In vivo and post-mortem detection of amyloid plaques with a non-specific gadolinium contrast agent

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INTRODUCTION
Alzheimer’s disease (AD) is the most common type of neurodegenerative disease, but no cure has yet been found. Amyloid deposits, one of the characteristic lesions, constitute the main target for diagnostics and therapeutics and have previously been identified in mouse models using magnetic resonance (MR) microscopy1,2. These plaques typically appear as hypointense spots on T2-weighted MR images and their size ranges from 50 µm to 200 µm. One of the challenges in imaging plaques is to achieve high-enough resolution and contrast to detect 50-µm large lesions. While most high-field MR systems can reach high resolution, the lack of contrast between the plaques and the parenchyma often impedes their detection. Some investigators have used targeted contrast agents that selectively bind to amyloid plaques, but they are not readily available and cannot be used clinically. We have hypothesized that the use of a non-specific Gd contrast agent coupled with a very high resolution would allow us to identify amyloid deposits in AD transgenic (Tg) mouse brains. We first tested our hypothesis in fixed and stained brains3,4 at 23 x 23 x 90 µm and the method was then extended to live anesthetized mice, using intracerebroventricular (ICV) injections of a contrast agent for in vivo imaging at 50 x 50 x 200 µm resolution.

METHODS
We used 6 APP Tg mice (24 months old) for the ex vivo study and 3 APP/PS1 Tg mice (8 months old) and 7 wild-type (WT) mice for the in vivo study. All MR images were acquired on a 7T clinical magnet (Siemens, Syngo MR VB15) with gradients’ strength of 80 mT/m and a slew rate of 4000/54 ms, RARE = 7, scan time = 2 h 28 min to avoid susceptibility artifacts found on gradient-echo sequences. For the ex vivo study, the brains were imaged before and after staining at very high (23 x 23 x 90 µm) and intermediate (65 x 65 x 200 µm) resolutions with a 3D gradient-echo-based sequence (FLASH, TR/TE = 100/19.4 ms, scan time = 12 h and 1 h). Histology sections were obtained from all brains and stained with congo red and cresyl violet for MR/histology plaque colocalization. The anesthetized animals were bilaterally injected with 1 µl of gadopentetate dimeglumine (Gd-DTPA, 0.5 M) via an ICV route and imaged before and after injection at a resolution comparable to the ex vivo study (50 x 50 x 200 µm). We used a 3D turbo spin-echo sequence (TR/TE = 4000/54 ms, RARE = 7, scan time = 2 h 28 min) to avoid susceptibility artifacts found on gradient-echo sequences.

RESULTS
Without CA, the ex vivo images showed low parenchymal SNR (11:1) and no detectable plaques (Fig.1a) even at very high resolution. After addition of CA, the SNR increased by a factor of 2. Although some plaques were visible at the intermediate resolution (Fig.1b) with a high CNR of 18, most of them were not resolvable enough to be quantified. Only the very high resolution images distinctly showed a large number of plaques (CNR ~ 11) and could be visually counted (Fig.1c-e). The in vivo study on WT animals showed an initial significant 3-fold increase in the parenchymal signal just after injection of CA which then remained elevated for at least 5 h (graph Fig.2), indicating good penetration of Gd into the brain tissue distant from the injection sites. The images of Tg animals showed that no plaques were detectable in the cortex or hippocampus before ICV injection, whereas some plaques were visible after injection (Fig.2). The CNR between the plaques and the parenchyma was about 2-fold lower than in fixed brains (table1), but was sufficient to detect plaques at the resolution used.

Table1: CNR measurements between plaques and parenchyma.
<table>
<thead>
<tr>
<th></th>
<th>23x23x90 µm² (no CA)</th>
<th>65x65x200 µm²</th>
<th>50x50x200 µm²</th>
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<tr>
<td>Ex vivo</td>
<td>NA</td>
<td>18.7 ± 4.1</td>
<td>NA</td>
<td>11.3 ± 0.4</td>
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<tr>
<td>ICV vivo</td>
<td>NA</td>
<td>NA</td>
<td>6.6 ± 2.9</td>
<td>NA</td>
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CONCLUSION
This study demonstrates that amyloid plaques can be detected both ex vivo and in vivo using a non-specific contrast agent delivered through simple passive diffusion in fixed brains, or through ICV injections in anesthetized AD Tg mice. This level of detection should allow us to follow changes in plaque load in pharmacology studies in these animals.

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REFERENCES