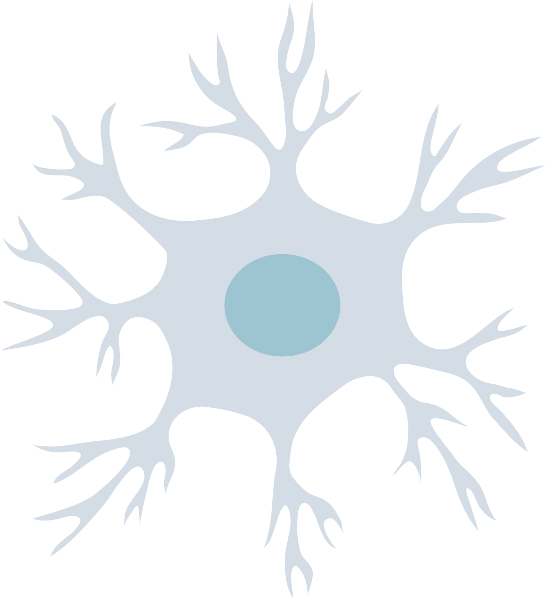

Simulation of neuronal networks coupled to microelectrode arrays

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Physical Models of Living Systems

(a.y. 2022/2023)

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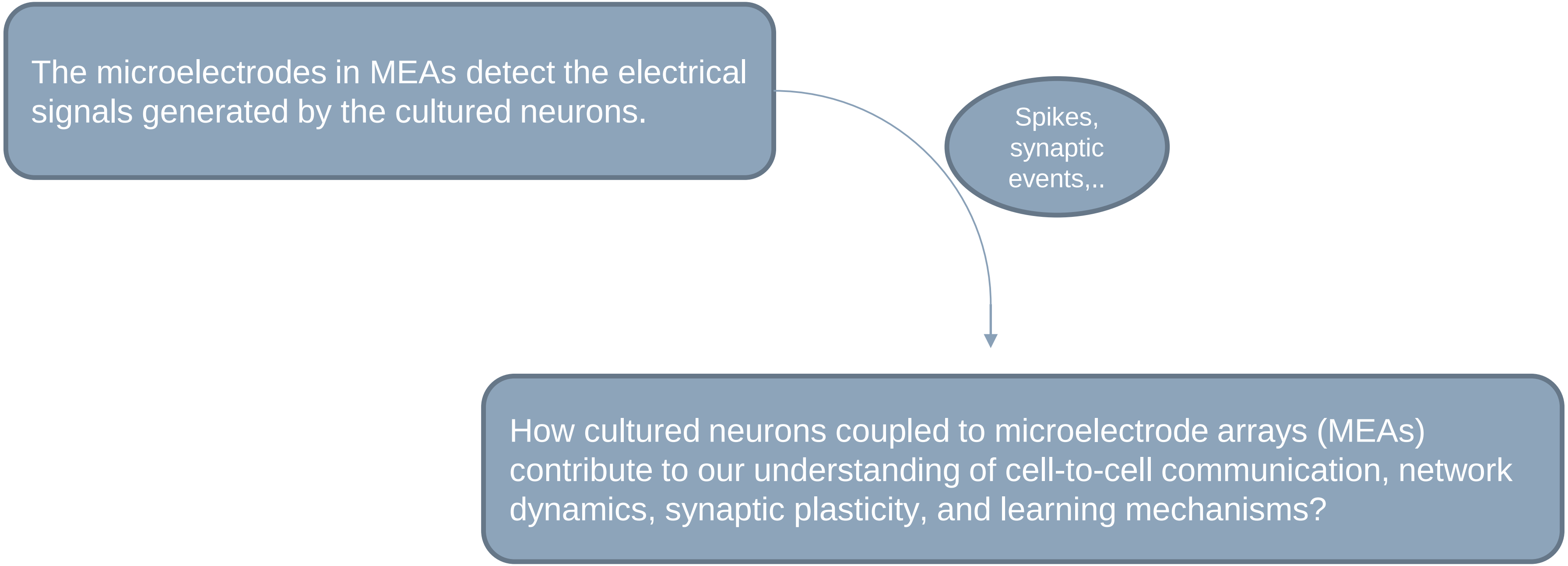
Introduction: Questions



The microelectrodes in MEAs detect the electrical signals generated by the cultured neurons.

Spikes, synaptic events,..

How cultured neurons coupled to microelectrode arrays (MEAs) contribute to our understanding of cell-to-cell communication, network dynamics, synaptic plasticity, and learning mechanisms?

Introduction: Goal of the project

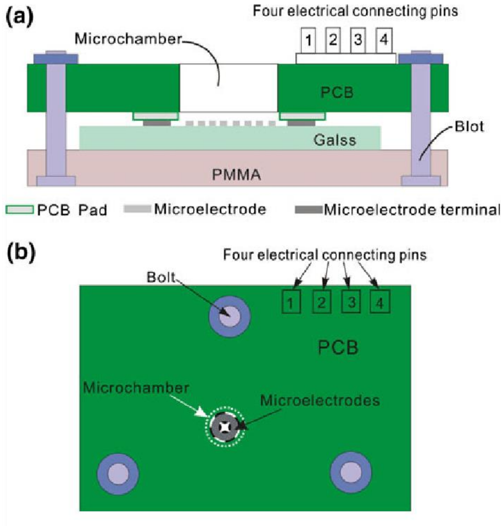
- Study and replicate the dynamics of a specific neuronal network model described in the paper. By simulating the network and implementing the proposed connectivity rules.

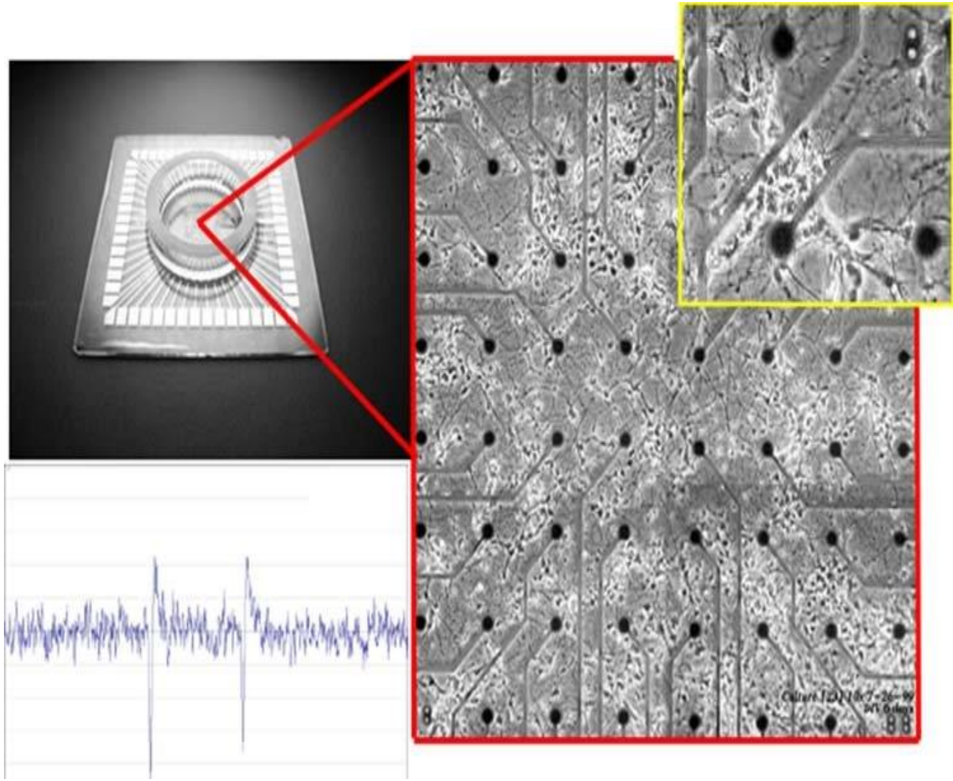
- The project seeks to validate the model's ability to capture the network dynamics and provide insights into the underlying mechanisms of neuronal communication and information processing.

- Additionally, the project aims to evaluate the impact of different connectivity rules (OTM and OTN) on the network's behavior, further investigating their role in shaping the network dynamics.

Introduction: Microelectrode arrays

- Microelectrode arrays (MEAs) are tiny devices with an array of closely spaced electrodes used to record electrical signals from cells or tissues.

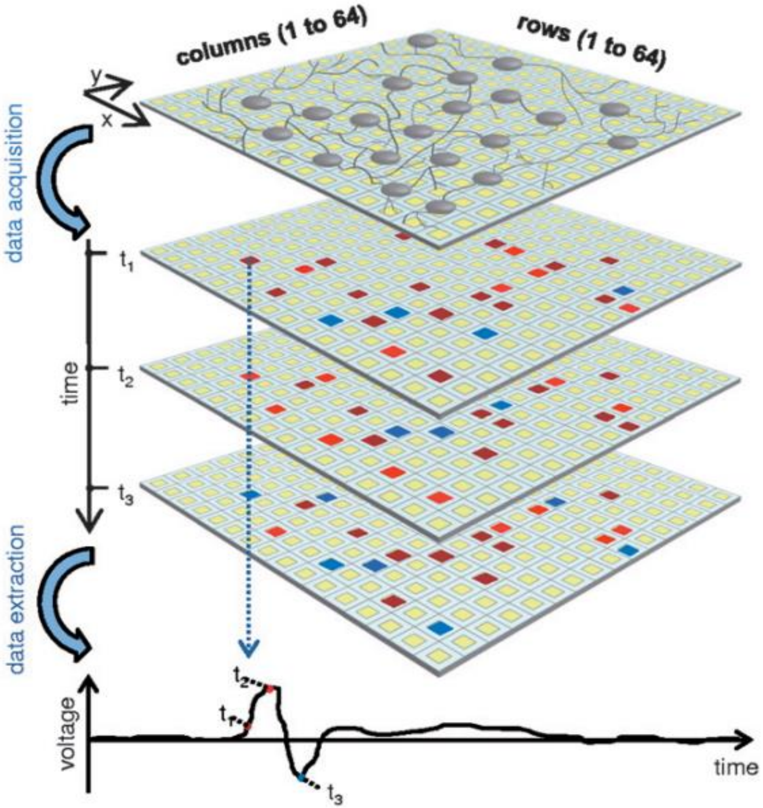




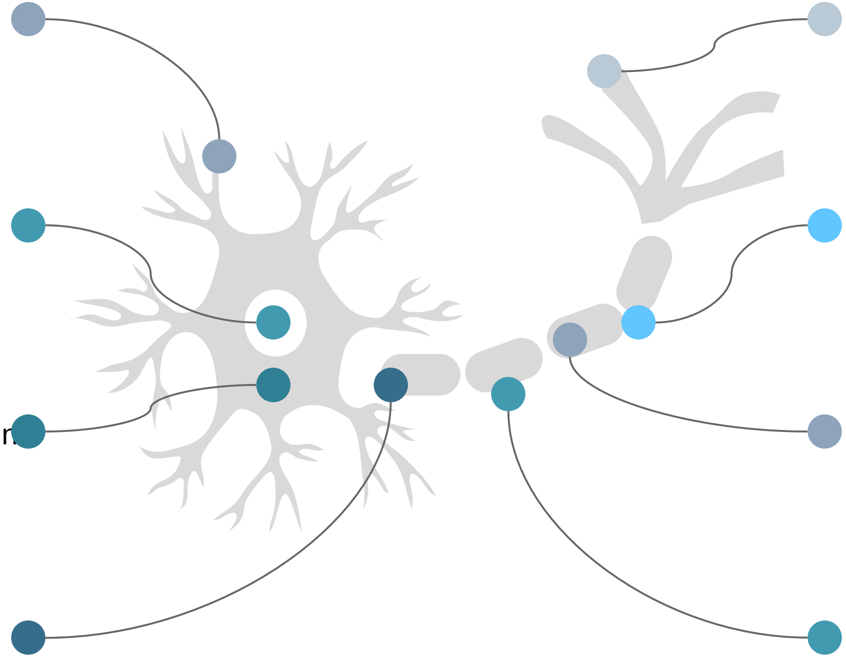
Introduction: Microelectrode arrays

Working principle of the high resolution platform

Neurons grow and develop chronically on the high-resolution microelectrode array. Fast acquisition of extracellular electrophysiological signals is performed as a sequence of frames by encoding extracellular voltage signals as pixels data. By using a false color map, this enables the video observation of the overall network activity as well as local activity on the basis of single pixel data. Single microelectrode raw data is reconstructed by combining single pixel data from sequential frames.



Introduction: Neuron infographics



Dendrite

Neuron's branch-like extension for receiving and transmitting signals.

Nucleus

Control center of a cell, containing its genetic material and regulating its activities.

Cell body

Main part of a neuron that contain the nucleus and other cellular components necessary for its functioning

Axon

Neuron's signal-transmitting projection.

Axon terminal

End of an axon where it forms a synapse with another neuron or target cell to transmit the electrical signal.

Node of Ranvier

Gap on the axon facilitating rapid signal transmission.

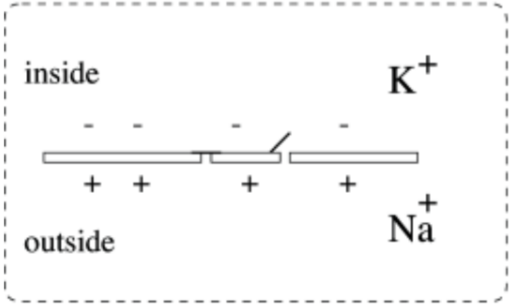
Schwann cell

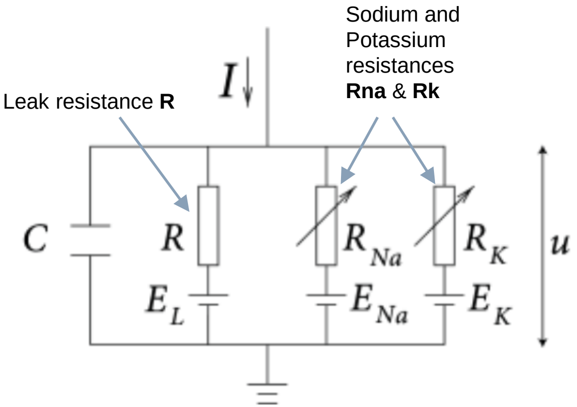
Axon's myelin-forming helper.

Myelin sheath

Axon's speed-enhancing insulation.

Theoretical background: model of neuron - Hodgkin-Huxley model




Sodium and Potassium resistances **Rna** & **Rk**

Leak resistance **R**

- Describes the opening and closing of ion channels in response to changes in the membrane potential → generation and propagation of action potentials.

- Sodium (Na+), potassium (K+), and chloride (Cl-).

- Describe the membrane currents and voltage dynamics.

- $I(t) = I_c(t) + \sum_k I_k(t)$

- $C \frac{dU}{dt} = -\sum_k I_k(t) + I(t)$

Theoretical background : model of neuron

- Hodgkin-Huxley model describes three types of channel. All channels may be characterized by their resistance or, equivalently, by their conductance:

$$\begin{cases} I = Cm\frac{du}{dt} + \overline{g_k} n^4 (u - V_k) + \overline{g_{Na}} m^3 h (u - V_{Na}) + \overline{g}_l (u - V_l) \\ \frac{dn}{dt} = \alpha_n(u)(1-n) - \beta_n(u)n \\ \frac{dm}{dt} = \alpha_m(u)(1-m) - \beta_m(u)m \\ \frac{dh}{dt} = \alpha_h(u)(1-h) - \beta_h(u)h \end{cases}$$

Theoretical background : model of synapse

The model of the synapse follows the approach proposed which based on the first-order kinetic scheme:

$$R + T \overset{k_1,k_2}{\Leftrightarrow} TR^*$$

The fraction of the bound receptors r is described by the equation:

$$\frac{dr}{dt} = k_1\,[T](1-r) - k_2\,r$$

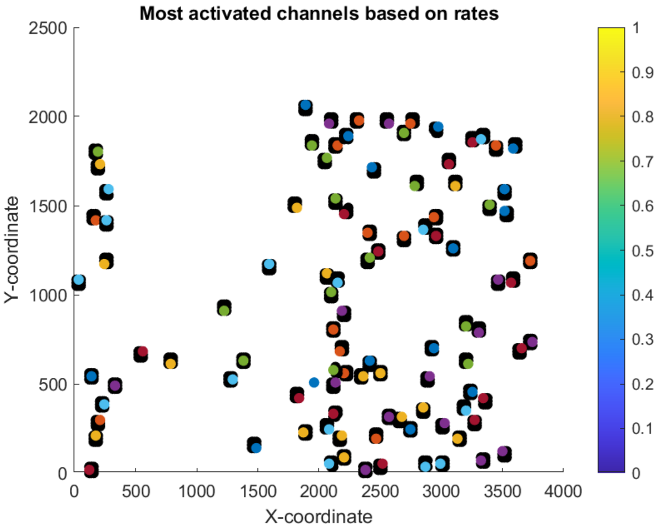
This modelling approach allowed the synaptic events to be represented by equations with the same structure of the H–H equations.

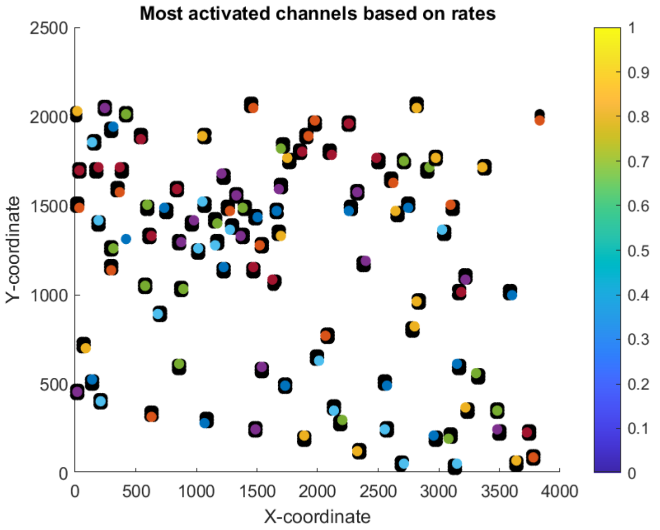
Theoretical background : Neuronal network

- The network model is made up of 60 meta-neurons arranged according to the MEA layout.

- Each neuron can establish synaptic connections.

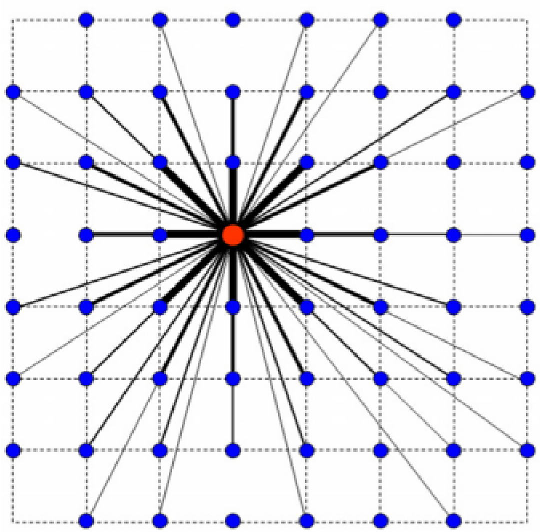
- Synapses are placed on the soma and on the dendrites.



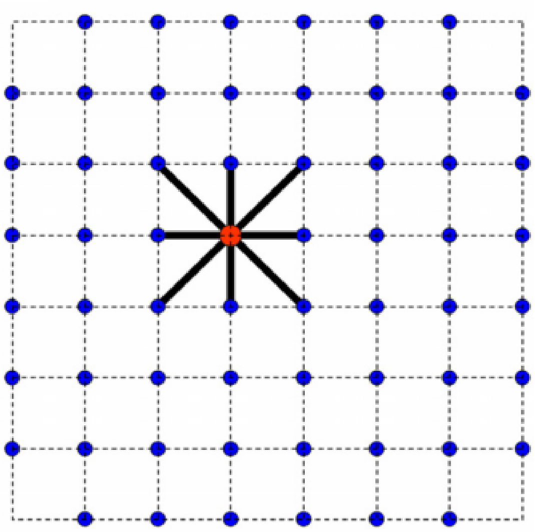


Theoretical background : Connectivity rules of neuronal network

One-to-many (OTM) rules states that each meta-neuron can establish several random connections.

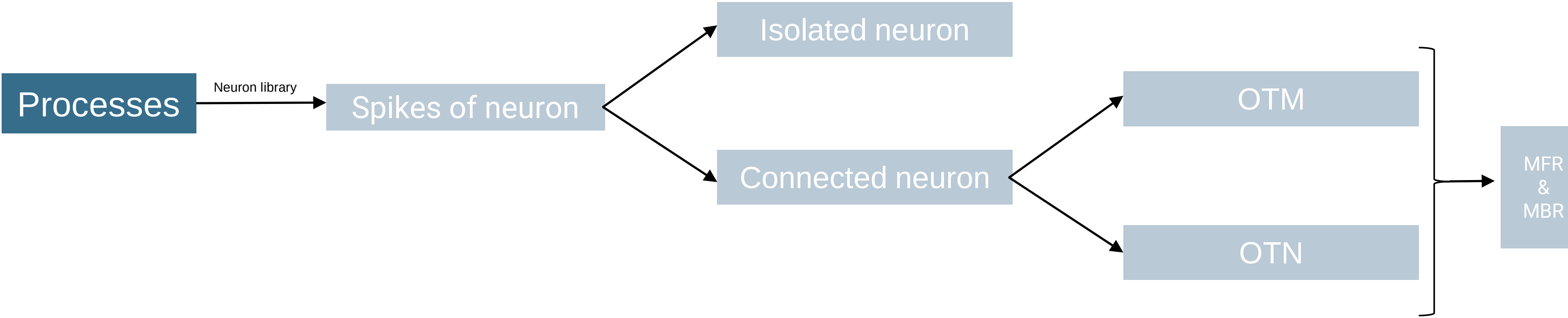


One-to-neighbors (OTN) rules states that each meta-neuron is only connected to its nearest neighbors (up to eight meta-neurons)



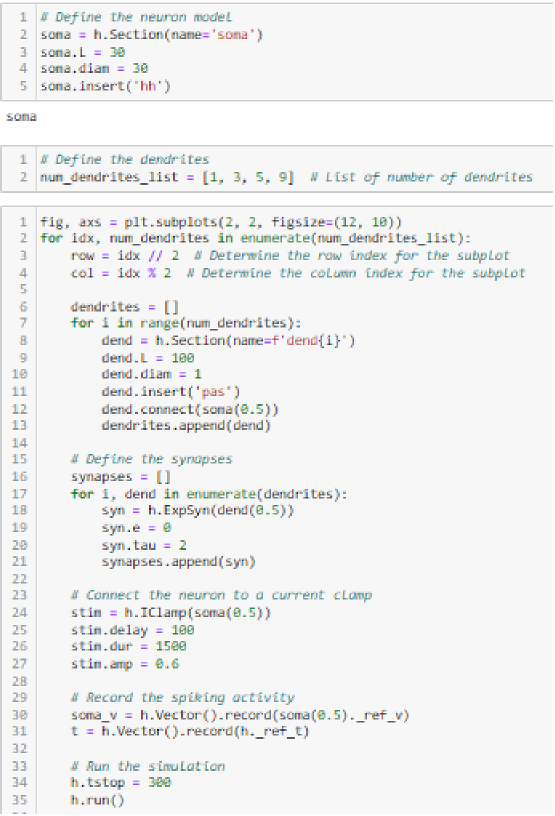
Methods

The project is implemented in python.



Methods

Simulation of isolated neuron



```python
# Define the neuron model
soma = h.Section(name='soma')
soma.L = 30
soma.diam = 30
soma.insert('hh')
```

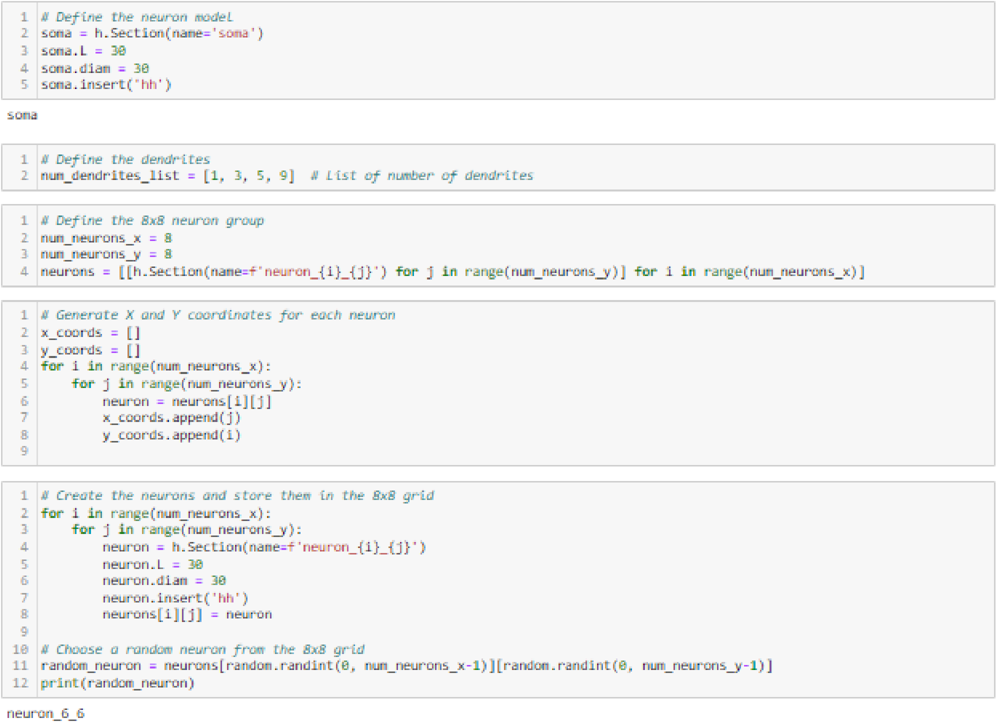
```
soma
```

```python
# Define the dendrites
num_dendrites_list = [1, 3, 5, 9]  # List of number of dendrites
```

```python
fig, axs = plt.subplots(2, 2, figsize=(12, 10))
for idx, num_dendrites in enumerate(num_dendrites_list):
    row = idx // 2  # Determine the row index for the subplot
    col = idx % 2  # Determine the column index for the subplot

    dendrites = []
    for i in range(num_dendrites):
        dend = h.Section(name=f'dend{i}')
        dend.L = 100
        dend.diam = 1
        dend.insert('pas')
        dend.connect(soma(0.5))
        dendrites.append(dend)

    # Define the synapses
    synapses = []
    for i, dend in enumerate(dendrites):
        syn = h.ExpSyn(dend(0.5))
        syn.e = 0
        syn.tau = 2
        synapses.append(syn)

    # Connect the neuron to a current clamp
    stim = h.IClamp(soma(0.5))
    stim.delay = 100
    stim.dur = 1500
    stim.amp = 0.6

    # Record the spiking activity
    soma_v = h.Vector().record(soma(0.5)._ref_v)
    t = h.Vector().record(h._ref_t)

    # Run the simulation
    h.tstop = 300
    h.run()
```

Simulation of connected neuron in 8x8 neurons group

To simulate the condition of spontaneous activity, we added a Gaussian noise source to the leakage channels of each component of the neuron models. Neurons are intrinsically noisy and several sources were identified. The most dominant source of such electrical noise is channel noise, i.e., electrical currents caused by the random opening and closing of the ion channels.



```python
# Define the neuron model
soma = h.Section(name='soma')
soma.L = 30
soma.diam = 30
soma.insert('hh')
```

```
soma
```

```python
# Define the dendrites
num_dendrites_list = [1, 3, 5, 9]  # List of number of dendrites
```

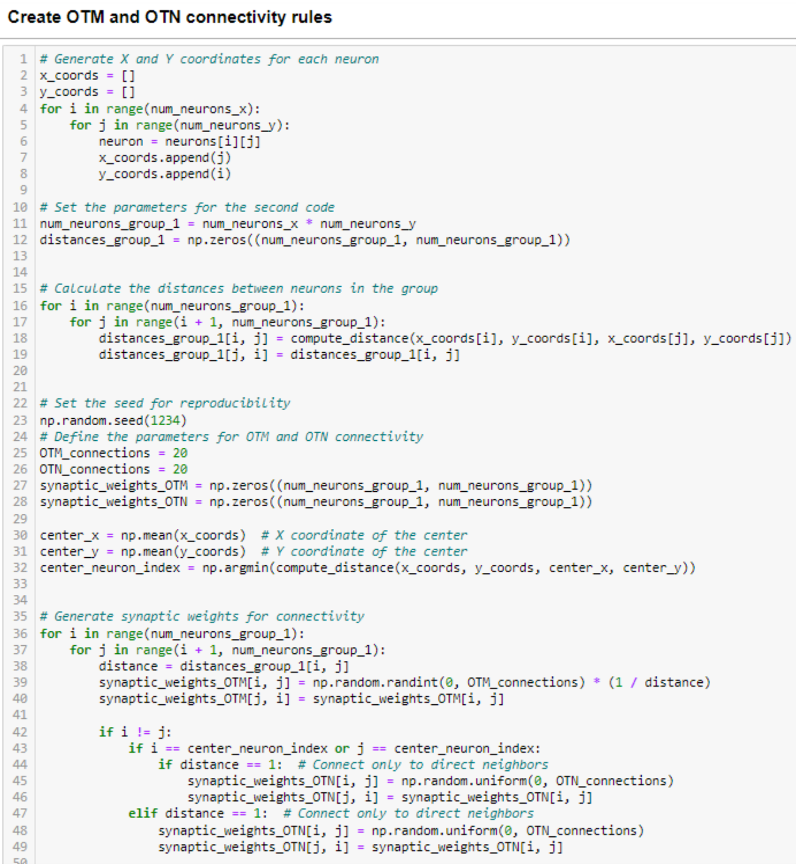
```python
# Define the 8x8 neuron group
num_neurons_x = 8
num_neurons_y = 8
neurons = [[h.Section(name=f'neuron_{i}_{j}') for j in range(num_neurons_y)] for i in range(num_neurons_x)]
```

```python
# Generate X and Y coordinates for each neuron
x_coords = []
y_coords = []
for i in range(num_neurons_x):
    for j in range(num_neurons_y):
        neuron = neurons[i][j]
        x_coords.append(j)
        y_coords.append(i)

```

```python
# Create the neurons and store them in the 8x8 grid
for i in range(num_neurons_x):
    for j in range(num_neurons_y):
        neuron = h.Section(name=f'neuron_{i}_{j}')
        neuron.L = 30
        neuron.diam = 30
        neuron.insert('hh')
        neurons[i][j] = neuron

# Choose a random neuron from the 8x8 grid
random_neuron = neurons[random.randint(0, num_neurons_x-1)][random.randint(0, num_neurons_y-1)]
print(random_neuron)
```

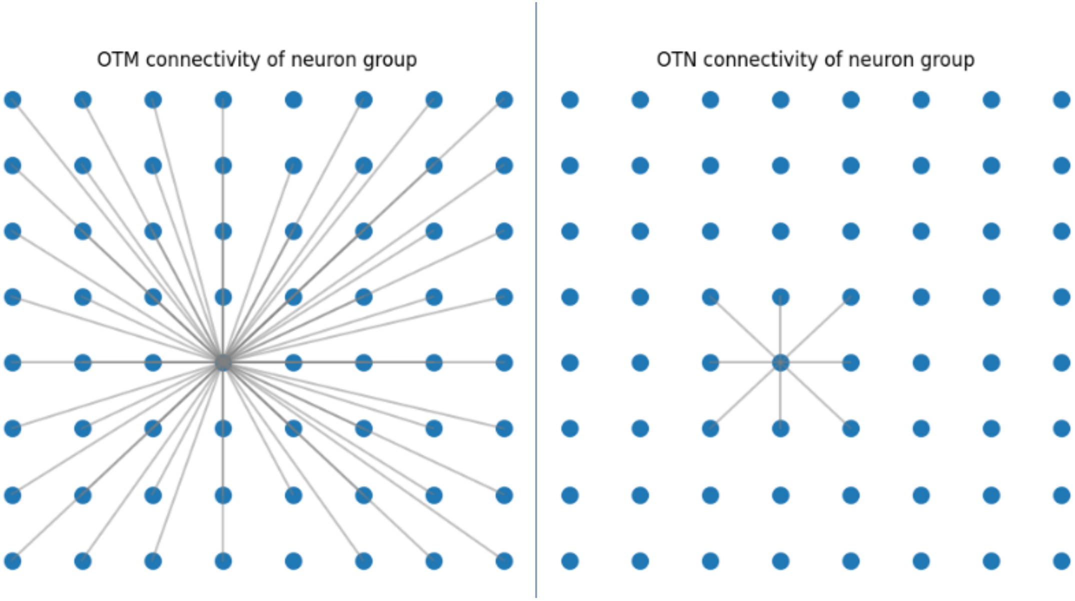
```
neuron_6_6
```

Methods



Create OTM and OTN connectivity rules

```
# Generate X and Y coordinates for each neuron
x_coords = []
y_coords = []
for i in range(num_neurons_x):
    for j in range(num_neurons_y):
        neuron = neurons[i][j]
        x_coords.append(j)
        y_coords.append(i)

# Set the parameters for the second code
num_neurons_group_1 = num_neurons_x * num_neurons_y
distances_group_1 = np.zeros((num_neurons_group_1, num_neurons_group_1))


# Calculate the distances between neurons in the group
for i in range(num_neurons_group_1):
    for j in range(i + 1, num_neurons_group_1):
        distances_group_1[i, j] = compute_distance(x_coords[i], y_coords[i], x_coords[j], y_coords[j])
        distances_group_1[j, i] = distances_group_1[i, j]


# Set the seed for reproducibility
np.random.seed(1234)
# Define the parameters for OTM and OTN connectivity
OTM_connections = 20
OTN_connections = 20
synaptic_weights_OTM = np.zeros((num_neurons_group_1, num_neurons_group_1))
synaptic_weights_OTN = np.zeros((num_neurons_group_1, num_neurons_group_1))

center_x = np.mean(x_coords)  # X coordinate of the center
center_y = np.mean(y_coords)  # Y coordinate of the center
center_neuron_index = np.argmin(compute_distance(x_coords, y_coords, center_x, center_y))


# Generate synaptic weights for connectivity
for i in range(num_neurons_group_1):
    for j in range(i + 1, num_neurons_group_1):
        distance = distances_group_1[i, j]
        synaptic_weights_OTM[i, j] = np.random.randint(0, OTM_connections) * (1 / distance)
        synaptic_weights_OTM[j, i] = synaptic_weights_OTM[i, j]

        if i != j:
            if i == center_neuron_index or j == center_neuron_index:
                if distance == 1:  # Connect only to direct neighbors
                    synaptic_weights_OTN[i, j] = np.random.uniform(0, OTN_connections)
                    synaptic_weights_OTN[j, i] = synaptic_weights_OTN[i, j]
            elif distance == 1:  # Connect only to direct neighbors
                synaptic_weights_OTN[i, j] = np.random.uniform(0, OTN_connections)
                synaptic_weights_OTN[j, i] = synaptic_weights_OTN[i, j]
```



OTM connectivity of neuron group

OTN connectivity of neuron group

Methods

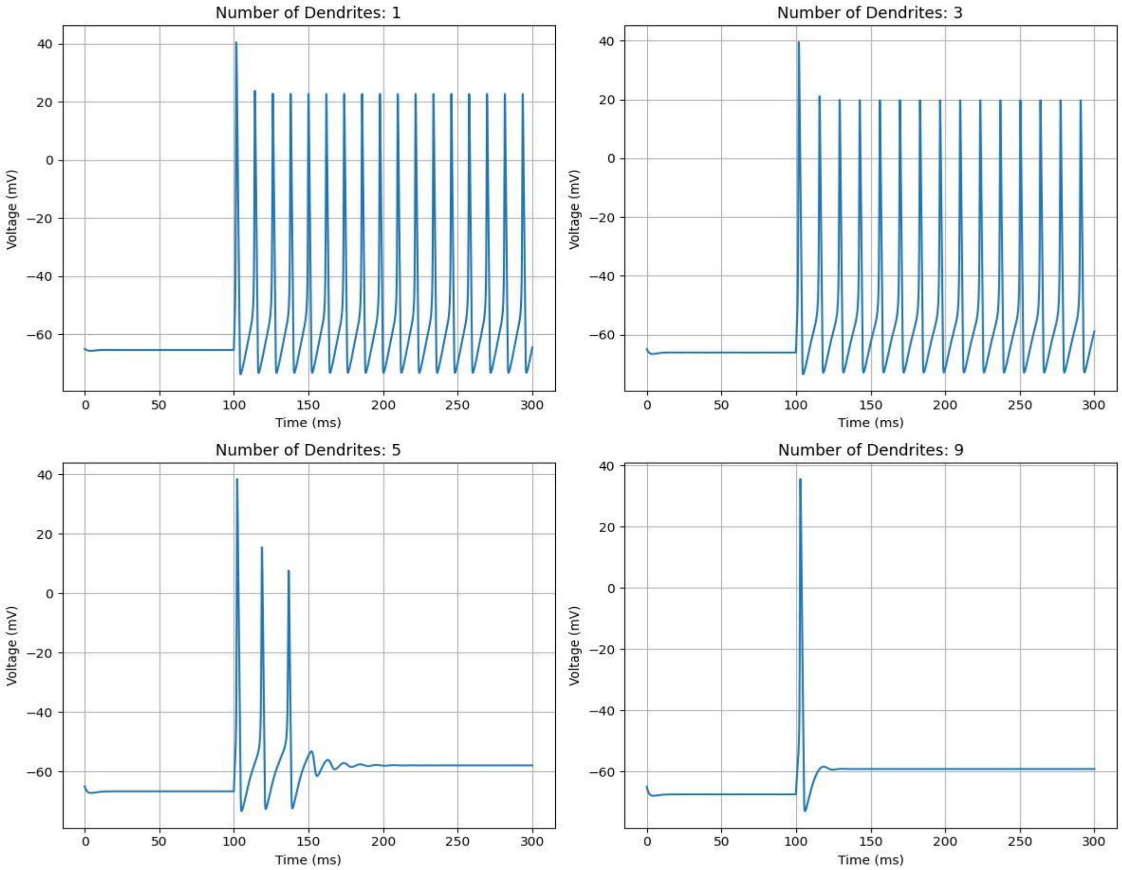
Mean Firing Rate: represents the average rate at which spikes are generated by a neuron or a population of neurons (spikes/s).

$$\boldsymbol{\nu}_{\mathbf{MFR}} = \frac{n^{sp}}{T}$$

Mean Bursting Rate: measures the average rate at which bursts of action potentials occur in a neuron or neuronal population. Bursts are characterized by a series of closely spaced spikes followed by a period of relative quiescence (bursts/min).

$$\boldsymbol{\nu}_{\mathbf{MBR}} = \frac{n^{b}}{T/60}$$

Results: Simulation of the neurons in their isolated form


Number of Dendrites: 1

Number of Dendrites: 3

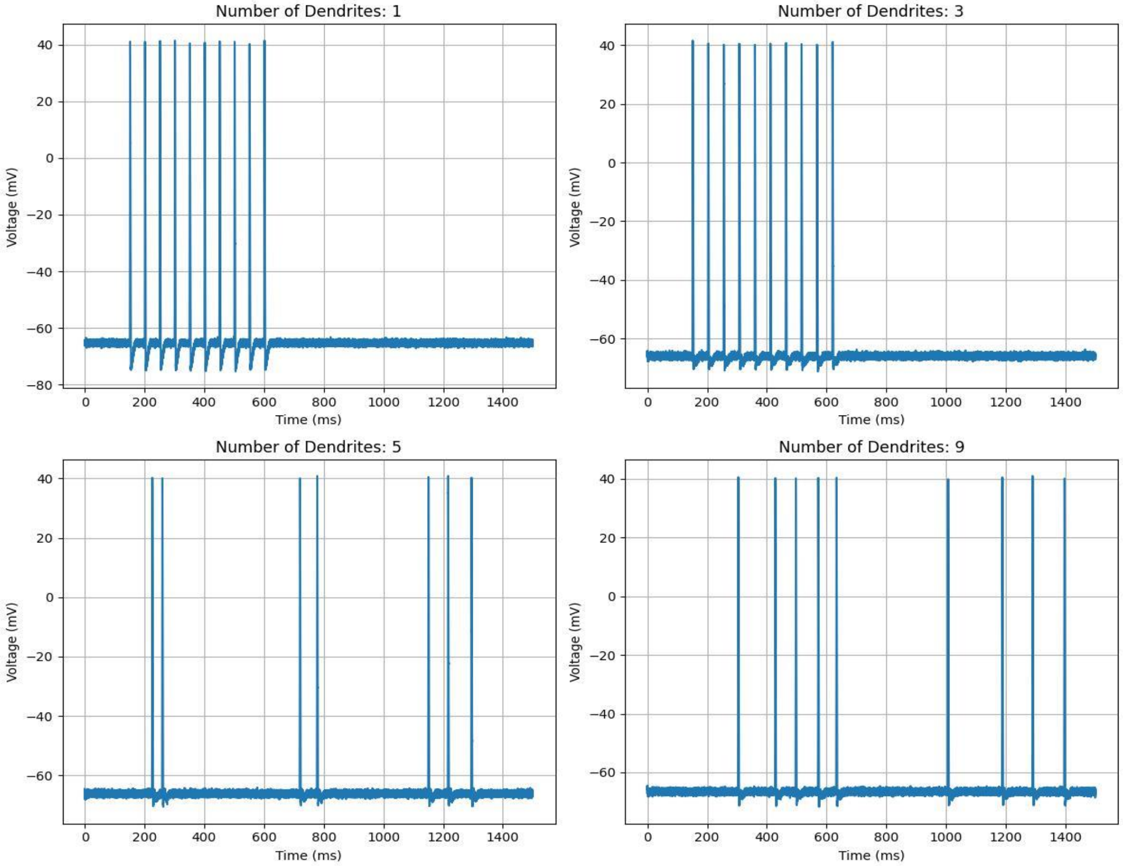
Number of Dendrites: 5

Number of Dendrites: 9

The simulated spiking activity of the soma of the neurons in a configuration with 1, 3, 5 and 9 dendrites.

The complexity of the dendritic tree is enhanced, the firing frequency decreases

Results: Simulation of spontaneous activity



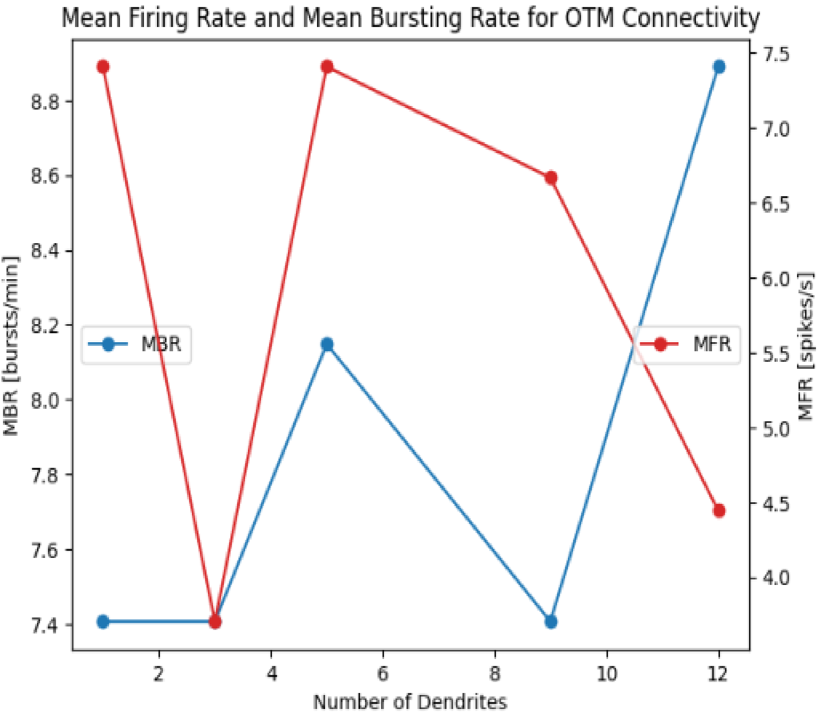
The electrophysiological behavior of the neurons changes dramatically when they are connected to make a network.

Increasing dendrites n.o makes the network behavior change dramatically: from isolated spikes (1 dendrite) → tonic spikes (3 dendrites) → clustered spikes (5, 9 dendrites).

improve the structure of the meta-neurons, it is feasible to transition from spiking to bursting behavior.

The electrophysiological patterns observed in the network are closely tied to network development and maturation.

Results: Complexity of the meta-neuron morphology is correlated to the development of the network

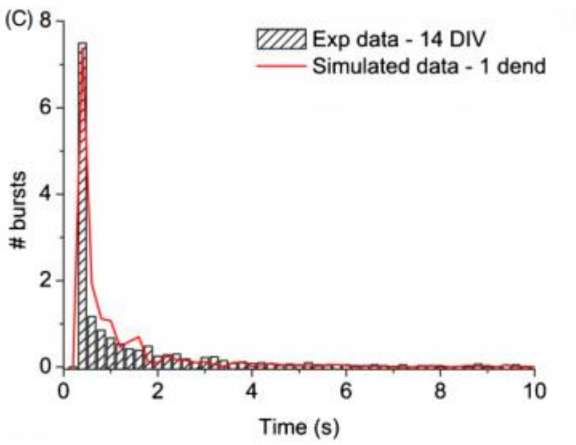


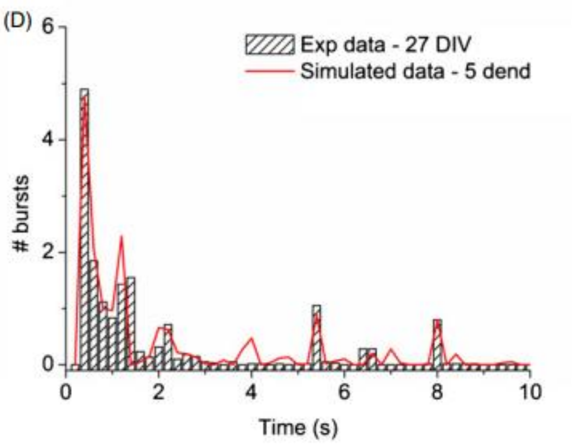
MBR and the MFR of the simulated network (OTM connectivity rule) as a function of the complexity of the dendritic tree.

MFR reaches high values in the case of neurons with 4 or 5 dendrites, but it decreases to 12 spikes.

Results: Complexity of the meta-neuron morphology is correlated to the development of the network

To make a comparison with the actual patterned neuronal networks (OTM), the simulated neuronal dynamics by means of the IBI distribution was characterized.



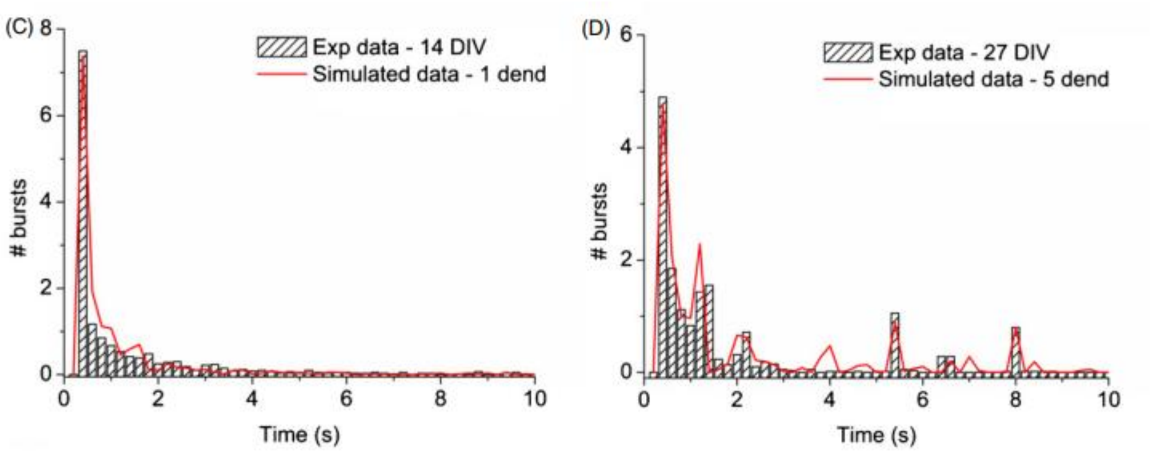


C. A mixed activity characterized by random spikes and bursts arises as shown by the IBI distribution.

D. A maturation of the neuron structure (neurons made up of 5 dendrites) makes the network more bursting.

Results: Complexity of the meta-neuron morphology is correlated to the development of the network

To make a comparison with the actual patterned neuronal networks (OTM), the simulated neuronal dynamics by means of the IBI distribution was characterized.



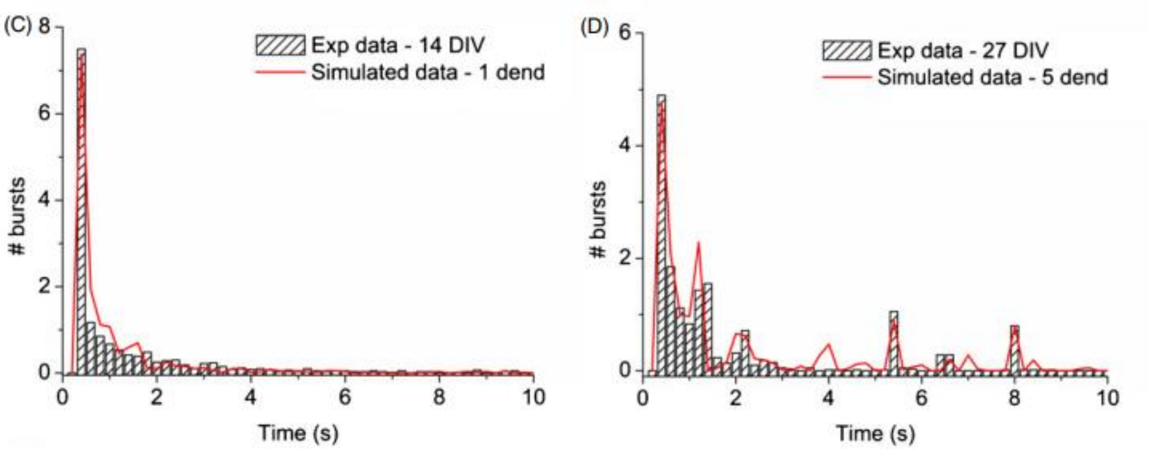
We found that: synaptic weights (1-50) and delays (1-4 ms) → very low firing frequency was achieved.

→ the bursting activity was totally absent.

→ several neurons silent: the entire simulation duration (number of active neurons <5).

Results: Complexity of the meta-neuron morphology is correlated to the development of the network

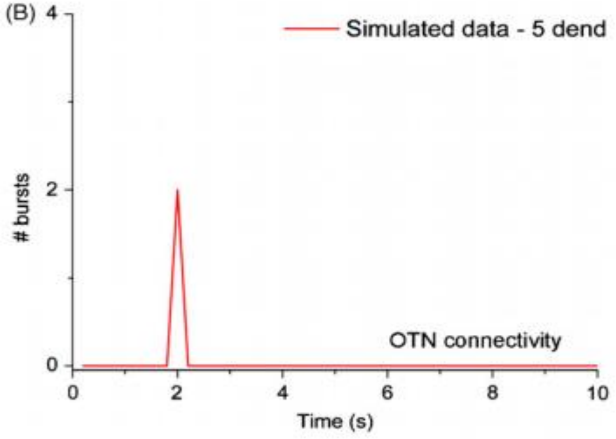
To make a comparison with the actual patterned neuronal networks (OTM), the simulated neuronal dynamics by means of the IBI distribution was characterized.



→ low-current values: network keeps on remaining silent /firing at low frequency.

→ high-frequency values: tonic firing appears and all the neurons are active.

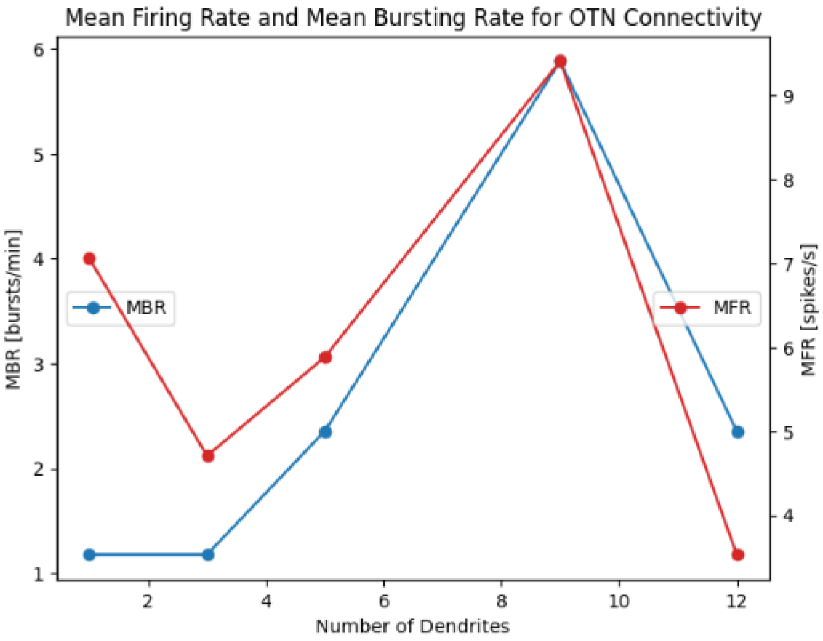
Results: Complexity of the meta-neuron morphology is correlated to the development of the network



The IBI distribution (OTN), meta-neurons each with 5 dendrites, shows a different trend from those experimentally found.

Only one peak is presented → quite different and not very realistic dynamics for OTN

Results: Complexity of the meta-neuron morphology is correlated to the development of the network



MBR and the MFR of the simulated network (OTN connectivity rule) as a function of the complexity of the dendritic tree.

The main result is that this kind of network architecture makes the role of the dendritic less relevant.

Conclusions

- Successfully simulated network model made up of meta-neurons with appropriate morphology (number of dendrites, length of neurites) and connectivity.

- Found out the a specific morphology (i.e., a certain number of compartments) and connectivity rule (i.e., OTM) are necessary to implement a suitable network model.

- Enhancing the complexity of the meta-neurons morphology reflects the variations of the network dynamics as a function of the network development (compare with the real data).

- Direct connections (by means of neurite bundles) among not-neighboring microelectrodes are also necessary.

Backup slides

- Cultured neurons coupled to microelectrode arrays (MEAs) refer to a technique in neuroscience where individual neurons are grown in laboratory conditions and connected to an array of tiny electrodes. This method allows researchers to study the electrical activity of neurons and investigate their interactions within a controlled environment.

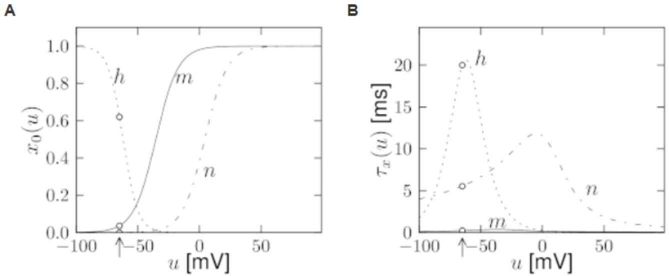
- MEA technology consists of a flat substrate with an array of microelectrodes, typically made of conductive materials such as gold or platinum. Neurons are cultured directly on top of these arrays, allowing their growth and development in close proximity to the electrodes. As the neurons mature, they establish connections with each other and form functional networks.

Backup slides

$$\sum_k I_k = Cm\frac{dV_m}{dt} + \overline{g_k}n^4(u-V_k) + \overline{g_{Na}}m^3h(u-V_{Na}) + \bar{g}_l(u-V_l)$$

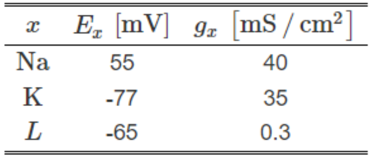
Three gating m, n, h evolve according to differential equation:

$$\dot{x} = -\frac{1}{\tau_{x(u)}}(x - x_0(u))$$ Where x stands for m, n, or h

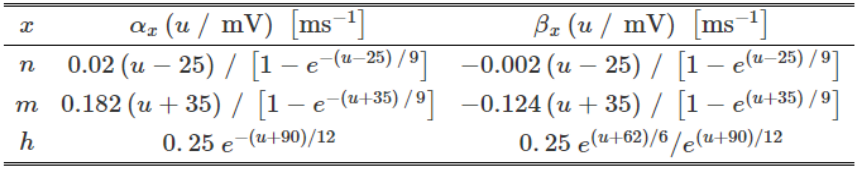


The Hodgkin-Huxley model. **A.** The equilibrium functions for the three variables m, n, h in the H-H model. **B**. The voltage dependent time constant.

The parameters E_{Na}, E_{K}, and E_L are the reversal potentials.



x	E_x [mV]	g_x [mS / cm^2]
Na	55	40
K	-77	35
L	-65	0.3



x	$\alpha_x\,(u\,/\,\mathrm{mV})$ [ms^{-1}]	$\beta_x\,(u\,/\,\mathrm{mV})$ [ms^{-1}]
n	$0.02\,(u-25)\,/\,\left[1-e^{-(u-25)\,/\,9}\right]$	$-0.002\,(u-25)\,/\,\left[1-e^{(u-25)\,/\,9}\right]$
m	$0.182\,(u+35)\,/\,\left[1-e^{-(u+35)\,/\,9}\right]$	$-0.124\,(u+35)\,/\,\left[1-e^{(u+35)\,/\,9}\right]$
h	$0.\,25\,e^{-(u+90)/12}$	$0.\,25\,e^{(u+62)/6}/e^{(u+90)/12}$

Voltage is measured in mV and the membrane capacity is C = 1μF/cm^2

Backup slides

The model of the synapse follows the approach proposed which based on the first-order kinetic scheme:

$R + T \overset{k_1,k_2}{\Leftrightarrow} TR^*$ → describes the binding of the neurotransmitter molecules T.

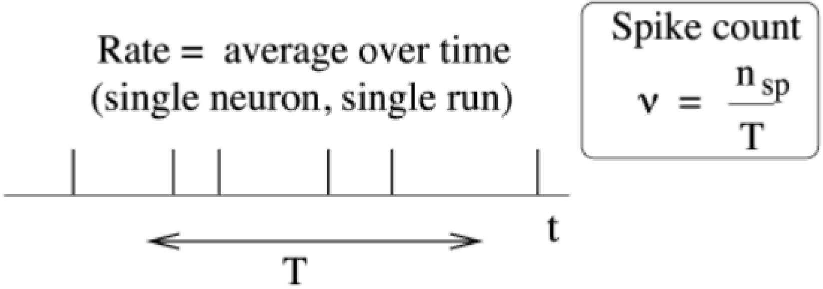
- R and R∗ are the unbound and bound forms of the postsynaptic receptor.
- k_1 and k_2 are the forward and backward rate.

The fraction of the bound receptors r is described by the equation:

$$\frac{dr}{dt} = k_1\,[T](1 - r) - k_2\,r$$

This modelling approach allowed the synaptic events to be represented by equations with the same structure of the H–H equations.

Backup slides



The spike count measure: Definition of the mean firing rate by temporal average.

- T: time of the window
- n^{sp}: the spike count
- In practice, to get sensible averages, several spikes should occur within the time window.