Background

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 enhanced neuronal activity on tau pathology, and assess connections between amyloid-beta accumulation and tau pathology 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 on these constructs, please refer to 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:

1. Acute optogenetic stimulation of the LEC

We will optically stimulate neurons in the LEC for 2s every minute for 4 hr for 24 hours. We will collect ISF samples. Using microdialisys techniques we will measure Aβ and tau [1]. In addition, we will sample CSF to measure CSF Aβ tau concentration.

- 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.
- Next, we will measure relative ISF and CSF Aβ and tau levels every hour, starting 2 hours before the optical stimulation up to 4 hours after stimulation. We will investigate %ISF and CSF Aβ and tau levels by plotting %ISF and %CSF levels every hour. We will analyze concentrations change over time [1][2].
- Although it has been established that tau spreads from cell to cell through neuronal connections, facilitated by Aβ, researchers are still actively studying the release of tau in the extracellular space [3]. During the course of our experiment, we will attempt to understand these mechanisms. We will compute 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 also compute the ratios CSF Aβ/ ISF Aβ, CSF tau/ISF tau in the infected mice and the W-T mice. We will compare these ratios over the time of the experiments (CSF Aβ/ ISF Aβ, CSF tau/ISF tau curves).
- We will 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 [4]. For this step, we will inject radiolabeled soluble Aβ , via a small cannula attached to the microdialysis probe, into the hippocampal ISF. We will then measure the ability to recover the radiolabeled ISF from the ISF and the CSF. We will check whether drop in soluble ISF Aβ relates to increase in amyloids deposits [5].
- We will repeat the same experiments with younger mice and draw conclusions from the various data scores and ratios defined above which we expect to show that Aβ and tau pathologies are age-dependent and change with age.
- We will unilaterally stimulate the left hemisphere of the hippocampus and compare Aβ and tau pathologies between left and right hemisphere.

2. Chronic optogenetic stimulation

We will increase the period of optical stimulation; we will stimulate the LEC of older mice for 2s every 24 Hr. up to 5 months and we will run the same analyses detailed above.

Finally, hippocampal and brain extracts will be collected and sections will be analyzed by immunofluorescence of c-Fos and SSFO-EYFP:

- We will use brain extracts to visualize amyloid plaque and bands of tau aggregates
- We will stain mouse brain slices with anti-Aβ antibody to quantify Aβ burden and anti-tau antibodies to measure tau levels.

In conclusion, we are investigating dynamics between Aβ deposits in the ISF and CSF and its connection to tau pathology using optogenetic stimulation in specific neuronal pathway.

Reference:

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[3] J. W. Wu *et al.*, “Neuronal activity enhances tau propagation and tau pathology in vivo,” *Nat. Neurosci.*, vol. 19, no. 8, pp. 1085–1092, Aug. 2016, doi: 10.1038/nn.4328.

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[5] S. Hong *et al.*, “Dynamic Analysis of Amyloid β-Protein in Behaving Mice Reveals Opposing Changes in ISF versus Parenchymal Aβ during Age-Related Plaque Formation,” *J. Neurosci.*, vol. 31, no. 44, pp. 15861–15869, Nov. 2011, doi: 10.1523/JNEUROSCI.3272-11.2011.