Background

In the past assignment, following acute opsin light activation of the LEC, $A\beta_{42}$ level in the hippocampal interstitial fluid increased about 25%. For this follow-up experiment, we want to show that tauopathy in the brain is aggravated by increased accumulation of APP fragments leading to an increase of phosphorylated 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 an optogenetically induced mice, investigate enhanced neuronal activity effects on 1) tau pathology, 2) Aβ/tau synergy in ISF and CSF [1], and 3) on the "glymphatic" pathway [2].

For the research, we will use the Tg EC-Tau/hAPP mouse, which overexpresses a mutant amyloid precursor protein (hAPP) and entorhinal cortex (EC) tau pathology in the brain. This mouse was created to model functional interactions between hAPP/Aβ and hTau pathologies.
We will have two groups of Tg EC-Tau/hAPP 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 3-, 6-, and 12-month-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 [3]. In addition, we will sample CSF to measure CSF Aβ and tau concentrations.

- First, we will determine using zero flow method the in-vivo concentration of monomeric ISF tau in the Tg EC-Tau/hAPP mice at different age in both groups. We hypothesize that monomeric ISF tau levels decrease with age and faster for the optically stimulated mice due to increased tau aggregate formation and accumulation.
- Then, we will measure CSF tau concentration for both groups of mice. We will expect to see CSF tau concentration increasing with age and we will evaluate the impact of the optical stimulation on CSSF tau concentration.
- 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 compute %ISF Aβ, %CSF Aβ, %ISF tau, %CSF tau levels every hour. With the data, we will analyze concentration changes over time for both groups of mice [3][4].
- It has been established that tau spreads from cell to cell through neuronal connections, facilitated by Aβ [5], future insight is needed to better understand this relationship : to that effect, we will compute the ratios CSF Aβ/ ISF Aβ, CSF tau/ISF tau, for the two groups of mice. We will compare these ratios over time (CSF Aβ/ISF Aβ, CSF tau/ISF tau curves).
- 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 exchanged between the brain ISF and the CSF leading to a decrease of Aβ rate clearance [6]. 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 and how the optical stimulation affects this decrease [7].

2. Chronic optogenetic stimulation

We will increase the period of optical stimulation; we will stimulate the LEC of 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 want to show that chronic optogenetic activation augments 1) tau aggregation in relation to an increase of Aβ deposits in the ISF and CSF and 2) its impact on the brain waste clearance pathway.

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