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Week 2 – Techniques to study Neural Circuits

1) Propose at least two possibilities to achieve conditional expression of exogenous genes in specific cell types of the mouse brain. For one of these possibilities, assume using wild-type mice, and for the other – assume using transgenic mouse line(s). Please explain the rationale (mechanism) and discuss pluses and minuses of both proposals.

2) Please list the viral vector tools that you know which can be used for anterograde and retrograde labeling approaches. Describe the general principle/properties for each tool.

3) It is known that lateral amygdala (LA) receives auditory inputs from both the auditory cortex (Au) and the auditory thalamus (MGM). Using a wild-type mouse as a model system and AAV as a tool, propose an experimental design (specific AAV vectors and locations of injections), with which one would specifically label those LA neurons that receive cortical inputs with a red fluorescent protein (tdTomato), and those LA neurons which receive thalamic inputs - with a green fluorescent protein (eGFP). How would you modify your design if you need to label with a blue fluorescent protein (eBFP) only those LA neurons which are innervated by both the Au and MGM (if any)?

4) Explain the main advantages of the optogenetic and chemogenetic approaches used to manipulate neuronal activity over extracellular stimulation. Please also compare optogenetics with chemogenetics and discuss the main strength/weakness of both approaches.

5) Explain the key principles of extracellular recordings of neuronal activity in a “single unit” mode: how recordings are done, what is sampled and what does it reflect at the single-cell level, and what are the main steps in the data processing?

6) Explain the principle of intracellular Ca^{2+} measurements using fluorescent Ca^{2+}-indicators. Derive the steady-state (equilibrium) equation for a Ca^{2+}-bound fraction of fluorescent indicator as a function of free intracellular calcium concentration ($[Ca^{2+}]_i$).

Explain the meaning of dissociation constant (K_D) of the calcium indicator and how it influences the measurements of transient changes in $[Ca^{2+}]_i$.

7) ***Read and discuss the following paper:***

Mahn, M., Saraf-Sinik, I., Patil, P., Pulin, M., Bitton, E., Karalis, N., Bruentgens, F., Palgi, S., Gat, A., Dine, J., et al. (2021). **Efficient optogenetic silencing of neurotransmitter release with a mosquito rhodopsin.** *Neuron* 109, 1621-1635.e8.

https://doi.org/10.1016/j.neuron.2021.03.013.