- 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, a last isolated compartment connected only to 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. Therefore we keep the design of the microfluidic chambers of the initial model. It has been observed that active network of neurons as it grows, produces, action potentials concomitantly with increases in the number of synapses. The overall pattern of firing gradually evolves during the formation of these networks with initially isolated action potential to localized train of spikes or bursts. If the nature of the stimuli has an impact of neuroplasticity, we will be able to measure it by characterizing the action potentials (spikes) or bursts 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 will use three different excitatory (depolarizing) opsins actuators: the fast temporal kinetics blue light-sensitive ChETA with a peak response 490 nm, one red-shifted opsin Chrimson (peak response of 590 nm) and Chronos (peak response of 500 nm), a blue light-and green light-sensitive. They will be delivered using an AAV Vector. In addition, note that this combination allows to have 3 different ranges of light activation of neuronal spiking without detectable crosstalk in mouse brain slices.

- Calcium indicator like Rho-2 may not the most appropriate indicator for this model for which we seek to target specific subpopulations of neurons, to measure spiking dynamics and subthreshold voltage fluctuations. Furthermore, genetically voltage indicators (GEVIs) can detect neuronal depolarization and hyperpolarization when Ca^{2+} does not decrease below cytosolic concentration. Genetically encoded Ca^{2+} indicators Ace-mScarlet is a red fluorescent GEVI that fuses Ace2N voltage sensitive inhibitory rhodopsin with mScarlet, a bright fluorescent protein. With paired with the appropriate optics, it can report voltage from dozens of neurons and capable of readouts of single action potentials firing at frequencies as high as 100 Hz. Upon depolarization, this electrochromic (eFRET), Ace2N acts as a FRET acceptor, quenching a proportion of mScarlet's emitted photons via FRET. The peak fluorescence response of Ace-mScarlet decreases nearly as a function of membrane voltage. So, we will replace Rho-2 by the Ace-mScarlet indicator in the central chamber.

- To obtain the electrophysiology of the neurons we will measure the ratio $\Delta F / F$ which kinetics should closely track action potentials. $\Delta F / F$ is defined as the ratio between the difference between the fluorescence measured in compartment 4 during excitation of neurons on the peripheral chambers and the fluorescence at rest in compartment 4 over the fluorescence at rest in compartment 4. To record the fluorescence of the Ace-mScarlet, we will use 2-photon Fluorescent Lifetime Imaging Microscopy (FLIM) FRET techniques. In contrast to confocal microscopy, 2-photon imaging microscopy provides better deep-tissue imaging, less light scattering and absorption, and less photobleaching allowing longer time analysis, and Ace-mScarlet has demonstrated under the FLIM technique high FRET efficiency.

- To detect the burst, we will look for a burslet: a sequence of at least 4 spikes with interspike intervals less than a threshold which will be estimated based on historical analysis. Any group of burslets that overlaps in time is a burst, and we will fix a minimum duration of 10 ms for a burst to eliminate noise recording. Minimum burst duration will be enforced to eliminate noise. Peak spike rates during each burst and time associated with that peak will be estimated form a smoothed histogram of spike count. We will investigate a spike train distance which defines the distance (dissimilarity) between two spike trains as the minimum cost of transforming one spike train into the other following a series of edit

operations (insertion, deletion, or temporal shifting). The cost of shifting a spike in time will be adjusted by a cost which will take into account the cost of shifting non-coincident spikes. We will be, then, in position to compare the similarity between bursts in chamber 4 depending the nature of the information in compartment 1, 2 and 3.

Victor, J. D., and Purpura, K. P. (1996). Nature and precision of temporal coding in visual cortex: a metric-space analysis. *J. Neurophysiol.* 76, 1310–1326.

In this metric spikes are binned into 1 ms intervals and convolved with an exponential kernel with time constant τ_R (e.q. 2.1 and 2.2 in van Rossum, 2001). The choice of the exponential kernel was originally motivated in van Rossum's work by its causal properties and correspondence to the shape of postsynaptic currents. The time constant of the exponential, τ_R, determines the precision with which this distance metric is sensitive. Following convolution with the exponential kernel we then calculated the similarity between the two convolved spike trains as a Pearson correlation to produce a similarity estimate, S_R, that is bounded from 0 to

1 (dissimilar to similar) that can then be compared directly with Victor-Purpura's measure producing a similar range of values.

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 neurons[1]

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

https://elifesciences.org/articles/25690

AAV5-CaMKIIα-ChETA-YFP
https://www.pnas.org/content/116/34/17051
https://www.jneurosci.org/content/36/39/9977
https://www.frontiersin.org/articles/10.3389/fncel.2019.00052/full
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0219886
"This reflects the difference in extinction coefficient and quantum yield between these two proteins and mCherry and demonstrates how mScarlet-I is a better overall acceptor that will provide more signal and greater dynamic range for intensiometric green-red based FRET experiments."