We saw in the previous optogenetic experiment about 25% increase in the hippocampal interstitial fluid of $A\beta_{42}$ level immediately following acute opsin light activation of the LEC. In this experiment which is a follow-up experiment, we want to show that tauopathy in the brain is aggravated by increased accumulation of APP fragments leading to an increase phosphorylation of tau and increased induction of neurofibrillary tangles (NFTs). Mature tau pathology in turn spreads and could aggravate Aβ-associated neuronal dysfunction and aberrant signaling, leading to a harmful feedback loop.

Aim
In optogenetically induced mice, investigate implications of aberrant functional specific neuronal circuitry on tau pathology, and assess the relationship between amyloid-beta accumulation and tau pathology and its propagation in ISF and CSF.

For the research, we will use the Tg EC-Tau/hAPP mice, which overexpress a mutant amyloid precursor protein (hAPP) and entorhinal cortex (EC) tau pathology in the brain.
Initially we will have two groups of mice, one group injected with AAV-SSFO and the other group with AAV-EYFP (for details see last week experiment), these mice will be at least 9 months old. After some period of time following the injection, we will follow the same protocol used in last week experiment:

- Acute optogenetic stimulation of the LEC

We will optically stimulate neurons in the LEC for 2s every minute for 4 hr for 24 hours. ISF samples will be then collected and tau concentration will be measured using tau microdialisys [1]. In addition, we will sample CSF to measure CSF Aβ with Aβ microdialysis and CSF tau concentration using tau microdialisys technique. We will then measure relative ISF and CSF Aβ and tau levels every hour, starting 2 hours before the optical stimulation up to 4 hours after stimulation. These measurements will allow to investigate how ISF and CSF Aβ and tau levels are related by plotting % ISF and CSF levels every hour. First, to assess the robustness of our experiments, we will verify previous studies establishing ISF tau is 10-fold higher concentrated in brain interstitial fluid than in CSF, and how these concentrations change over time [1][2]. Then we will analyze potential correlations between ISF and CSF Aβ, ISF and CSF tau, ISF Aβ and tau, ISF Aβ and CSF tau, CSF Aβ and tau. We will repeat the same experiments with younger mice and draw conclusions which will show that Aβ and tau pathologies are age-dependent but also, we will study how they change comparatively with age.
Hippocampal and brain extracts will be collected so:

- We will perform Immunohistochemistry and morphometric analyses of Aβ depositions and quantification of Aβ burden
- And fractions of brain extracts will be used to be able to visual bands of tau and quantify tau aggregates

- Chronic optogenetic stimulation

Instead of applying an acute optical stimulation, we will stimulate the LEC of older mice once for 2 every 24 Hr for at least 5 months and we will run the same analyses detailed above.

We will then study the clearance pathway in wild-type mice and the Tg EC-Tau/hAPP mice. Results in previous studies have shown that increased brain Aβ plaque burden in AD patients, is accompanied by a reduction in the amount of soluble Aβ exchanging between the brain ISF and the CSF leading to a decrease of Aβ rate clearance [3]. We will repeat the measurements described above with optical stimulation of the Tg EC-Tau/hAPP mice and will measure ICF and CSF Aβ and tau in a control group of W-T mice and an experimental group of Tg EC-Tau/hAPP mice.

Reference:

[1] K. Yamada *et al.*, “In Vivo Microdialysis Reveals Age-Dependent Decrease of Brain Interstitial Fluid Tau Levels in P301S Human Tau Transgenic Mice,” *J. Neurosci.*, vol. 31, no. 37, pp. 13110–13117, Sep. 2011, doi: 10.1523/JNEUROSCI.2569-11.2011.

[2] L. D. Evans *et al.*, “Extracellular Monomeric and Aggregated Tau Efficiently Enter Human Neurons through Overlapping but Distinct Pathways,” *Cell Rep.*, vol. 22, no. 13, pp. 3612–3624, Mar. 2018, doi: 10.1016/j.celrep.2018.03.021.

[3] E. L. Boespflug and J. J. Iliff, “The emerging relationship between interstitial fluid-cerebrospinal fluid exchange, amyloid β and sleep,” *Biol. Psychiatry*, vol. 83, no. 4, pp. 328–336, Feb. 2018, doi: 10.1016/j.biopsych.2017.11.031.