Rapid appearance and local toxicity of Aβ plaques in a mouse model of Alzheimer’s disease

Melanie Meyer-Luehmann1, Tara L. Spires-Jones1, Claudia Prada1, Monica Garcia-Alloza1, Alix de Calignon1, Jessica Koenigsknecht-Talboo2, David M. Holtzman2, Brian J. Bacskai1 and Bradley T. Hyman1

1MassGeneral Institute for Neurodegenerative Disease, Department of Neurology, Alzheimer’s Disease Research Laboratory, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA USA.
2Washington University School of Medicine, Department of Neurology, St. Louis, MO USA.

To whom correspondence should be addressed. E-mail: bhyman@partners.org

This file includes:

1) Figures and legends for Supplementary Figure 1-7
Supplementary Figure 1

Amyloid plaque formation can be monitored in vivo.

(a-c) Low magnification images provide an overview of the areas of potential plaque formation. The angiogram (red, Texas Red), amyloid deposition (blue, methoxy-XO4) and neurons (green, YFP) are easily identified on the initial day of surgery (a) as well as 1 week (b) and 2 weeks later (c) allowing re-imaging of the same sites over different imaging sessions. No new parenchymal amyloid deposition was identified 1 week (b) or 2 weeks (c) after the first imaging at this site.

Scale bar, 100 μm.
Supplementary Figure 2

Amyloid plaque formation in Tg2576 mice.

(a-d) Two examples of plaque formation in 11 month old Tg2576 transgenic mice. New plaques were obtained 1 week after the initial imaging session (arrows). Methoxy-XO4 labeling is seen in amyloid plaques as well as in vessels (blue), with the image color inverted from the original fluorescent micrographs for clarity.

Scale bar, 100 μm.
Supplementary Figure 3

Distribution of amyloid-β deposits after methoxy-XO4 staining.

(a-c) Plotted are the mean plaque sizes (area μm²) of plaques measured either after in vivo imaging (a) or postmortem (b). The histogram in (c) shows the distribution of plaque sizes from animals that were not exposed to methoxy-XO4 in vivo but stained postmortem with methoxy-X04. Note that All 3 histograms show a similar distribution.

(d,e) The tables display the numbers of plaques in different area and diameter ranges as well as the corresponding % of total plaque numbers.
Supplementary Figure 4

Dystrophic neurites do not precede but appear after plaque formation and change over time.

(a,b) New dystrophic neurites in B6C3-YFP mice were identified around 11 out of 13 newly formed plaques but were absent at earlier imaging time points. Arrowheads indicate the site of a new neuritic dystrophy. (c-f) Dystrophic neurites were observed in B6C3-YFP mice at an initial time point and then re-imaged 1 week later. However, no dense core plaque occurred at the area of high amount of swellings. Instead, individual dystrophic neurites changed their appearance. Arrowheads indicate dystrophic neurites
that were stable after one week (c and d) and others that were eliminated over the course of a week (e and f).

Scale bars, 15 μm (a,b), 20 μm (c,d) and 50 μm (e,f).
Supplementary Figure 5

*Plaque-associated dystrophic neurites are both dendritic and axonal in origin.*

Plaques (a, e, i labeled with methoxy X-O4) have associated dystrophic neurites (shown in YFP filled neurons b, f, j). Immunostaining for axonal neurofilament Smi312 (c, d) shows that some of these dystrophies (arrowheads) are axonal in origin. Some dystrophic neurites also stain with synaptic marker synaptophysin (g, h). The majority of large dystrophic neurites co-stain with dendritic marker Smi32 (k, l).

Scale bar, 30 μm.
Supplementary Figure 6

**Aβ deposits in B6C3-YFP mice are mainly compact.**

(a-d) Low and high magnification views of neocortex from a 5 month old B6C3-YFP mouse following staining with 3D6 antibody (a and c) and methoxy-XO4 labeling of dense core plaques (b and d), show that there is not a significant amount of diffuse amyloid staining around dense plaques.

Scale bars, 200 μm (a,b) and 20 μm (c,d).
Supplementary Figure 7

*Schematic illustration of how plaques may be a source of bioactive molecules.*

We propose that plaques act as a local source of soluble Aβ. Either plaques themselves induce neuritic alterations or the high concentration of surrounding soluble Aβ.