6th	0.7%	1.8%	3.1%	4.7%	7.4%	8.1%
7th	1.1%	1.9%	3.8%	5.4%	8.4%	9.3%

Supplemental Table 2: Extended table of FSL-FIRST and Freesurfer measures of hippocampal asymmetry grouped by decade. Asymmetry is defined by absolute difference in left to right hippocampal volumes with respect to total hippocampal volume. (2nd decade N=28, 3rd decade N=331, 4th decade N=613, 5th decade N=601, 6th decade N=344, 7th decade N=53)

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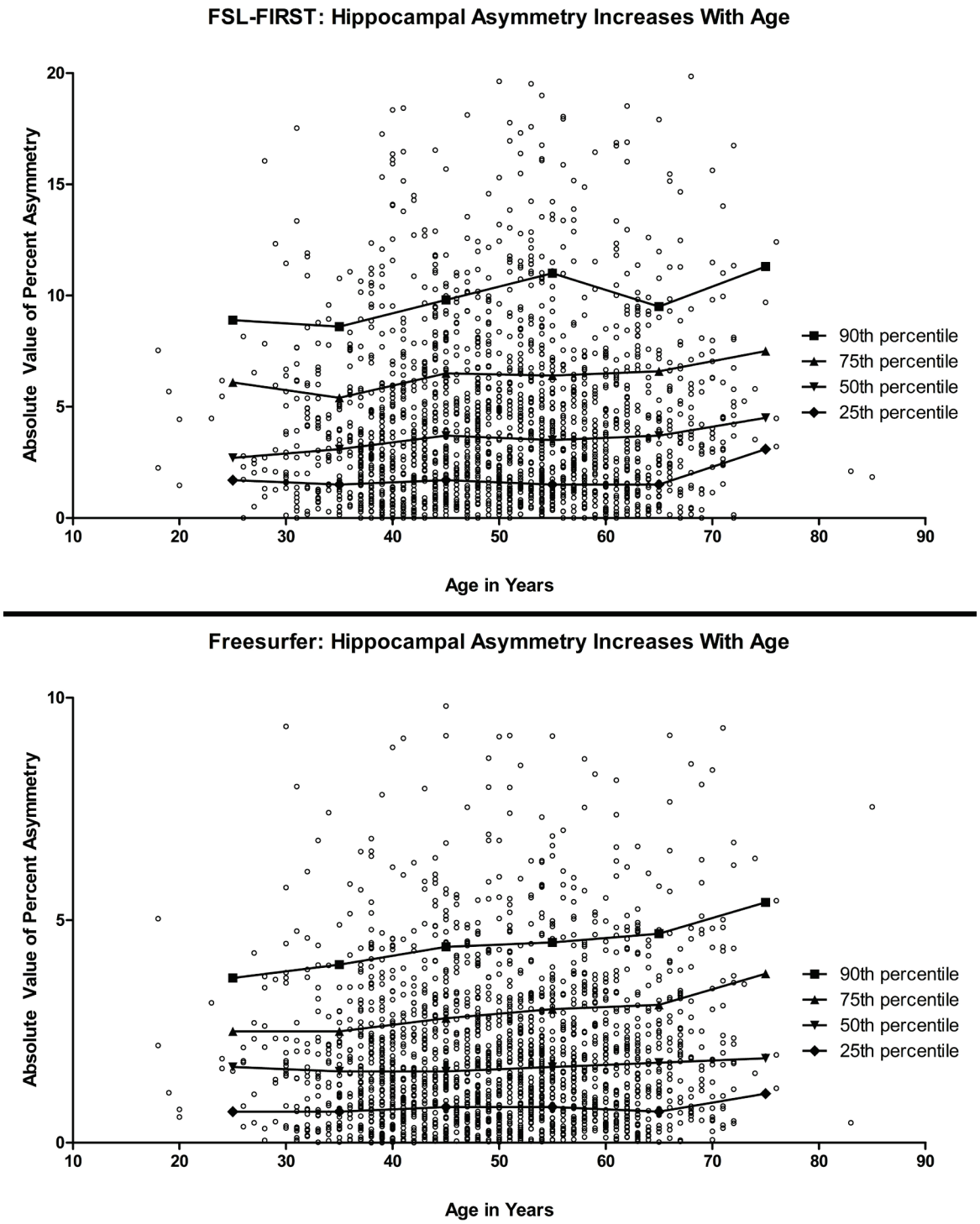

Figure 1: Hippocampal asymmetry increases with age as measured by FSL-FIRST (top) and Freesurfer (bottom). (Less than 1% of points are not plotted to preserve scale. 2nd decade N=28; 3rd decade N=331; 4th decade N=613; 5th decade N=601; 6th decade N=344; 7th decade N=53)

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