Sampling extracellular Tau in human Tau transgenic mice: optimization of push/pull in vivo microdialysis

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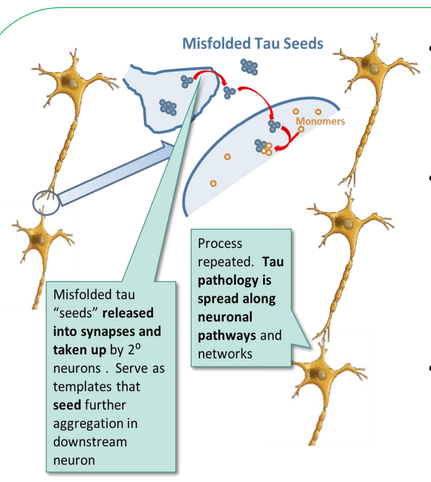
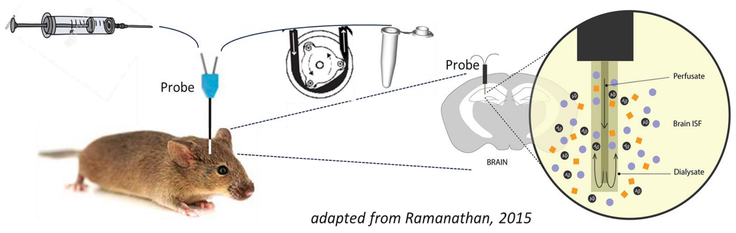
BACKGROUND



- Prion-like propagation of Tau might play a key role in the progression of Alzheimer's disease (AD).
- Thus, monitoring extracellular Tau species in transgenic mouse models of tauopathy might facilitate the understanding of AD pathogenesis.
- *In vivo* microdialysis is the state-of-the-art method to sample extracellular molecules in animals over time.

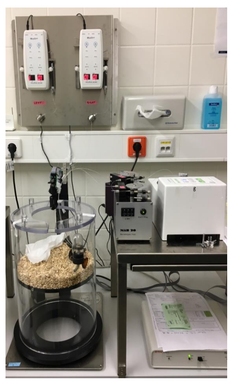
METHODS

Schematic representation of the microdialysis principle



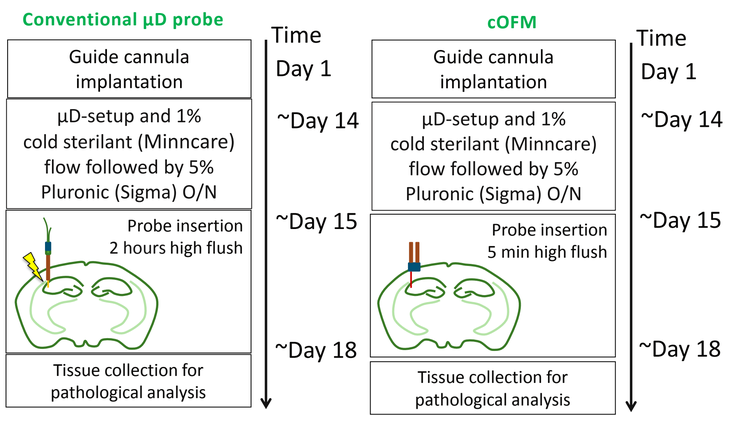
adapted from Ramanathan, 2015

State-of-the-art swivel-free push/pull *in vivo* microdialysis for 2 probes/animal



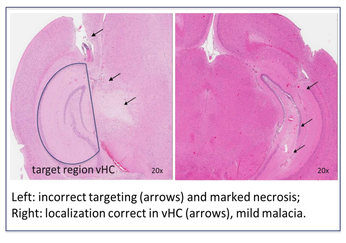
Animals: PS19 (4 & 9 mo) and TG4510 (2 & 7 mo)
Stereotaxic coordinates for hippocampus: 3.2/3.1/4.7 (bilateral) guide cannulas EICOM PEG-4 or cOFM guides (Joanneum)
Probe: Atmos LM (Eicom) (1MDa cut-off) 2mm membrane or cOFM (Joanneum)
Push pump: CMA 402 (CMA)
Pull pump: MAB20 (Microbiotech)
Tubing: FEB-Tubing inner diameter 0.12mm (Microbiotech) - Length -> inlet: 60cm; outlet: 60cm (1.2µL/10cm); for pull pump: Santopren tubing (Microbiotech)
Perfusion medium: aCSF, 0.2% BSA (Sigma)
Vials: polystyrene (Microbiotech)
Pre-filling of vials: aCSF/0.2%BSA/0.55%Tween (total volume: ~60µl)
Flow rate: 1µl/min
Sampling Intervall: 60-90 min

Workflow



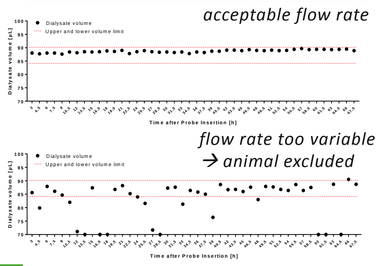
Establishment of quality criteria

Refinement of surgery protocol



Left: incorrect targeting (arrows) and marked necrosis; Right: localization correct in vHC (arrows), mild malacia.

Constant monitoring of flow rate

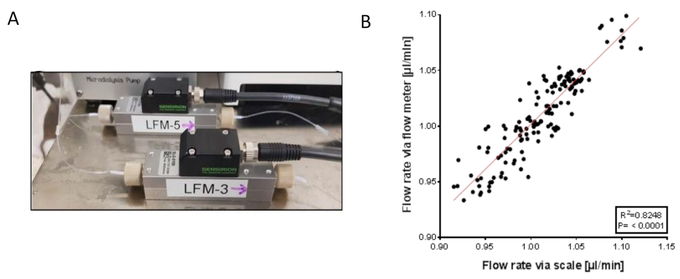


Sample Analysis

Tau levels in microdialysis samples were analyzed by the commercially available MSD VPlex human Tau assay.

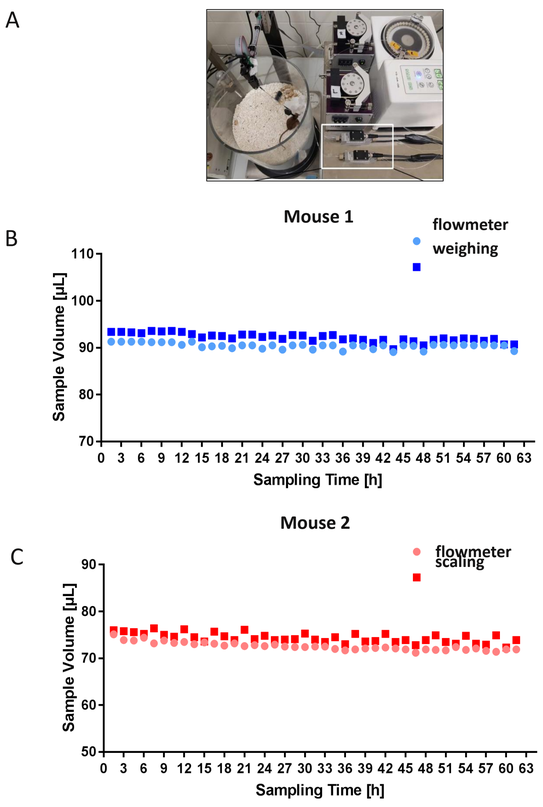
RESULTS

Figure 1 – Validation of real-time flow monitoring system *in vitro*.



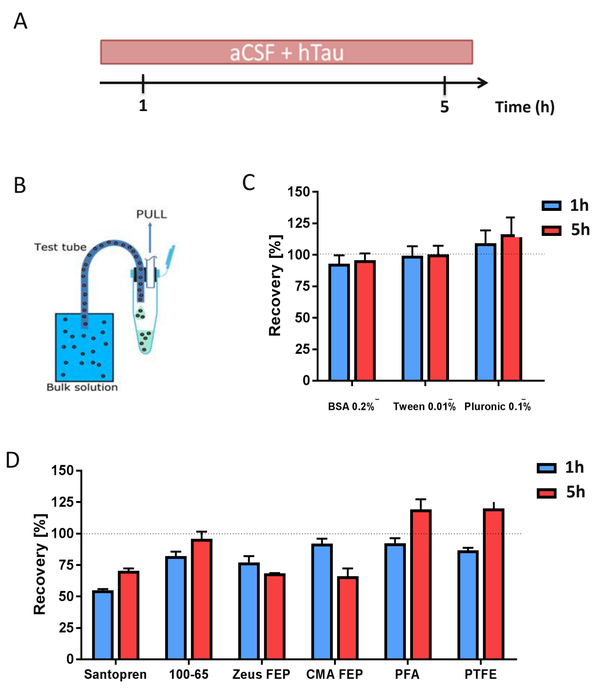
[A] Two online flow meters (Sensirion). Flowmeters implementation led to a significant saving of time and real-time resolution (msec). [B] Correlation between flow rate estimated by manual weighing of samples and flow rate determined by the novel online flow sensors.

Figure 2 – Microdialysis flow rate monitored by online flow sensors can replace manual weighing



[A] Picture of microdialysis setup. [B] & [C] Comparison between weighing of vials and flowmeter volume measurements during microdialysis of mouse 1 [B] and mouse 2 [C].

Figure 3 – Tau recovery is affected by tubing material and composition of perfusion fluid



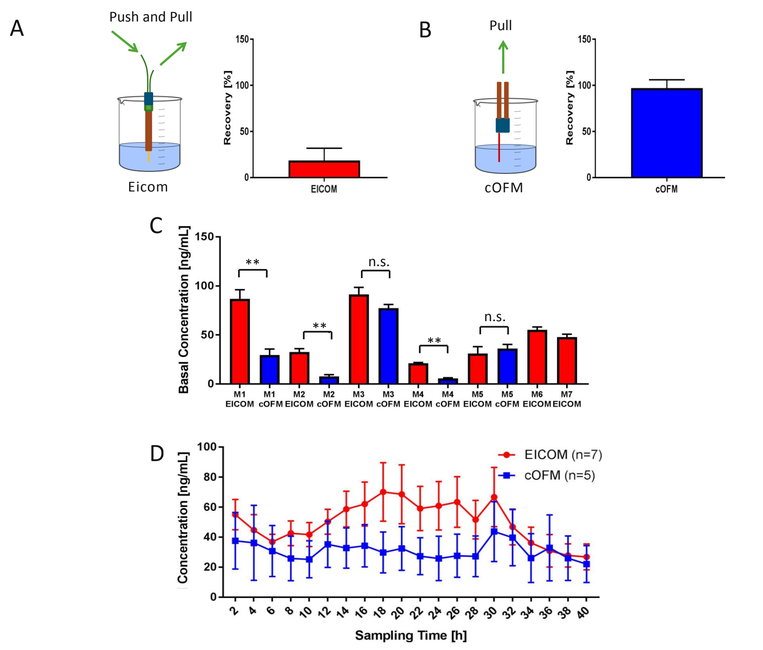
[A] Workflow scheme. [B] Schematic illustration of *in vitro* recovery experiments. [C] *In vitro* recovery rates of human Tau compared between different additives at different time points (1h and 5h). [D] *In vitro* recovery rates of human Tau in aCSF/0.2% BSA compared between different tubing materials at different time points (1h and 5h).

CONCLUSIONS

- **Implementation of novel flow sensors into the microdialysis system can replace the manual weighing of samples resulting in significant savings in hands-on experimental time and, if needed, higher time resolution.**
- **PFA and PTFE tubings showed ~100% Tau *in vitro* recovery. Different additives to the perfusion fluid (BSA, pluronic, Tween) revealed a similar Tau *in vitro* recovery of ~100%.**
- ***In vivo* microdialysis for Tau was successfully setup. Tau levels can be stably measured over a duration of 40h with both cOFM and conventional µD probe (Eicom).**
- ***In vivo* microdialysis experiments in different mouse models of tauopathy revealed that extracellular Tau decreases during Tau pathology progression and is enhanced by K^+-evoked neuronal depolarization.**

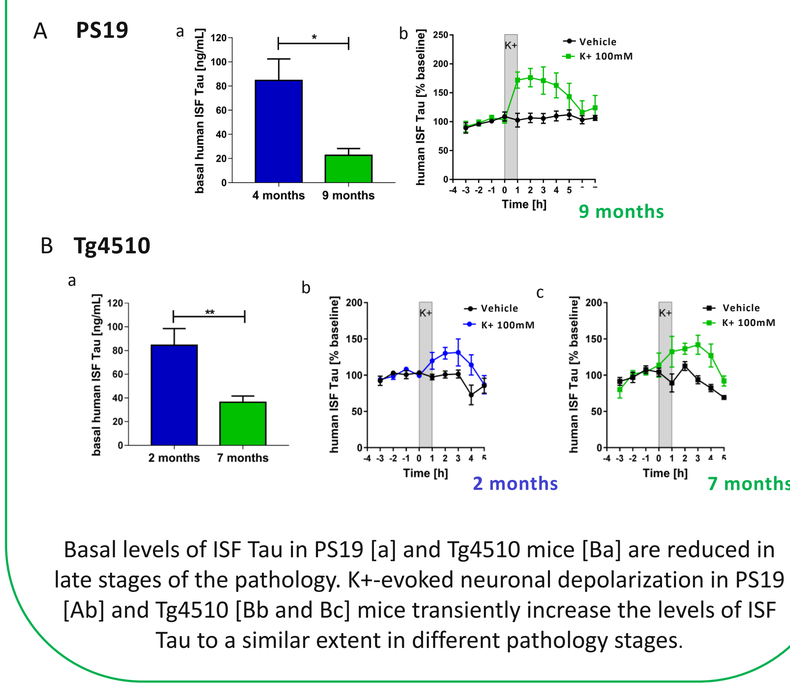
RESULTS

Figure 4 – Tau levels can be stably measured over a duration of 40h with cOFM and Eicom probes



[A] & [B] *In vitro* recovery of EICOM [A] and cOFM [B] probe. Note the differences in setup: push/pull for the Eicom probe, only pull for the cOFM probe. [C] Comparison of basal concentration of Tau in different animals using cOFM (right hemisphere) and EICOM probe (left hemisphere). [D] Time course of Tau levels sampled with cOFM and EICOM probe.

Figure 5 – Extracellular Tau decreases during Tau pathology progression and is enhanced by K^+-evoked neuronal depolarization



Basal levels of ISF Tau in PS19 [a] and Tg4510 mice [Ba] are reduced in late stages of the pathology. K+-evoked neuronal depolarization in PS19 [Ab] and Tg4510 [Bb and Bc] mice transiently increase the levels of ISF Tau to a similar extent in different pathology stages.

DISCLOSURE

EB, MM, JH, GP, FP, IM, MM, HJM, LG and KB are employees of AbbVie. TAK and TB are employees of the Joanneum Research GmbH. The design and study conduct were provided by both Joanneum Research GmbH and AbbVie. The financial support for this research was provided by AbbVie. Both AbbVie and the Joanneum Research GmbH participated in the interpretation of data, review, and approval of the publication.