- Dominic Giannangeli designs a custom MEMS device which allows to investigate neuroplasticity, the spatial and network reorganization of neurons in response of other neurons feedback. The device has three compartments, cells in one chamber is connected to the two others, the two other chambers are only connected to the central one. Primary rat cortical neurons are grown in each microfluidic chamber, each side extending their axons through micro-channels with the other side.
  Electrical stimuli are applied to the neurons of one of the extreme chambers and not to neurons in the other one, thus creating neural feedback in only one direction: from one of the peripheral chambers to the central chamber. Neuronal activity and physiological changes in the connections between each well are then studied and compared.

- Organotypic cells compared to cell cultures maintain cytoskeleton and are less limited in term of axon growth or synapse formation. We keep the interconnection of the three chambers used in the initial model, but instead of cortical neurons, rat cortical slices of about 350 μm will be cut using a MacIlwain tissue chopper and placed onto petri dishes with three polydimethylsiloxane (PDMDS) wells separated by microchannels. Cultures will be covered with a well-established culture medium[1], medium will be changed regularly. The cultures will be placed into a tissue incubator at 37°C. Each slice will be perfused with artificial cerebro-spinal fluid (ASCSF) and drugs. Electrical activity will be stimulated by electrodes with carefully calibrated pulses placed in the slices to generate an action potential, the neuronal response will be recorded by microelectrodes placed in the slices of the central and peripheral slices. An electrode is attached to the neurons of the dish with no explicit stimuli to record if neural activation sent by one of the peripheral wells, is transmitted through the central well to the other peripheral compartment, for a modelling of the entire neural pathway. Signals detected by the electrodes will be amplified, digitized and recorded for analysis.

- The previous model is simple but does not fully describe the complexity of interactions between neurons. 2-D cultures may affect the spatial growth of the neural network. In addition, a 3-D cultures like organoids have better cell-cell interactions and simulate cellular functions and neural signaling which replicate the ones in-vivo. In this model, we are no longer using the three-chamber device. We will start by using high-quality hPSC cells with favorable culture conditions[2]. Organoids will be embedded within an ECM hydrogel matrix such as Matrigel. We will measure the sensory response of a specific neuron to a laser ablation using a femtosecond laser. We will also use to detect neuronal network activity, small florescent molecules like ANEP [6] or ANNINE-6plus dyes (after checking toxic effects to the cells), with a careful design protocol to have good permeation to the cell membrane and avoid photobleaching. Optical equipment setup is key in this experiment and we will mimic to some extent specific steps described in previous experiments[3] except we will be using organoids instead of line cells.

- Lastly, the various models previously discussed will show significant limitations to understand the neural network reconstruction which happens for example in stroke or brain injury where some components of the microenvironment are beneficial like blood capillaries, oxygen and neurotrophic factor or damaging like neuroinflammatory. In order to mimic closer, the in-vivo environment in which these cells evolve, the design of a more complex model like organ-on-a-chip is ultimately required.

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[1] 50% MEM/HEPES (Gibco), 25% heat-inactivated horse serum (Gibco/Lifetech, Austria), 25% Hanks' solution (Gibco), 2 mM $NaHCO_3$ (Merck, Austria), 6.5 mg/ml glucose (Merck, Germany), 2 mM glutamine (Merck, Germany), pH 7.2 Stoppini et al.

[2] Neural induction products like: N2 supplements, Heparin saline solution; induction supplements: N2, 2-mercaptoethanol, insulin; maturation of mature cortical neurons: Vitamin A, retinoic-acid

[3] Steps to look into for the model: equipment setup, loading neurons with voltage-sensitive dye, optical recordings and data analysis described in: Voltage-sensitive Dye Recording from Axons, Dendrites and Dendritic Spines of Individual Neurons in Brain Slices

1) Yevgeny Berdichevsky et al., Lab on Chip, Issue 8, 2010 - Building and manipulating neural pathways with microfluidics
2) Michael P. Schwartz et al., 2015-10-06, Human pluripotent stem cell-derived neural constructs for predicting neural toxicity, https://doi.org/10.1073/pnas.1516645112
3) Kimura, H., Sakai, Y. (2018). Organ/body-on-a-chip based on microfluidic technology for drug discovery**.** Drug Metabolism and Pharmacokinetics. Vol. 33, Issue 1. pp. 43-48.
4) Wang, Z., Wang, S., Xu, T., et. al. (2017). Organoid technology for brain and therapeutics research. CNS Neuroscience and Therapeutics. Vol. 23, Issue 10.
5) Quadrato et al., 2017, Cell diversity and network dynamics in photosensitive human brain organoids, doi:10.1038/nature22047
6) https://assets.thermofisher.com/TFS-Assets/LSG/manuals/mp01199.pdf
7) 3D Organoid Culture: New In Vitro Models of Development and Disease