# Quantitative cytoarchitectural mapping of the parahippocampal region

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**Background.** Classical cortical mapping relies on the visual identification of distinct architectonic features on histology. Extraction of intensity line profiles allows for an objective quantification of cytoarchitectonic borders [1]. Unlike many neocortical areas that have no distinguishing features other than topography [2], the parahippocampal region displays characteristic cytoarchitecture resulting from the laminar organization and connectivity of its components. Our purpose was to delineate cytoarchitectonic borders of major parahippocampal region structures based on their laminar cortical pattern on ultra-high resolution MRI of *postmortem* brains.

**Methods.** We studied a single hemisphere obtained from 3 neurologically healthy males (mean age, 65). The antero-medial temporal lobe containing the parahippocampal gyrus was dissected and scanned on a 7T MRI using a solenoid coil. We acquired 100 µm isotropic images using a fast, low-angle shot protocol with multiple flip angles ($5^0$ to $25^0$, steps of 5), yielding a total of 15 scans (3 for each flip angle) synthesized into one volume [3]. Tissue blocks were then sectioned at 25 µm in the coronal plane perpendicular to the hippocampal long axis, mounted on slides and stained for cell bodies (Nissl). In each case, we analyzed 10 consecutive MRI slices. We sought to delineate the borders between perirhinal cortex (Pc), entorhinal cortex (Ec), para-subiculum (Para), pre-subiculum (Pre) and subiculum (Sub) at the level of the mid hippocampal head. Ec/Pc border was also examined at the level of the amygdala and anterior hippocampus. *Pre-processing:* On each slice, GM/CSF and GM/WM interfaces were outlined manually, creating 2 surfaces. The Laplace equation was applied to generate series of nested equipotential contours. Lines (profiles) orthogonal to each streamline were then produced. Grey level intensities (GLI) were extracted along the profiles at 100 uniformly spaced points. *Feature extraction:* The mean and the first 4 central moments of the frequency curve were computed from the GLI and from the absolute value of the first derivative of the GLI. To assign equal weight to each feature, we z-transformed them with respect to all profiles. Finally, the 10 features were combined into one vector. The Mahalanobis distance (MD), a measure of dissimilarity, was calculated between all pairs of neighboring cortical areas for blocks of 6 to 22 profiles using a sliding window technique. *Cytoarchitectonic borders:* MD maxima were tested for statistical significance using Hotelling's $T^2$ test with Bonferroni correction for multiple comparisons. Significant maxima not exceeded by another significant maximum within a neighborhood of defined size were accepted as areal borders only if spatially corresponding on consecutive slices and verified on histology.

**Results.** Significant maxima coinciding with the cytoarchitectonic borders of the parahippocampal region structures were found in all three samples. The detection rate within the three examined levels ranged from 40% to 100% (see Table). Among the 16 block sizes studied, the overall average frequency of significant maxima was 45% (i.e., 7/16 block sizes showing the same profile position).

**Table.** % detection rate of parahippocampal borders over 10 slices.

| | level | Pc/Ec | Ec/Para | Para/Pre | Pre/Sub |
|---|---|---|---|---|---|
| **Case 1** | 1 | 100% | | | |
| | 2 | 50% | | | |
| | 3 | 70% | 80% (EC/PRE) | | 100% |
| **Case 2** | 1 | 100% | | | |
| | 2 | 100% | | | |
| | 3 | 100% | 70% | 40% | 100% |
| **Case 3** | 1 | 100% | | | |
| | 2 | 100% | | | |
| | 3 | 100% | 60% | 50% | 70% |

Levels: amygdala (1); hippocampal head (anterior -2- and mid -3-)

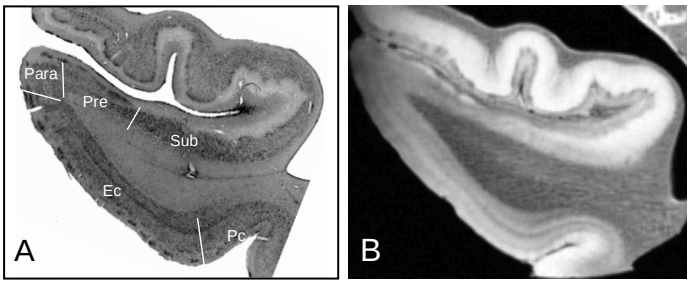


***Figure 1.*** *Coronal Nissl-stain (A) and MR (B) sections at the level of the mid hippocampal head.*

*Lines on histology section show the borders between parahippocampal structures.*

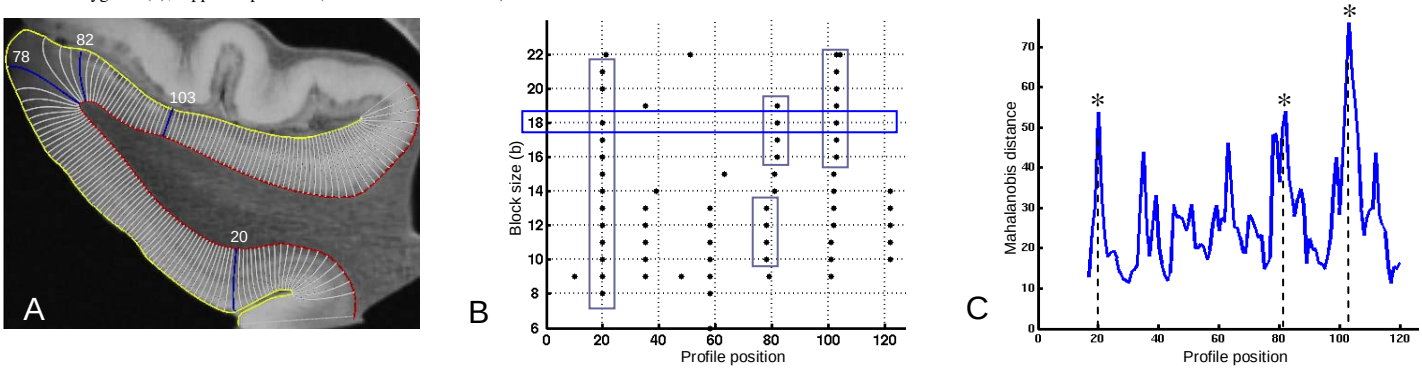


***Figure 2. (A)*** *Profiles on MRI. Numbers indicate maxima corresponding to borders in Fig. 1A.* ***(B)*** *Frequency of maxima against profile position. Example: Pc/Ec border at position 20 is detected by 13/16 block sizes (vertical frames). Horizontal frame at b=18 corresponds to the Mahalanobis distance in C, where significant maxima are shown by *.*

**Conclusion.** Quantitative analysis of cortical laminar distribution of the human brain can be performed on MRI. Combined with multivariate statistics, this method allowed an objective distinction of cytoarchitectonic borders between parahippocampal subregions that are currently invisible to conventional means of analysis. Application of this technique to neurological disorders such as Alzheimer's disease and temporal lobe epilepsy may provide a better understanding of the contribution of parahippocampal pathology to these conditions.

**References:** 1) Schleicher, *et al.* (2000), J Chem Neuroanat. 2) Van Hoesen, *et al.* (2000), Annals of NYAS. 3) Augustinack, *et al.* (2005), Annals of Neurology.