MAGNETIC RESONANCE IMAGING AND BEHAVIOURAL TEST COMPARISONS IN A MOUSE MODEL OF ALZHEIMER’S DISEASE

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Alzheimer’s disease (AD), the most common form of dementia, affects thought, memory and language but cannot be diagnosed with certainty until after death. Currently the only sure diagnosis is pathology of brain tissue taken post-mortem. Beta-amyloid (Aβ) plaques are a signature pathological feature of AD. Only recently have these been imaged in living, transgenic mice using Magnetic Resonance Imaging (MRI) [1-5].

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, 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.

THEORY

Beta-amyloid is a fragment of a larger protein called the Amyloid Precursor Protein (APP). In its complete form, APP spans the fatty cell membrane, extending from the inside to the outside of a brain cell. While the precise physiological role of APP is unknown, recent experiments indicate roles for APP fragments in axonal transport, cell adhesion, cell survival, cholesterol metabolism, gene transcription, and cognitive processes [6]. When APP is activated (to perform its physiological role), it is cut by enzymes to form separate fragments. Under some circumstances, one of the fragments produced is the insoluble beta-amyloid protein. Beta-amyloid accumulates in microscopic plaques inside the brain. According to the amyloid hypothesis, beta-amyloid plaques clog cell-to-cell communications, disrupting cells and activating immune responses that kill and remove disabled cells [7]. If the amyloid hypothesis is correct, in vivo visualization of Aβ plaques will advance the current understanding of AD’s progression in the brain and aid in both the diagnosis and treatment of this debilitating disease.

Aβ plaques have a diameter ranging from 5-200 μm in transgenic mice [1,4]. In MRI, the basis for contrast between individual plaques and normal background tissue is related to the higher iron content of the plaques [8,9], which affects the T2 and T2* parameters of MRI. In T2- and T2*-weighted images, plaques appear as dark spots devoid of MRI signal.

Prior to this work, plaques have been successfully identified in mice ex vivo [1,8-13] and in vivo [2-4] without contrast agents. Other in vivo visualization methods have utilized contrast agents [1,5,14-16]. With methods applied to human patients, it is important to be as non-invasive as possible, especially considering their age and possible symptoms related to AD. Thus, there are obvious benefits in having a method that does not require exogenous contrast agents to visualize Aβ plaques.

Performing behavioural tests in conjunction with imaging can determine whether plaques precede memory deficits, supporting the amyloid hypothesis, or follow memory deficits, in which case, Aβ plaques might be a side effect of some larger physiological process. The MWM is a behavioural task to test spatial reference memory, which depends on proper functioning of the hippocampus [17]. According to the amyloid hypothesis, transgenic mice should perform more poorly than control mice, due to the presence of beta-amyloid plaques in the hippocampus.

METHODS

Transgenic Mouse Model of Alzheimer’s Disease

In order to develop visualization and testing methods for AD, we used a double transgenic APPSwe/PS1 mouse model, strain 00462, from Jackson Laboratories (Bar Harbor, Maine). PS1 is a mutant form of human presenilin 1 linked to early-onset AD. APPSwe is the "Swedish"
mutation of APP. Both mutations have been linked to increased levels of Aβ. These mice develop Aβ deposits by 6 months of age and have substantial plaque burden by 12 months of age.

Two groups of mice were studied. Group 1 included six male mice (four APP/PS1 and two control mice) that were imaged from 6 to 13 months of age but did not undergo MWM tests. Group 2 included six female mice (three APP/PS1 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 (Fast Low Angle Shot (FLASH) gradient-echo imaging sequence, 15 degree flip angle, 5 averages, 1.7 x 1.7 x 1.7 cm³ field of view, 128 x 128 x 128 matrix size, 133 μm isotropic resolution without zero padding, TR = 73 ms, TE = 4 ms, 99 minute acquisition time) spanning the entire brain were acquired monthly on an 11.7 T Bruker Avance spectrometer running Paravision 3 with a send/receive surface RF coil. 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 (spin-echo imaging sequence, 5 slices, 8 echoes, 2 averages, 2.5 x 2.5 cm field of view, 256 x 256 matrix size, 98 x 98 x 750 μm³ resolution, TR = 2.1 s, TE = 27 ms, 21 minute acquisition time) spanning the hippocampus were acquired on a 7 T Bruker Avance spectrometer running Paravision 2 with a send/receive quadrature RF coil.

Mice were placed in custom-built holders to restrict movement during imaging. Temperature and respiration were monitored. Mice were anesthetized with 1-2.5 % isofluorane in oxygen (Group 1) and in oxygen and nitrous oxide (Group 2).

**Morris Water Maze (MWM)**

Mice in Group 2 were placed in an opaque pool of water (81 cm diameter) with a platform (5 cm diameter) 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 with 4 trials/day. Memory retention (without platform) was then tested over 3 days with 4 trials/day. Swimming paths were tracked and analyzed using a custom MATLAB script. One control mouse was removed from the MWM study as it 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 and Histology**

Histological staining showed that plaques with neuritic cores were present in the cerebral cortex and hippocampus. Apparent plaques in the MR images appeared in the same location as those found in histology (Figure 1). In Group 1, single voxels with hypointense signal were identified with increasing frequency as the animals aged (Figure 2). At 13 months, histological staining showed plaques in the same locations as those found in the MRI. We hypothesize that the low signal is due to the presence of

**Fig. 1** Presence of plaques confirmed in both T2-weighted MR images (a,b) and histology stained with the modified Bielschowsky silver method (c,d) of a 10 month old APP/PS1 mouse. Plaques ranged in size up to 50 μm in diameter in the hippocampus (e) and cortex.

**Fig. 2** More Aβ plaques are visible in APP/PS1 mice each month as they age from six to 13 months (Group 1). Representative sections of axial slices of the brain from *in vivo* 3D 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.
plaques, which are roughly the size of the in-plane voxel image, accepting that for Group 2, volume averaging through the slice thickness degrades the true ability to image individual plaques. Not all plaques found in the histology show up in the MR images. This may be related to limitations in the resolution, volume averaging effects, and differences in iron deposition in the plaques.

**Morris Water Maze**

During acquisition, both APP/PS1 and control mice showed improvement, with control mice consistently finding the platform in under 20 seconds on the 5th day (Figure 3). During retention, mouse paths and various scalar metrics suggest APP/PS1 mice might have impaired spatial reference memory due to the presence of Aβ plaques (Figures 4 and 5), although the difference is not statistically significant.

**CONCLUSION**

Beta-amyloid plaques were visualized in living transgenic mice 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 diminished when 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 and to further investigate the amyloid hypothesis. To gain statistical significance, the number of time points for the Morris water maze and the overall number of subjects needs to be increased. Other behavioural tests, such as the Barnes maze and object recognition tasks, can be performed to verify memory deficits. Improved in vivo imaging and image processing will help quantify the number and size of plaques and better track their progress over time.
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