Automatic Computational Method for the Measurement of Neuronal Cell Loss in Transgenic Mouse Model of AD

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Introduction

Alzheimer's disease (AD) neuropathology is characterized by key features that include the deposition of the amyloid-beta peptides into senile plaques, the formation of neurofibrillary tangles, and the loss of neurons and synapses in specific brain regions. Here we report a measure of AD evolution based on the use of MR images of the APP transgenic mouse brain to visualize neuronal loss. A novel semi-automatic segmentation method is used to quantify the regional differences in 3D images of the pyramidal cell layer in hippocampal CA1 subfield (PLCA1) in wild type (WT) control and 5xFAD [1] transgenic mice, a very rapid progression AD model. The proposed method uses unsupervised support vector machines (SVM) [2] to discriminate the PLCA1 voxels, and their distance to the classification hyperplane is used for a detailed analysis of PLCA1 characteristics.

Methods

Transgenic Mice: 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 [1]. *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 a 10 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 35µm x35 µm x35 µm.

PLCA1 Analysis: The voxels of MR Image I are described using 3D "sheetness" features that match the PLCA1 MR appearance and are based on the Laplacian and Hessian matrix of the volume intensity function combined with isotropic Gaussian blurring. Laplacian L(I) = div (grad (I)) is used to model the neuronal layer core, 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. The other feature is the largest positive eigenvalue of the Hessian matrix $H_{jk}(I)= D_jD_k(I)$, the square matrix of second-order partial derivatives. The Hessian eigenvalues provide a curvature analysis that is independent of the data coordinate system and they have been used previously to determine voxel's vessel likelihood [3]. For classification, we define the CA1 pyramidal cell layer as an outlier detection problem of one-class [4] SVM (OCSVM). OCSVM is an extension of the two classes SVM, which estimates a classification function that encloses a majority of the training prototypes in a feature space. We use µ-SVM, an OCSVM implementation that computes a hyperplane to separate a specified fraction (1- µ) of data with the maximum distance to the origin. SVM classification uses kernel methods to project the original data space into a high dimensional feature space, and a linear classification in the latter is equivalent to a nonlinear classification in the former. We use Radial Basis Function (RBF) kernel, $k(x; x_i) = e^{-\gamma\|x-x_i\|^2}$, where γ determines the kernel width [5]. Our method has two training steps. First, an OCSVM model is created by training on a 10 M 5xFAD mouse dataset where the CA1 cell layer loss is most visible. This model will classify as PLCA1 any voxel that has features different than the delineated brain region in the training dataset. Then, a two class SVM is trained on a wild type 10 months old mouse dataset where the PLCA1 is clearly visible The training is performed using the labels created by the OCSVM classifier. The resulting classifier is then applied to all datasets.

Results and Discussion

We applied our algorithm to images of 12 excised mouse brains: six 5xFAD mice (age 2 (n=2), 4 (n=2) and 10 (n=2) months) and six control mice (same ages). For each dataset the volume of interest (VOI) around the PLCA1 was delineated manually. For each VOI voxel the algorithm generates two measures: the PLCA1 label (0 or 1) and the distance (SVMDist) to the SVM separation hyperplane (positive values for PLCA1 labeled voxels). SVMDist combines both classification features and measures the neuronal cell loss as indicated by the MR contrast. The total PLCA1 volume and its average SVMDist for each dataset are compared in Figure 1. Cerebral amyloid deposition begins in 5xFAD transgenic mice at 2 months of age and reaches a very large burden by 9-10 months of age, especially in the subiculum and deep cortical layers. Our results show that this age dependant evolution of plaques is matched by the MR signal loss in the PLCA1. Figure 2 shows the PLCA1 labeled voxels qualified with the SVMDist index. The distribution of the neuronal cell loss within the PLCA1 is shown in Figure 3, which may be a useful tool to understand the mechanism of cell loss in AD. Similar behavior of the PLCA1 volume and SVMDist distribution has been observed in MR images of live animals. Although our initial histology validation shows that the MR signal loss is related to cell packing pattern visible in PLCA1, a more thorough stereological analysis is needed to correlate the presented MR signal loss with neuronal cell loss. Unlike senile plaques, neuronal cell loss shows strong correlation with cognitive decline in AD. Therefore, the presented technique could be used for tracking AD evolution and asses emerging AD therapies.

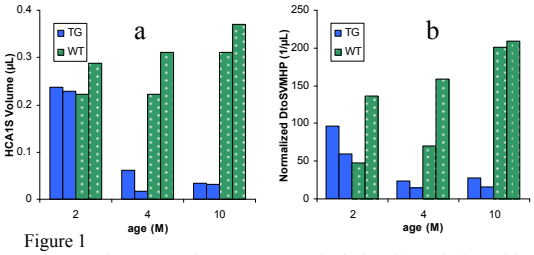

Figure 1
PLCA1 volume (a) and average SVMDist index (b) evolution with age is opposite for 5xFAD (solid blue) and WT (dotted green) mice. Note that the PLCA1 MR contrast increases with age in WT animals, even if this is less visible in the PLCA1 volume alone.

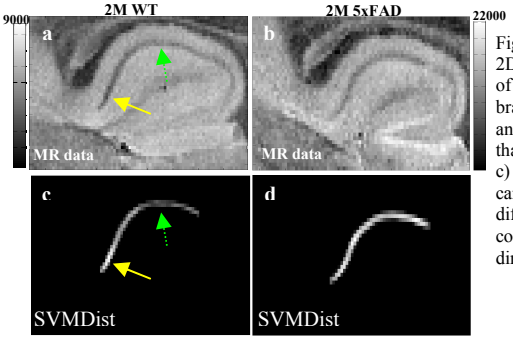

Figure 2
2D sagittal section through the 3D MR image of a wild type (a) and 5xFAD (b) mouse brain. Corresponding SVMDist distribution (c and d) within the PLCA1 labeled area shows that voxels with high (yellow arrow in a and c) and low (green-dotted arrows) MR contrast can be differentiated Furthermore, despite the different MR signal ranges in a and b, the corresponding SVMDist values can be directly compared between different datasets.

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Acknowledgement: Alzheimer Association HRG-06-27578 & NIH S10 RR13880

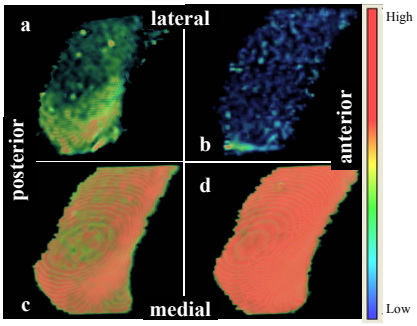

Figure 3
Volume rendering of the SVMDist index within the PLCA1 classified volume of a 2M TG (a), 10 M TG (b), 2M WT (c) and 10 M WT (d) mice. The neuronal loss measured by the SVMDist index is already apparent in the 2M TG when compared to the control 2M WT (Figure 1b), even though the PLCA1 volumes are similar (Figure 1a). The SVMDist distribution further decreases at 10 M for the TG mouse in contrast with the increase in the 10 M WT mouse.