An Automatic Computational Method for the Measurement of Amyloid Plaque Load in the APP Transgenic Mouse Brain

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Introduction

Alzheimer's disease (AD) is linked to increased brain deposition of amyloid-beta peptides in senile plaques. Successful visualization of amyloid deposits in the APP transgenic mouse brain with MR imaging has been reported by several investigators recently. There is no suitable method, however, to measure plaque burden in the mouse. The number of plaques, their size and brain distribution depend on the transgenic mouse line and vary with age. We present a novel method based on simulated flooding [**1**] to segment the AD plaques in mouse MR brain images, and show how the method can be used for a detailed analysis of plaque characteristics.

Methods

Transgenic Mice: Tg2576 mice overexpressing human APP695 with the "Swedish" mutation were purchased from Taconic. 5XFAD transgenic mice coexpressing a total of five FAD mutations [APP K670N/M671L (Swedish) + I716V (Florida) + V717I (London) and PS1 M146L + L286V] were generated in a collaborator's laboratory [**2**]. Amyloid deposition begins in the Tg2576 mouse brain at 14 months and by 18 months of age, a large number of plaques may be found. Plaques begin to appear in the 5XFAD mouse brain much earlier, at 2 months, and level off at 9 months.

MR imaging: Brains fixed in 4% paraformaldehyde were used for imaging. During imaging, brains were immersed in Fomblin (a perfluorinated liquid) to prevent dehydration and reduce magnetic susceptibility gradients. All imaging experiments were performed on a Bruker Avance 14.1T imaging spectrometer fitted with a 100G/cm gradient using a10 or 20 mm resonator tuned to proton frequency (600MHz). 3D images were acquired using a fast spin-echo (RARE8) pulse sequence and the following imaging parameters: TR/TE*eff* 2500ms/40ms; pixel size 35µx35µx35µ.

Plaque Analysis: The automatic plaque segmentation algorithm has two steps: (1) Simulated flooding (watersheds [1]) is used to extract regions with low intensities completely surrounded by higher intensity neighbors. The result, WS(I), is a map of the MR image I whose labels define the catchments basins of the MR local minima. Watersheds are ignored since they are places of local maxima. (2) Image Laplacian L(I) = div (grad (I)) is used to model the plaque cores, defined as regions with small derivatives, surrounded by neighbors with rapidly increasing intensity. L(I) is the degree to which the gradient vector field flow behaves like a source or a sink. It is a global measure of intensity variation, independent of the baseline MR levels, which, unlike local minima, allows direct variability comparison between different regions. The background level of L(I) is estimated using the image statistics. Plaques are segmented by selecting the WS(I) regions that have maximum Laplacians larger than the background noise. The segmentation results P(I) is a map of I that shows each pixel's membership to a plaque.

Results and Discussion

We applied our algorithm on images of four excised mouse brains: Two 5XFAD mice (aged 2 and 9 months), one Tg2576 (22 months) and a normal B6 mouse (7 months). For each dataset the subiculum was segmented manually to assess the plaque distribution within that brain structure. Several measures were derived from P(I): individual plaque average profile AP (intensity profiles along any directions can be averaged after alignment to the plaque core) (Figure 1 c); plaque load PL (ratio between the volume of the segmented plaques and the volume of the structure of interest); plaque distribution - frequency distribution of plaques PD, their pixels distribution PPD (Figure 2 a and c), or distances in different orientations; and plaque grade assessment (higher L(I) means larger and more space consistent intensity variation). The algorithm was evaluated visually (Figure 1) and by comparing the PL values to known plaque characteristics of the mouse strains. The plaque load results show a PL increase with age for the 5XFAD mice from 8% at 2 months to 17% at 9 months. In contrast, the 22-month Tg2576 showed a PL of 28%, while the normal mouse had a PL of nearly 0% as expected.

Our results are consistent with known characteristics of amyloid plaques in the subiculum of 5XFAD mice [**2**] as well as dense cored plaque ultrastructure in aged Tg2576 mice [**3**]. A more comprehensive validation would require a comparison with histology data. Our method makes no assumption on the plaques shape and size. This makes it useful to analyze data for different AD stages, where plaque variability should be expected. The segmentation gives us not only the plaque labels, but also the position of their core. This allows the correct alignment of profiles along random directions, to compute the AP. The algorithm can be used for the analysis of individual plaques and plaque distribution within brain structures.

Figure 1. Plaque segmentation (Tg2576): (a) Sagittal MR image; (b) detail of the subiculum, arrow shows the place of the plaque; (c) AP and the associated profiles along the three major axes; volume rendering (d) and surface rendering (e) of the segmented plaque show the contour matches the profiles, being slightly elongated on the medial–lateral axis, volume rendering color map below is over-imposed on image histogram.

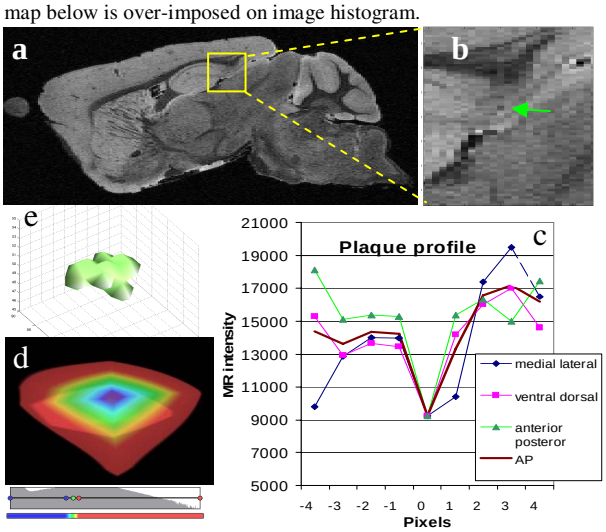
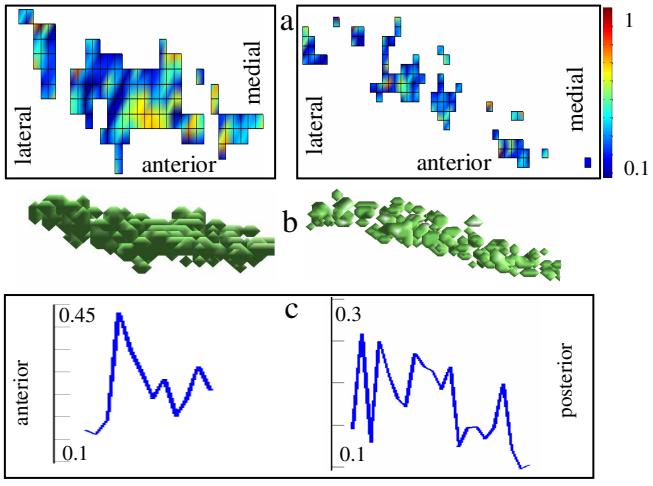


Figure 2. Tg2576 (left column) and 9 months old 5XFAD (right column). PPD, computed as ratio between the plaques and subiculum volume; (a) PPD in the transversal plane for the Tg2576 mouse shows increased plaque distribution (red-yellow) in the anterior slices; 5XFAD shows a distribution more uniform in values but sparser in space; (b) Corresponding 3D surface rendering of the plaques, looking along the dorso-ventral axis, towards the ventral side; (c) PPD along antero-posterior axis, showing larger concentrations in the anterior slices for both mice.



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