PROBING AMYLOID-β DYNAMICS IN APP KNOCK-IN MICE AND *IN VITRO* USING STABLE ISOTOPE LABELLING AND MALDI IMAGING MASS SPECTROMETRY

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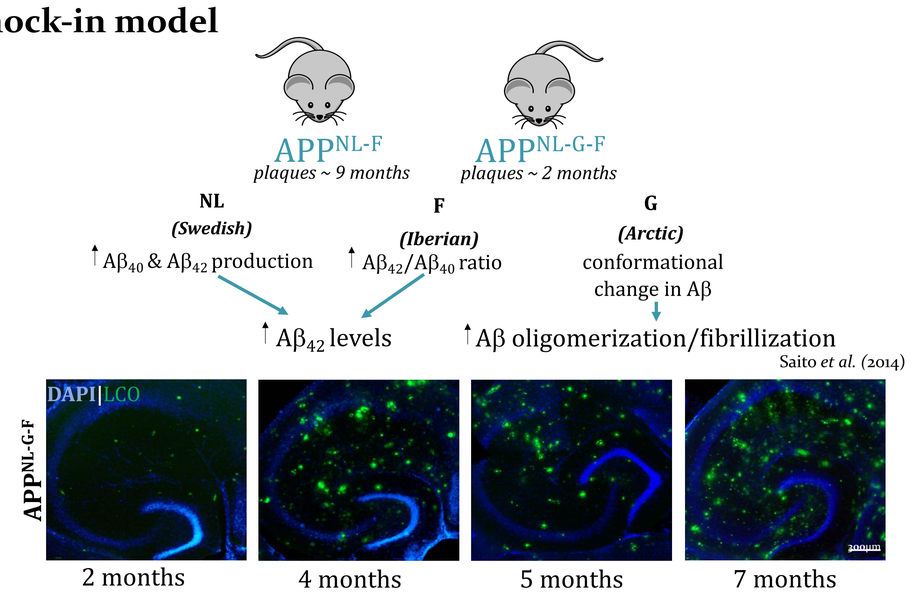
Introduction

Amyloid beta (Aβ) plaque deposition is a major pathological feature of Alzheimer's disease (AD); however, this exact process remains unclear. Microglia, the immune cells of the brain, have been shown to interact with plaques but their significance to Aβ deposition is unknown.

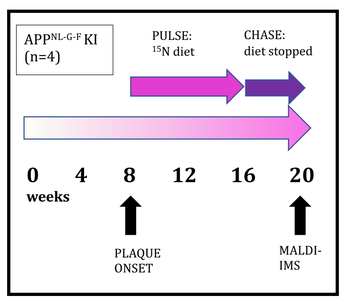
We aim to assess Aβ deposition and plaque composition in detail both *in vivo* and in an organotypic hippocampal slice culture (OHSC) model obtained from amyloid precursor protein (APP) knock-in mice (APP^{NL-F} and APP^{NL-G-F}), and also assess the interaction with microglia. Specific aggregation dynamics of individual Aβ species and plaque-associated lipids will be analysed on a nanometer level using stable isotope labelling and novel in situ mass spectrometry (MS) methods.

Experimental methods

APP knock-in model



Stable isotope labelling kinetics (SILK) for monitoring Aβ deposition

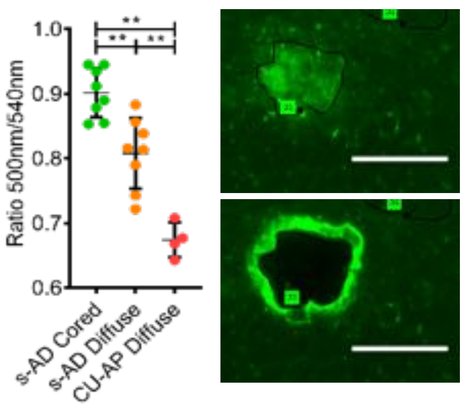


15N-labelled feeding pattern for mice.

- APP^{NL-F} and APP^{NL-G-F} mice are fed ^{15}N-labelled food from a pre-plaque timepoint until the earliest plaques have started to form.
- Mice are then switched back to a normal diet for a 'chase' period whilst plaques continue to grow.

Chemical imaging setup for analysis of Aβ plaque composition

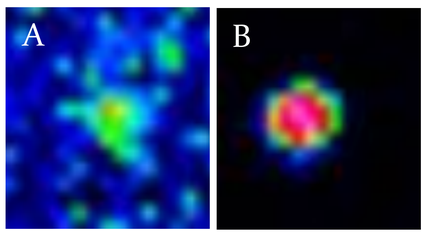
1. Laser microdissection/matrix-assisted laser desorption/ionisation (MALDI)-MS



- Amyloid staining with LCO (q-FTAA and h-FTAA) distinguishes between cored and diffuse plaques based on hyperspectral emission ratios.
- Individual plaques are laser microdissected out of the tissue section and subjected to immunoprecipitation for Aβ enrichment.
- MALDI-MS is used for quantification of individual Aβ species.

2. MALDI imaging-MS

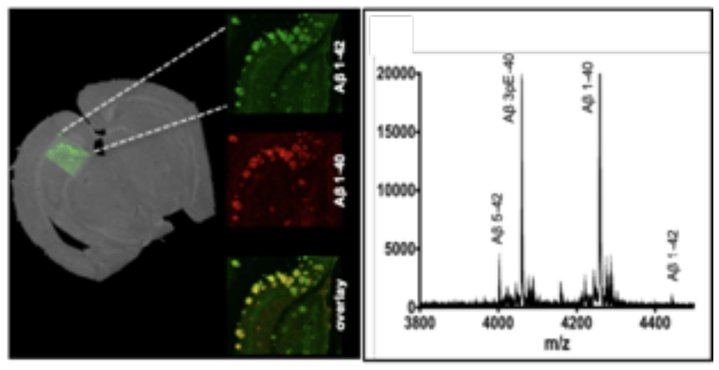
- MALDI-TOF-MS data is generated from a brain section coated in crystalline matrix.
- The spectra peak for the molecule of interest is used to visualise its distribution across the brain section. A pixel-by-pixel image is created, which is mapped against distinct coordinates of the original tissue section.



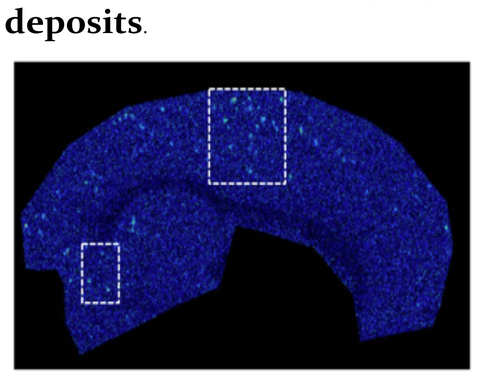
(A) Aβ1-42 in diffuse plaques and (B) Aβ1-40 in the centre of cored plaques.

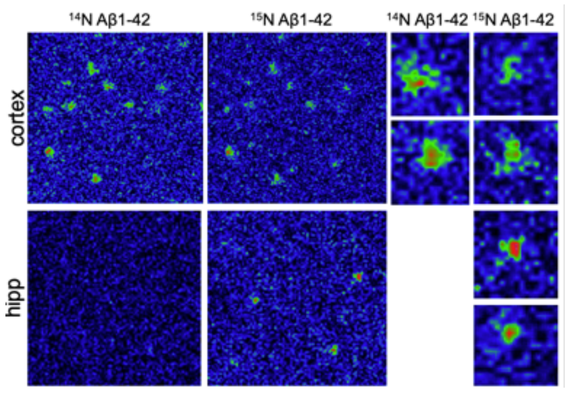
Results

1. Individual Aβ peptide species can be visualised and quantified using MALDI imaging-MS



2. Examining the proportion of ^{15}N labelled vs. unlabelled protein by MALDI imaging-MS reveals the pattern in which Aβ deposits.

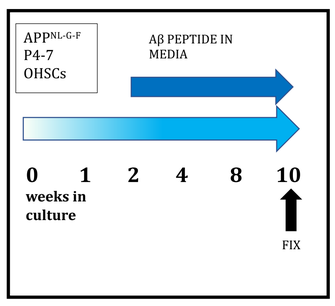




MALDI assessment of varying content in ^{15}N labelled Aβ1-42.

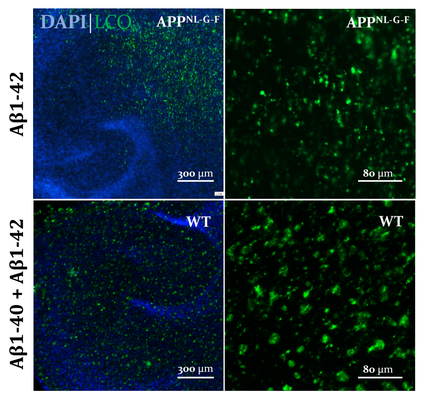
3. Metabolic labelling can be applied *in vitro*

OHSCs ($APP^{N-L-G-F}$ and WT) treated with exogenous Aβ1-40 or Aβ1-42 peptide develop small Aβ deposits after 10 weeks in culture.



Aβ peptide is continuously supplemented in the culture media for 8 weeks.

Adapted from Novotny *et al.* (2016)



Conclusions & future work

- MALDI techniques can distinguish between initial Aβ seeds and later deposition depending on the pattern of feeding labelled isotopes. This allows for assessment of what type of aggregates are formed, in what order, and their spatial relationship to each other.
- The type, number and activation states of microglia around different types of plaques (diffuse vs. cored), and the comparison between APP^{NL-F} and APP^{NL-G-F} mice, will be characterised.
- Electrophysiological recordings will be obtained from SILK mice to correlate synaptic dysfunction with the stage of pathology.

References

Novotny, R., Langer, F., Mahler, J., Skodras, A., Vlachos, A., Wegenast-Braun, B.M., Kaeser, S.A., Neher, J.J., Eisele, Y.S., Pietrowski, M.J., Nilsson, K.P.R., Deller, T., Staufenbiel, M., Heimrich, B. and Jucker, M. (2016). Conversion of Synthetic Aβ to *In Vivo* Active Seeds and Amyloid Plaque Formation in a Hippocampal Slice Culture Model. *The Journal of Neuroscience*, 36(18), pp.5084–5093.

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