The model Dominic is proposing is to correlate pattern of stimuli, e.g., different informational inputs to changes in neural plasticity. He designs a microfluidic chamber with 5 compartments: 3 compartments containing rat cortical neurons, injected with ChR2 opsin, are triggered optically and are connected to a central well in which neurons are loaded with Rhod-2 Calcium indicator to detect synapse activities, an isolated compartment with the central compartment is acting like a terminal neural pathway. The initiator neurons (compartment 1, 2, and 3) are injected with AAV2-ChR2V to be excited with light at different frequencies: constant, random and increasing-decreasing.

One comment here: there is a lack of critical details as how the Chr2 opsin is used for the neurons in the three chambers and yet they can be independently activated by light at different.

Dominic’s intention is to show the connection between potential for information and neural plasticity with a focus on synapse activity. We keep the design of the microfluidic chambers of the initial model However the intent of the new model is primarily to characterize the action potentials (spikes) detected in the central chamber in connection with the nature (constant, random, monotonically increasing-decreasing pulses of the activation lights) and the amount of information that can be transmitted from one chamber to the opposite chamber.

To avoid phosphorescence inference and have a fine independent optical control of the 3 populations of neurons in each “AP originator” compartments, we use three different excitatory (depolarizing) opsins actuators: the fast temporal kinetics blue light-sensitive ChETA opsin pAAV-Ef1a-DIO ChETA-EYFP with a peak response 490 nm, one red-shifted opsin Chrimson pAAV-ChrimsonR-tdT (peak response 590 nm) and, Chronos pAAV-Syn-Chronos-GFP (500 nm), a blue light-and green light-sensitive. This combination allows to have 3 different range of light activation of neuronal spiking without detectable crosstalk in mouse brain slices.

Chronos: longer pulse width (~700ms), 200 spikes/s in response to 100 pulses/s stimuli,

Chr2: pulse width (~350mss), excitation max. 460 nm

Red-shifted opsin indicators

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6944482/

<https://www.addgene.org/guides/optogenetics/>

ASAP1 and its derivative ASAP2f were used to characterize voltage responses in different neuronal compartments in response to visual stimuli under two-photon illumination in flies, demonstrating that voltage–calcium relationships differ between neurons1

<https://elifesciences.org/articles/25690> ASAP2s

https://elifesciences.org/articles/25690