Correlating Longitudinal In Vivo Images of Beta-Amyloid Plaques with Morris Water Maze Test Results in a Mouse Model of Alzheimer’s Disease

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Introduction
Alzheimer’s disease (AD), the most common form of dementia, affects thought, memory and language but cannot be diagnosed with certainty until after death. Beta-amyloid (Aβ) plaques are a pathological feature of AD. Using MRI, Aβ plaques can be visualized in live transgenic mice1,4,6 making MRI a potential diagnostic tool for early detection of AD. To determine if Aβ plaques occur early or late in the clinical progression of AD, behavioral tests need to be performed in parallel with imaging studies to establish whether Aβ plaques precede or follow memory deficits. This study follows Aβ plaques in transgenic APP/PS1 mice (amyloid precursor protein and presenilin 1 co-mutation; strain 00462 from Jackson Lab), and is the first to combine imaging over an extended period of time with Morris Water Maze (MWM) tests being run in parallel with MRI studies.

Methods
Two groups of mice were studied. Group 1 included six mice (four AD and two control mice) that were imaged from 6 to 13 months of age but did not undergo MWM tests. Group 2 included six mice (three AD and three control mice) that were imaged at 9 and 10 months of age and performed the MWM between imaging sessions. The local CCAC committees approved all experiments.

MRI Group 1: 3D T2*-weighted images (FLASH, 5 averages, (1.7 cm)2 FOV, 1282 matrix size (133 µm isotropic resolution) without zero padding, Te 73 ms, Tr 4 ms, 15 degree flip angle, acquisition time 99 minutes) were acquired monthly from the mice on an 11.7 T Bruker Avance spectrometer running Paravision 3. One control and one AD mouse died prematurely and thus did not complete the full seven months of imaging. Group 2: 2D T2*-weighted images (5 slices, 8 echoes, 2 averages, (2.5 cm)2 FOV, 2562 matrix size (98 x 98 x 750 µm3 resolution), Tr 2.1 s, Te 27 ms, acquisition time 21 minutes) spanning the hippocampus were acquired from the mice on a 7 T Bruker Avance spectrometer running Paravision 2. MWM The MWM tests spatial reference memory and has been described in detail previously5. Mice in group 2 were placed in an opaque pool of water (81 cm diam) with a platform (5 cm diam) just below the surface. They learned the location of the platform based on visual cues placed around the pool. Acquisition (with platform) lasted 7 days (4 trials/day = 1 block). Memory retention (without platform) was then tested over 3 days (4 trials/day = 1 block). Swimming paths were tracked and analyzed in Matlab. One control mouse was removed from the MWM study as she failed to attempt a search.

Histology
Mice were perfused with 4% paraformaldehyde in PBS within 48 hours of their last imaging session. The brain was removed and embedded in paraffin. Axial slices (6 microns thick) were sectioned spanning the hippocampus. Slides were stained with the modified Bielschowsky silver method to visualize amyloid plaque deposition.

Results
MRI Histologic staining showed that plaques with neuritic cores were present in the cerebral cortex and hippocampus (Figure 1). They had diameters of up to 50 µm. On MRI, single voxels with hypointense signal were identified with increasing frequency as the animals aged. We hypothesize that the low signal is due to the presence of plaques, which are roughly the size of the in-plane voxel image (2D study), accepting that volume averaging through the slice thickness degrades the true ability to image individual plaques.

Figure 1 (right). Presence of Aβ plaques confirmed with histological staining. White arrow in the T2 MRI (left panel) shows the position of a Aβ plaque corresponding to the plaque shown with the black arrow in the corresponding histological image (middle panel). 40x histological image of the rectangular region shows the same plaque (right panel).

Figure 2. More Aβ plaques are visible in transgenic mice each month as they age from six to 13 months. Sections of the brain from in vivo T2* images of a typical transgenic mouse are shown. Arrows in the figures indicate the presence of Aβ plaques in the cerebral cortex and hippocampus.

MWM

Figure 3. Transgenic mice (blue) learn and retain spatial information less quickly than control mice (yellow). While the differences were not statistically significant, the data indicate that the control mice find the location faster than the AD mice (a). This trend is not due to the control mice swimming faster (b). In retention, with no platform, the control mice spend more time in the quadrant that used to contain the platform (c) and on average are closer to the former location of the platform (d).

Discussion and Conclusions
Beta-amyloid plaques were visualized in vivo and through time using T2* and T2*-weighted MRI. The number of visible plaques increased with the age of the mice, and the presence of plaques was confirmed with histology. Spatial memory performance of APP/PS1 mice was lowered as compared with control mice in the MWM. This work lays the foundation for future combinations of imaging and behavioural studies to determine whether Aβ plaques are associated with AD symptoms.

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