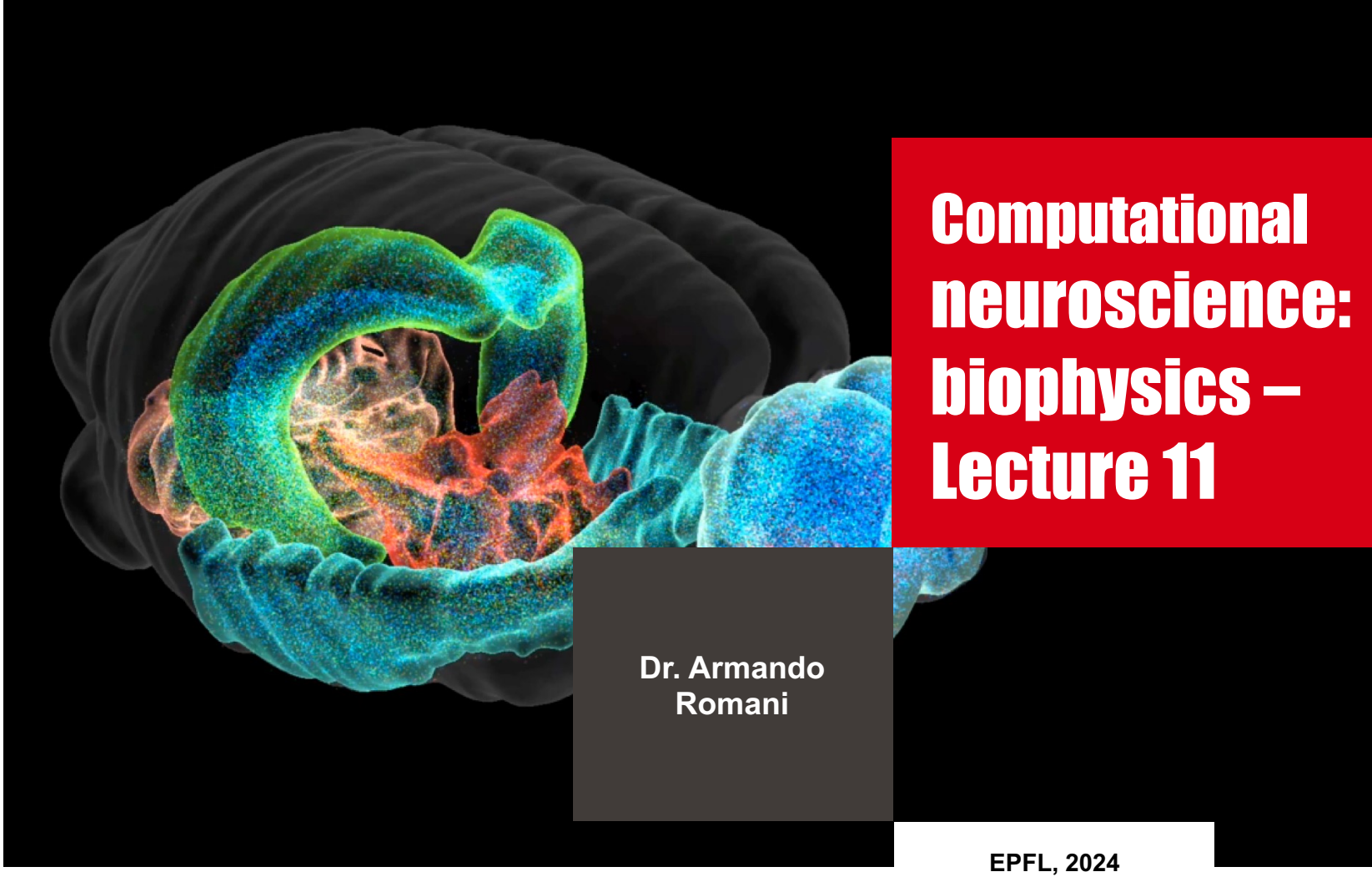


Computational neuroscience: biophysics – Lecture 11

Dr. Armando Romani

EPFL, 2024

Circuit 2: simulation experiments

Lecture Overview

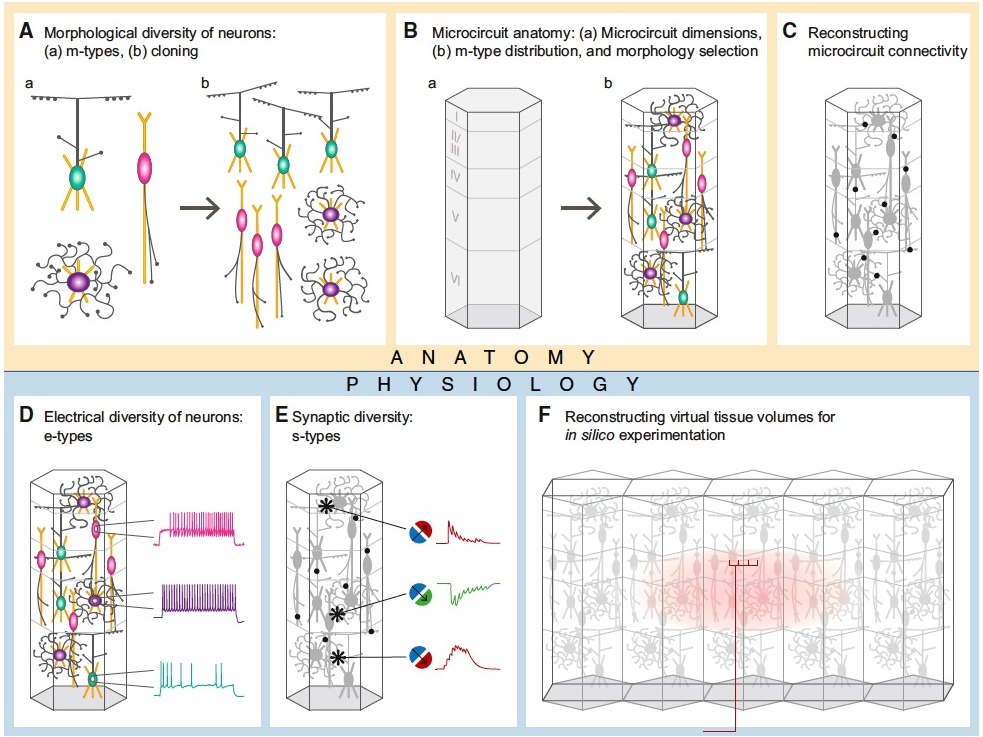
- Scope
- Approaches
- Applications

Lecture Overview

- **Scope**
- Approaches
- Applications

Network and network simulation

- Network is a compound model of several *building blocks* (ion channels, morphologies, synapses…)
- Simulating the network means solving the time dimension
- The network can be simulated under spontaneous or evoked activity, *in-vitro* or *in-vivo*



Markram et al., 2015

Spontaneous network activity

- Some brain regions contain neurons that are spontaneously and persistently active (pacemaker) and they can drive the network dynamics
- In the lack of pacemaker neurons, as it happens in SSCx and hippocampus, the network is driven by spontaneous synaptic activity
- Without pacemaker cells and spontaneous synaptic activity, the network should be silent (see later)
- Under spontaneous network activity, the network can show different dynamics depending on the region of interest
- Studying the spontaneous activity means studying the network at its resting state

Evoked network activity

- We can ask what happens if I perturb the network
- Internal dynamics contributes to the network activity, but regions are heavily interconnected, and a region that is only driven by internal mechanisms is far from the reality
- We can model external innervations (lecture 7). In SSCx (Markram et al., 2015) we implemented thalamic projections, while in the hippocampus (Romani et al.) we implemented CA3 projections or Schaffer collaterals
- We can mimic an external input by injecting currents in the somas
- We can inject an absolute amount of current or a percentage of the current necessary to make the cell fires (to bring the voltage to spike threshold) (Markram et al., 2015)

In-vitro simulations

- Most of the data are obtained *in-vitro*
- It is often useful to replicate *in-vitro* conditions. We may want to validate the network, get insights in some experimental results, extent some experimental findings
- *In-vitro* conditions may differ from *in-vivo* ones for several reasons
- The region of interest is cut and removed from its context. As a consequence, it does not receive most of the input from connected regions, and there is not the same background activity
- The external solution is different. It does not contain important molecules (ions, hormones, neuromodulators…)
- The solution is also altered on purpose to simplify the experiments. For example, an higher Ca^{2+} concentration is normally used to make the synapse responses stronger and more easily recordable

In-vivo simulations

- *In-vitro* conditions are useful to study the system, especially because we can access much more data to validate our model
- Anyway, the behavior of the network *in-vitro* may not occur *in-vivo*
- Capturing what would happen *in-vivo* is not trivial. We have to reproduce the extracellular solution and the background activity
- While we cannot reproduce exactly *in-vivo* condition, we can approximate it to have an idea in which direction the system is moving when passing from *in-vitro* to *in-vivo*
- Markram et al. (2015) approximate *in-vivo* condition by lowering extracellular calcium concentration and applying tonic depolarization around the rheobase (voltage at the spike threshold) (see later)

Set up a simulations

Define the simulations we want to run and set up the parameters accordingly

- Basic simulation parameters (duration, dt, seed…)
- Conditions (extracellular ion concentrations…)
- Stimuli (in any) (from projections or by injecting current)
- Recorded variables (spike times, voltages… See later)

Sensitivity analysis

- If the network model contains random processes (and in most of the cases, it should contain them), the simulation should be replicated several times with different random number seeds
- Network and network simulations depend on many parameters. We want to avoid that our results depend too much from the particular set of parameters used
- The variability in biology is quite high, and our results are not very strong if they are valid only for a very narrow space of parameters
- To address this problem we can replicate the simulations with slightly different parameters on insert some noise in the parameters (e.g. insert noise in the stimuli) to check the robustness of the results
- An additional option is creating different instances of the network model, where you change key parameters still in biological range (Markram et al. (2015) created 6 equivalent circuits)

Why do we want to simulate the network?

- Test the circuit
- Study the network regimes or answer general questions
- Answer region-specific questions

- Sanity/quality checks, validations
- Predictions, validations
- Predictions, validations

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Summary 1

- There are several reasons why we want to simulate the network
- In any case, first we have to *design* our simulations, that is making explicit aim of the simulations, conditions, parameters, inputs, recorded variables
- We have to set up the simulations accordingly
- In most of the cases, more than one simulation is necessary. We can try different random number seeds or slightly different conditions to make our results more robust

Lecture Overview

- Scope
- **Approaches**
- Applications

Simulation output

During the simulation, you can output a series of parameters:

- Spike times
- Voltages
- Currents
- Conductances
- Ion concentrations
- Any other variables of the model

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Analyses

Additional analyses are often required:

- Raster plot
- Instantaneous firing frequency
- Inter-spike interval (ISI) histogram
- Mean firing frequency (histogram)
- Voltage traces
- Visualization
- Local-field potential (LFP)
- Voltage-sensitive dye imaging (VSDI)

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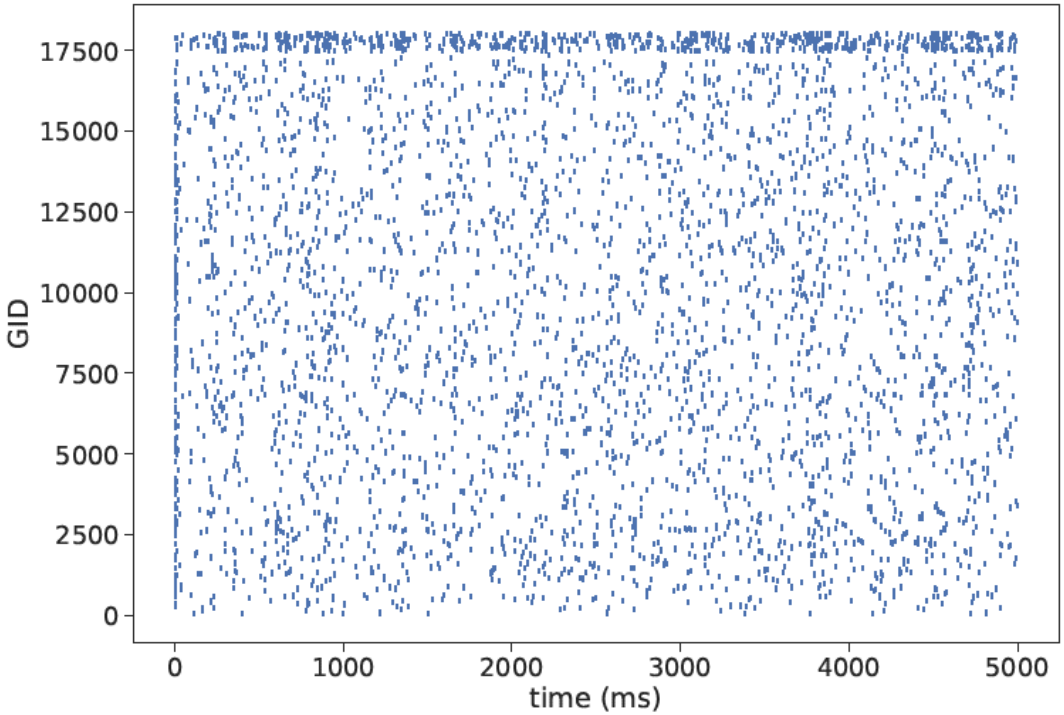
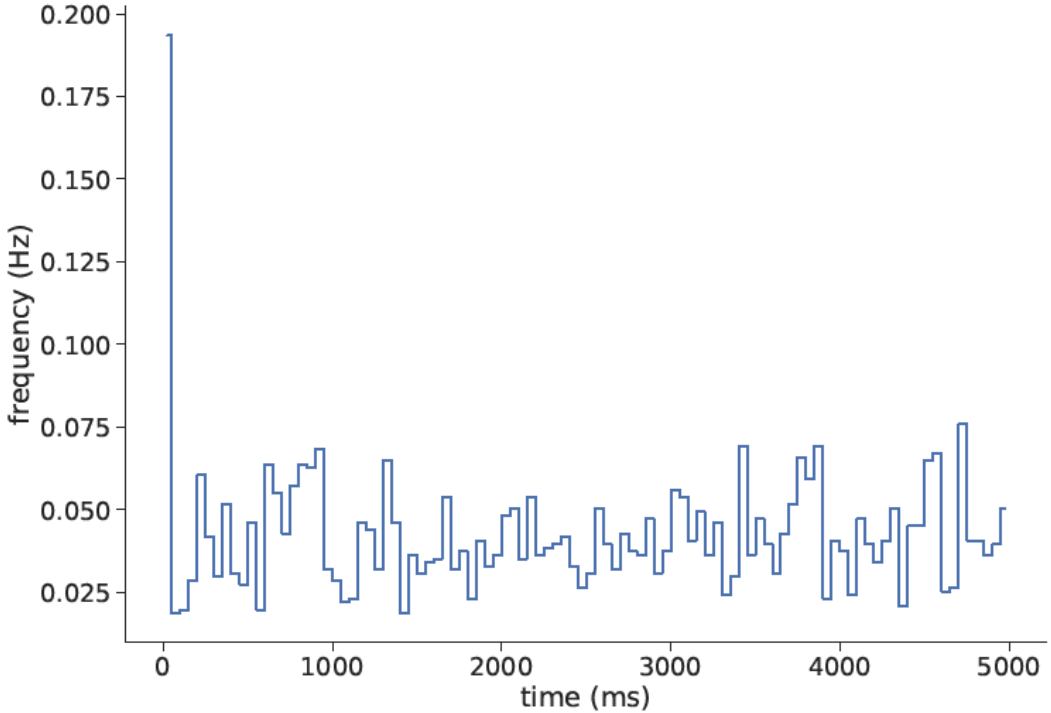
Raster plot



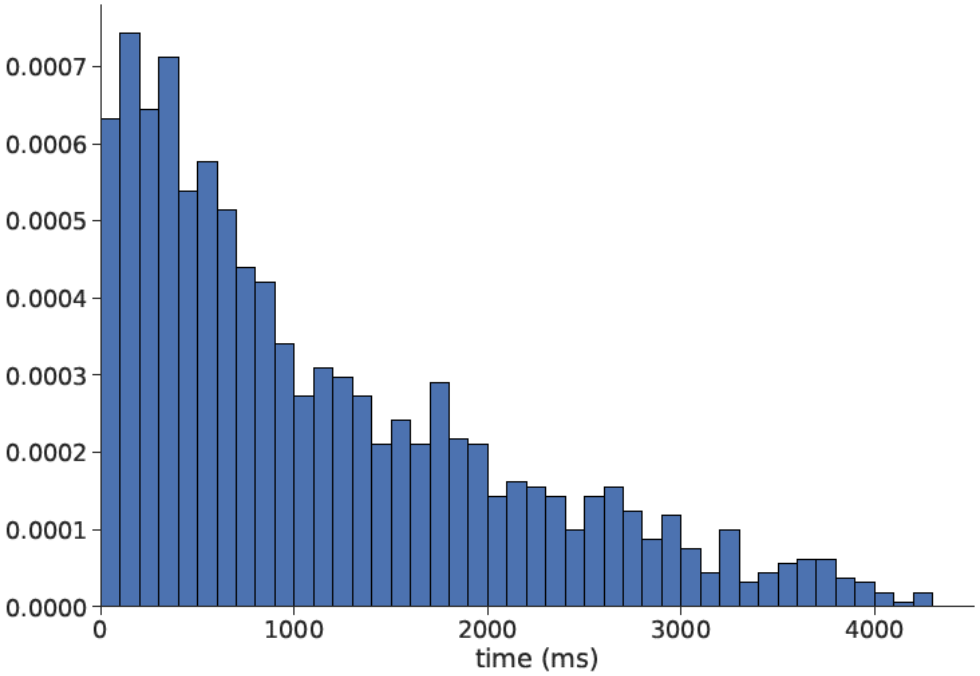
Plot of the spike times for each cell

Instantaneous firing frequency



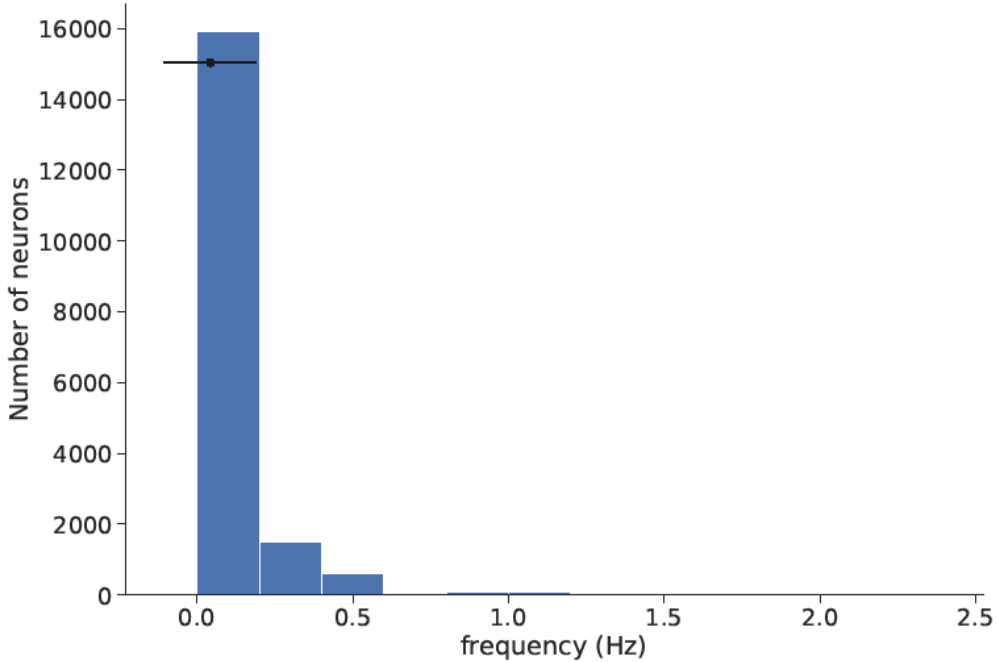
Bin of the number of spikes divided by the bin size (ms)

Inter-spike interval (ISI) histogram



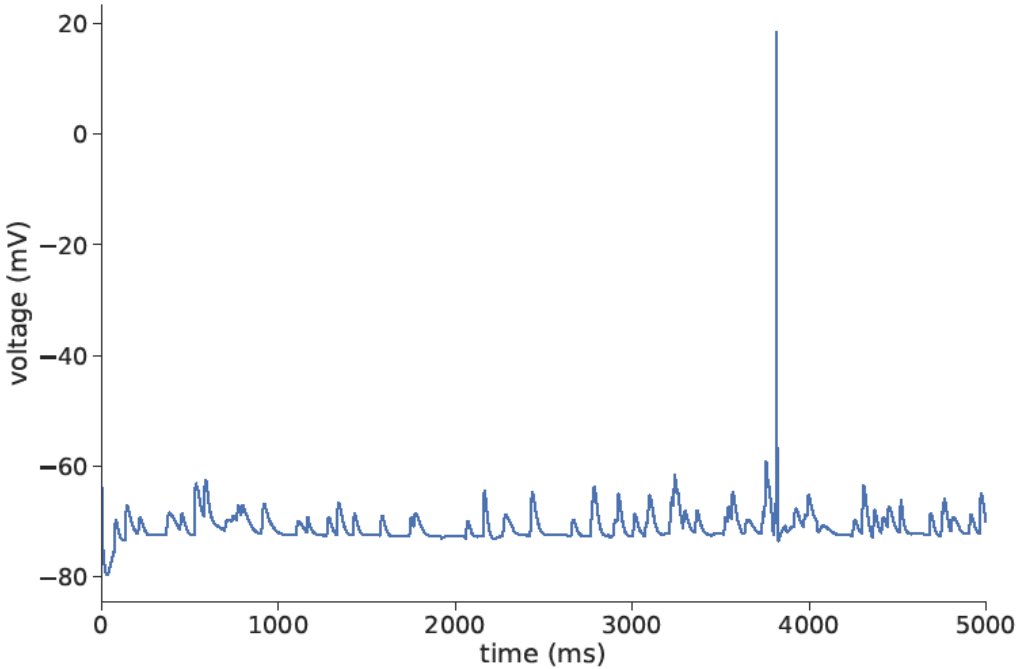
Distribution of the inter-spike intervals (ISIs) from all cells

Mean firing frequency distribution



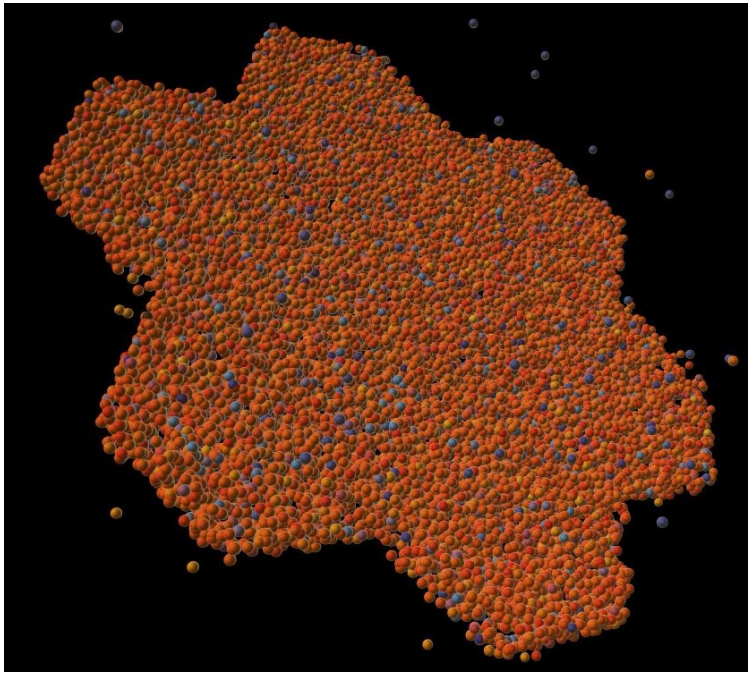
Distribution of mean firing frequency for each cell

Voltage traces



Somatic voltage over time from one cell

Visualization

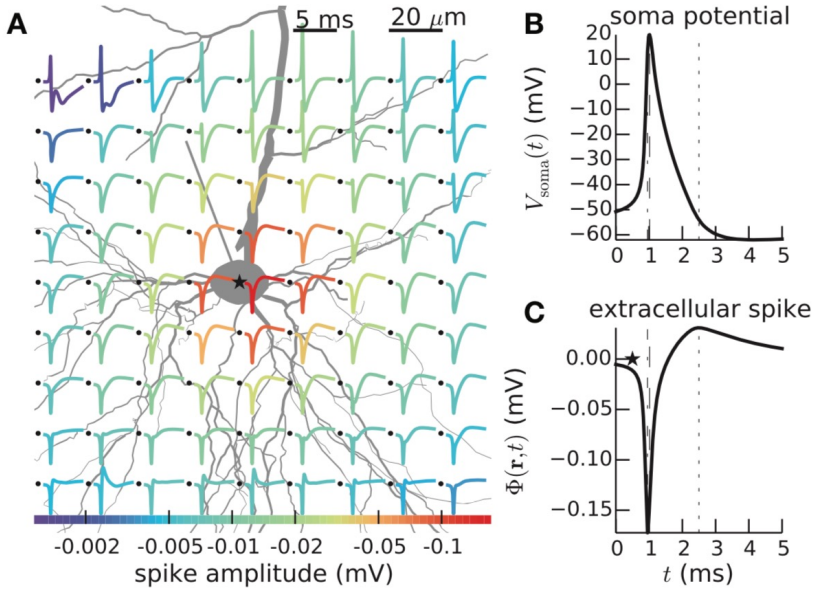


Frame during the simulation of the hippocampus microcircuit.

Colors represent voltages

Local-field potential (LFP)

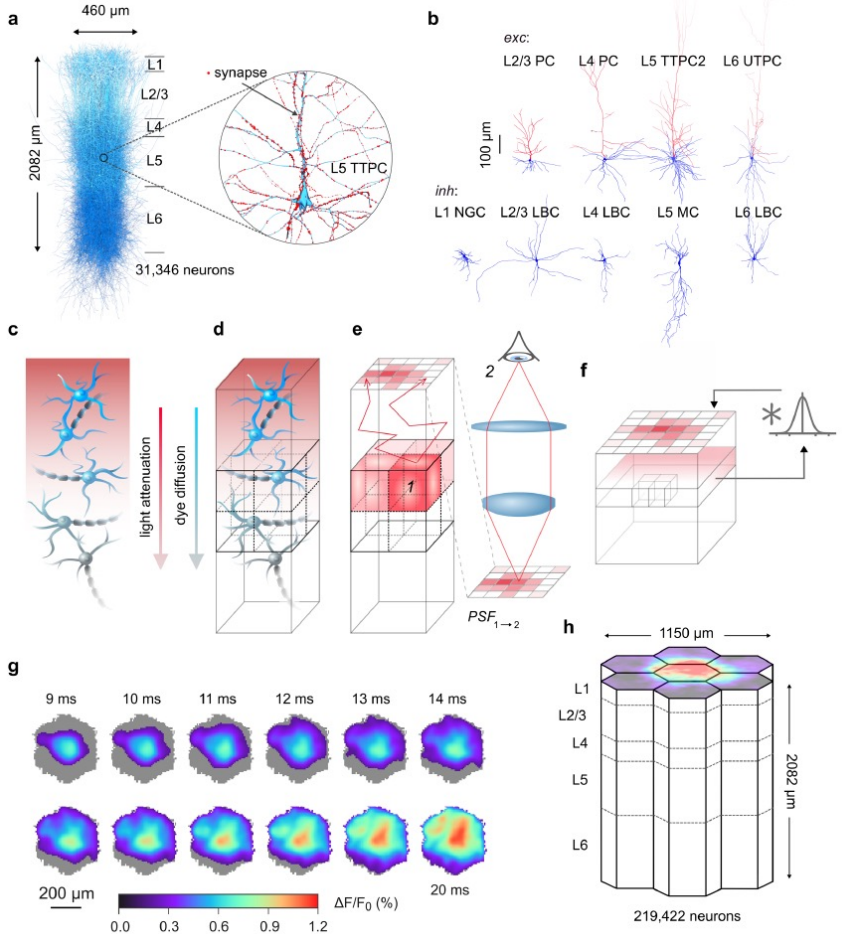
- The local field potential (LFP) refers to the electric potential in the extracellular space around neurons. The LFP is a widely available signal in many recording configurations, ranging from single-electrode recordings to multi-electrode arrays.
- Several methods are available to estimate LFP from network parameters (Reimann et al. 2013; Linden et al., 2014).



Linden et al., (2014)

Voltage-sensitive dye imaging (VSDI)

- Voltage-sensitive dyes are organic molecules or proteins which reside in a cell membrane and change their optical properties in response to a change in membrane potential. They have been used to follow population changes in membrane potential over large regions of the brain.
- Newton et al. (2021) developed a method to compute an in-silico VSD signal.



Newton et al., (2021)

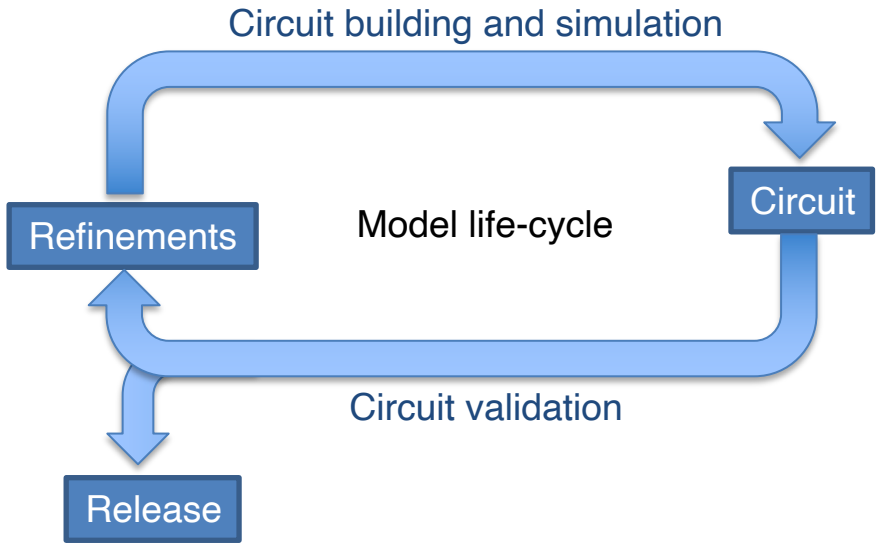
Simulate the network

- Test the circuit
- Study the network regimes or answer general questions
- Answer region-specific questions

- Sanity/quality checks, **validations**
- Predictions, **validations**
- Predictions, **validations**

Validate the network

- Select one or multiple experiments
- Reproduce (as much as possible) the same bath conditions (*in-vitro*) or the same *in-vivo* conditions
- Reproduce the same stimuli (if any)
- Reproduce the same analyses
- Compare and discuss results
- Validations often lead to improvements of the model and so the release of new versions

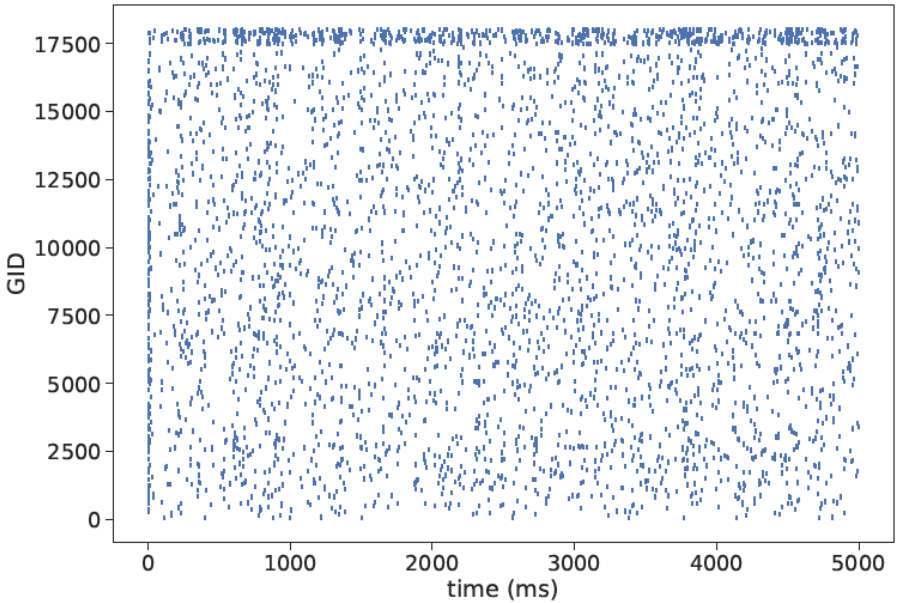


Test the network

- Even if each building blocks are apparently well-constrained, the correct behavior of the network is not granted
- The interaction of the different building blocks is often complex, and the overall behavior cannot be predicted by looking at each blocks
- Extensive testing of the network is essential
- It can unmask incorrect behavior of the building blocks and assumptions
- Some common tools and strategies can be described…

Let's switch on the network...

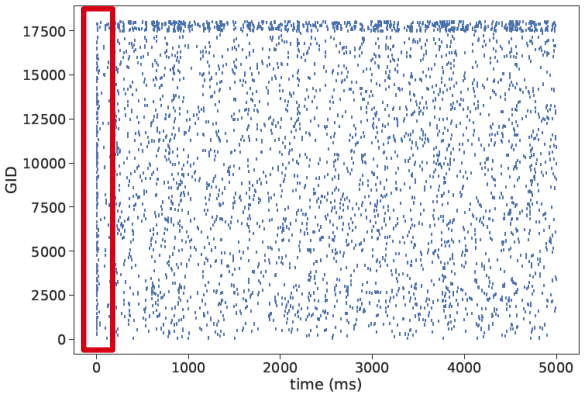
- The first check we want to make is running a test simulation with default parameters
- Check the spontaneous activity without applying any stimuli
- Obvious misfunctioning can already appear here

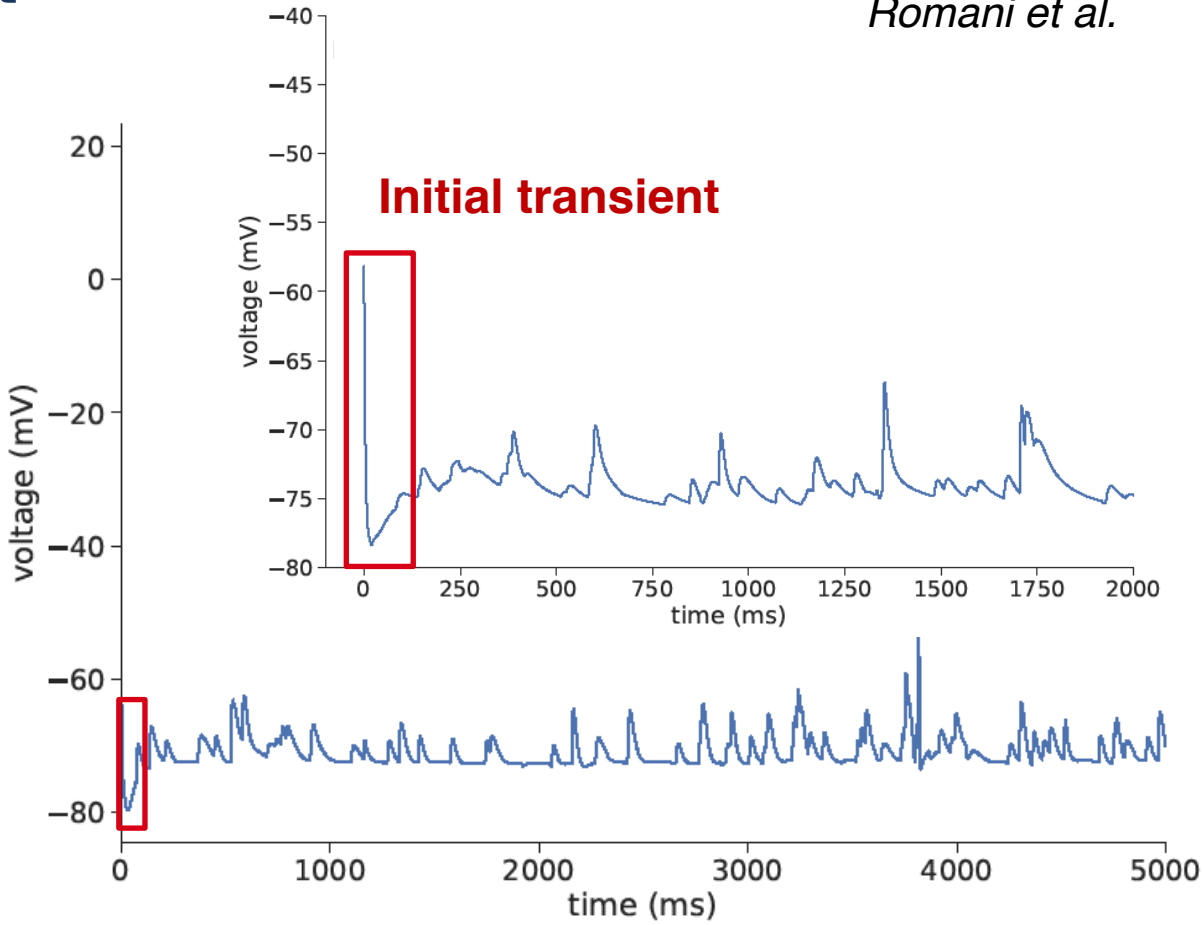


Romani et al.

Exclude initial transient

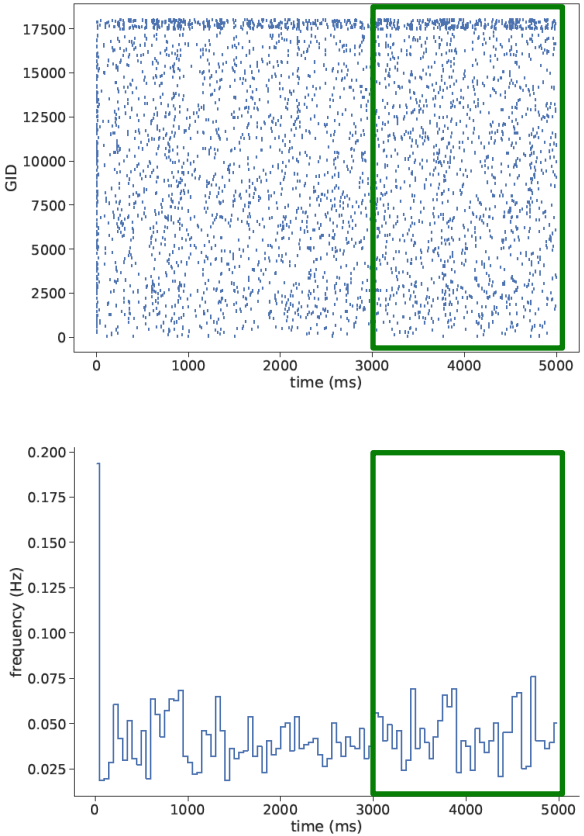
Romani et al.

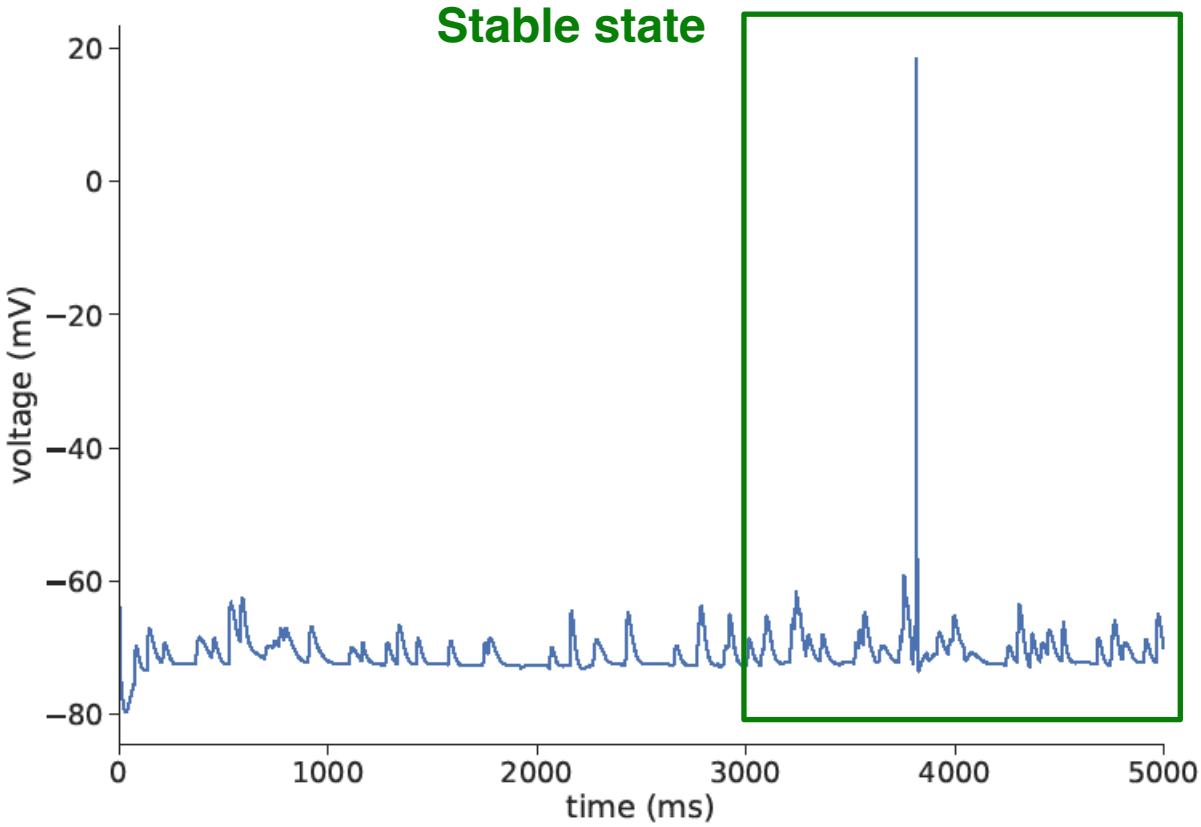




Look for stable state

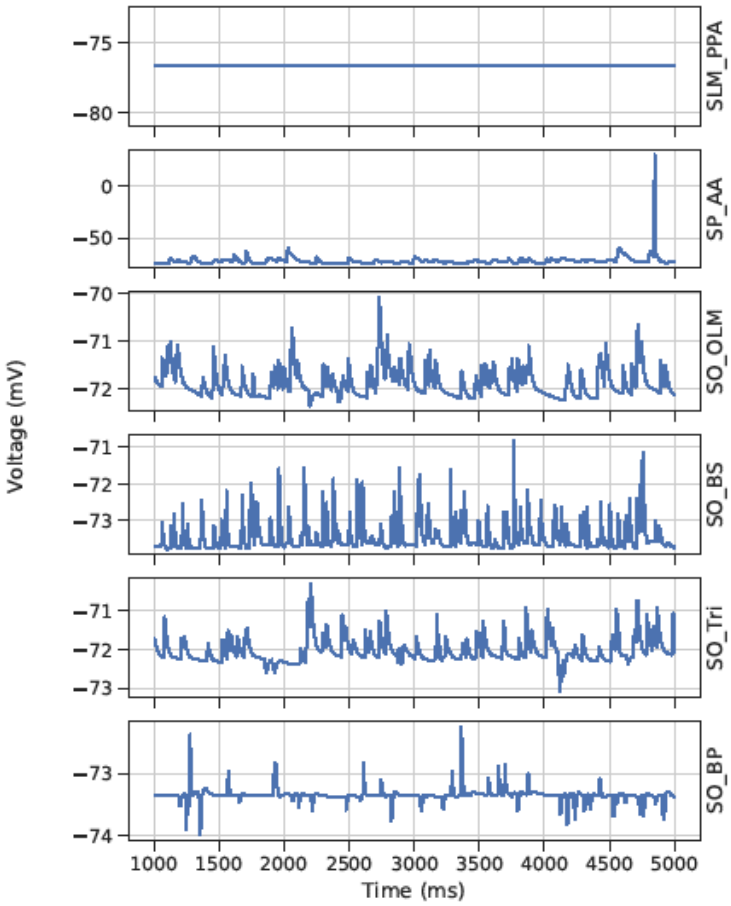
Romani et al.

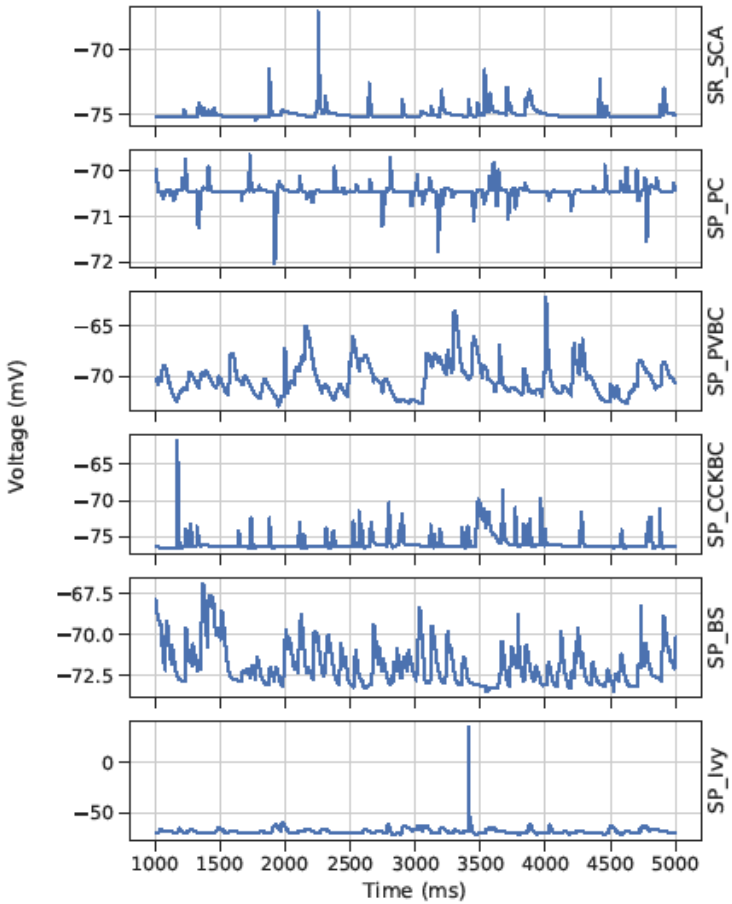




Inspect single traces

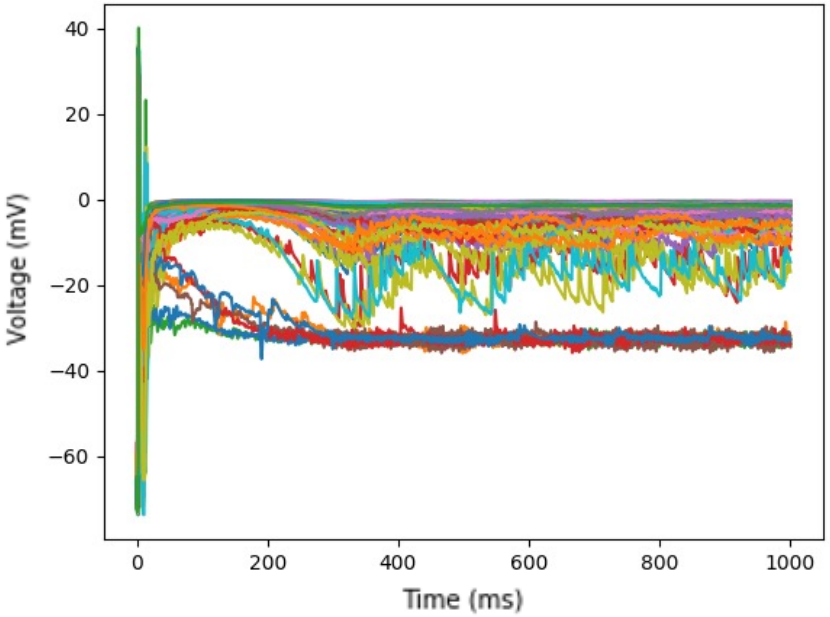
Romani et al.



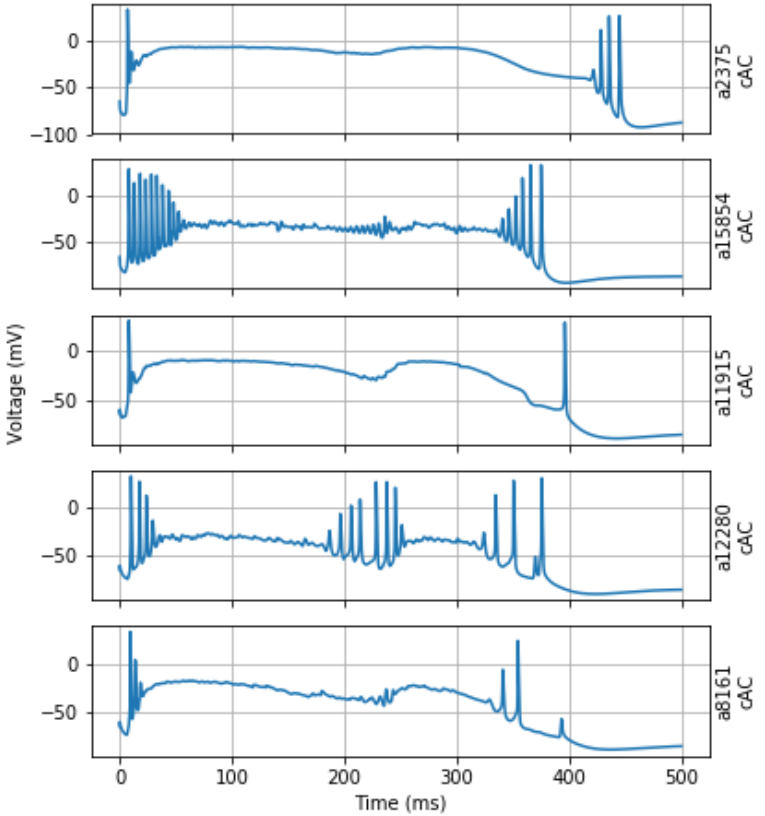


Inspect single traces

Stuck cells!



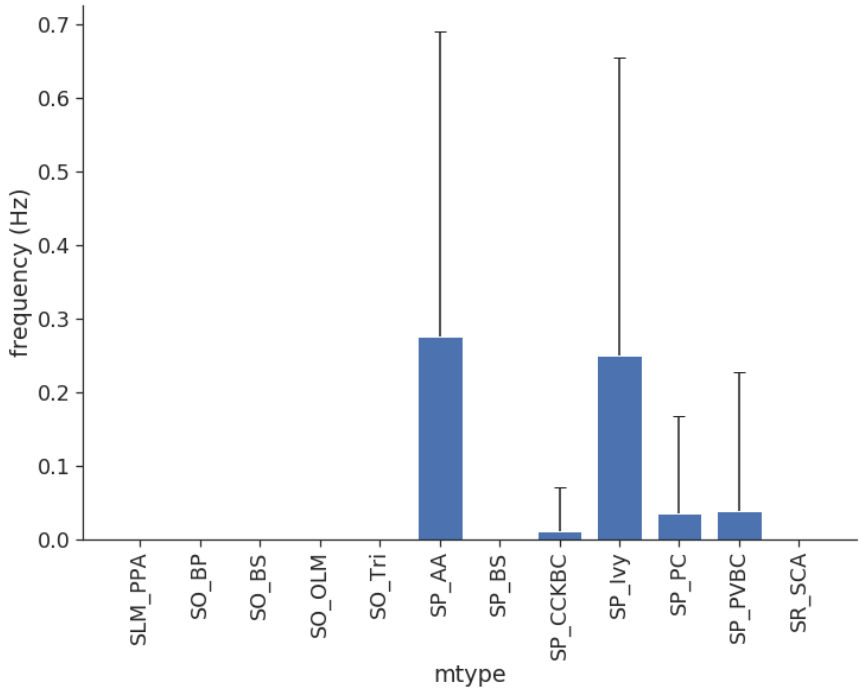
Depolarization block!



Romani et al.

Check (validate) mean firing rates

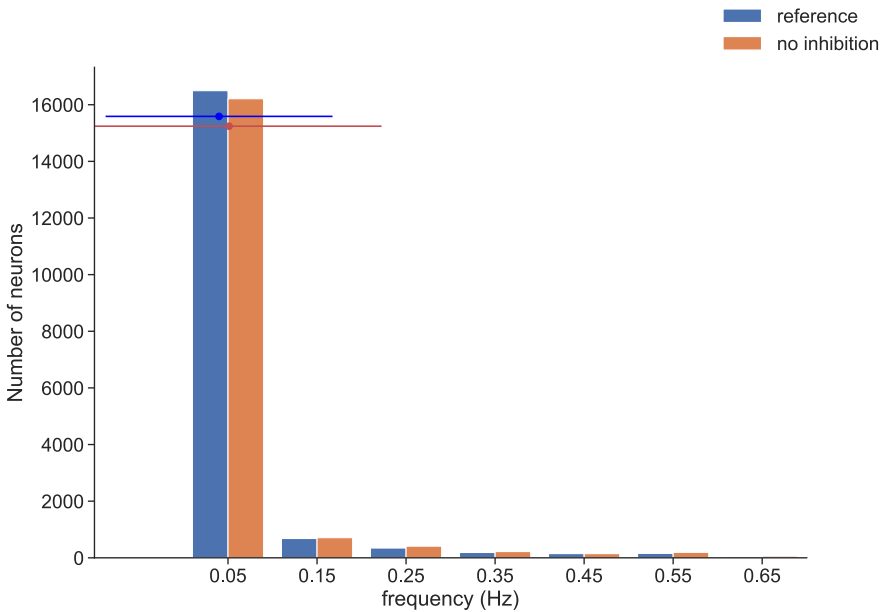
- The mean firing rates of the different cell types give an overall activity of the network and its different populations
- If data are available, this can be an useful validation
- In any case, the research should have an idea of the network activity under certain condition
- Cells too activity or silent could be an indication of a misfunctioning



Romani et al.

Disable inhibition

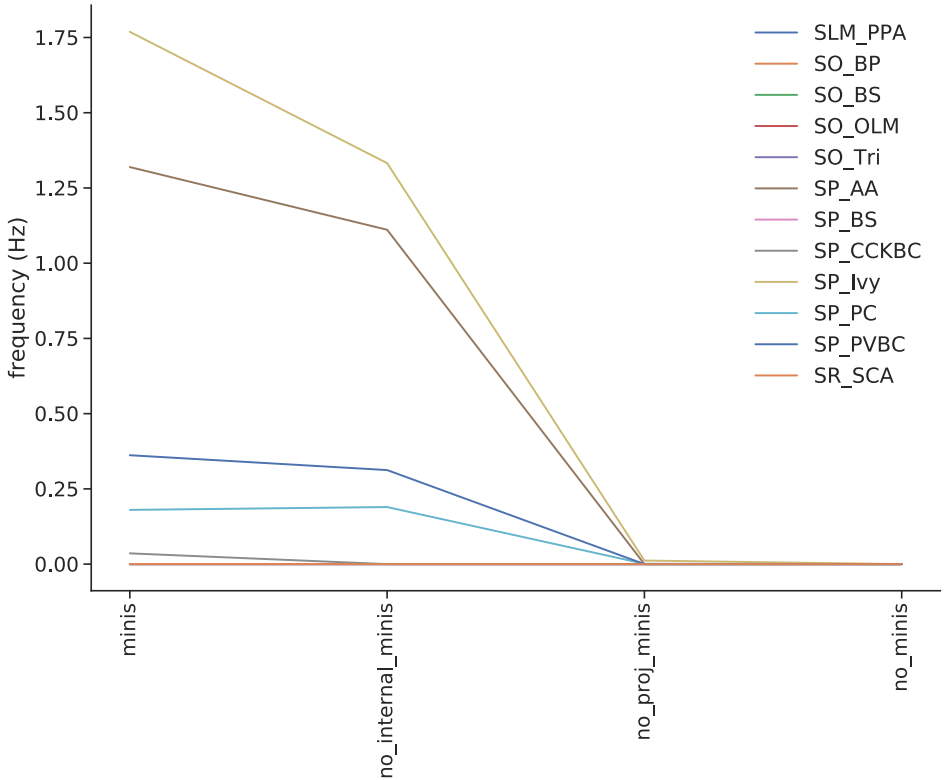
- Network manipulation is an useful approach to detect misfunctionings
- We apply simple manipulations (we change only one parameter at the time) and
- Manipulations for which we know the results, either quantitatively or qualitatively
- In the example, blocking the inhibition should increase the overall network activity



Romani et al.

Alter spontaneous synaptic events

- In this series of manipulations, we disable the spontaneous synaptic activity in groups of synapses
- The expected results is to have a reduced network activity



Romani et al.

Study the network regimes or answer general questions

- The network model is a system that can be quite complicated on its own
- Study the model to have insights into the real system
- Characterize the input-output (IO) function of the model
- Characterize in which different (steady) states the model can be
- It could be synchronous, asynchronous…
- We can look at spike correlation, LFP…

Answer region-specific questions

- Each brain region has its own specific roles and properties
- The research on each brain region has its own questions
- Once the model is extensively validated, we can use the model to make predictions
- We can support an existing theory, reveal the mechanism behind a given behavior, predict the behavior of the system in conditions that are not possible experimentally

Summary 2

- Closer is the model to the biophysical system, more complicated it will be to study it
- We can access all the variables of the model, and we have a vast repertoire of analyses to answer our questions
- Check the default model to spot any problems
- The model can be checked by altering it when the expected result is known
- Validate the emergent properties (that emerge from the interaction of model parts)
- Use the model to make predictions
- There is no magical recipe to check, validate and use the model to make prediction but we can learn from experience

Lecture Overview

- Scope
- Approaches
- **Applications**

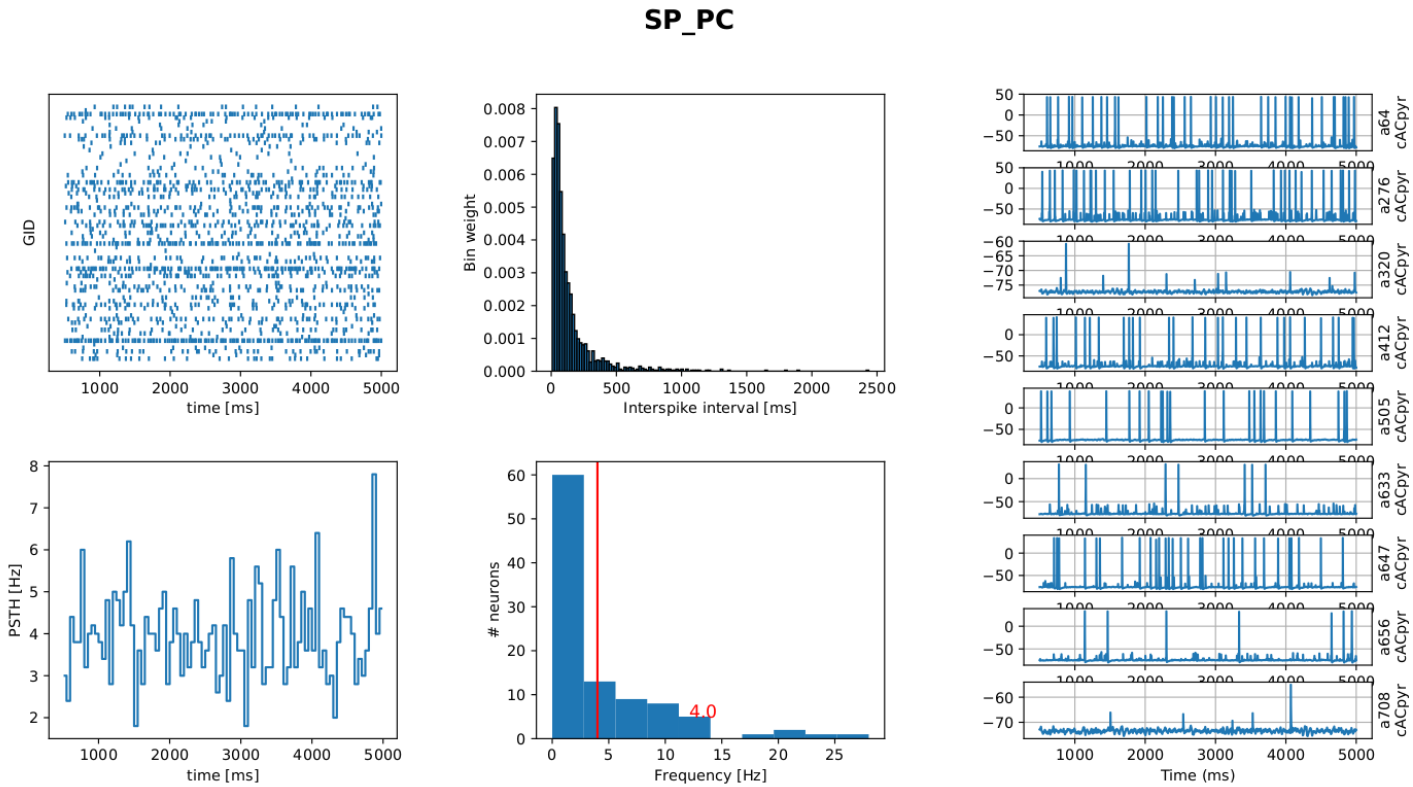
Simulate the network

- Test the circuit
- Study the network regimes or answer general questions
- Answer region-specific questions

Simulate the network

- **Test the circuit**
- Study the network regimes or answer general questions
- Answer region-specific questions

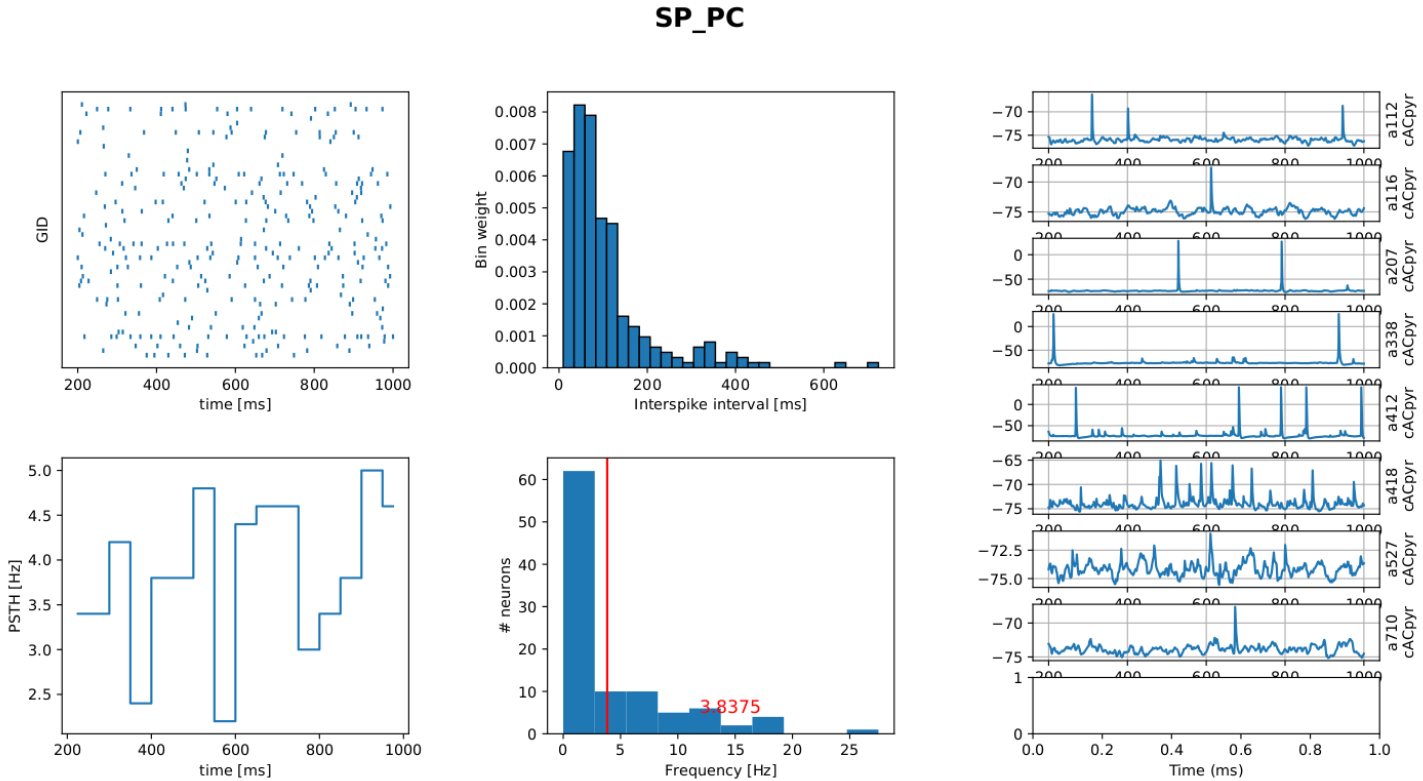
Testing the hippocampus model



Spontaneous activity in v5 model

Romani et al.

Testing the hippocampus model

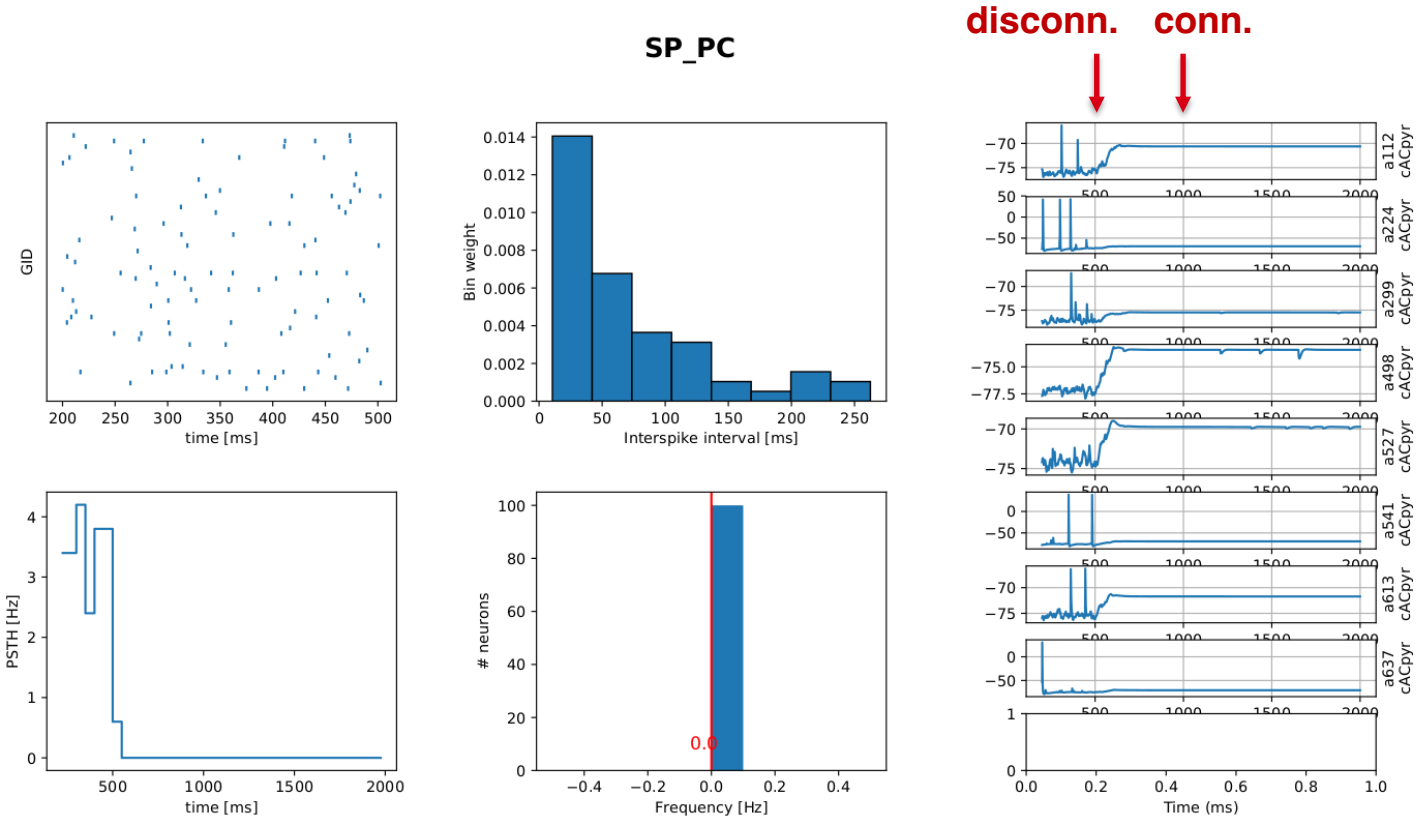


No spontaneous synaptic events (minis)

Romani et al.

Testing the hippocampus model

Romani et al.



no minis + PC are disconnected after 500 ms and reconnected after another 1000 ms

Testing the hippocampus model

- Under spontaneous activity the network is too active compared to experimental data
- Removing minis only decreases slightly the firing frequency. Without minis there is no ‘driving force’
- By disconnecting the cells, the activity fade out. Once, the cells are reconnected, there is no more activity
- It seems that the initial excitation (transient) reverberates through the network for the entire length of the simulation
- Connections between cells may be too strong or cells too excitable

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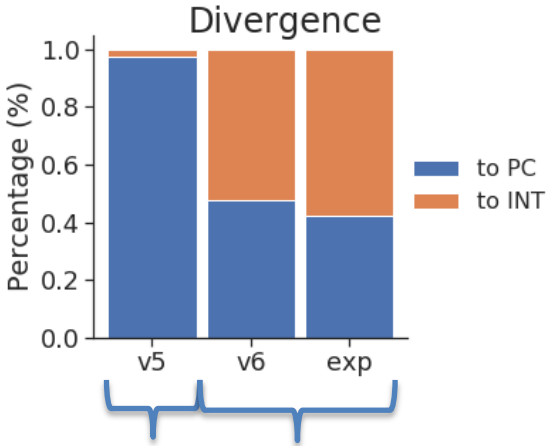
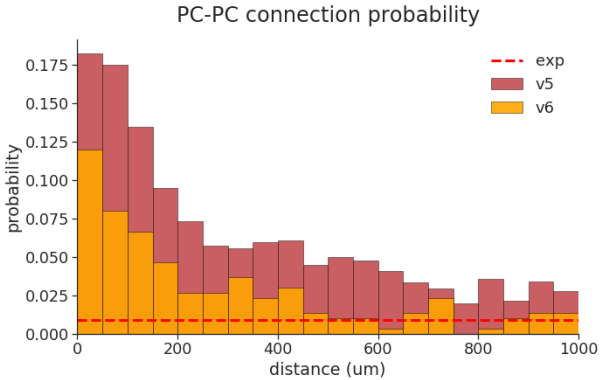
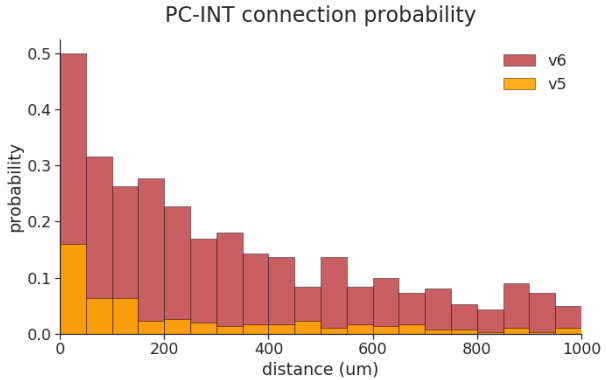
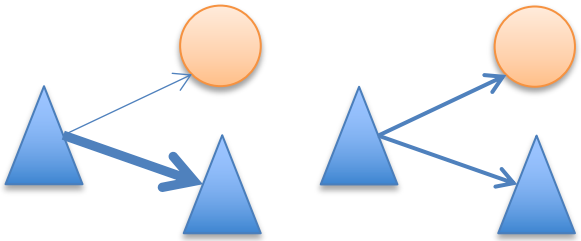
Testing the hippocampus model









v5: touch distance of 0.5 for INT and 2.5 for PC
v6: touch distance of 6.0 for INT and 1.0 for PC

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Testing the hippocampus model

- An important parameter we did not match was the divergence from PC to PC and INT
- By matching it, we could improve connection probability between PCs, and between PC and INT
- This was the major improvement in v6
- While this certainly improved the behavior of the network, it did not solve completely the problem and the network was still too active
- We explored our other hypothesis: the cells are too excitable

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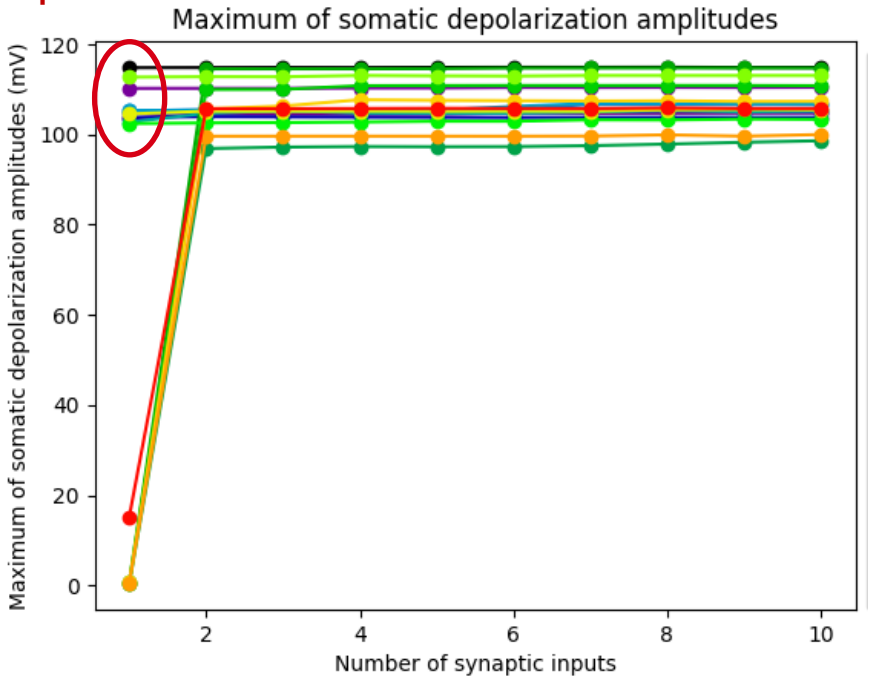
Testing the hippocampus model

- If few spikes of the initial transient can ‘survive’ in the network, it could mean that the EPSPs generate other spikes without loss of activity
- We tested cells by placing 1-10 synapses on different dendritic locations and stimulate them synchronously
- Some location leads to spike generation following a single EPSP only, that is not physiological

spike!



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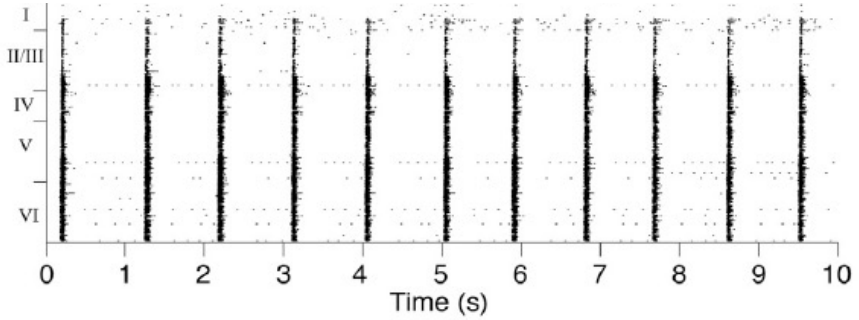
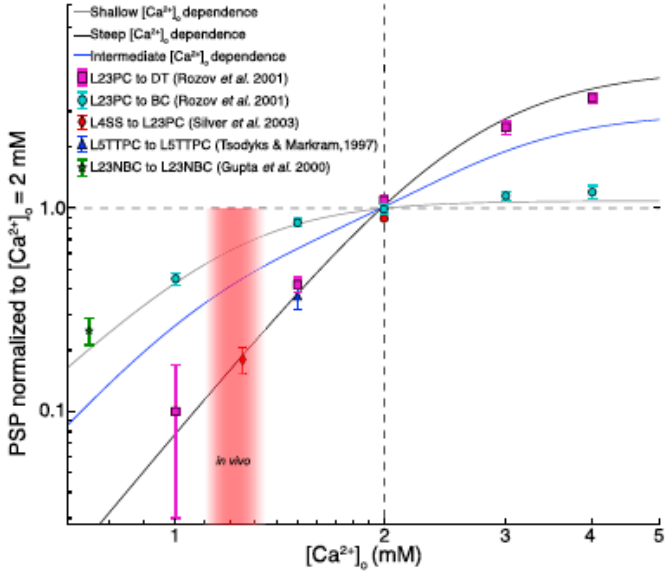
Testing the hippocampus model

- The single cell models were constrained using somatic features, that are the experimental data normally available
- Inside a big network, most of the activity occurs at level of dendrites, that should be constrained accurately
- We undertook a subsequent refinement of the single cell models
- We incorporated the new cell models into the network generating v7
- Now the behavior of the network is much more in line with the experimental findings

Testing the SSCx model

- The network shows bursts of activity that should be further investigated
- Most of the data come from (*in-vitro*) experiments that uses high $[Ca^{2+}]_o$ (and low $[K^+]_o$)
- *In-vivo* conditions have a lower $[Ca^{2+}]_o$ and higher $[K^+]_o$
- higher $[K^+]_o$ can be mimicked with a tonic depolarization
- lower $[Ca^{2+}]_o$ can be mimicked with a change in release probability



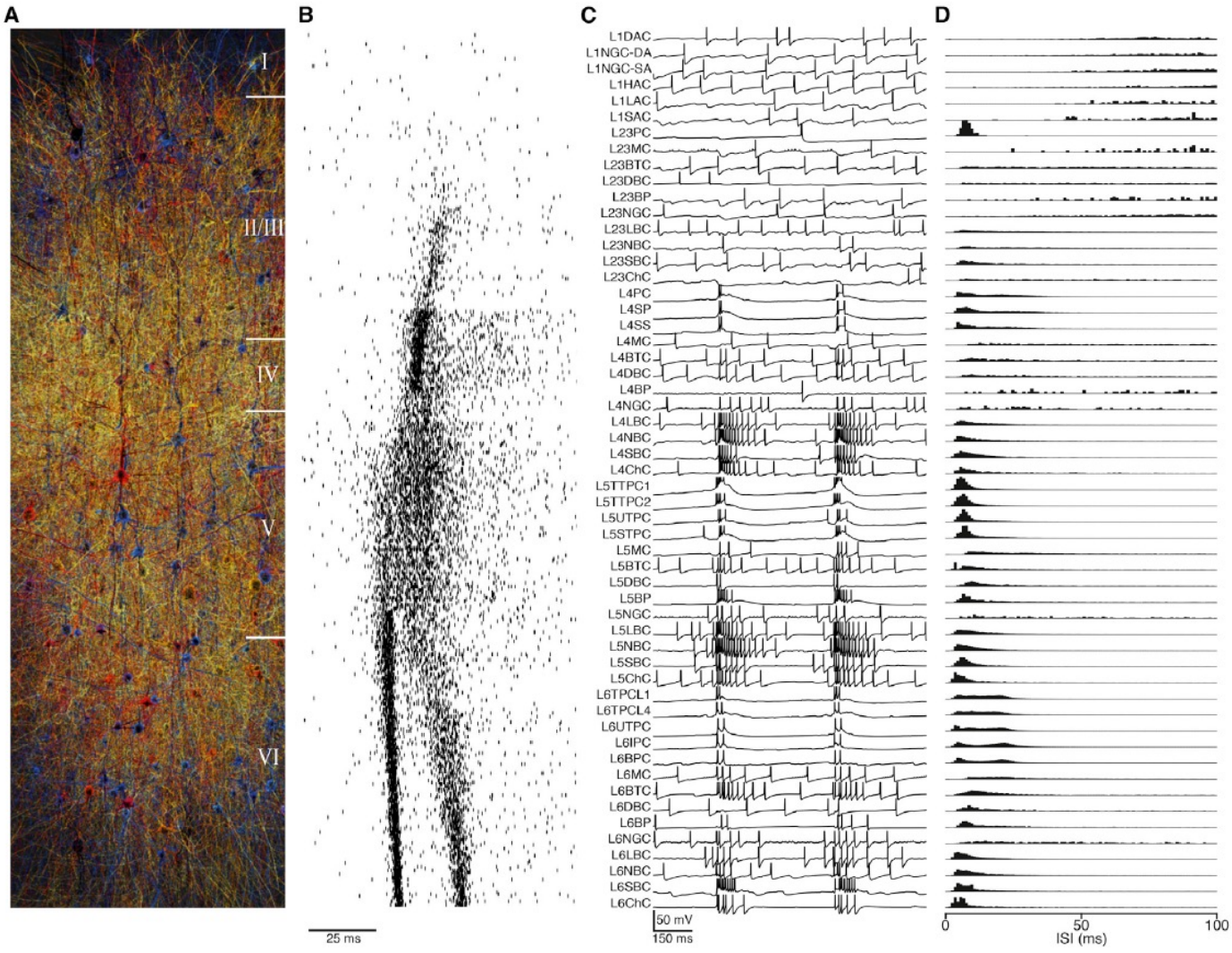


Markram et al., 2015

Simulate the network

- Test the circuit
- **Study the network regimes or answer general questions**
- Answer region-specific questions

Spontaneous activity



Markram et al., 2015

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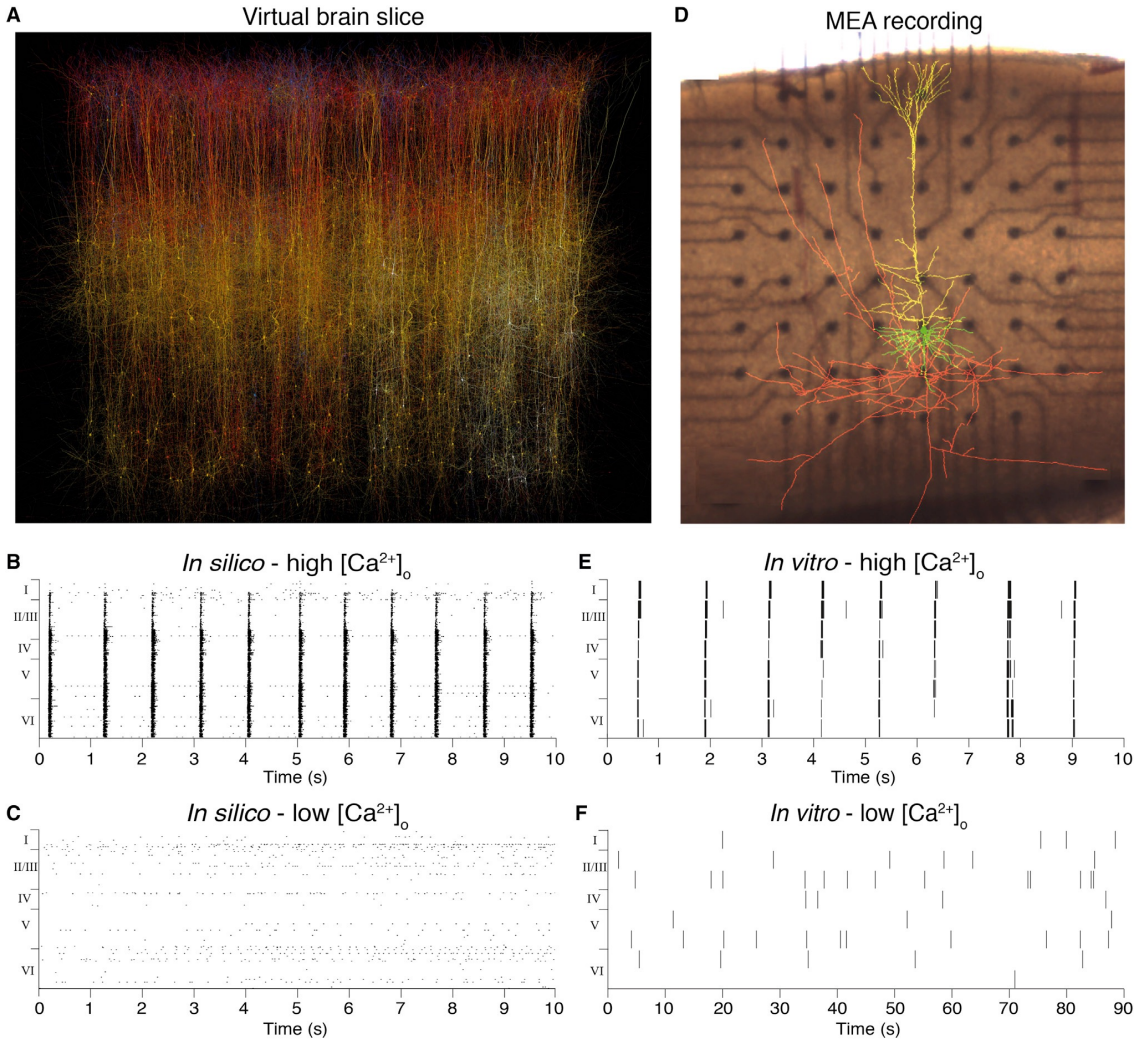
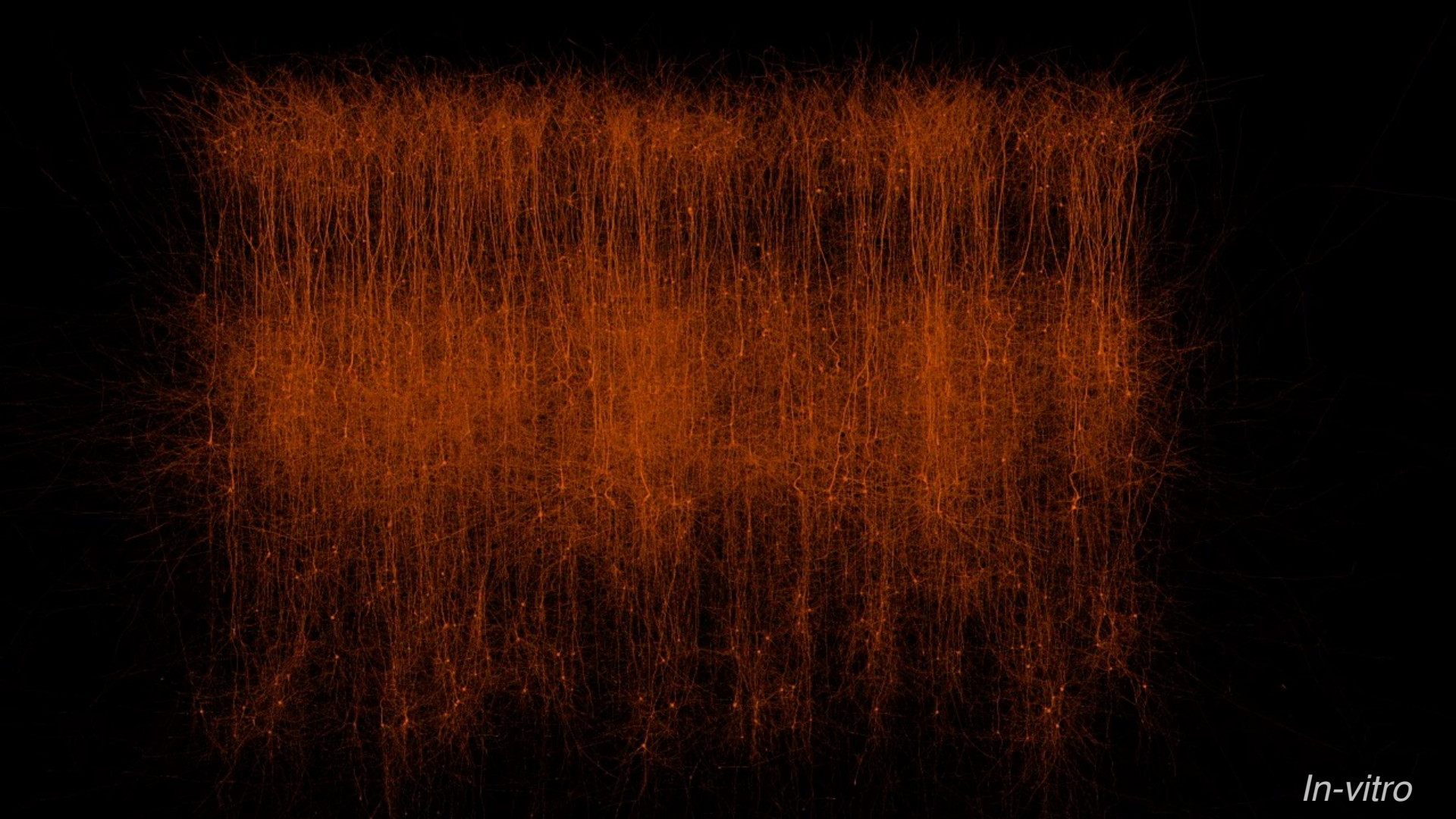
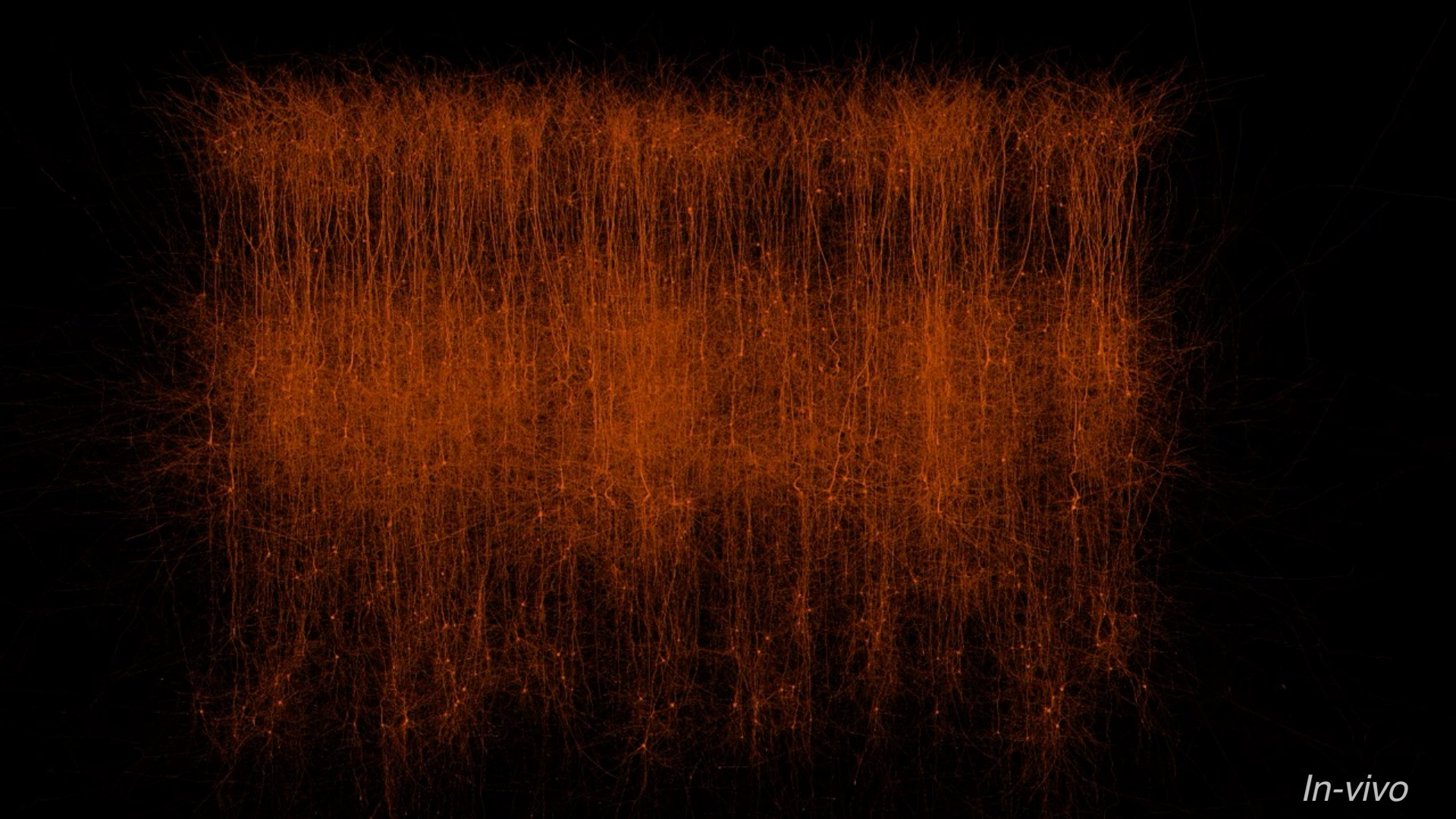
- Virtual brain slice of 7 microcircuits
- In vitro experiments are typically performed at 2 mM $[Ca^{2+}]_o$, while the level of $[Ca^{2+}]_o$ in vivo is reported to lie in the range 0.9-1.1 mM
- Slow oscillatory bursts in high $[Ca^{2+}]_o$
- asynchronous and irregular activity in low $[Ca^{2+}]_o$

Markram et al., 2015

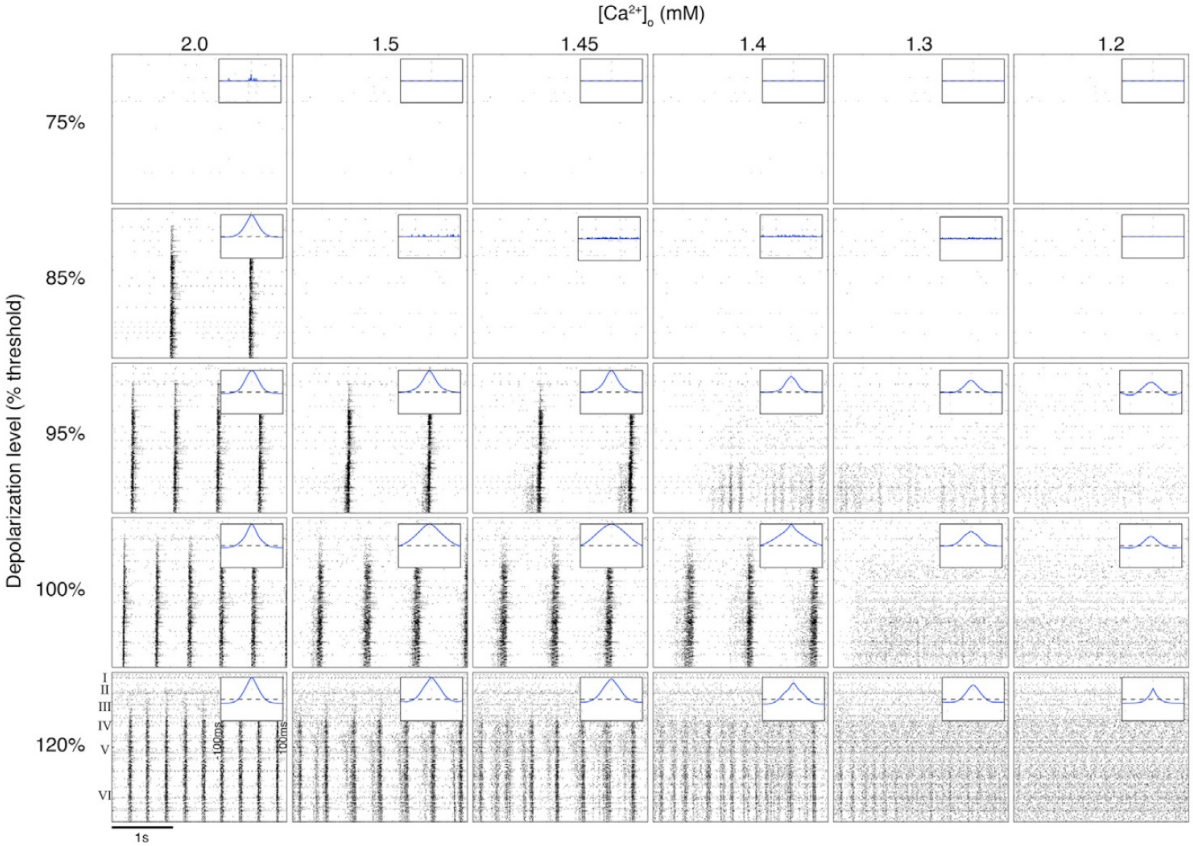



In-vitro

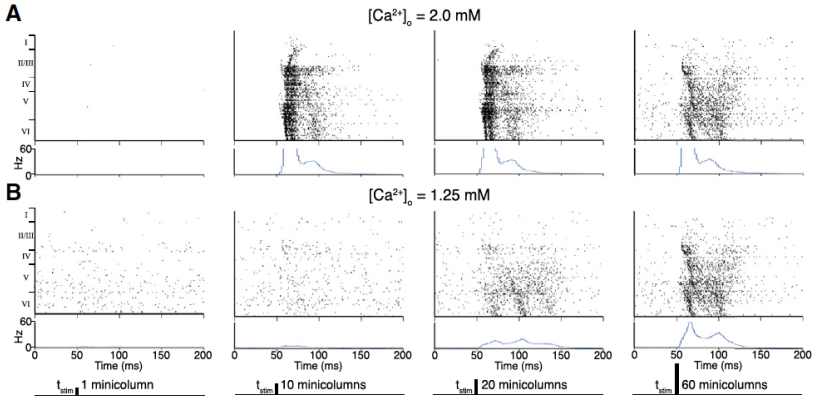

In-vivo

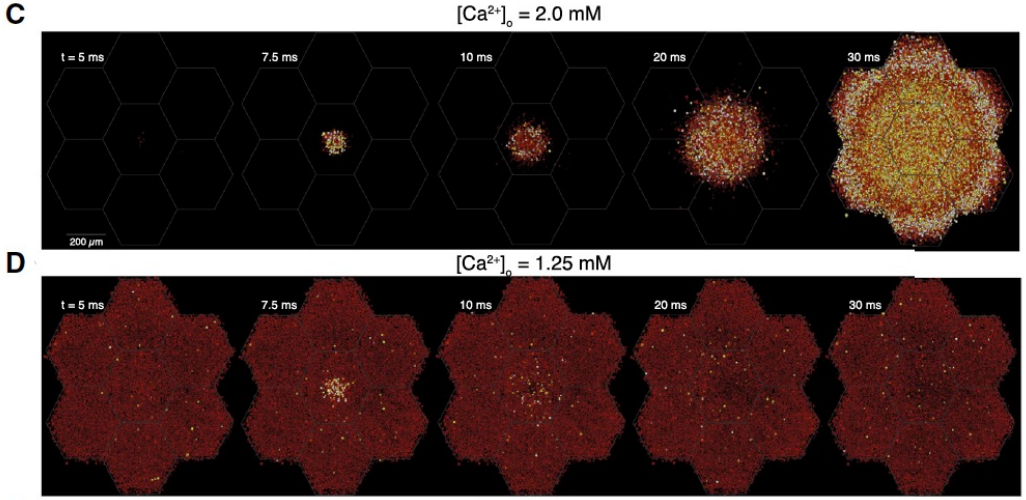
The mechanism underlying this sharp transition is likely to involve the differential Ca^{2+} sensitivities of inhibitory and excitatory synapse types. Indeed, we found that changing $[Ca^{2+}]_o$ from 2 mM to 1.3 mM alters the ratio between excitatory and inhibitory synaptic PSPs by a factor of ~3.5, in favor of inhibition. This suggests the existence of a threshold level of Ca^{2+} beyond which inhibition is insufficient to prevent a supercritical state.

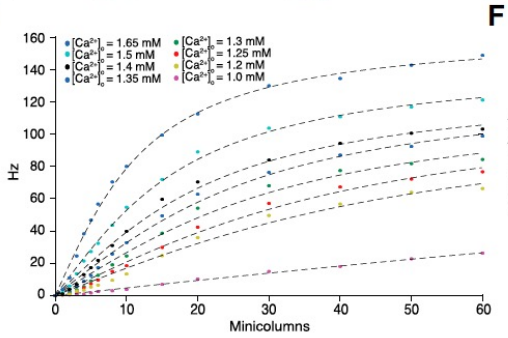
Markram et al., 2015

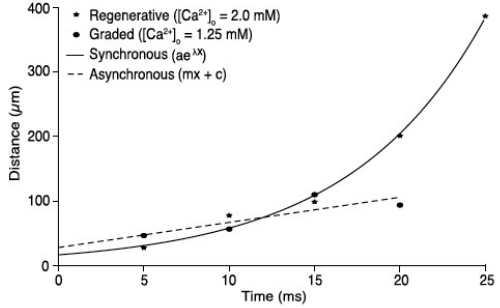


- Single synchronous spike to activate a progressively increasing number of fibers innervating the center of the mesocircuit.
- While under in-vitro-like conditions (Ca^{2+} 2 mM, 0 dep.), stimulating as few as four thalamic fibers produced all-or-none behavior, indicative of a regenerative state that spread across the whole mesocircuit, under in-vivo-like conditions (Ca^{2+} 1.25 mM, 100% dep.), the activity remained localized.



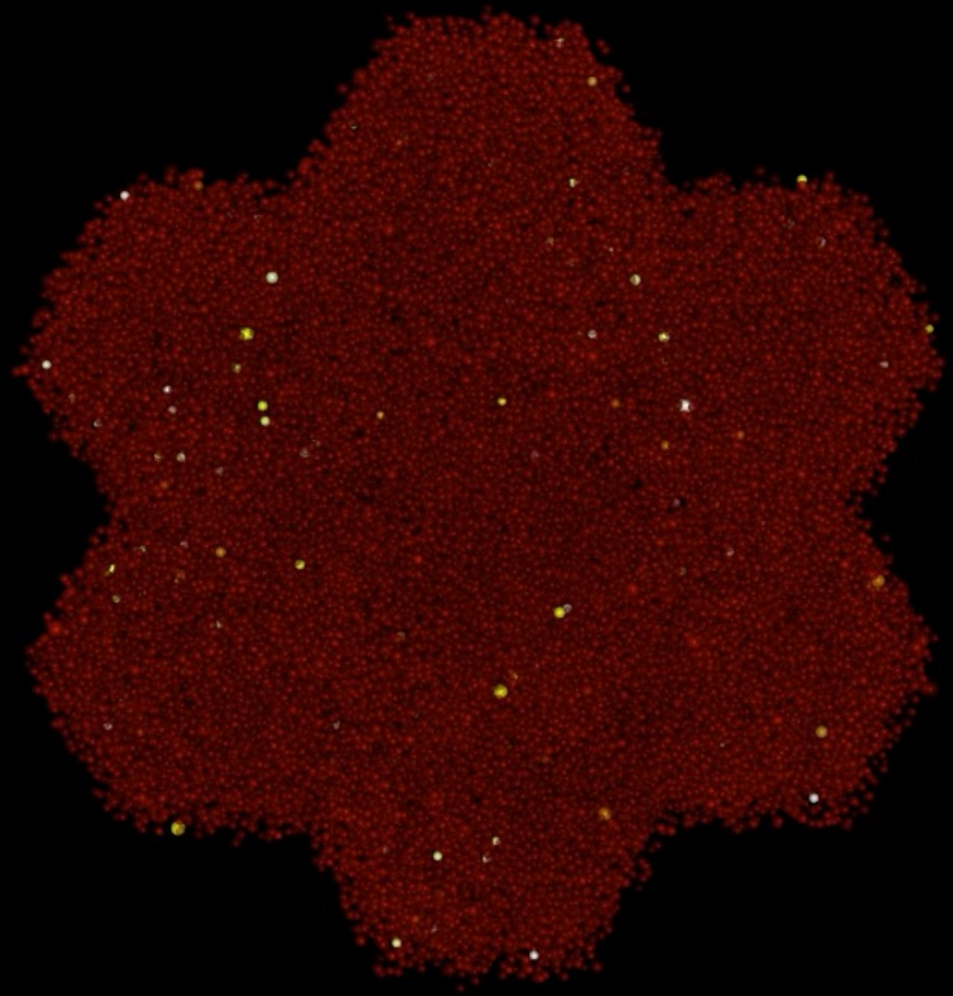






Markram et al., 2015


In-vitro


In-vivo

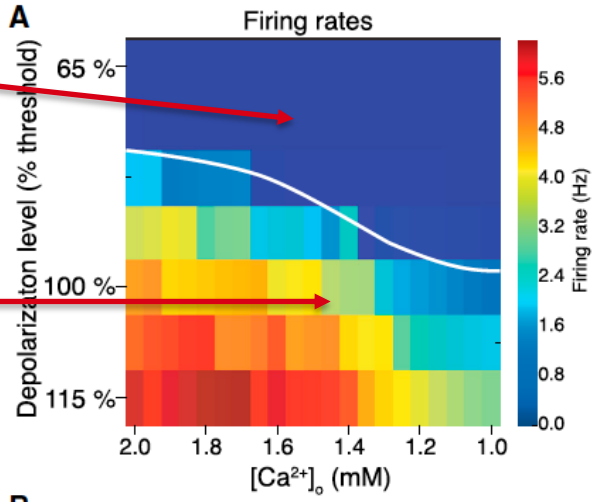
Activity should be evoked

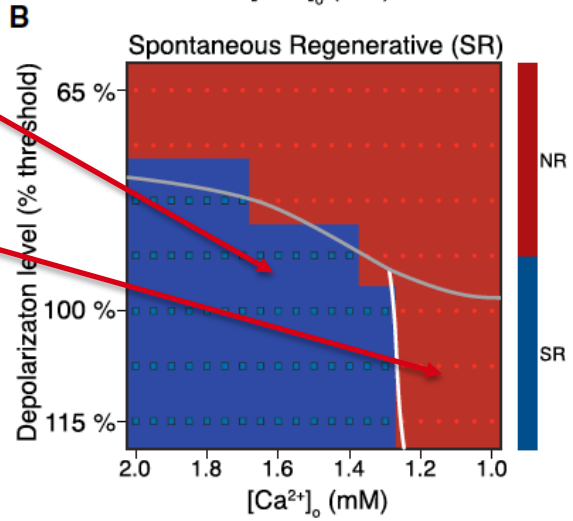
Activity is spontaneous

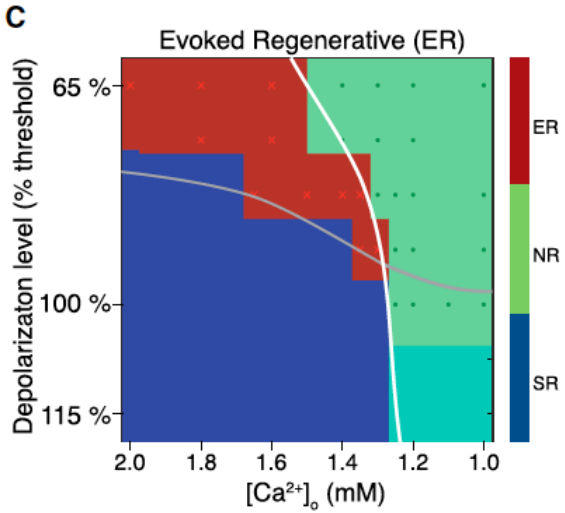
Bursting activity

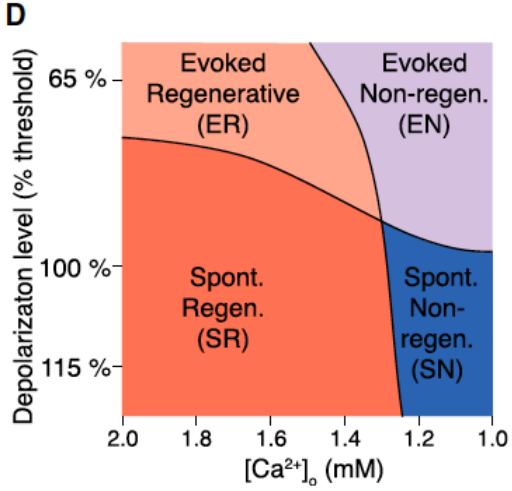
No bursting activity

Markram et al., 2015

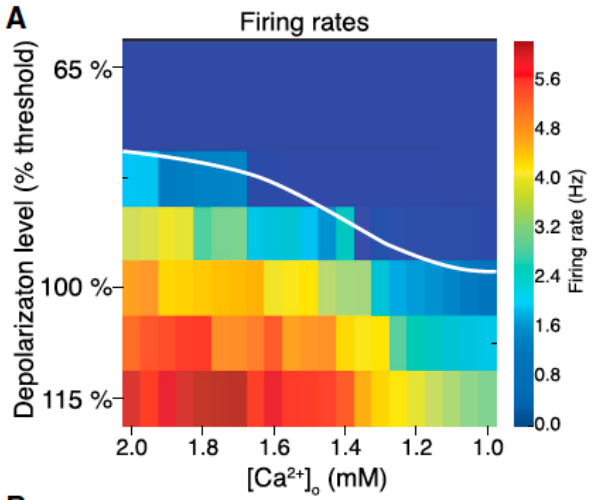


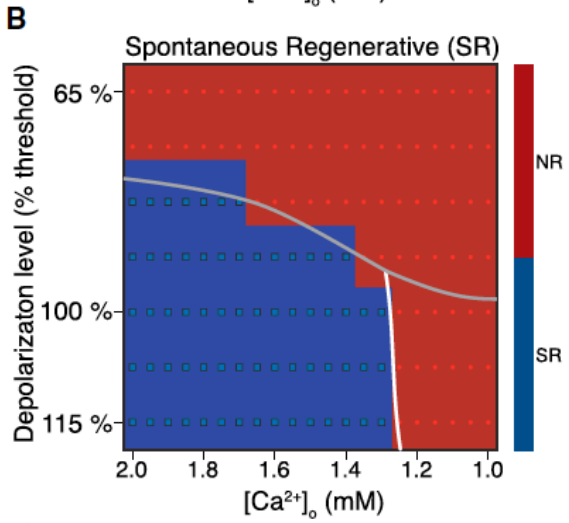


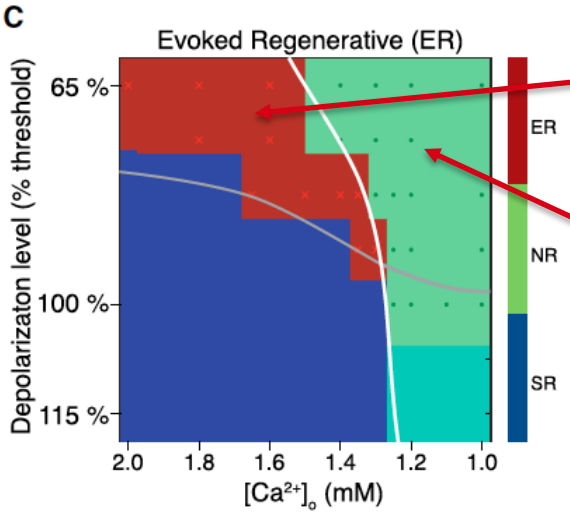




EPFL

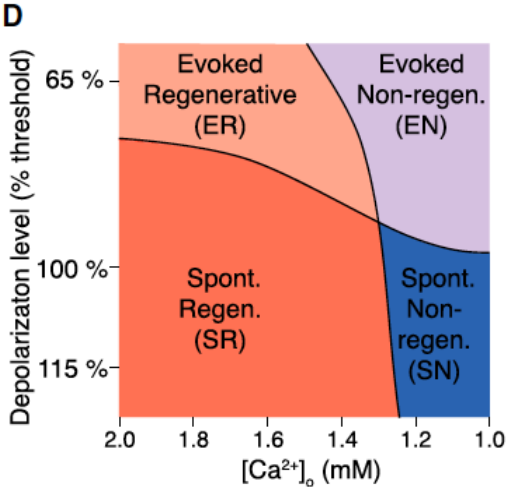






Activity propagates

Activity does not propagates



Markram et al., 2015

Simulate the network

- Test the circuit
- Study the network regimes or answer general questions
- **Answer region-specific questions**

Reproduce an experiment: select a paper/experiment

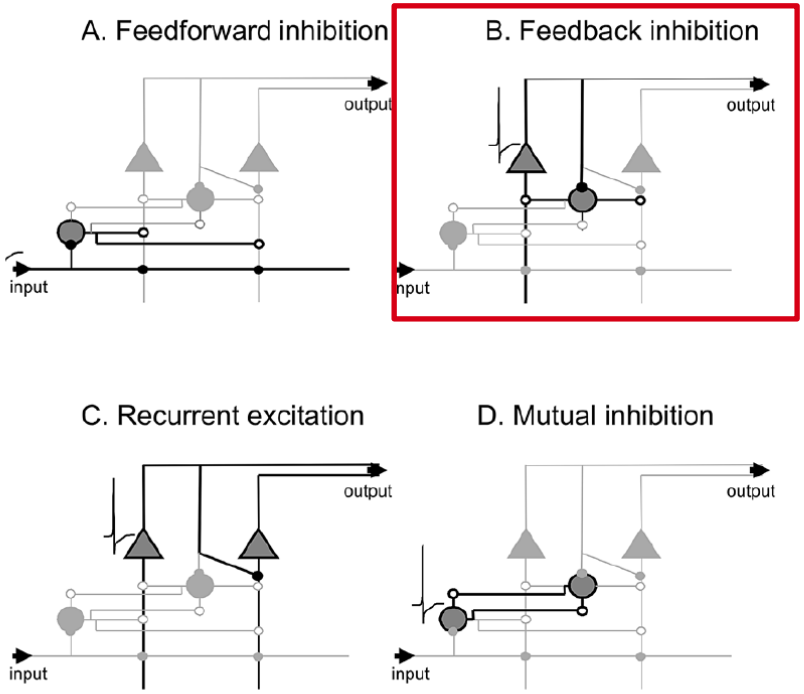
J Physiol 574.1 (2006) pp 195–208

Integrative spike dynamics of rat CA1 neurons: a multineuronal imaging study

Takuya Sasaki, Rie Kimura, Masako Tsukamoto, Norio Matsuki and Yuji Ikegaya

Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo 113-0033, Japan

Local connectivity patterns



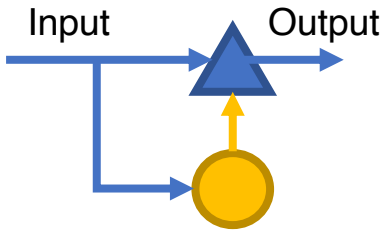
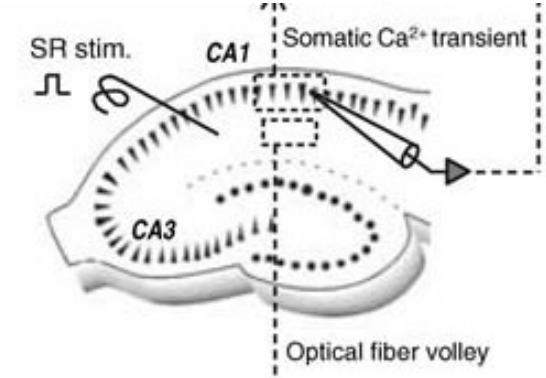


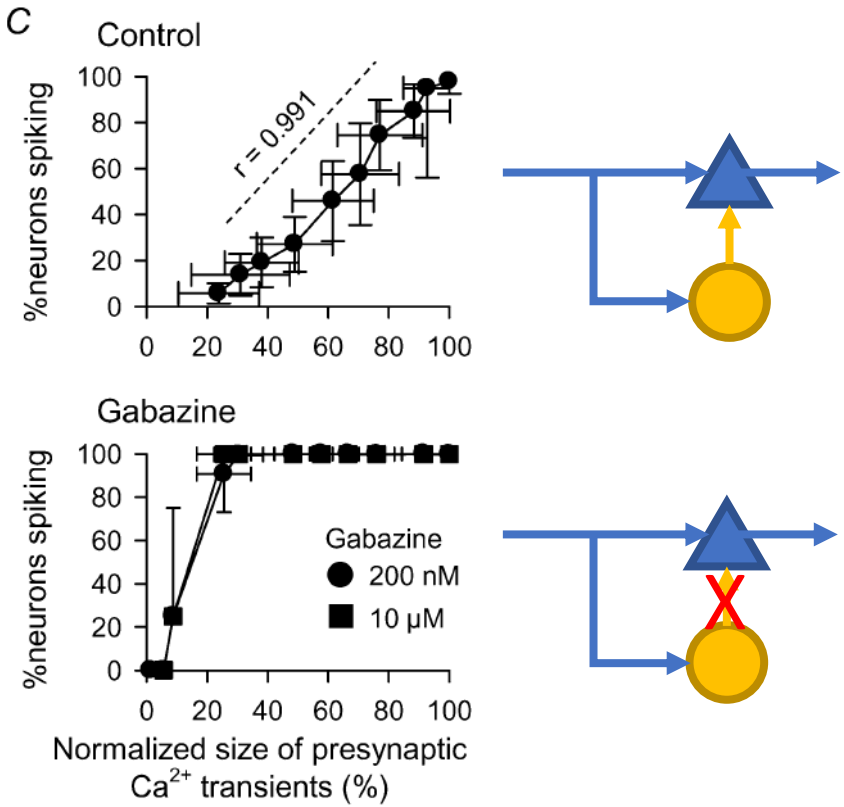
Figure 8–1. Basic local circuit interactions. *A*. Feedforward inhibition. Axon collaterals from excitatory afferent fibers contact local interneurons. The additional synaptic delay compared to direct afferent excitation onto principal cells provides a time-dependent sequence of excitation and inhibition from single afferent inputs. *B*. Feedback inhibition. Axon collaterals from local principal cells contact local interneurons, providing a period of inhibition of principal cell activity following the generation of an output. Interneuron populations involved in *A* and *B* are not always mutually exclusive. *C*. Recurrent excitation. Axon collaterals from local principal cells also contact other local principal cells, providing an excitatory mechanism for concerted, temporally coordinated population output. *D*. Mutual inhibition. Some interneuron subtypes contact other interneurons as well as principal cells, and some interneurons contact other interneurons exclusively. This pattern of connectivity can serve to impart spatiotemporally coordinated patterns of excitation and inhibition in the local circuit leading to rhythm generation. Filled circles, excitatory synapses; open circles, inhibitory synapses.

Andersen et al., 2007

Reproduce an experiment: understand the main point(s)

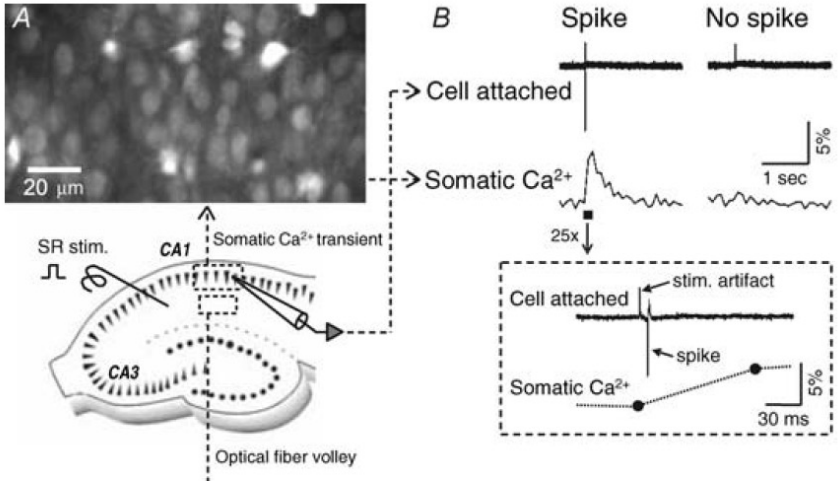
- Stimulation of SC (CA3 PC axons) and recording from CA1 cells (mainly PCs)
- I/O curve is quasi linear thanks to the feedforward inhibition
- When they apply a GABAAR blocker as Gabazine, the IO curve saturates very quickly





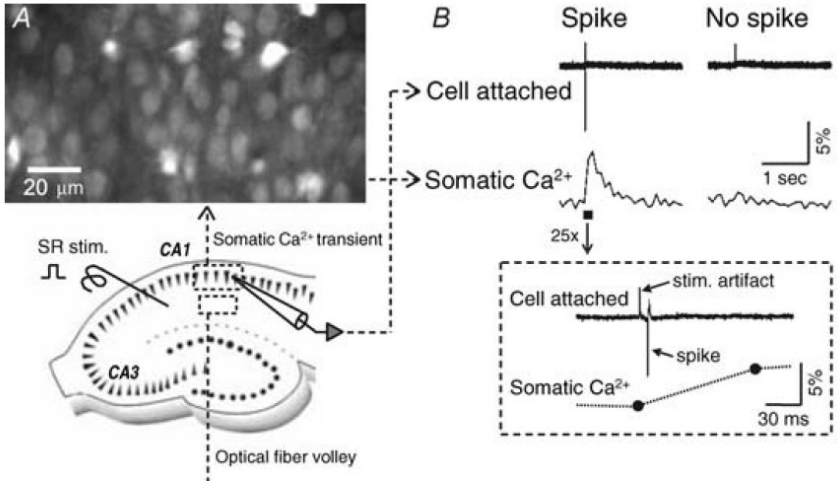
Reproduce an experiment: dive into the technical details

- P7 Wistar/ST Rat
- Slice cultures
- Horizontal slice of 300 μm
- Bath: 1.4 Mg^{2+}, 2.4 Ca^{2+}
- Ca^{2+} imaging
- 32°C
- Incision was made between the CA2 and CA3 regions and between CA1 and the subiculum



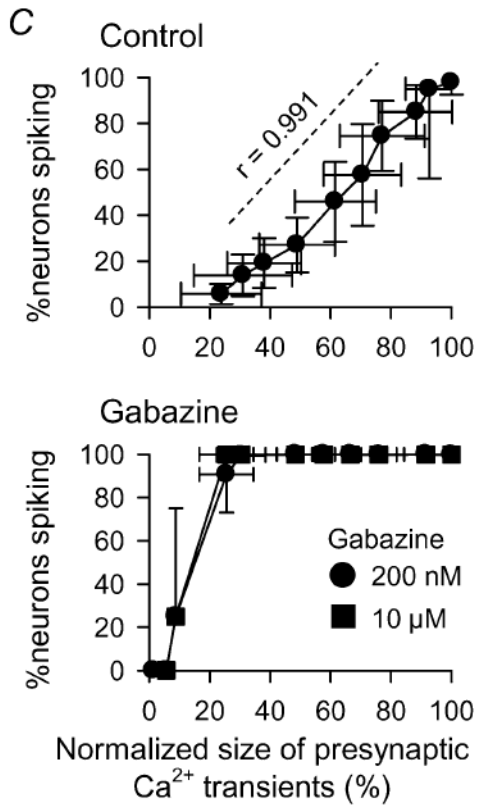
Reproduce an experiment: dive into the technical details

- Electrodes were placed in the CA1 stratum radiatum
- single pulse (50 µs, 60–270 µA) were applied every 30 s to activate Schaffer collateral axons



Reproduce an experiment: dive into the technical details

- Repeat the protocol with the addition of Gabazine, an inhibitor of GABAAR



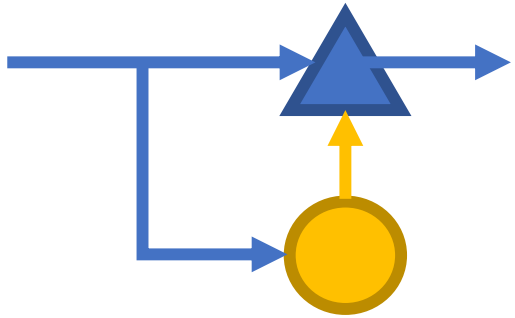
Reproduce an experiment: set up the simulation experiment

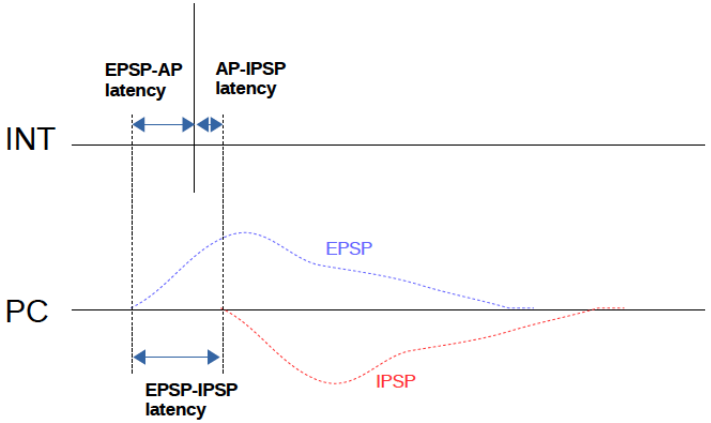
Experiment	Model
P7 Wistar/ST Rat	Adult rat
Slice cultures, 300 μm	Slice of 300 μm
Bath: 1.4 Mg^{2+}, 2.4 Ca^{2+}	Bath: 1.4 Mg^{2+}, 2.4 Ca^{2+}
Ca^{2+} imaging	Spikes
32°C	32°C
Single pulse (50 µs, 60–270 µA) were applied to activate Schaffer collateral axons	Activation of an increasing number of projections
Gabazine	Cut connections from INT (synaptic weigths = 0)

EPFL

Validation failure and revision of the SC

- The validation failed
- This induced the revision of the SC
- To have feedforward inhibition, the IPSP needs to arrive within a certain latency to decrease the EPSP
- We reoptimized the SC synaptic parameters to obtain a better timing



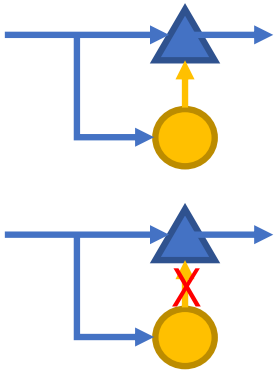


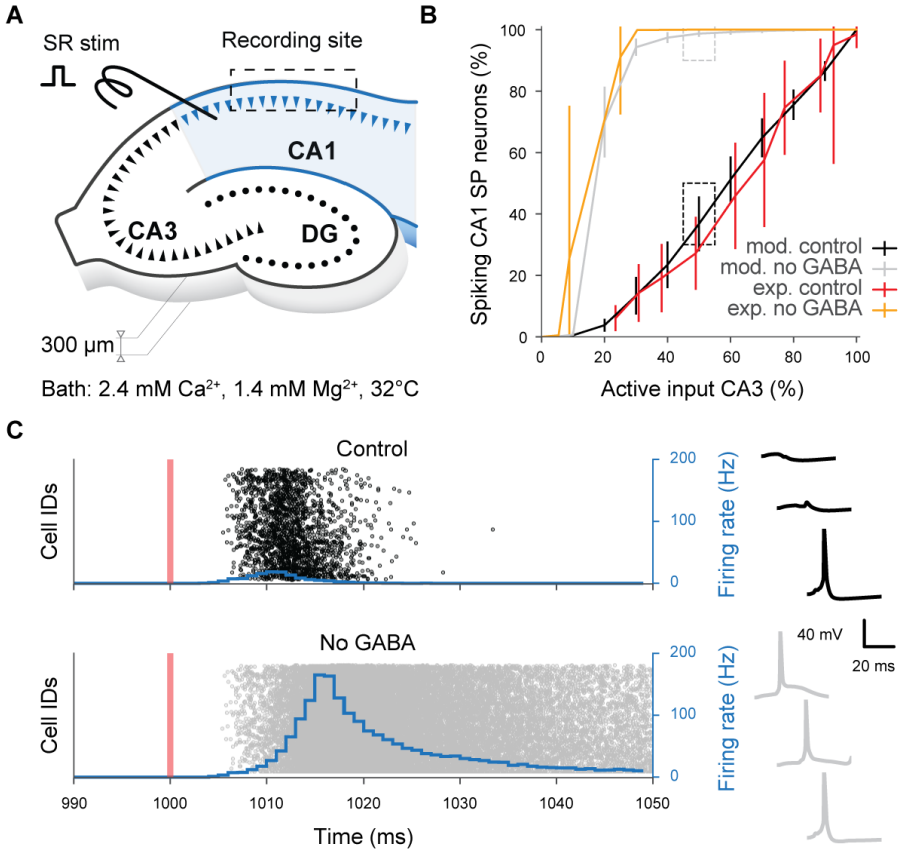
Validation failure and revision of the experiment setup

- The validation continued to fail
- One of the problem is the number of SC fibers to stimulate because this is unknown
- The normalization for the input and the output was also suspicious
- We could not easily obtain that all the neurons fire
- We came back to the experiment details
- 100% of the output corresponds to the point where all the 101 observed cells fire

=> We selected only 101 cells and the minimum stimulus that can fire them all and considered it 100%. Then we tested smaller percentages of the stimulus

Reproduce an experiment: we made it!





Romani et al., 2023

Summary 3

- Extensive testing and validation lead to improvements and legitimate the use of the model to make predictions
- Studying the properties of the network, its IO function, the different stable states, is fundamental for deciphering the role of the network in the brain
- Simulations should be set carefully to reproduce experimental conditions
- It is not always possible to perfectly match experimental conditions due to limitation of the model or incomplete knowledge of the experimental setup
- This should be taken into account and discussed when comparing simulation results and experimental observations

EPFL

Lecture Summary

- A network model should be checked and validated not only at level of its constituent but also as an whole
- Testing and validation build our confidence in the model and in the predictions we can drawn from it
- It is important we spend time to define carefully the simulation setup to better capture the experimental conditions we want to reproduce
- The model is an imitation of the system, but it allows maximum freedom to manipulate and analyze the system
- In this sense, in-silico experimentations are complementary to experiments

EPFL

What you have learnt

- Network, network simulations
- Different types of simulations
- Set up a simulation. Different outputs.
- Sanity/quality checks, validations, predictions