Aging promotes the neuroinflammatory response to amyloid-beta oligomers in mice

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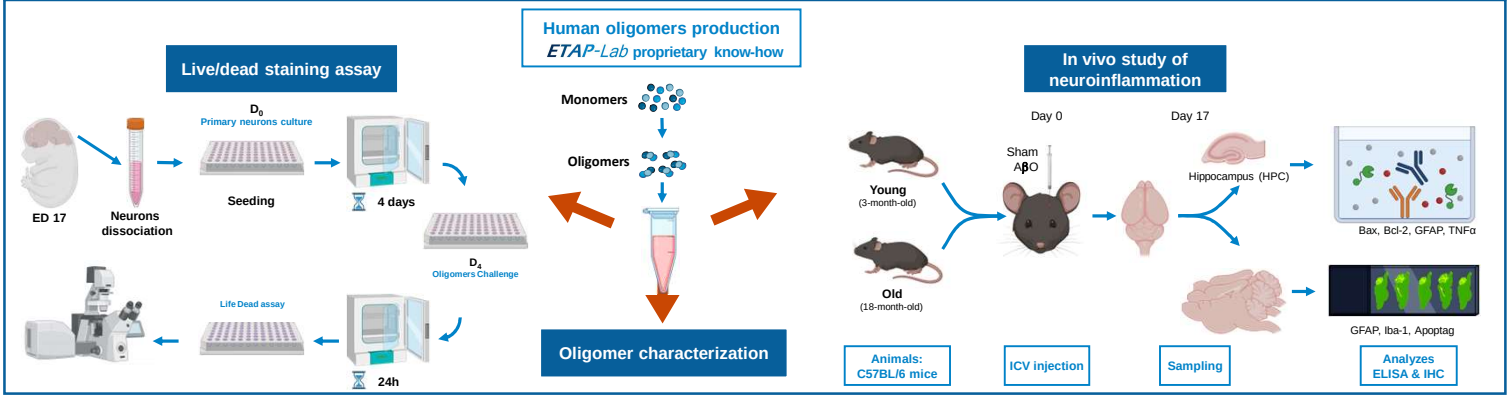
Introduction

Even though aging is the main risk factor for Alzheimer's disease (AD), little is known about the susceptibility of aging brain to key pathological proteins such as amyloid-beta oligomers (AβO). In a previous work, we demonstrated that aging potentiates the neurotoxic effects of a single intracerebroventricular (ICV) injection of AβO on cognitive performances. Here, we compared the effects of AβO on microglia and astrocyte activation in young and aged mice as neuroinflammation emerged as a key mechanism underlying the progression of neurodegenerative disease.

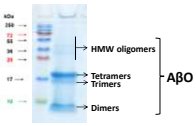
Methods



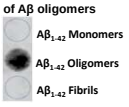
Results

Oligomers characterization

Coomassie blue staining in SDS-PAGE

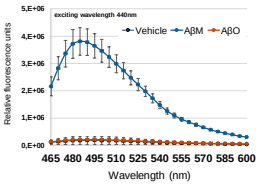


Dot-blot detection of Aβ oligomers



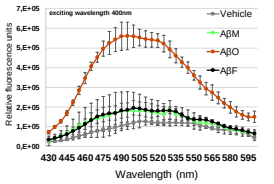
◀ Monomer, oligomer and fibril preparations were probed with A11 oligomer-specific antibody (dot blot assay). No oligomers were detected in monomer and fibril preparations.

Thioflavin T assay



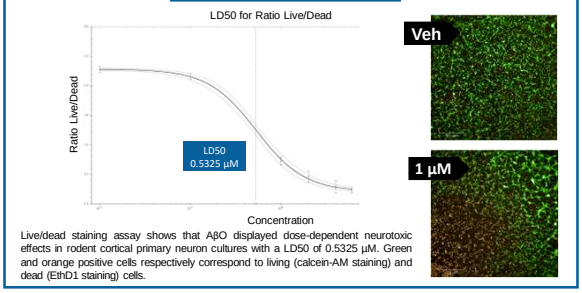
The spectra of our preparations incubated with Tht shows a fluorescence emission peak for AβM, but not AβO. These results demonstrate that untreated monomers spontaneously form fibrils while AβO remain stable when prepared with our technology.

8-Anilino-1-naphthalenesulfonic acid (ANS) assay



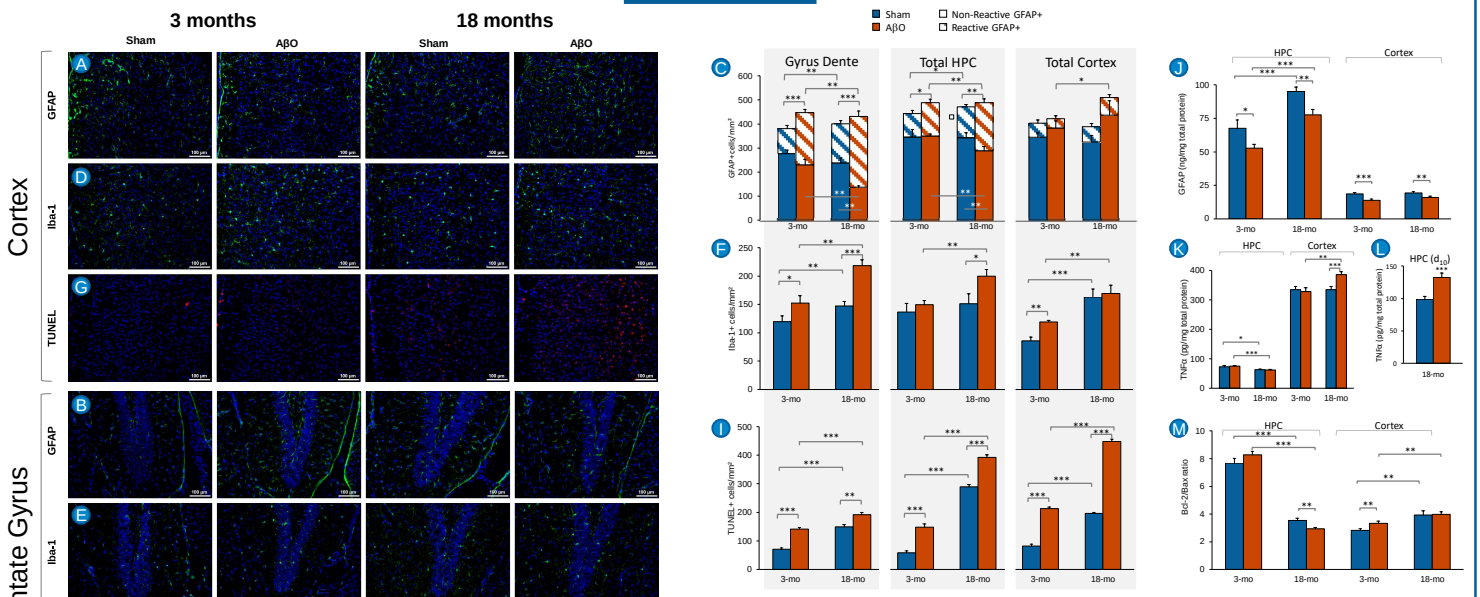
ANS tags oligomeric aggregates. The spectra show that only AβO preparations performed with our technology contains significant amounts of AβO.

Live/dead staining assay



Live/dead staining assay shows that AβO displayed dose-dependent neurotoxic effects in rodent cortical primary neuron cultures with a LD50 of 0.5325 µM. Green and orange positive cells respectively correspond to living (calcein-AM staining) and dead (EthD1 staining) cells.

In vivo study of neuroinflammation



Fluorescence detection of GFAP, a marker of activated astrocytes in cortex (A) and dentate gyrus (B); Quantification of the ratio of GFAP-reactive cells to the total GFAP-positive cells (C); Fluorescence detection of Iba-1, a marker of microglia in cortex (D) and dentate gyrus (E); Quantification of the number of Iba-1-positive cells (F); Fluorescence detection of ApopTag® TUNEL, a marker of apoptosis in cortex (G) and dentate gyrus (H); Quantification of the number of TUNEL-positive cells (I). Data are presented as means ± SEM, with n = 6/group.

Levels of GFAP in the HPC and cortex tissues determined by ELISA (J); Levels of TNFα in the HPC and cortex tissues determined by ELISA (K); Levels of TNFα in the HPC tissues determined by ELISA at D10 (L); Ratio of levels of Bcl-2 and Bax proteins in the HPC and cortical tissue determined by ELISA (M); Data are presented as means ± SEM, with n = 10-16/group.
*Statistical significance level: * P < 0.05, ** P < 0.01 and *** P < 0.001 (t-student test for treatment effect by age and for age effect by treatment).*

Conclusion

Our data demonstrate that AβO increase neuroinflammation in both young and aged mice. However, the effect was more striking in aged mice and clearly associated with worsened neuronal apoptosis. These results are in good accordance with our previous data showing a susceptibility of aged mice to the neurotoxic effects AβO on memory performance, suggesting an improved translational value of AD models in aged animals.

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This project is co-financed by the "Region Grand-Est" and the European Union through the operational programme "FEDER-FSE Lorraine et Massif des Vosges 2014-2020".