Shaping Dynamics in Neuronal Cultures through Engineering and Stimulation: Development & Plasticity

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Univ. Barcelona Institute of Complex Systems (UBICS)

Maturation and Plasticity in Biological and Artificial Networks
Cargèse, 25th October 2024

Universitat de Barcelona
UBICS Institute of Complex Systems
Ministerio de Ciencia, Innovación y Universidades
Agencia Estatal de Investigación
NEU-ChiP

NEU-ChiP

Neuronal networks from
Cortical human iPSCs for
Machine Learning
Processing

cnrs

Rémi

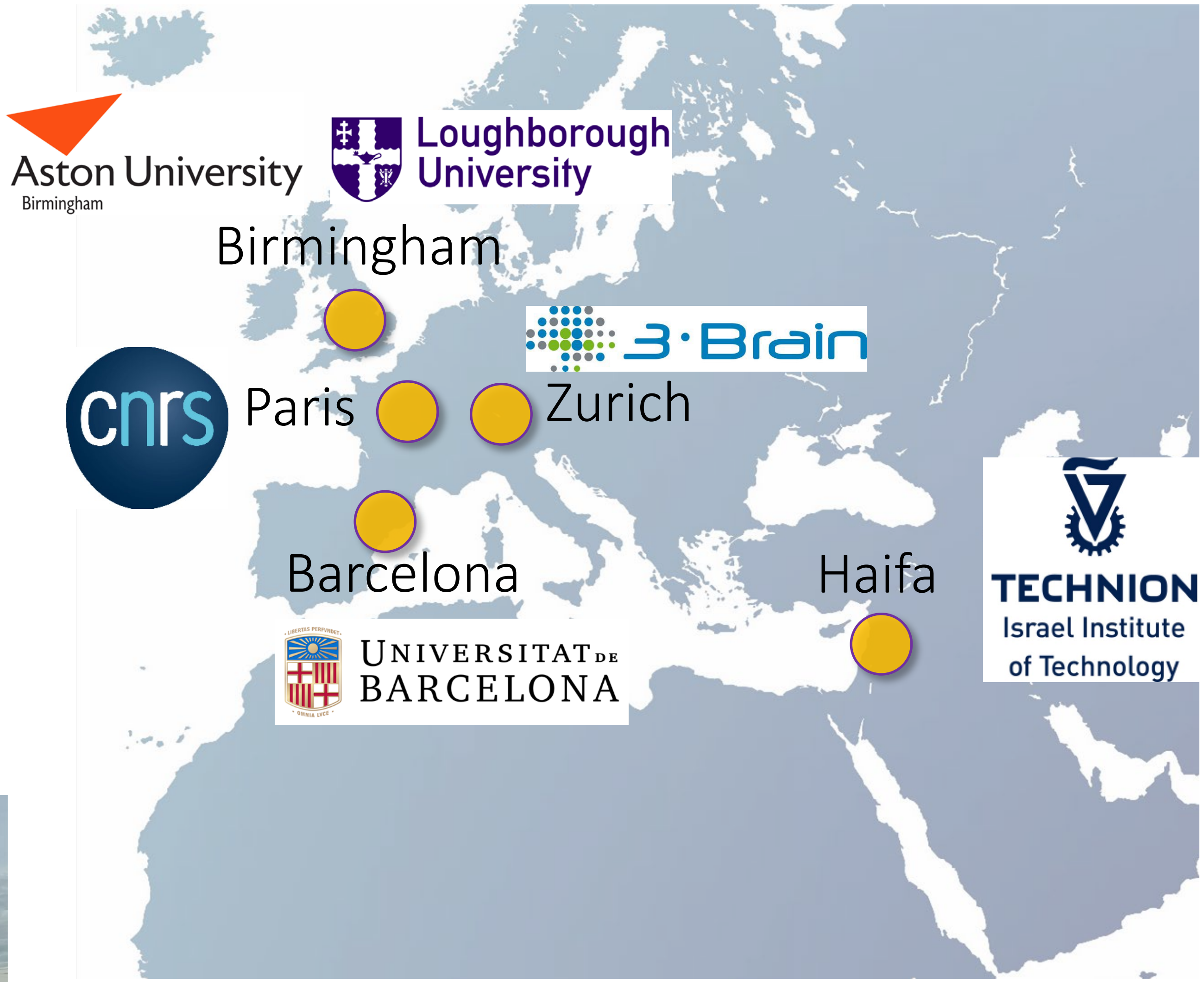
Francesco

UNIVERSITAT DE BARCELONA

Jordi +

Anna

Akke



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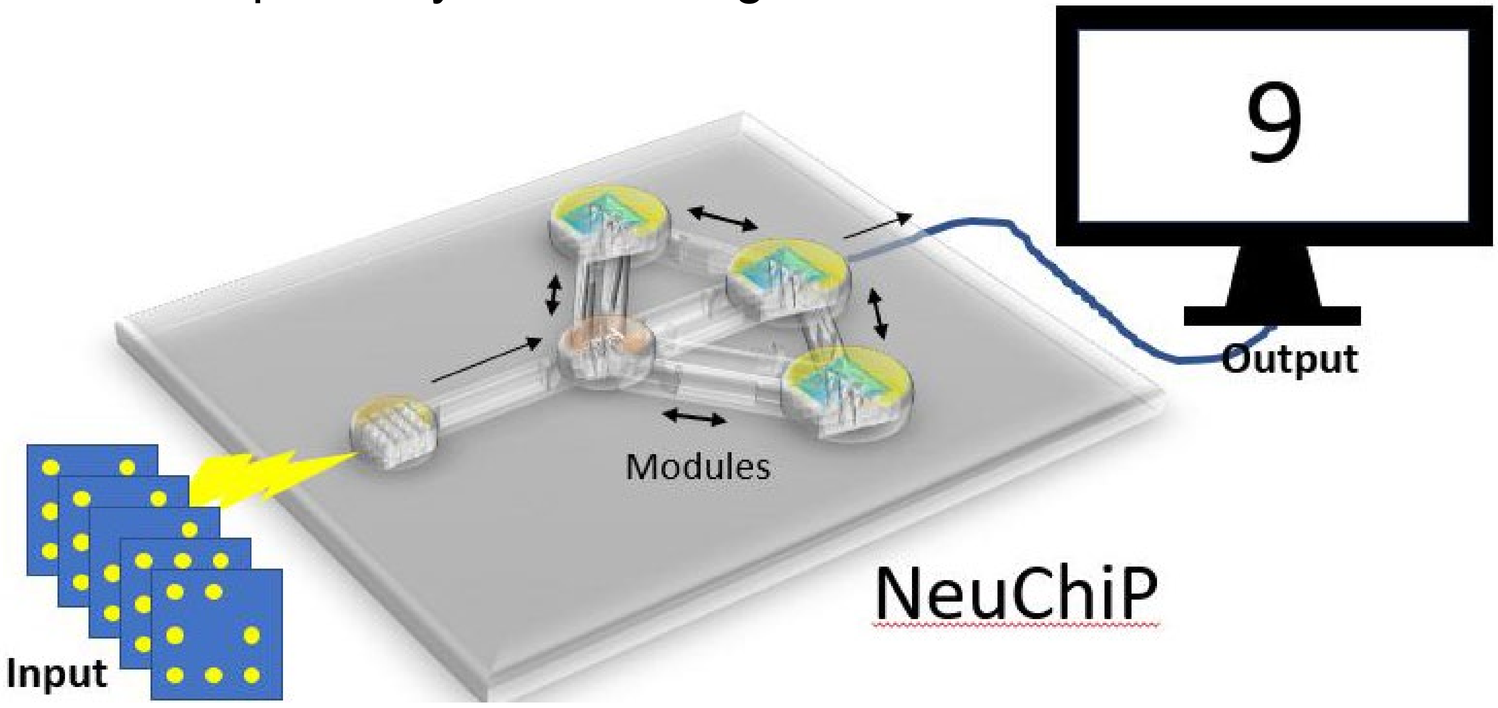
Neuronal networks from Cortical human iPSCs for Machine Learning Processing

- Emulate AI-based processing with **biological circuits in vitro**.
- Design and train neuronal cultures.
- Blend electronic and biological systems.
- Show evidences of task-oriented plasticity and learning.

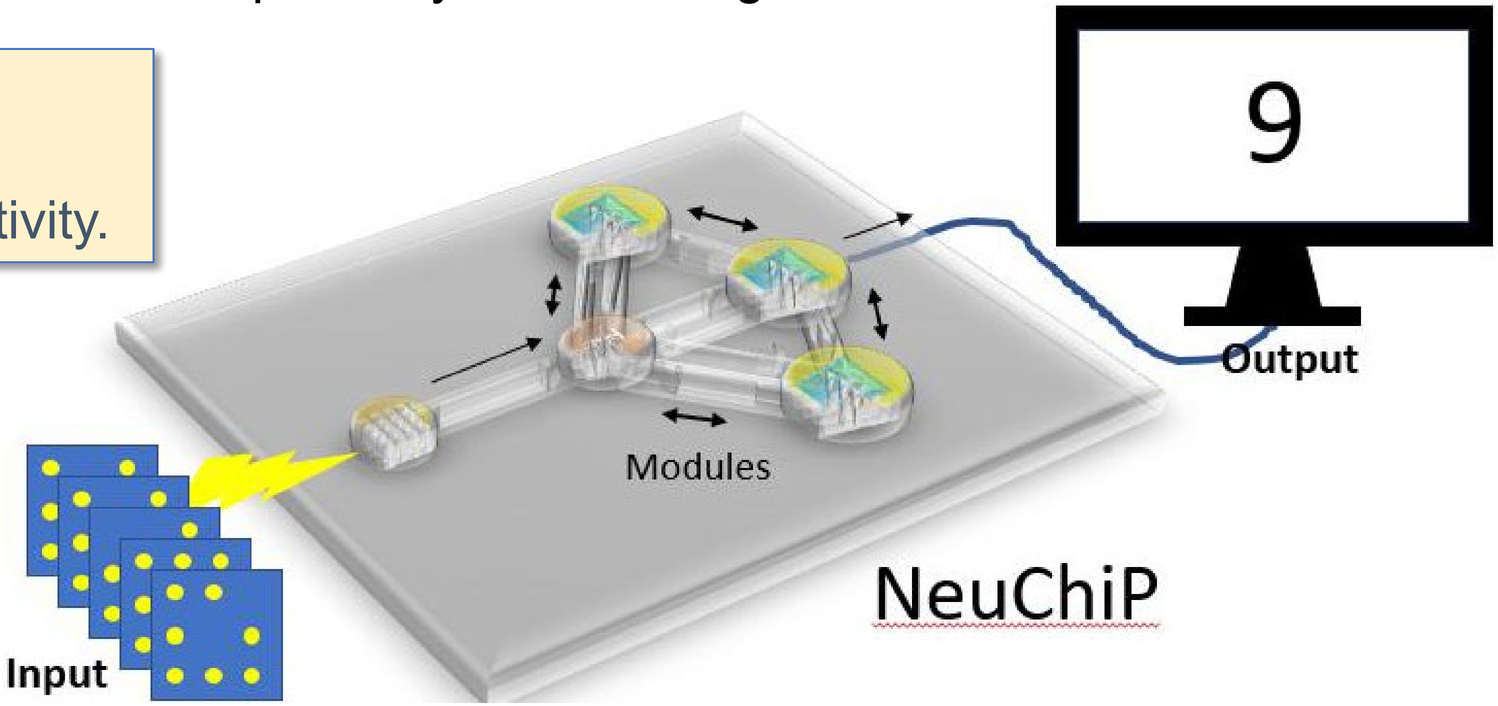


NEU-ChiP

Neuronal networks from
Cortical human iPSCs for
Machine Learning
Processing

- Emulate AI-based processing with **biological circuits in vitro**.
- Design and train neuronal cultures.
- Blend electronic and biological systems.
- Show evidences of task-oriented plasticity and learning.

But:
- Very difficult!
- Unknown role of spontaneous activity.



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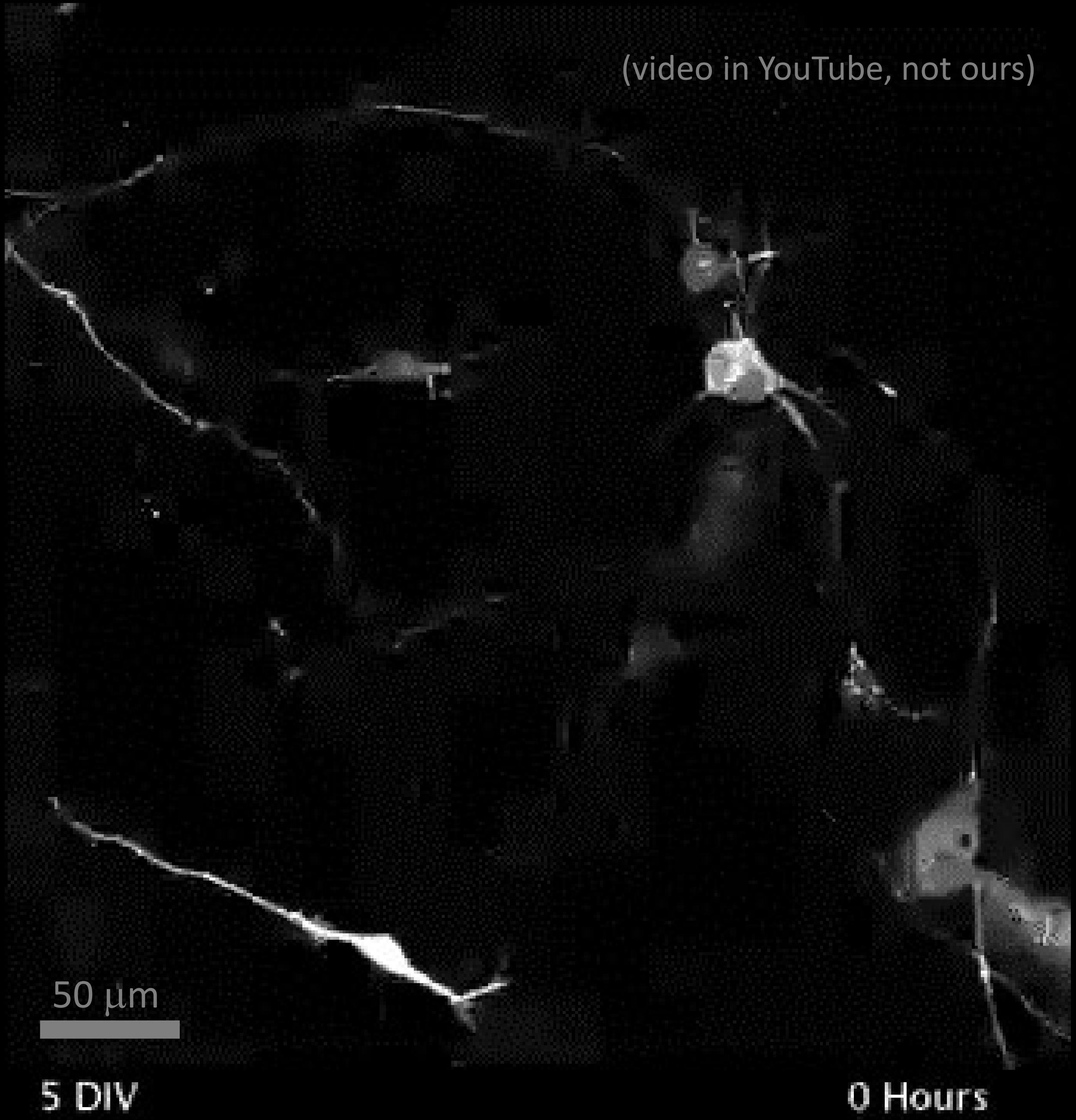
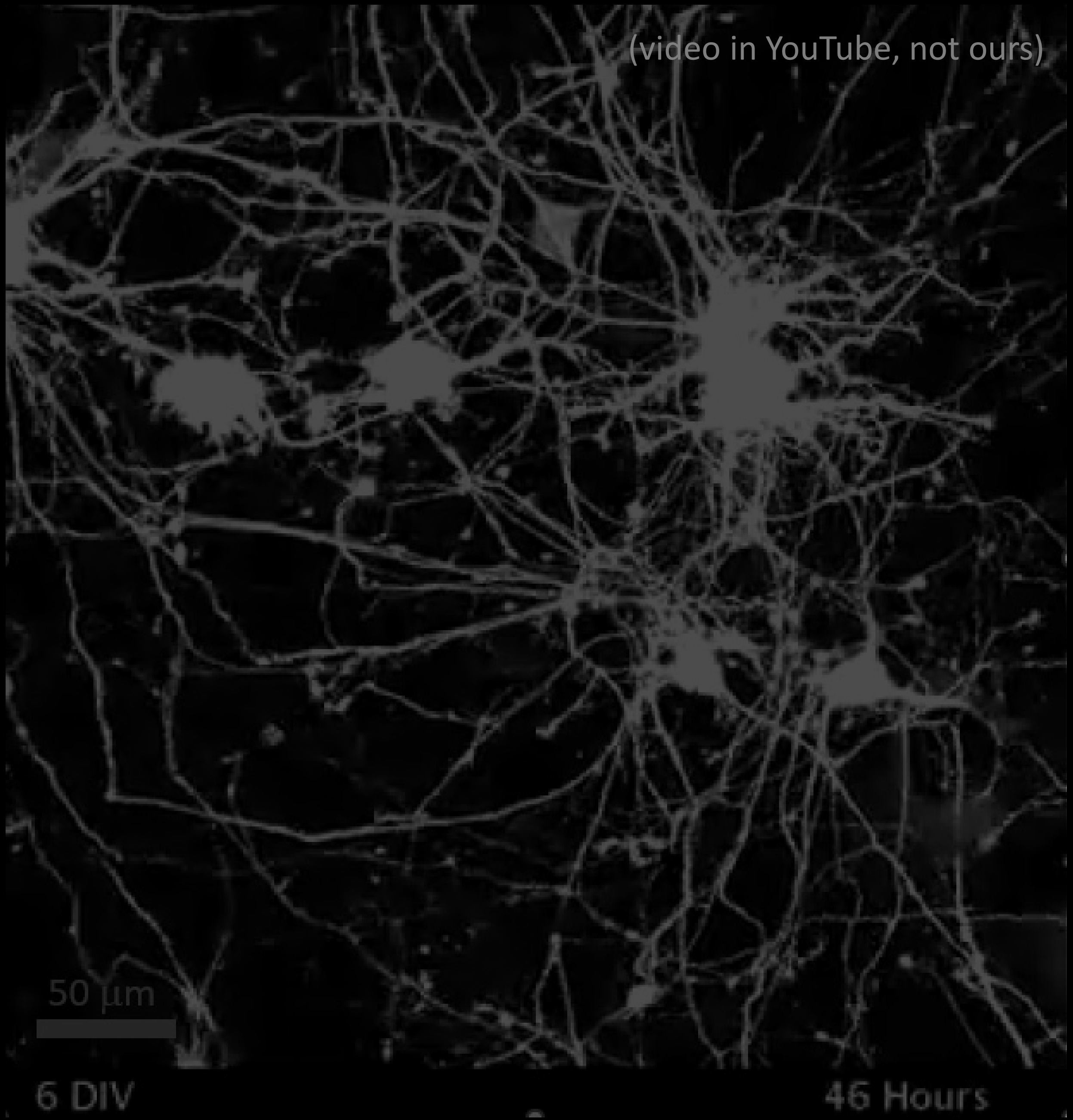
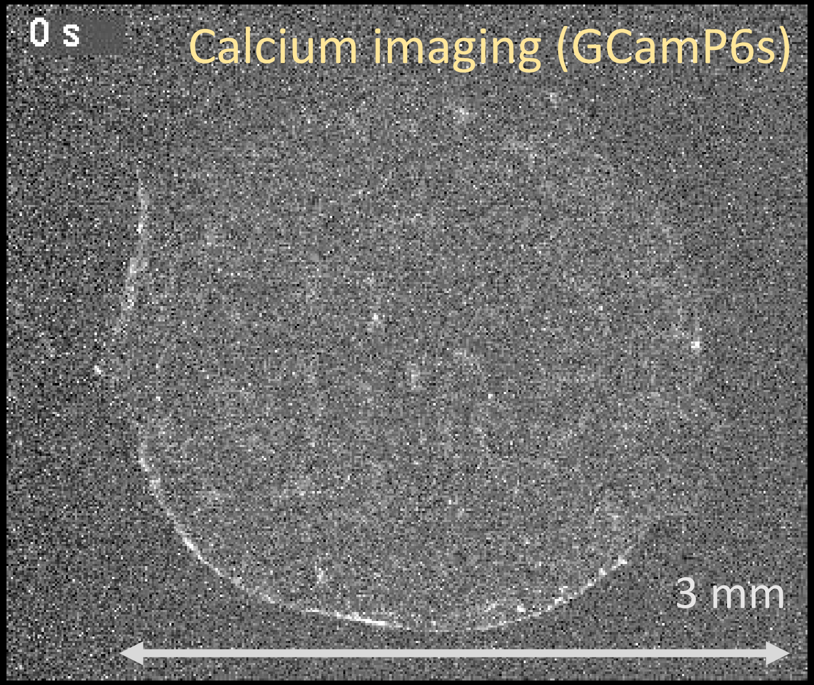
Quick overview of neuronal cultures

(video in YouTube, not ours)







In general:

- Excitatory & inhibitory neurons.
- Glia cells.

Review on neuronal cultures:
J. Soriano, Biophysica (2023)

High density MEAs and new technologies

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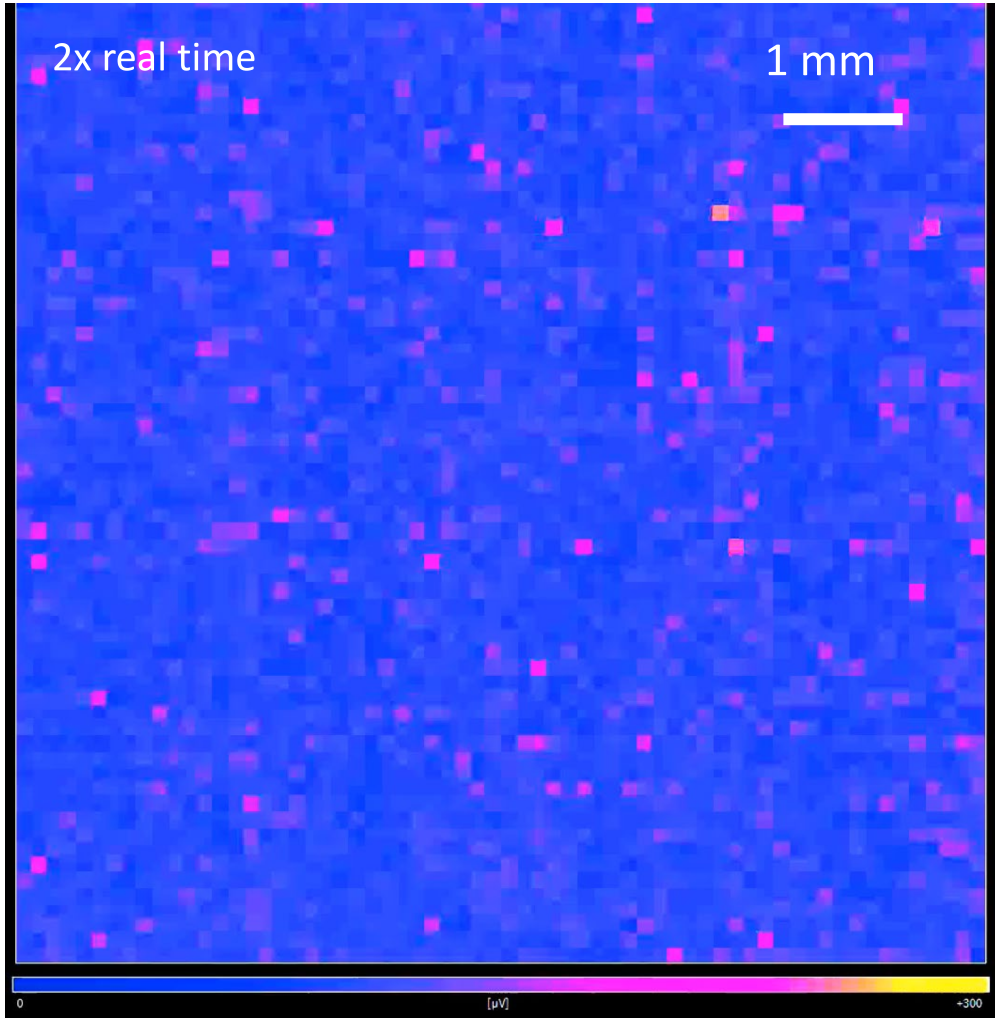
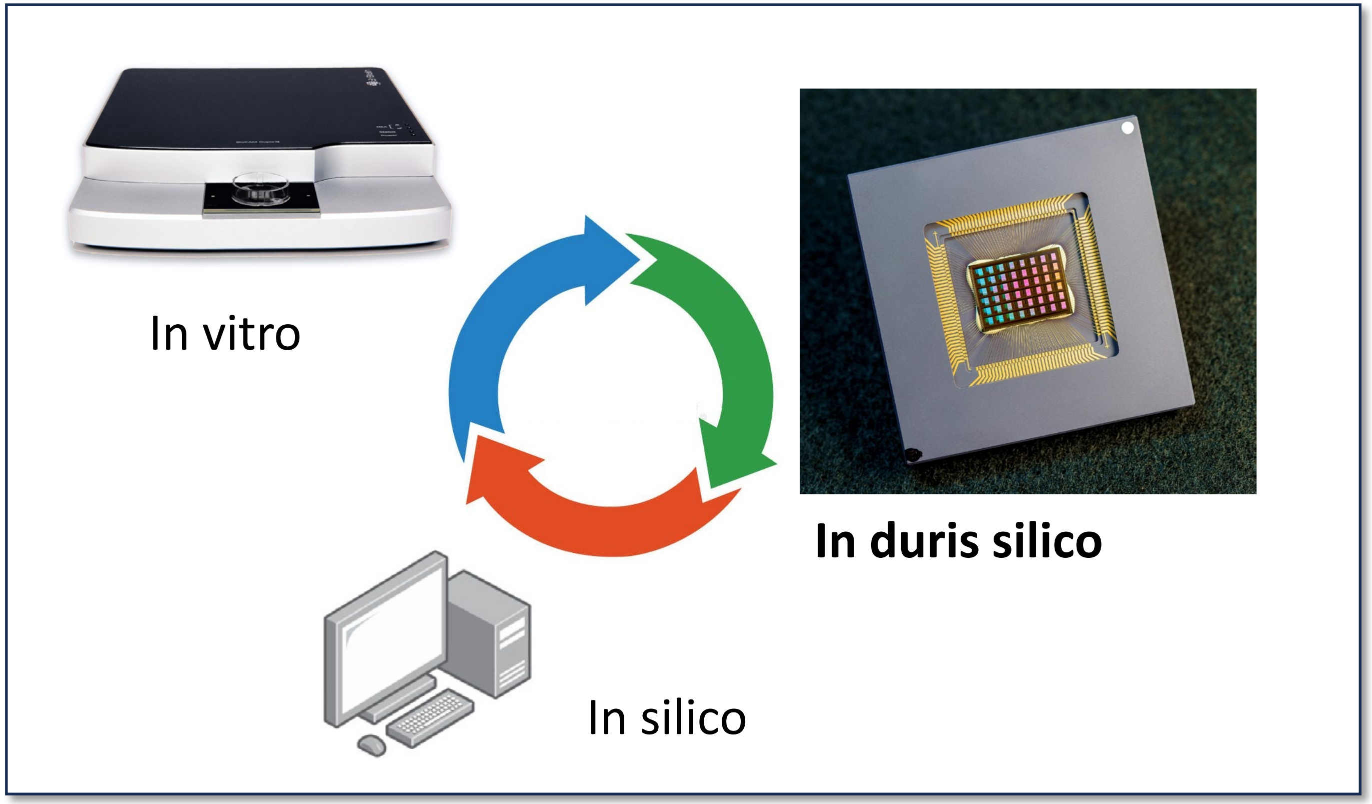
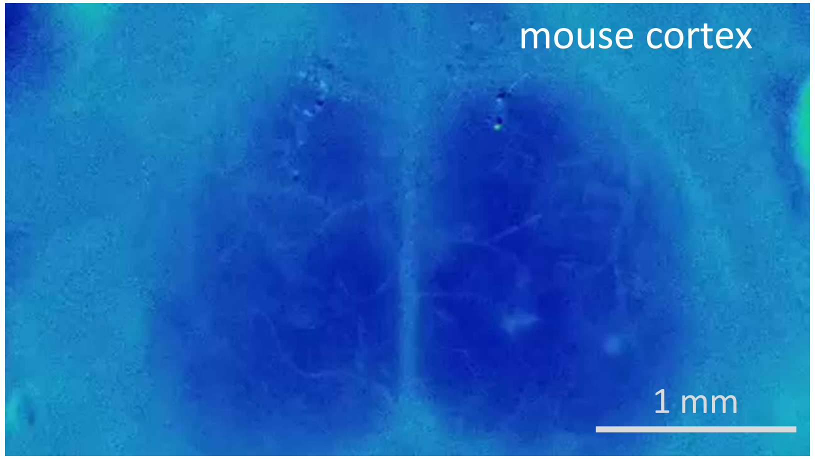


Vallejo-Mancero et al., Neural Networks (2024)

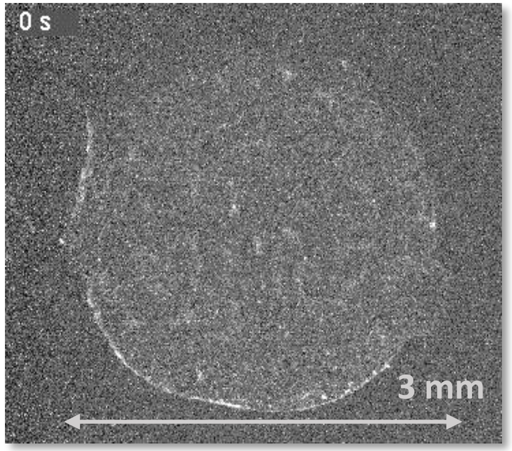
- Help designing neuromorphic chips.
- Biohybrid devices.
- Biological computation and AI.



Neuronal cultures and engineering

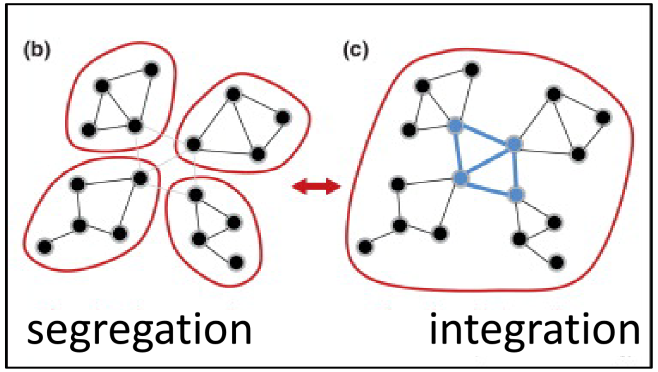


(Yale Uni., Youtube video)



(our lab)

SEGREGATION (local processing) ⇄ INTEGRATION (global communication)



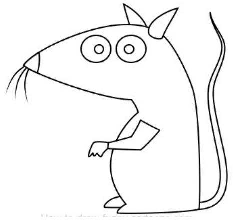
G. Zamora-López et al., Front Neurosci (2011).
O. Sporns, Curr. Op. Neurobiol. (2013).

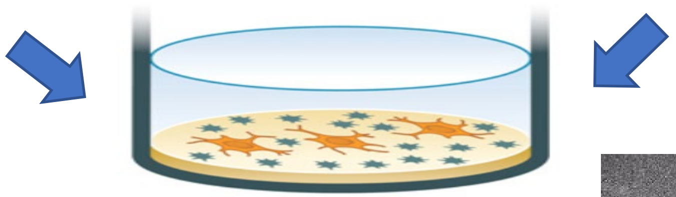
Neuronal cultures and engineering

Yamanaka 2012

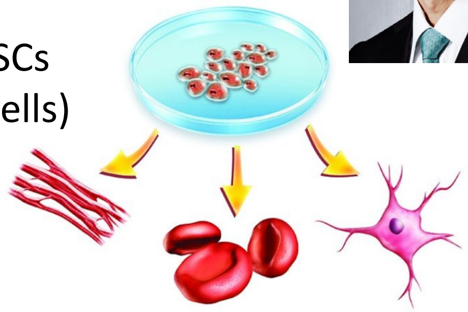
CELL SOURCE

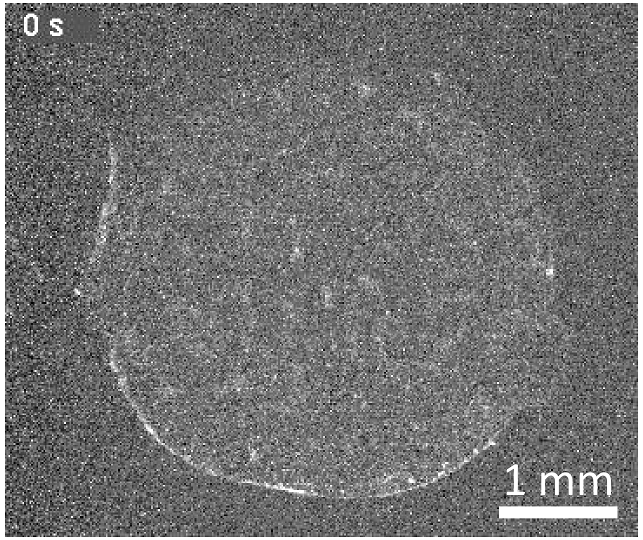
Rat primary cultures

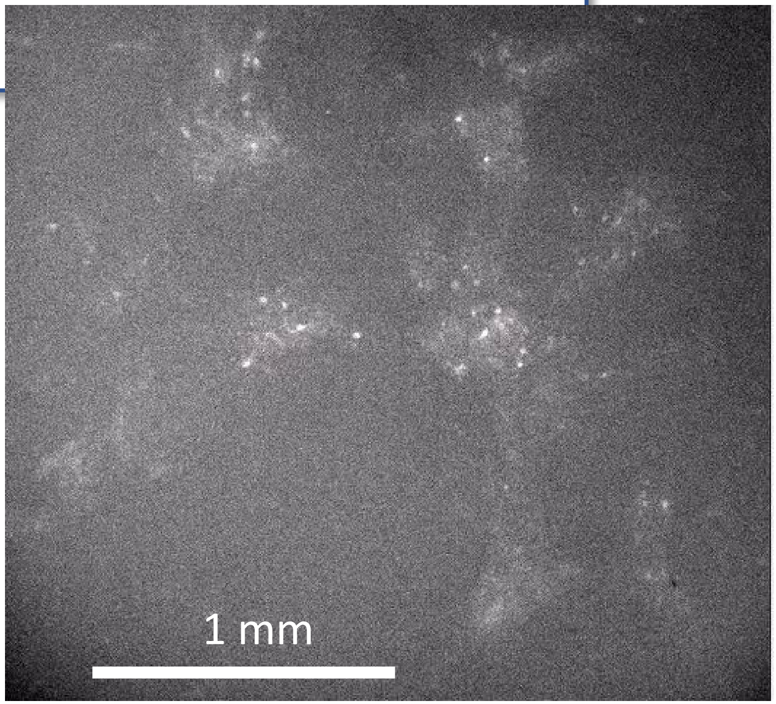


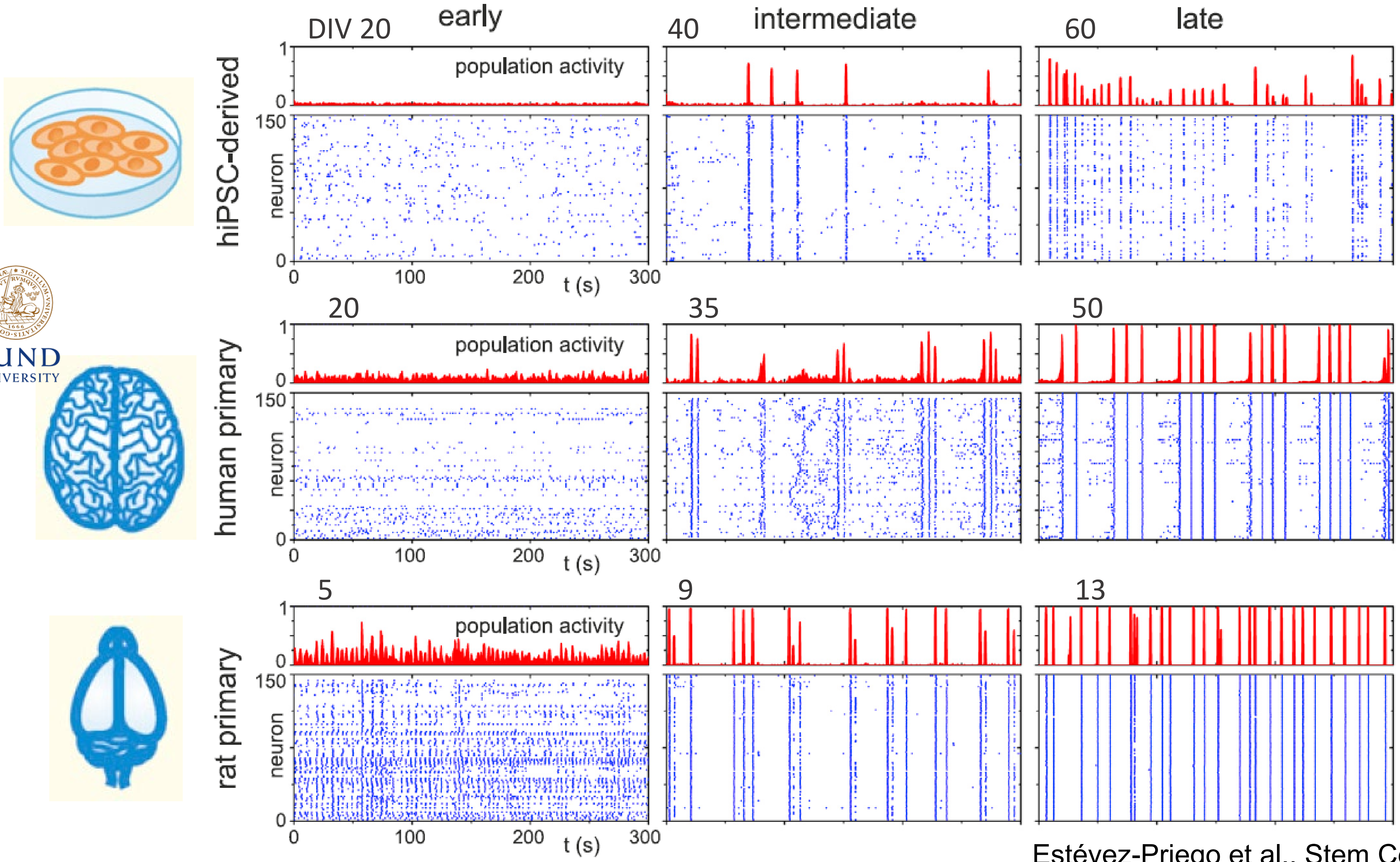


Human iPSCs
(induced stem cells)

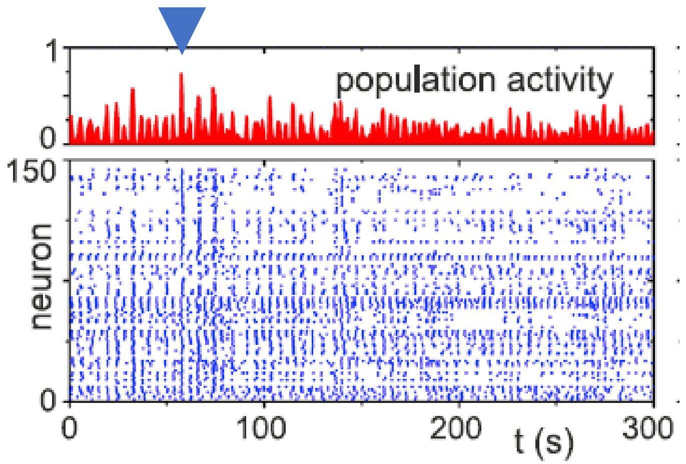


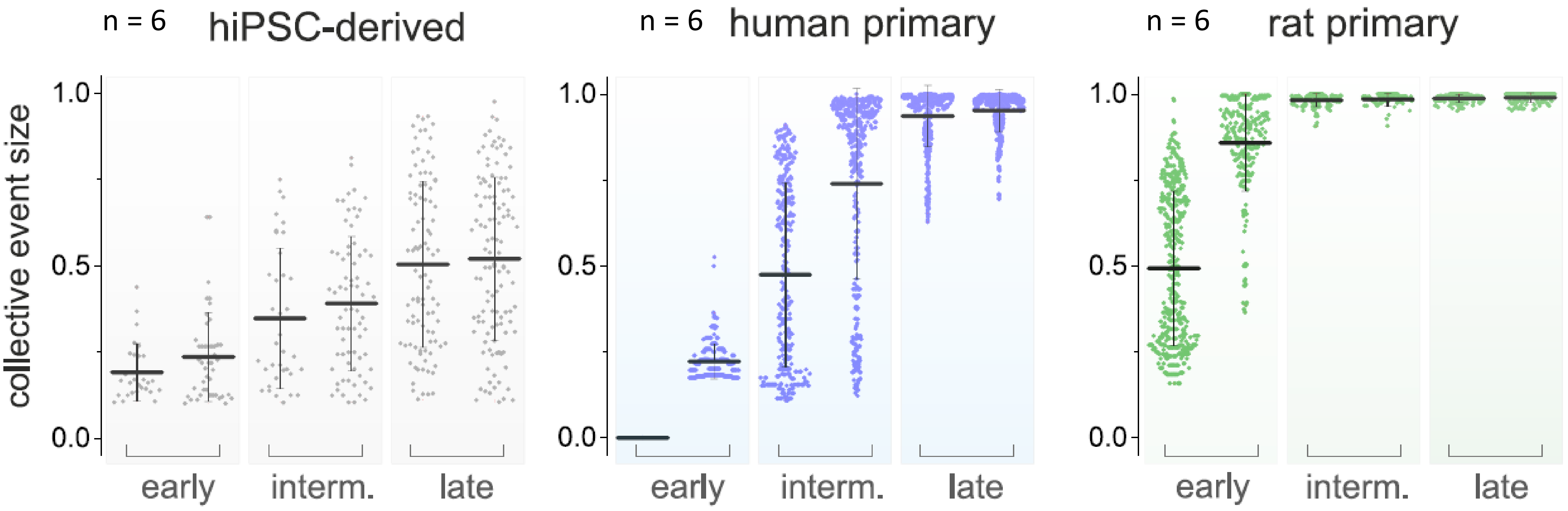






Estévez-Priego et al., Stem Cell Rep (2023)



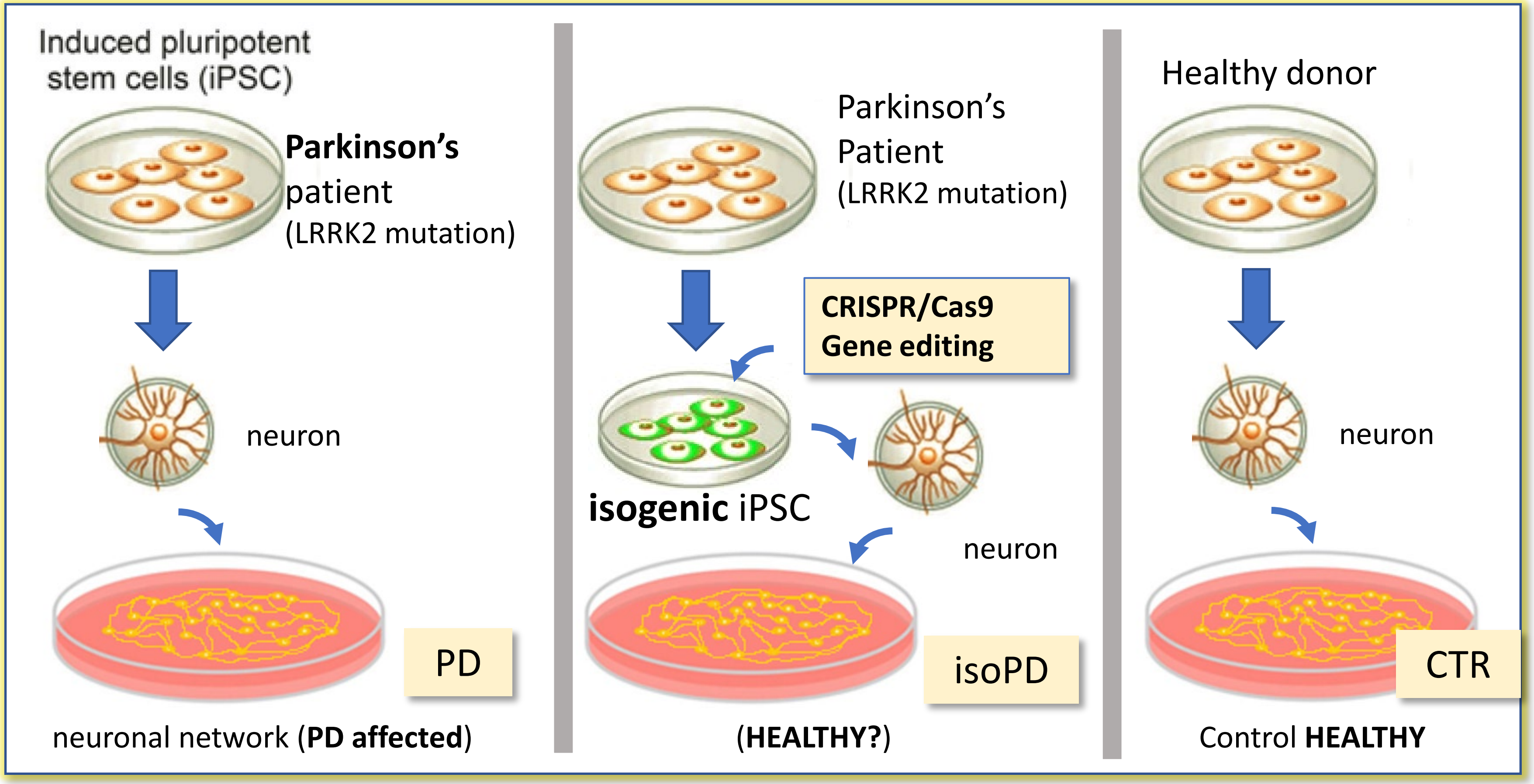


Estévez-Priego et al., Stem Cell Rep (2023)

Maturation in affected hiPSC cultures: Parkinson's disease

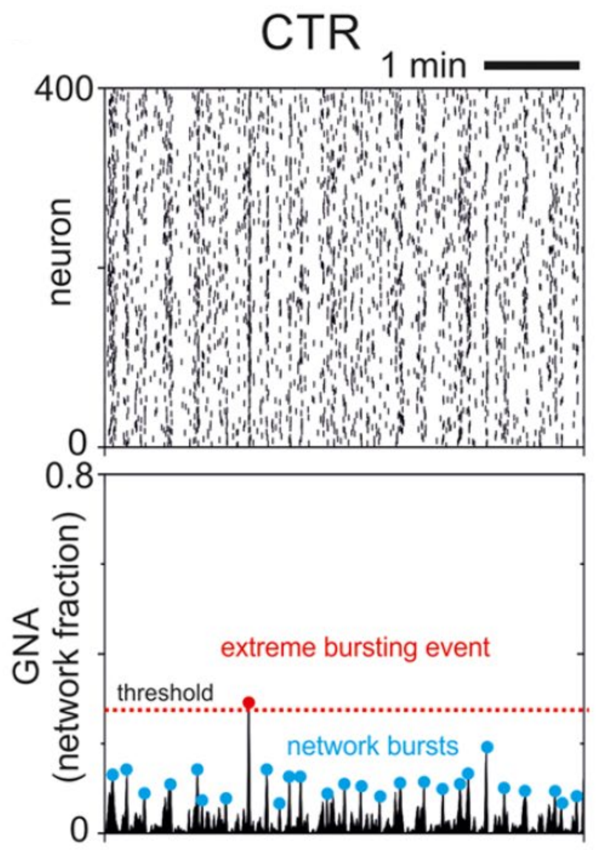
IDIBELL
Institut d'Investigació Biomèdica de Bellvitge
Á. Raya, A. Consiglio

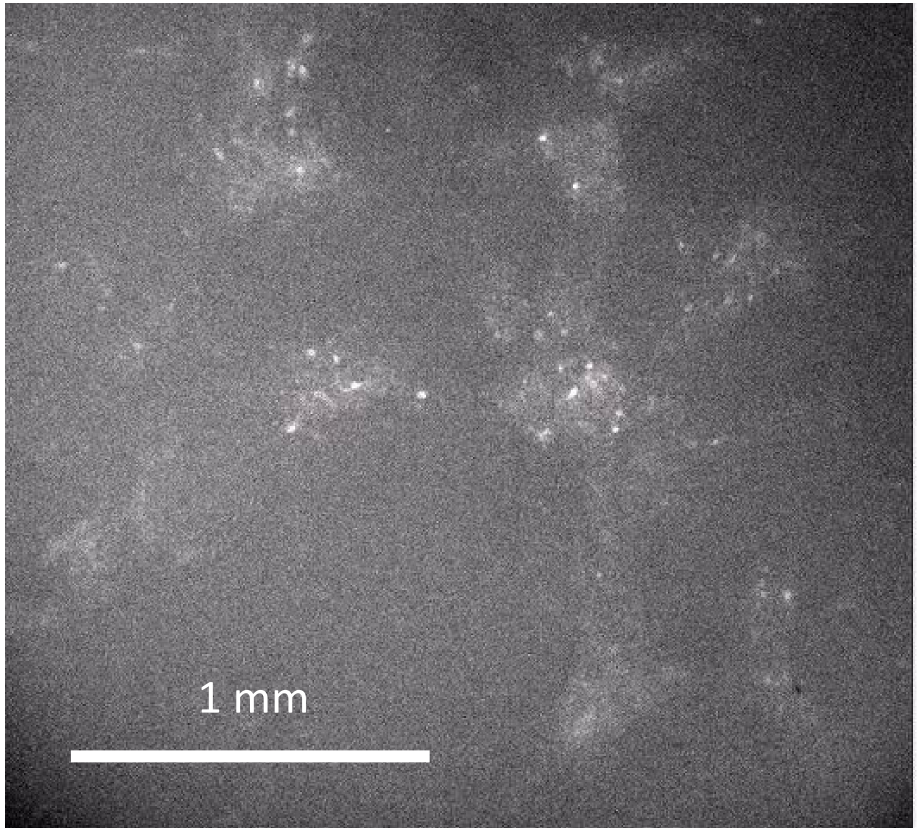
Degradation and death of **dopaminergic** neurons.



di Domenico et al., Stem Cell Reports (2018)
Carola et al., npj Parkinson's disease (2021)

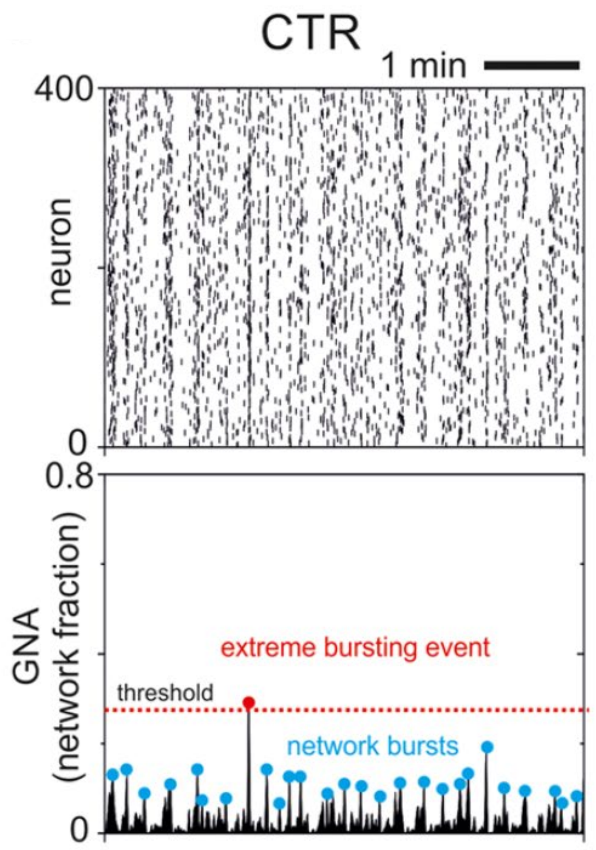
Maturation in affected hiPSC cultures: Parkinson's disease





Carola et al., npj Parkinson's disease (2021)

Maturation in affected hiPSC cultures: Parkinson's disease



Carola et al., npj Parkinson's disease (2021)

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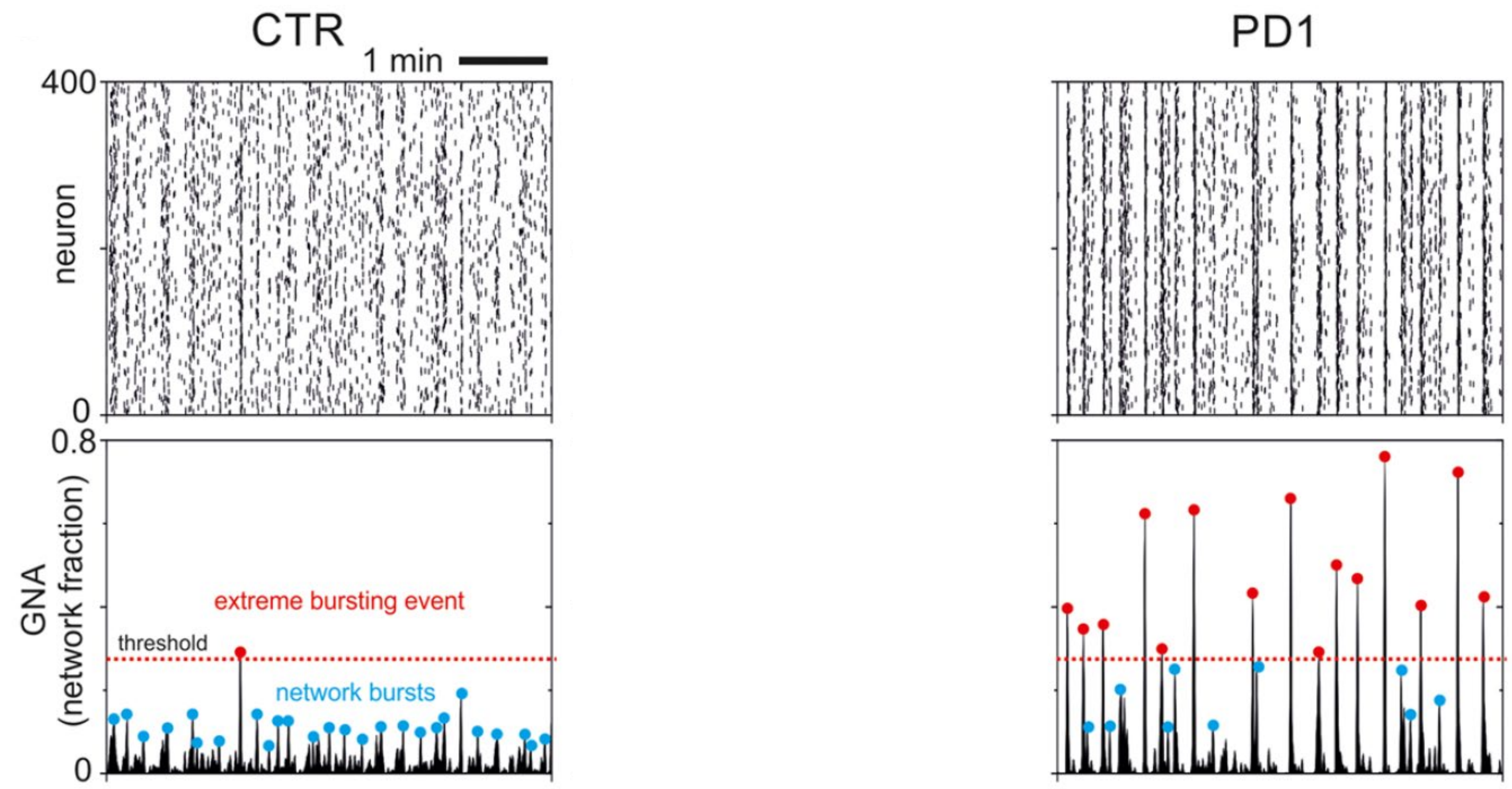

Carola et al., npj Parkinson's disease (2021)

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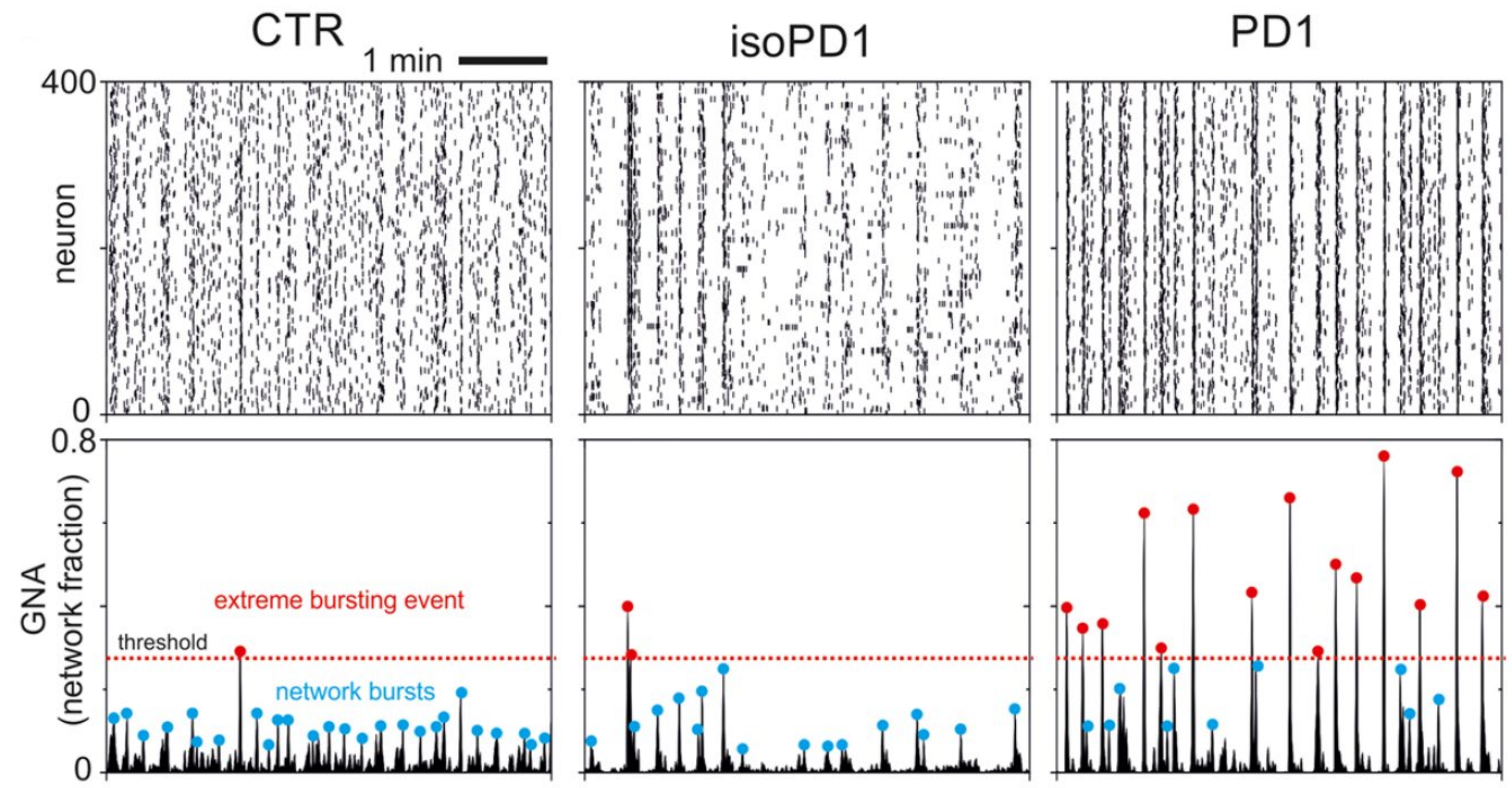
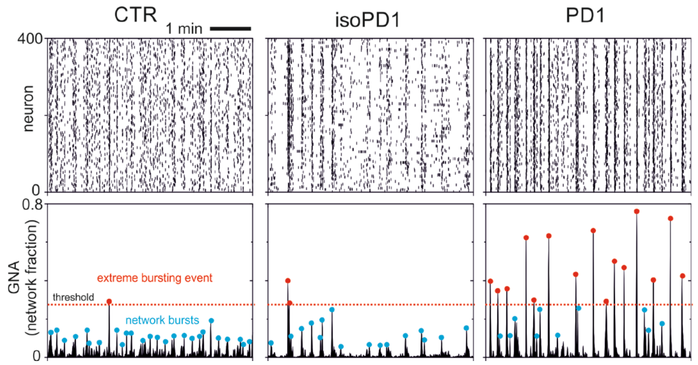
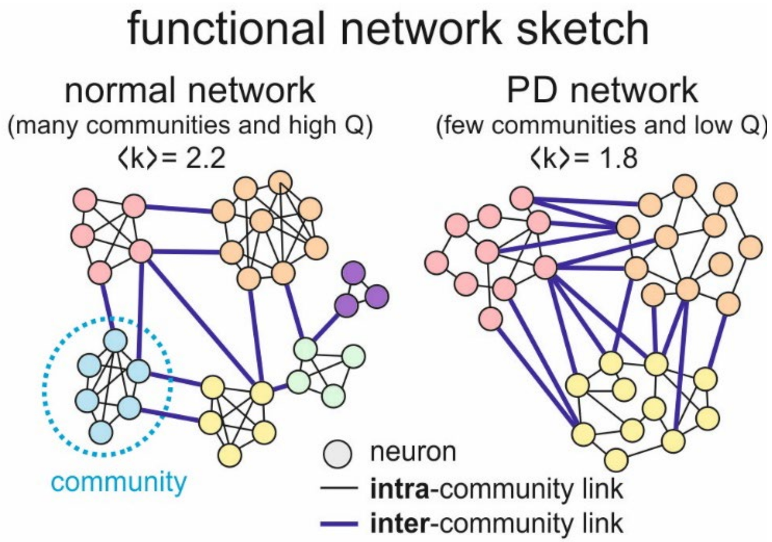
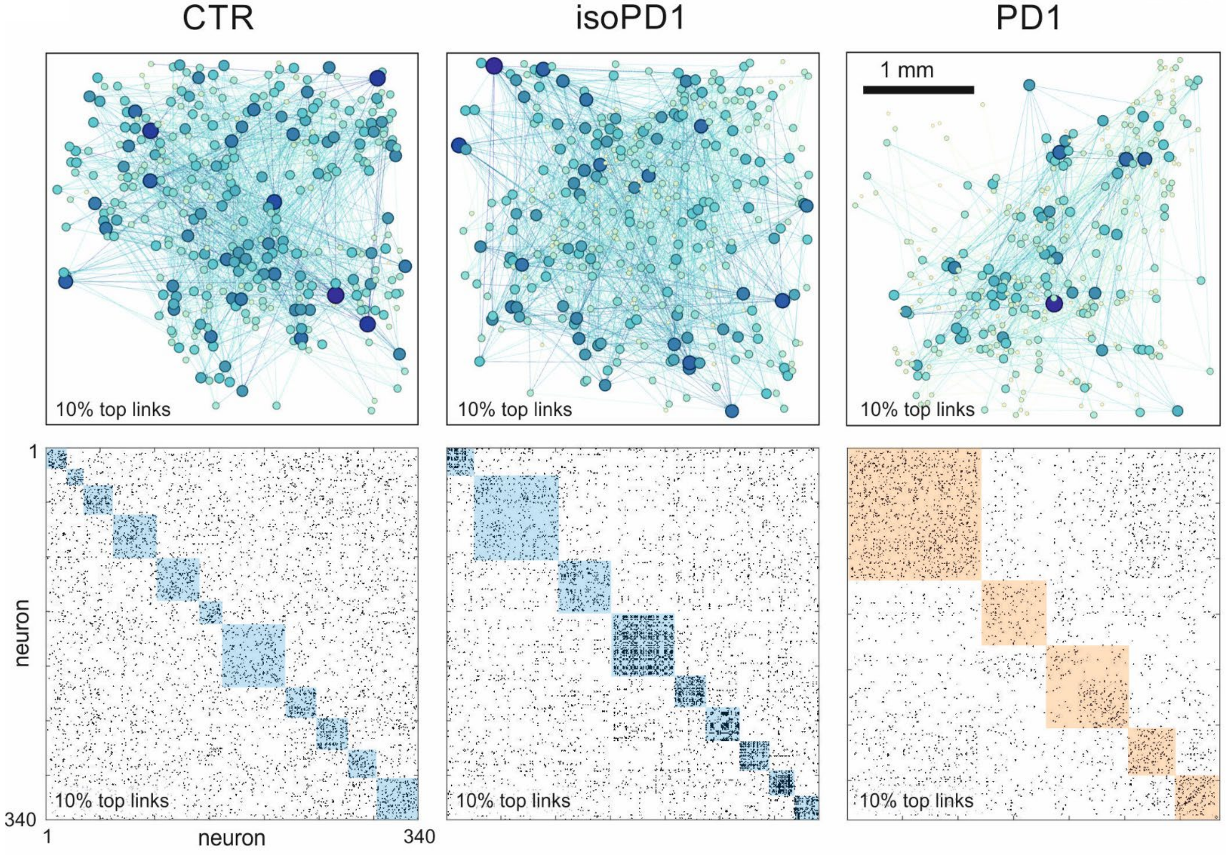


Carola et al., npj Parkinson's disease (2021)







Carola et al., npj Parkinson's disease (2021)

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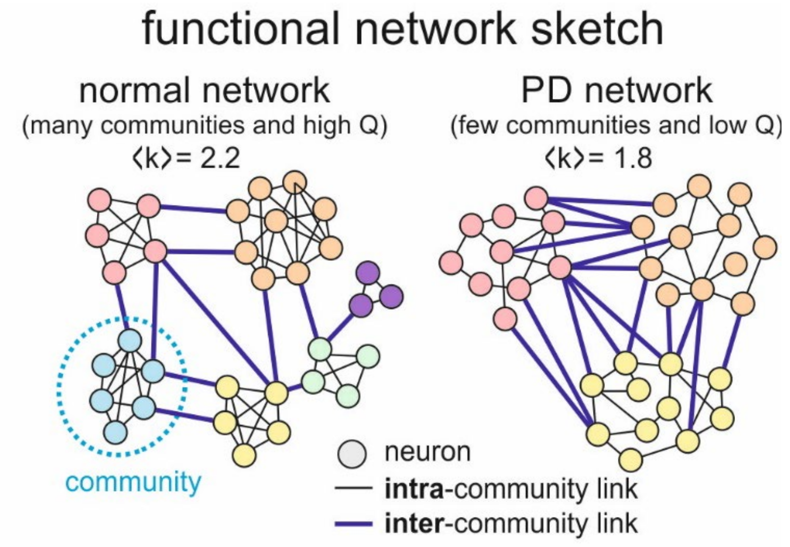
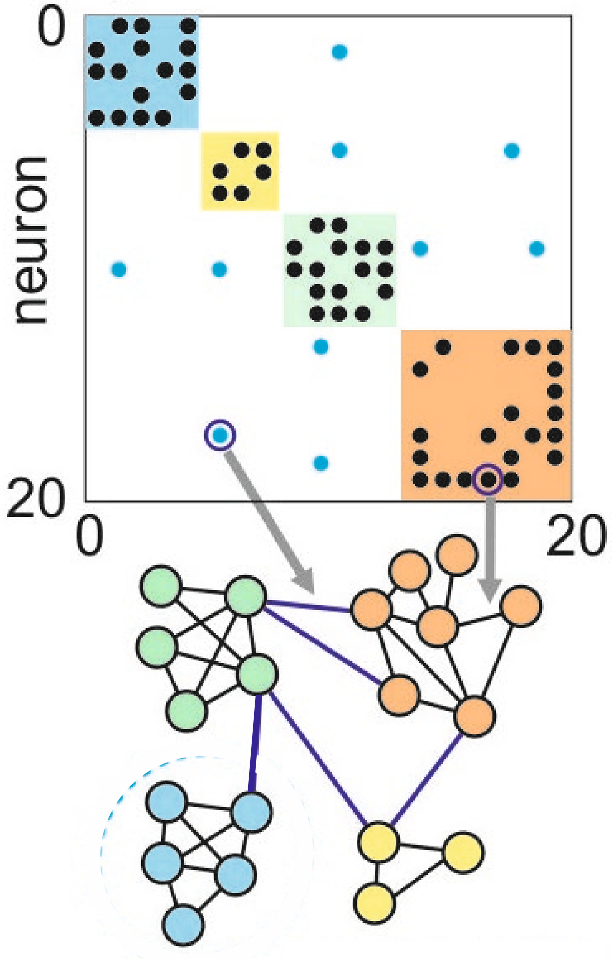


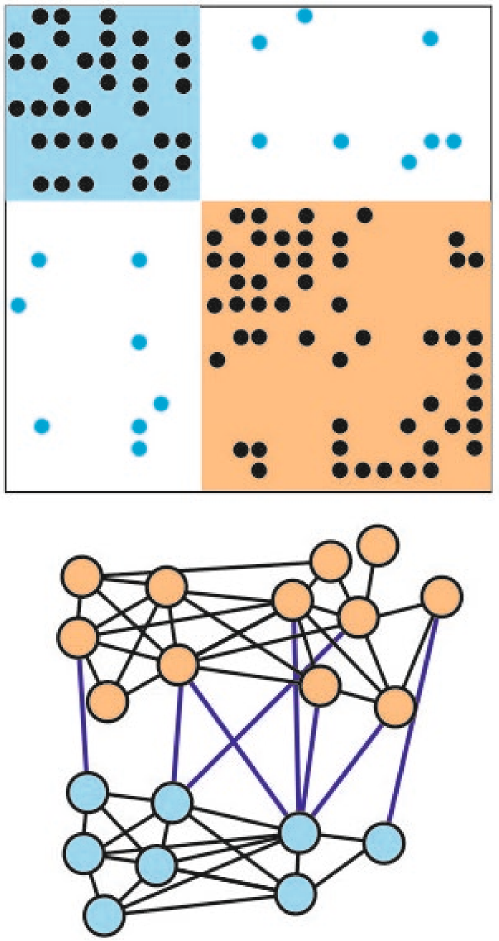
Complex networks and engineering

A bit of complex networks

Highly segregated



Strongly integrated



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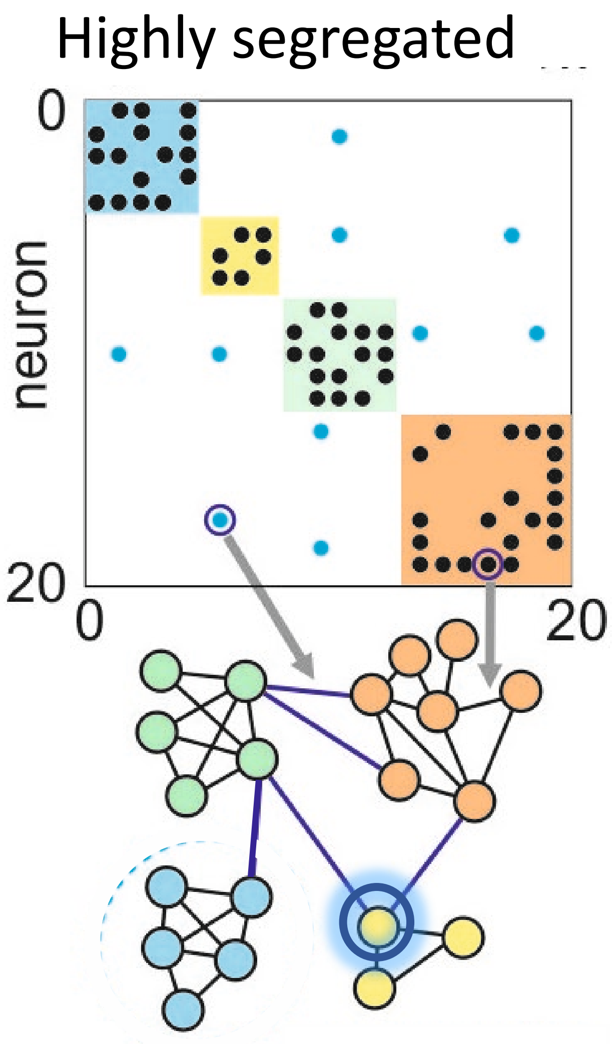
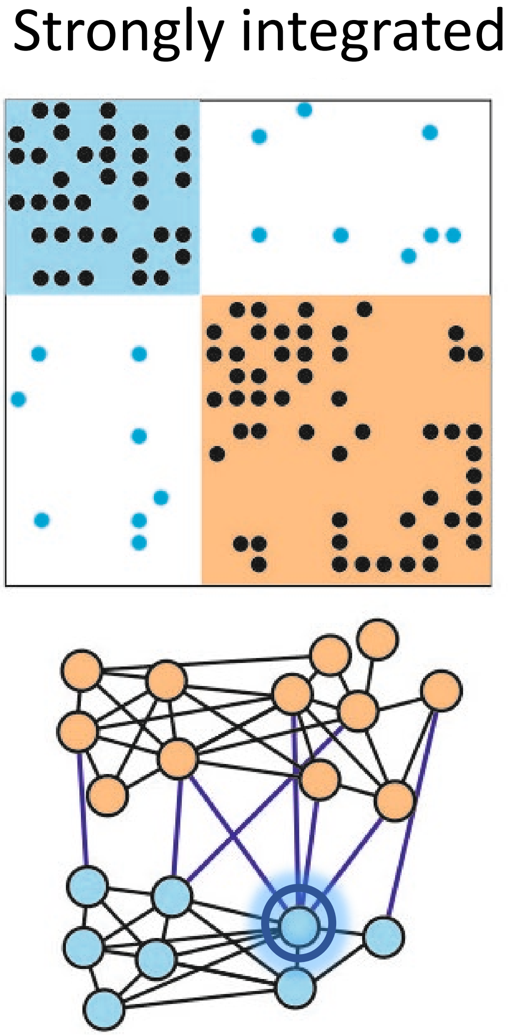


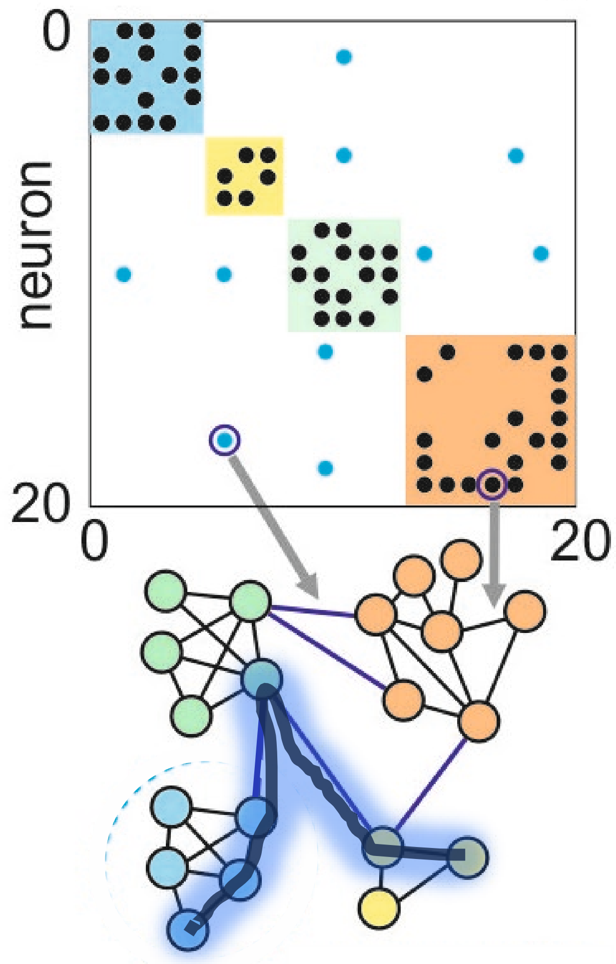


<k>= 3.3

<k>= 6.5

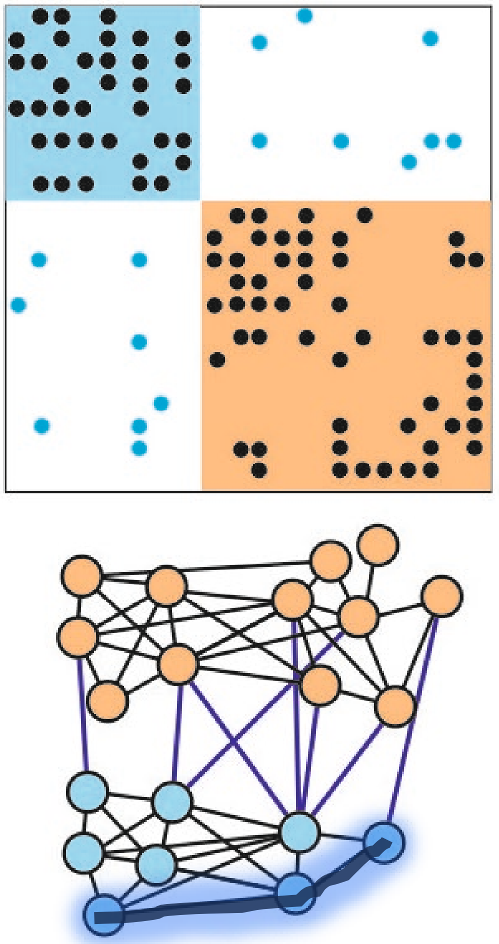
A bit of complex networks

Highly segregated



$\langle k \rangle = 3.3$, $\mathbf{G_E = 0.30}$

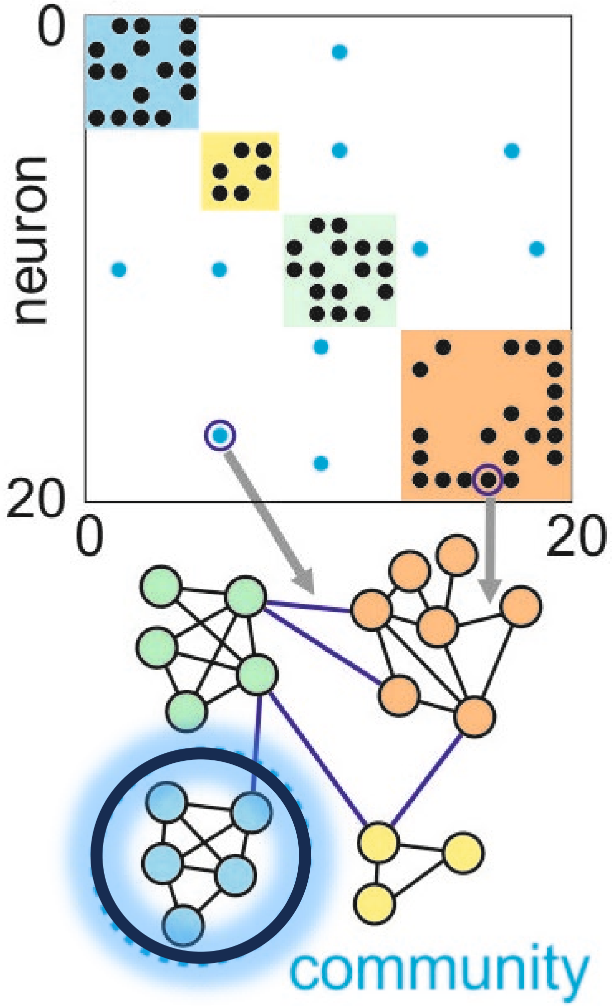
Strongly integrated



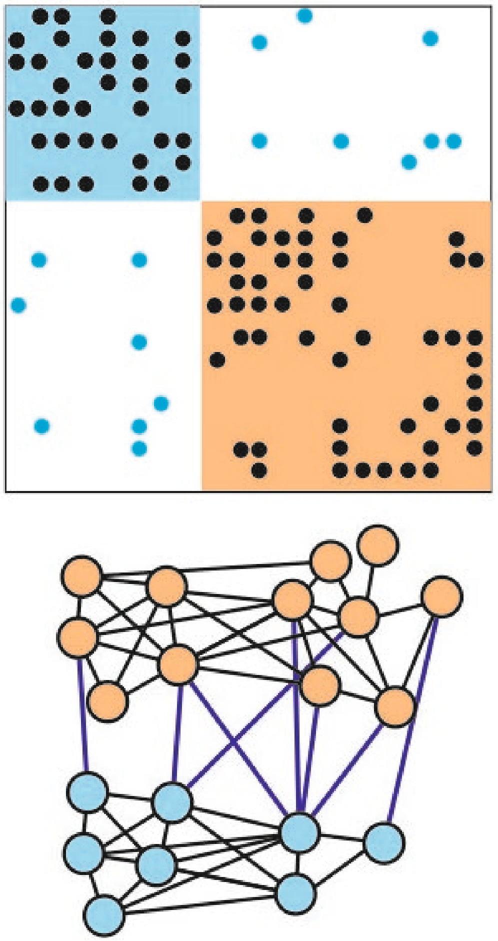
$\langle k \rangle = 6.5$, $\mathbf{G_E = 0.70}$

A bit of complex networks

Highly segregated



Strongly integrated

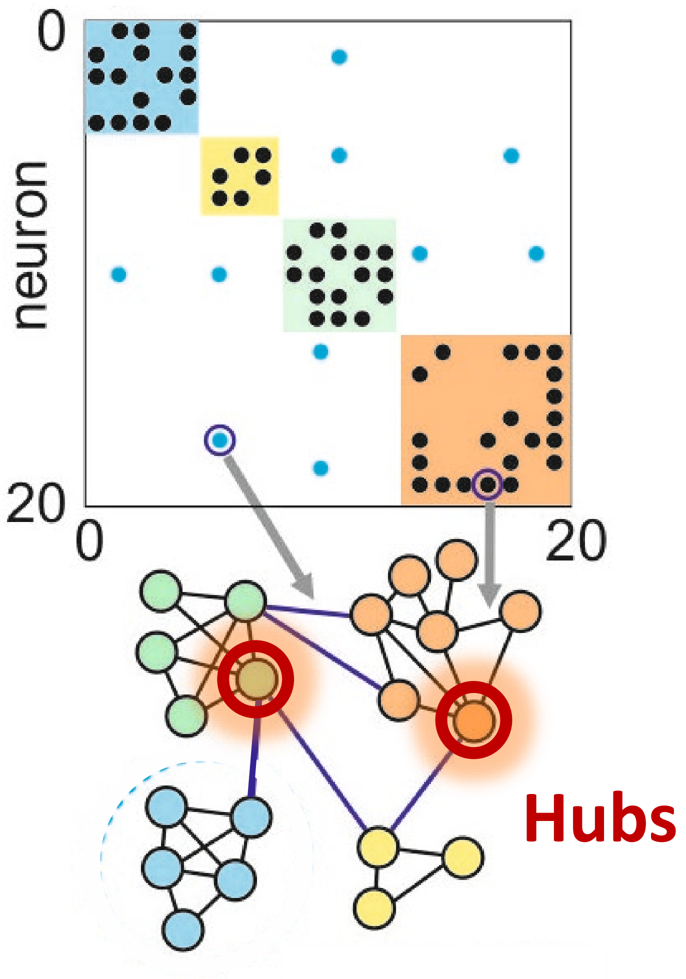


<k>= 3.3, G_E = 0.30, **Q = 0.45**

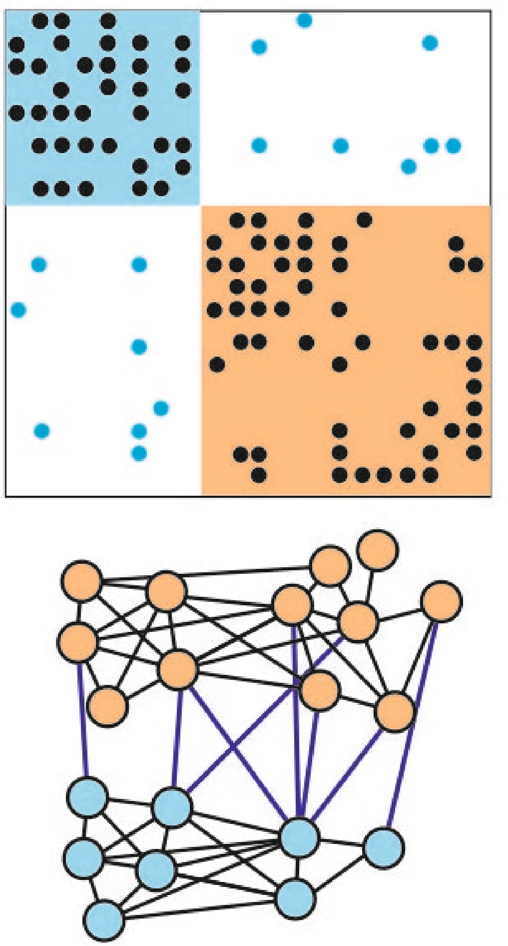
<k>= 6.5, G_E = 0.70, **Q = 0.25**

A bit of complex networks

Highly segregated



Strongly integrated



<k>= 3.3, **G_E = 0.30**

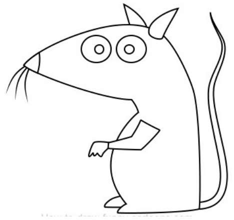
<k>= 6.5, **G_E = 0.70**

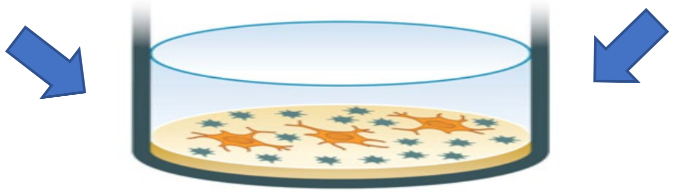
Neuronal cultures and engineering

Yamanaka 2012

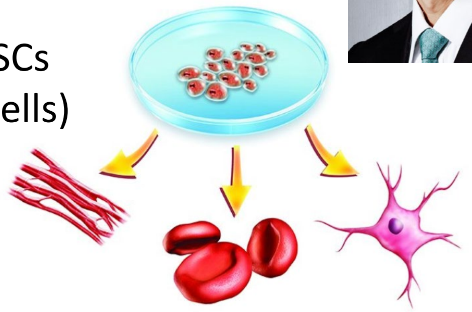
CELL SOURCE

Rat primary cultures

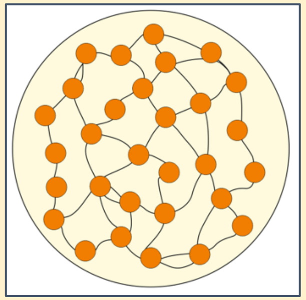




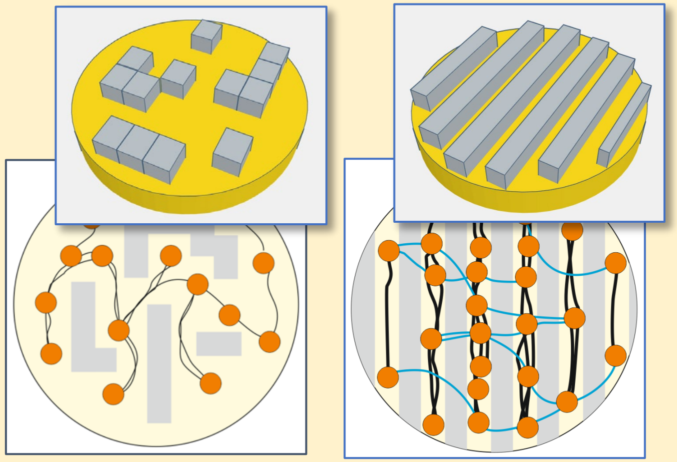
Human iPSCs
(induced stem cells)



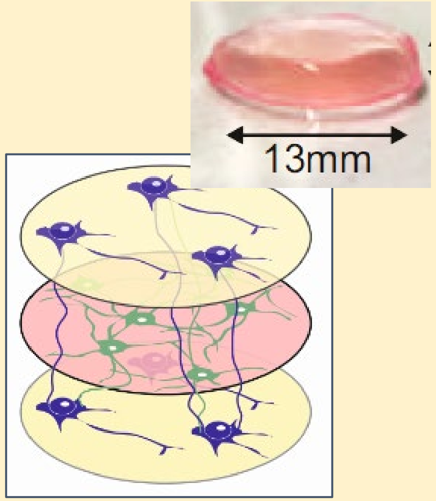
2D, homogeneous



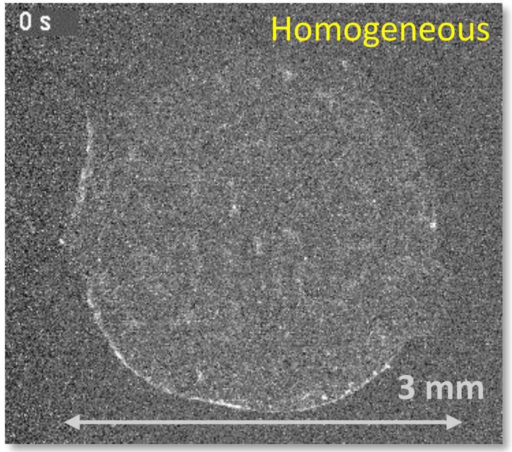
2D, topographical design
(**mesoscale patterning**)



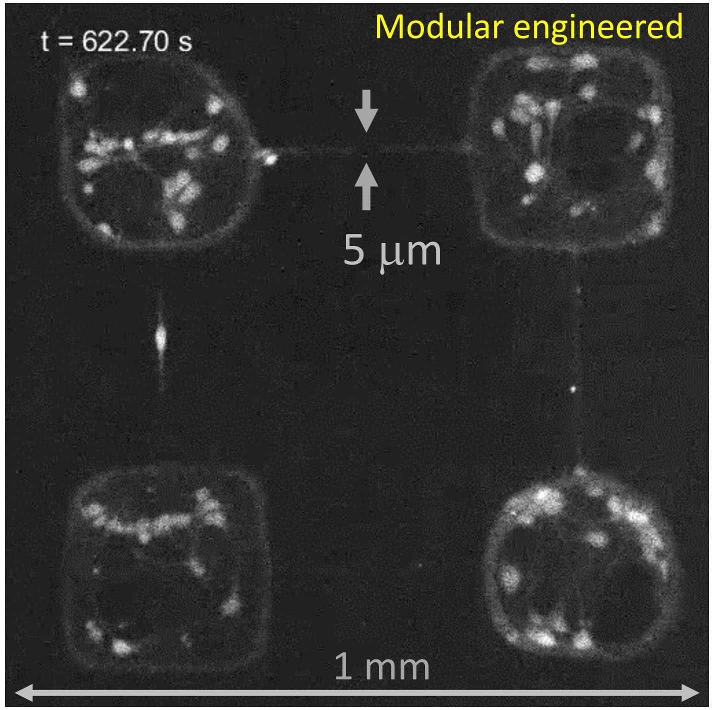
3D (hydrogels & organoids)



Our repertoire



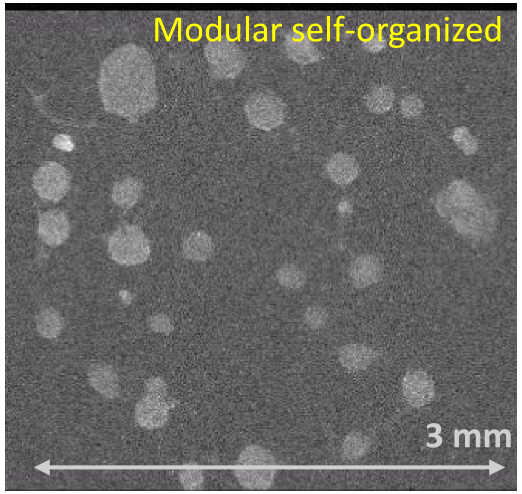
Orlandi et al., Nat Phys. 2013



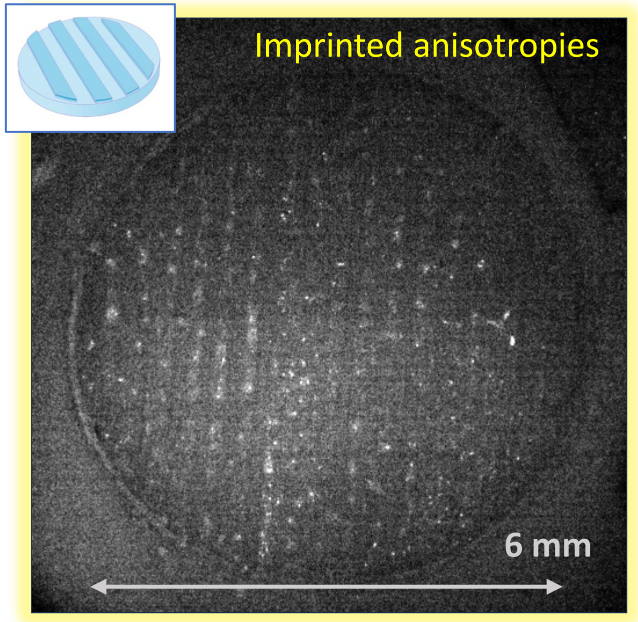
Profr. Hideaki Yanamoto

TOHOKU

Yamamoto,..., Soriano, Science Advance, 2018, 2023

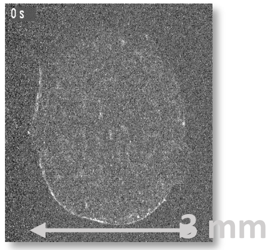


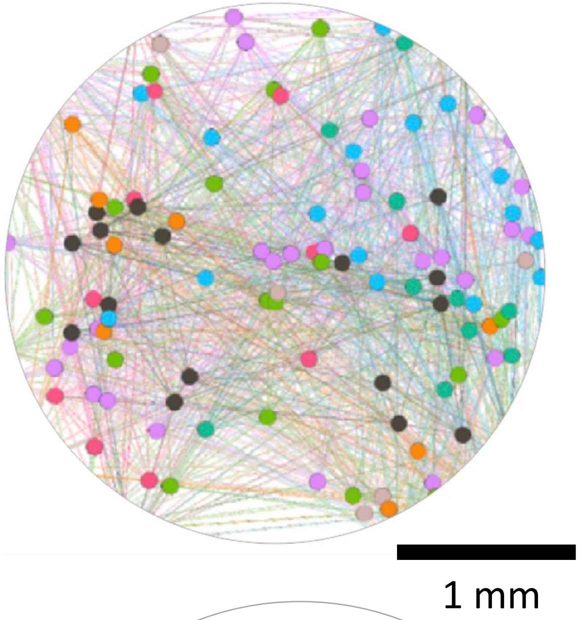
Teller et al., 2014, 2015, 2020

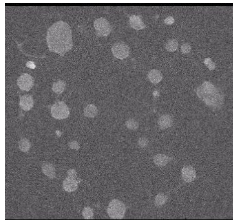


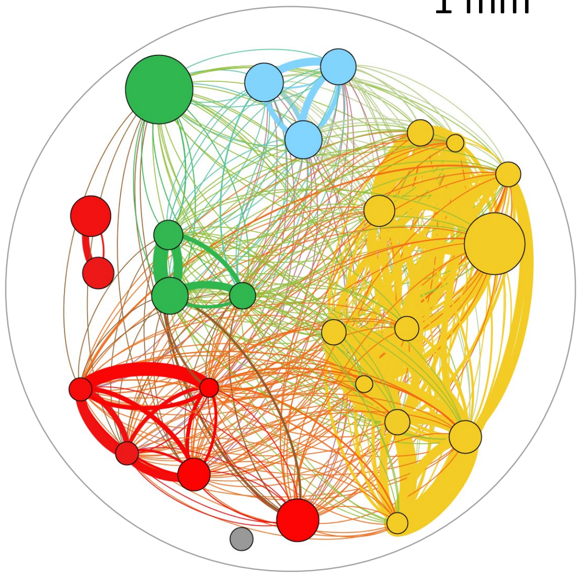
Montalà et al, iScience 2023

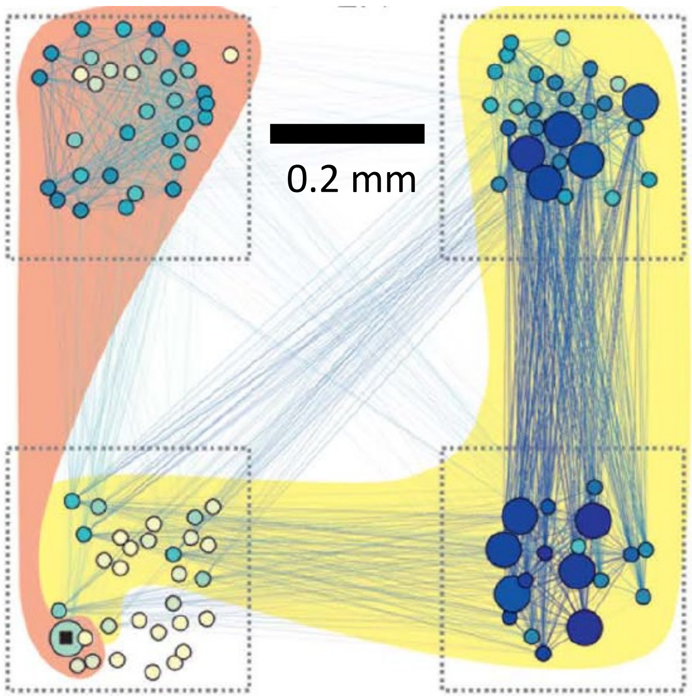
Our repertoire

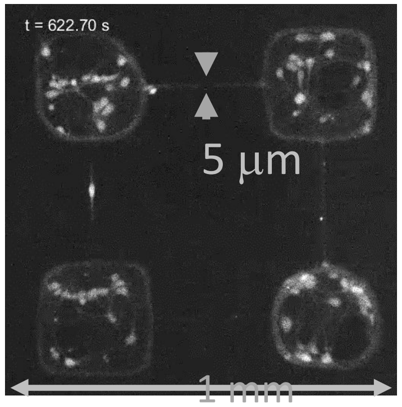


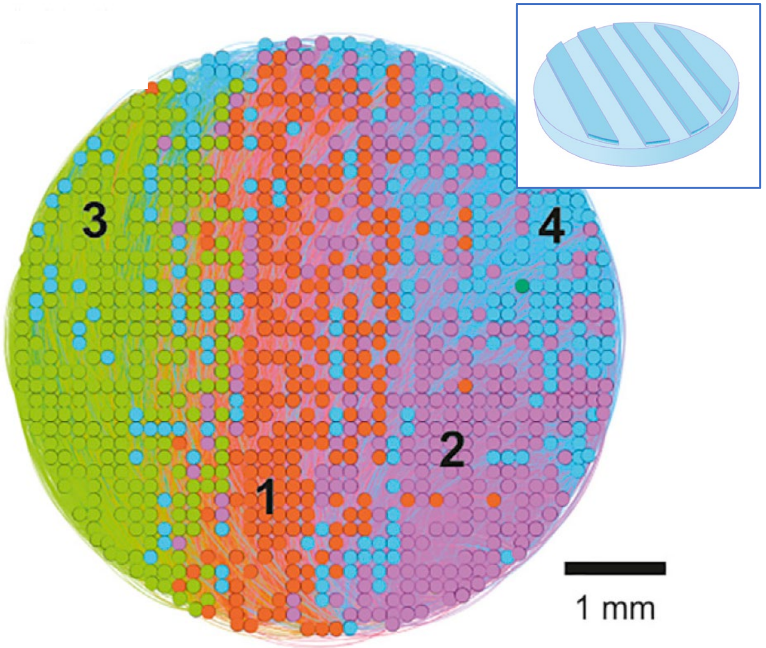


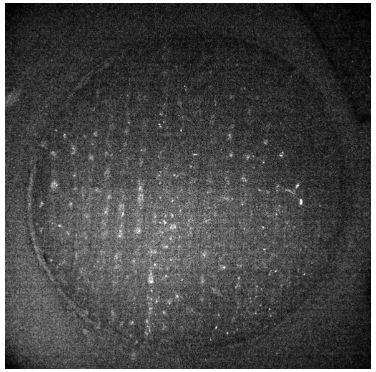


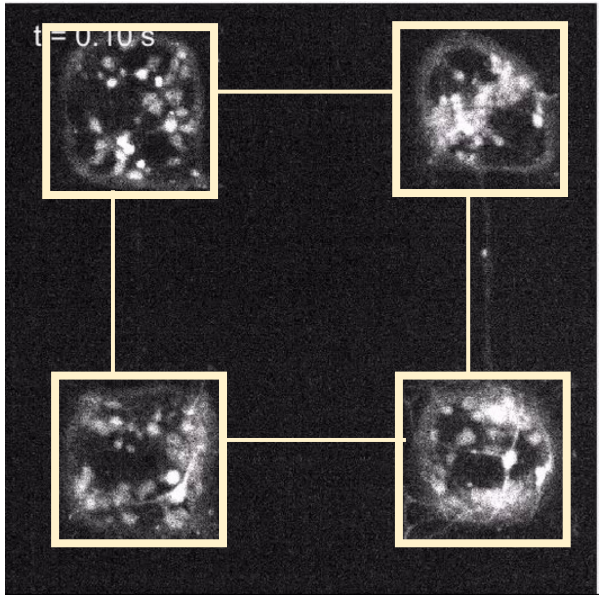








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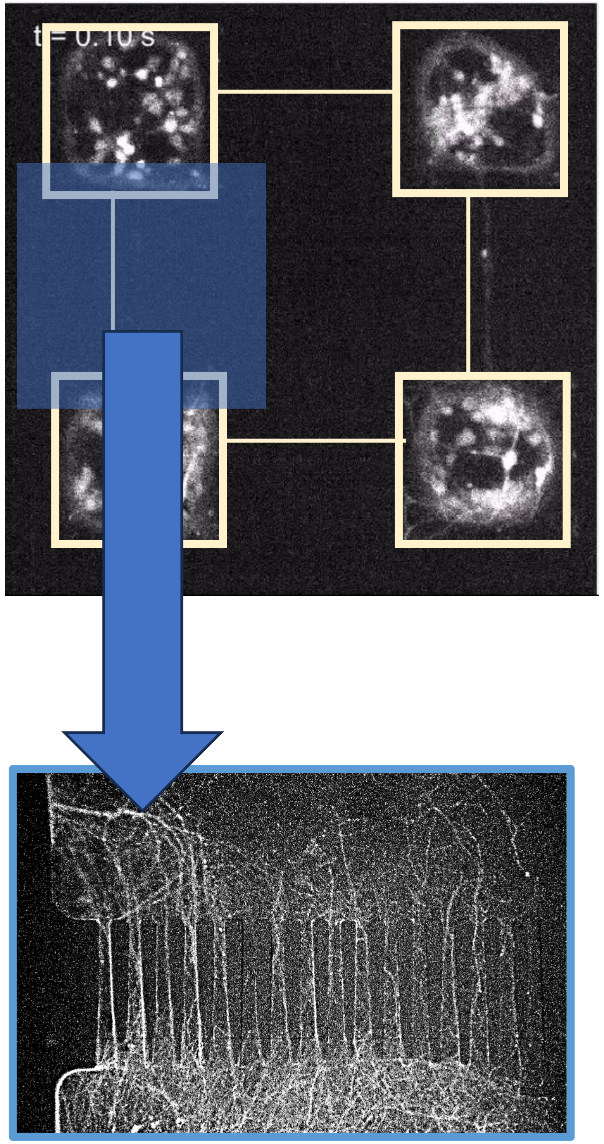
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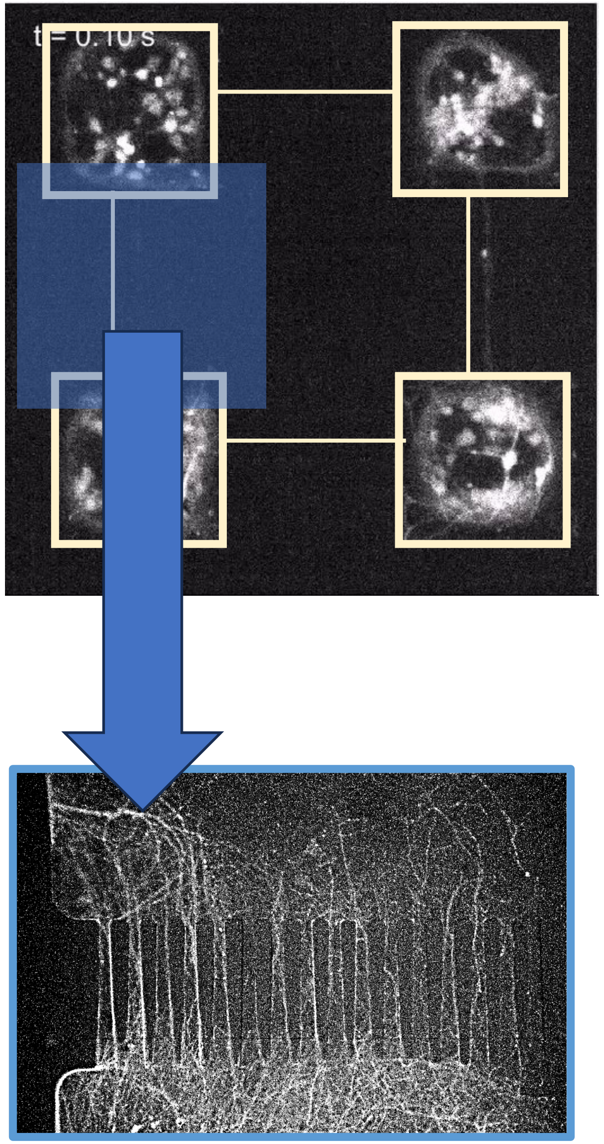
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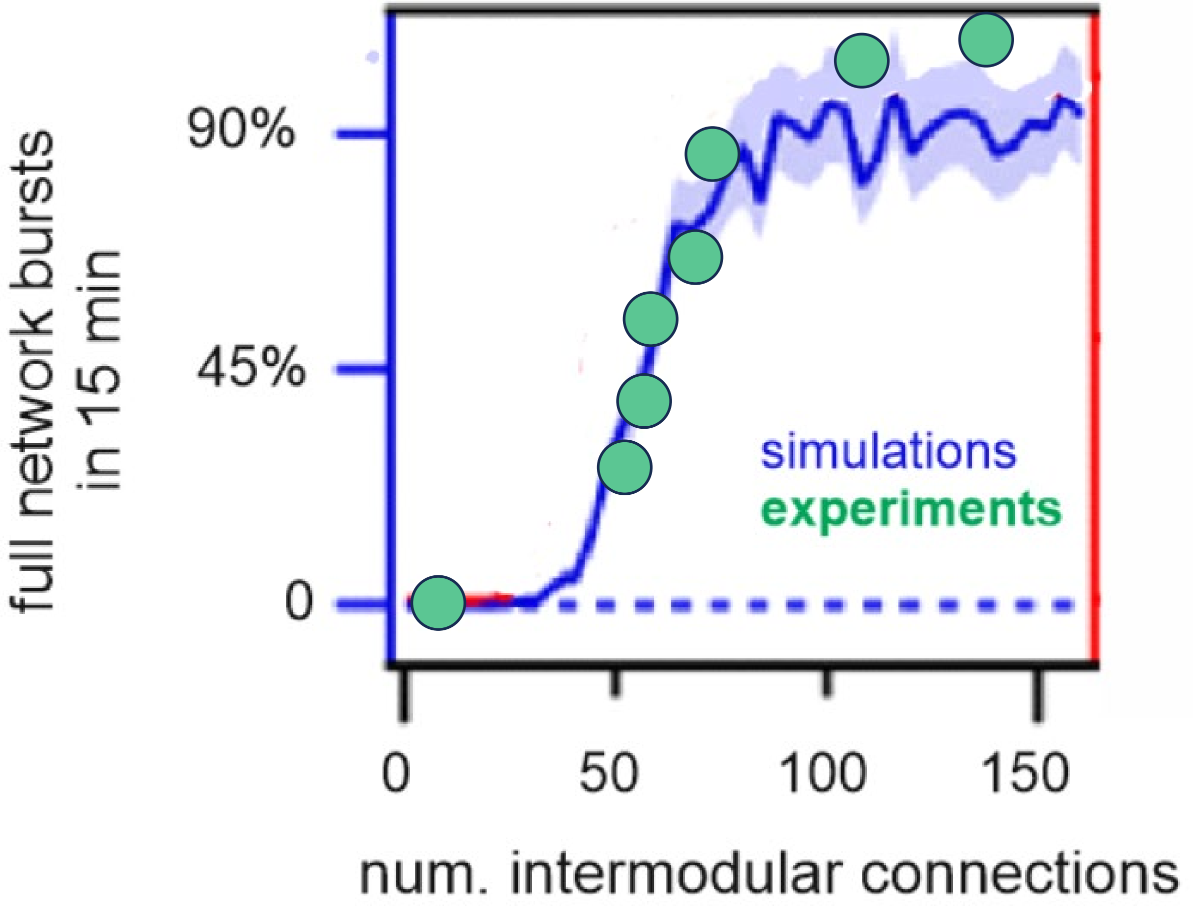
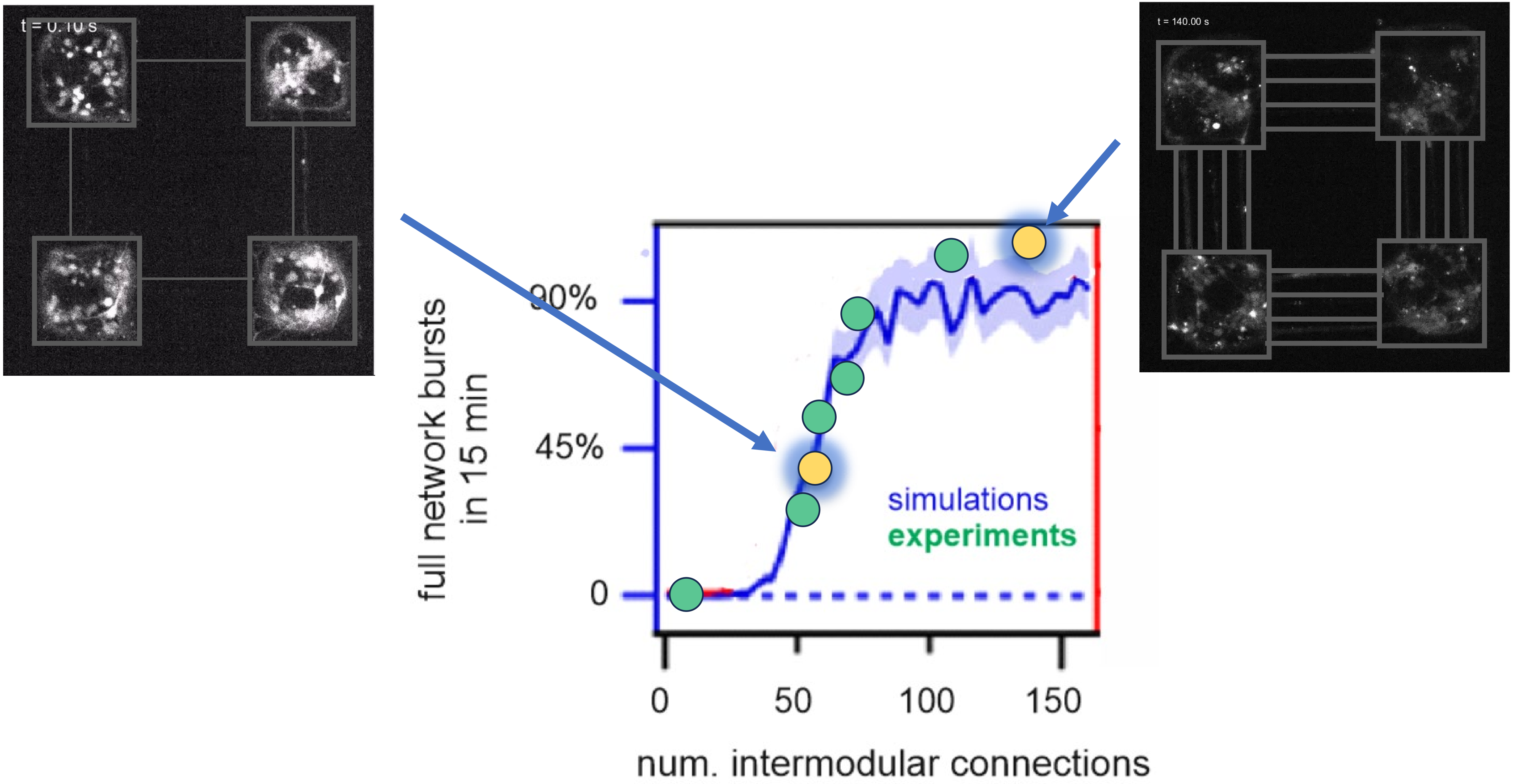
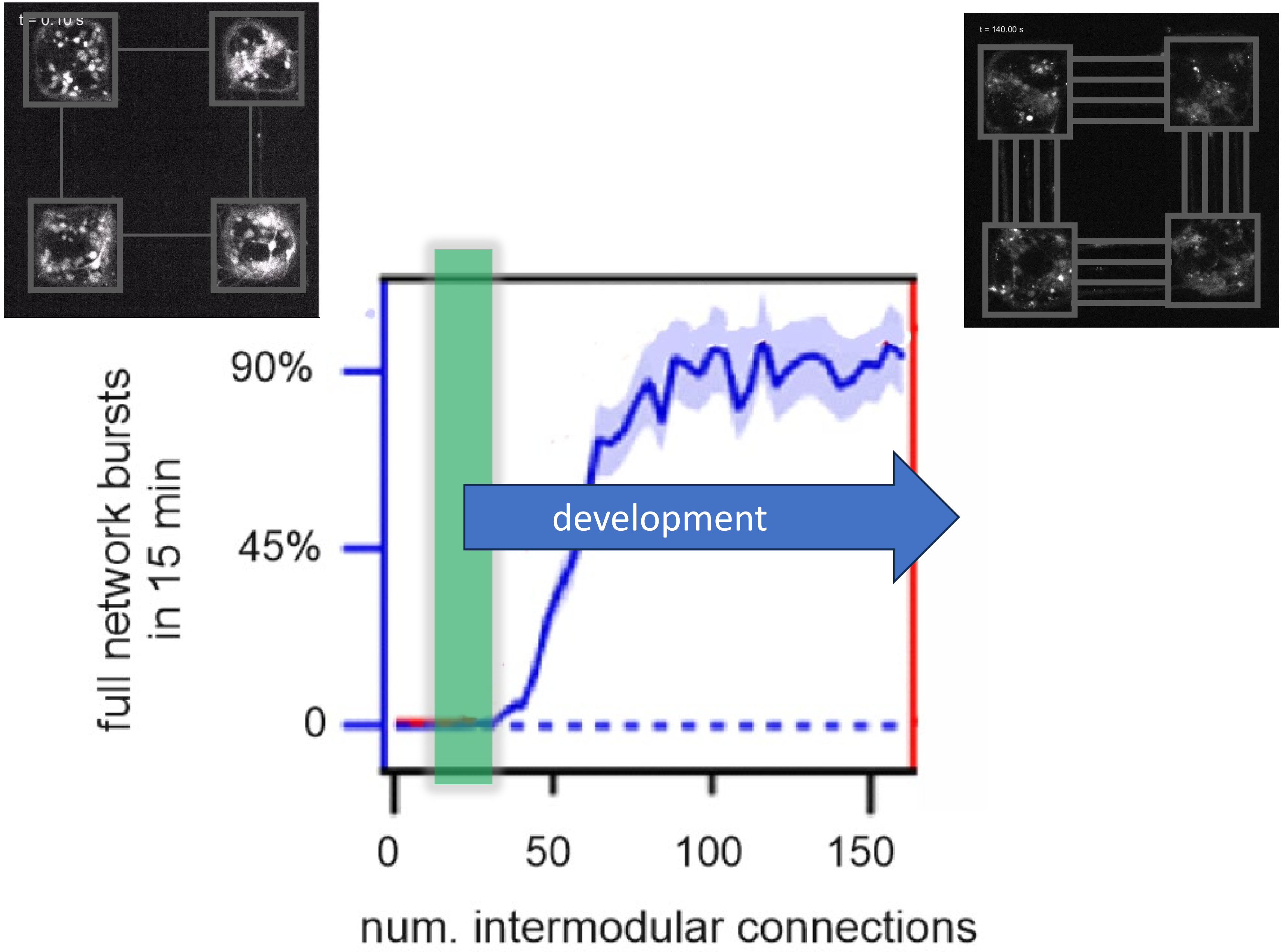

full network bursts
in 15 min
90%
45%
0
simulations
experiments
0
50
100
150
num. intermodular connections


t = 140.00 s
full network bursts
in 15 min
90%
45%
0
simulations
experiments
0
50
100
150
num. intermodular connections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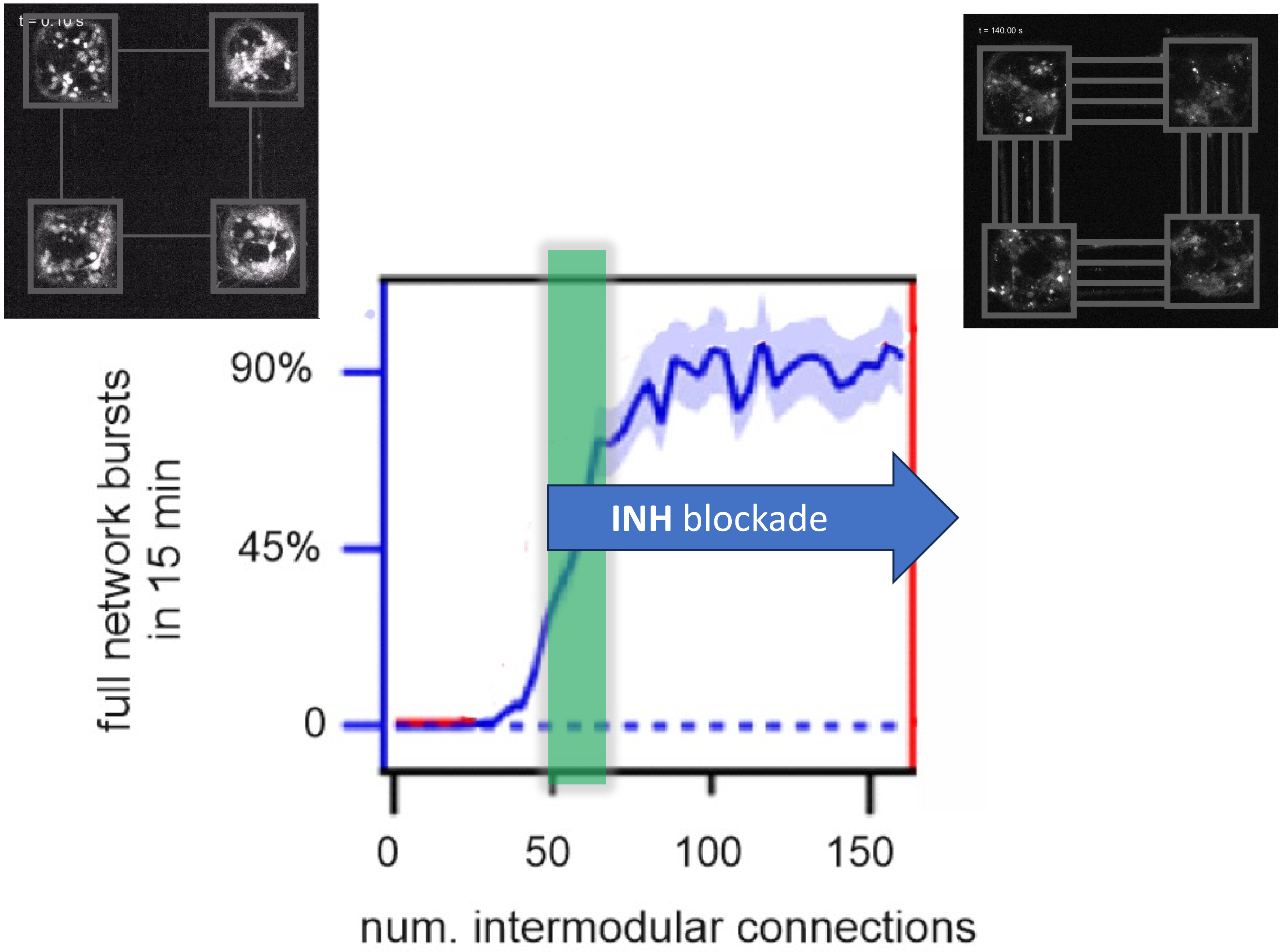
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full network bursts
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90%
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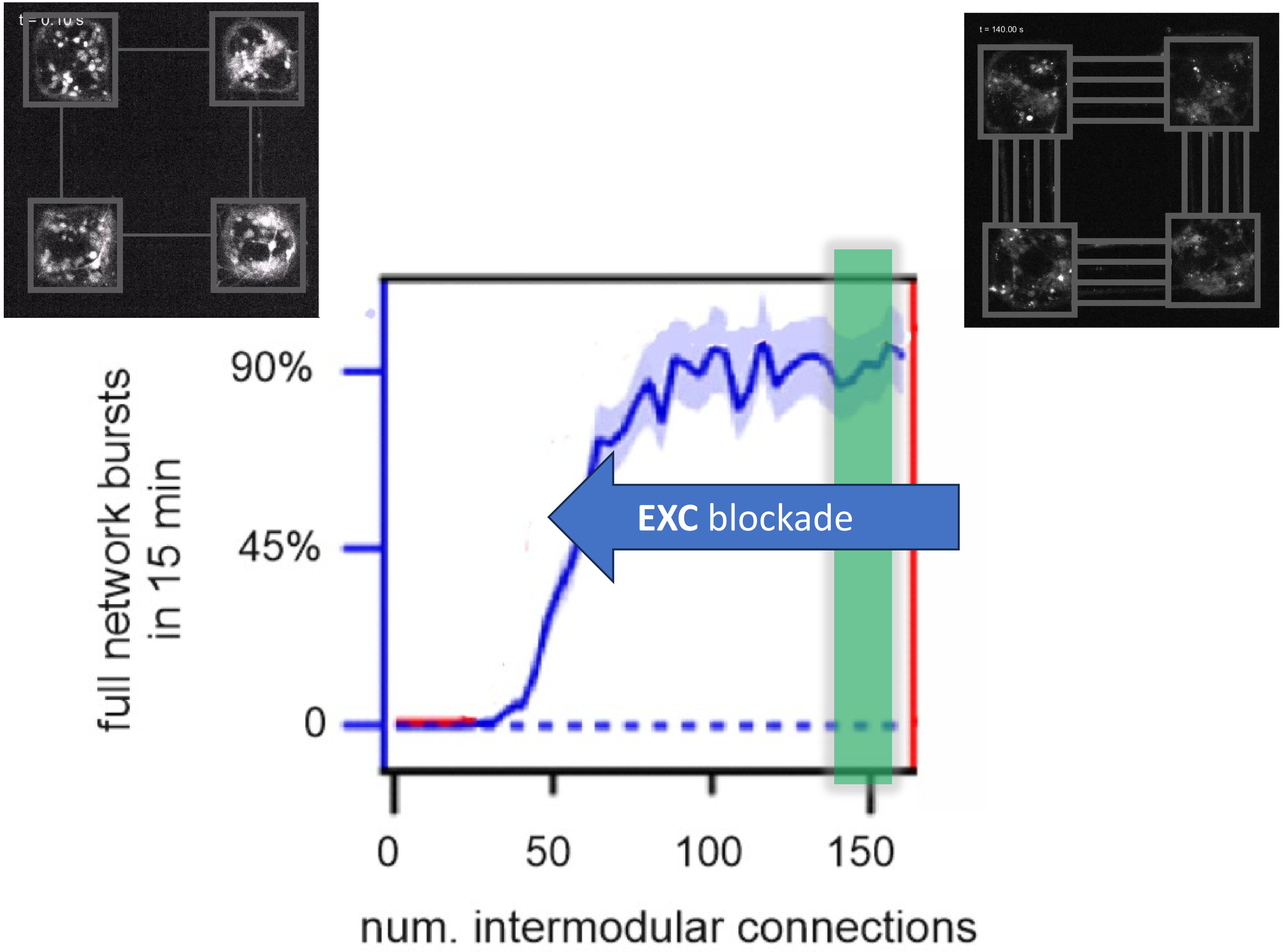
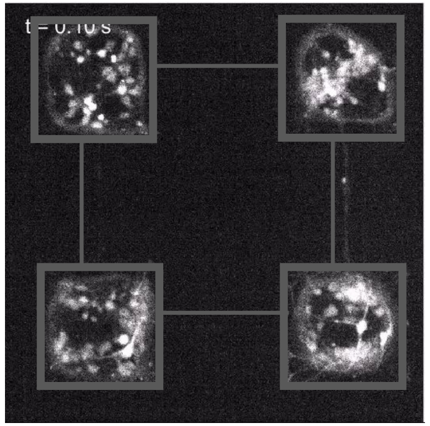
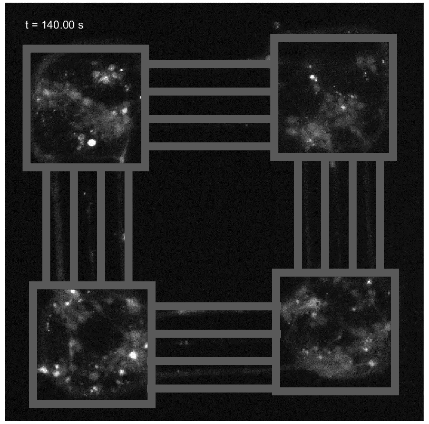
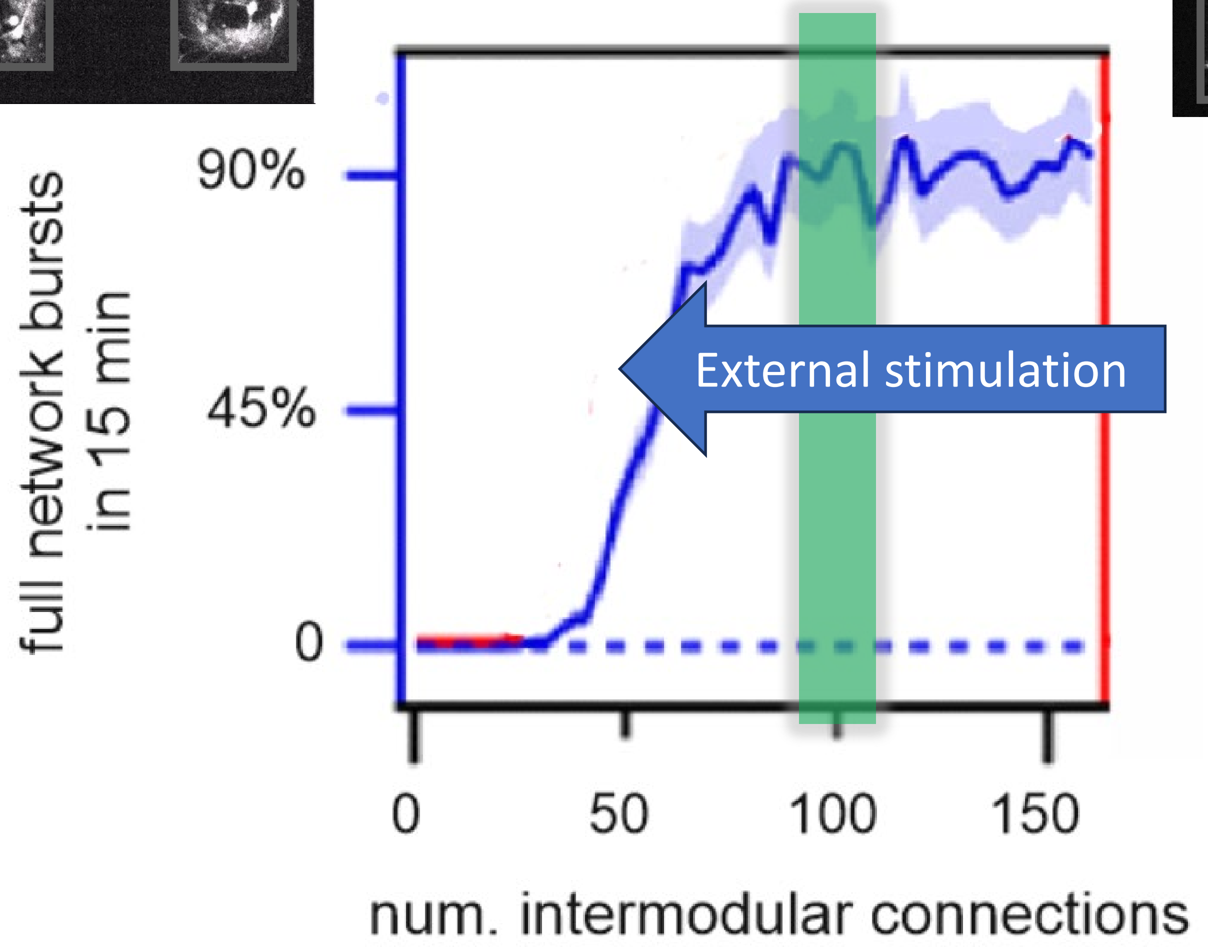
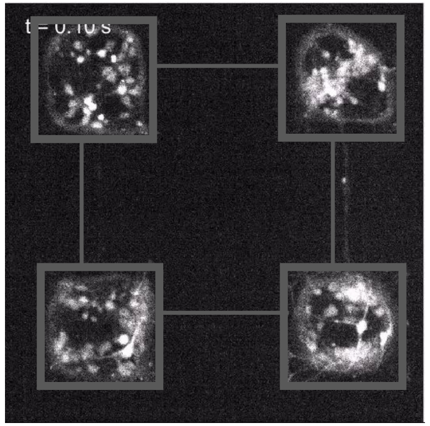
0
0
50
100
150
num. intermodular connections
INH blockade

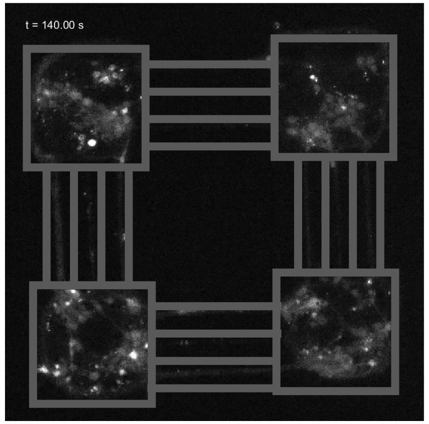

t = 140.00 s
full network bursts
in 15 min
90%
45%
0
EXC blockade
0
50
100
150
num. intermodular connections

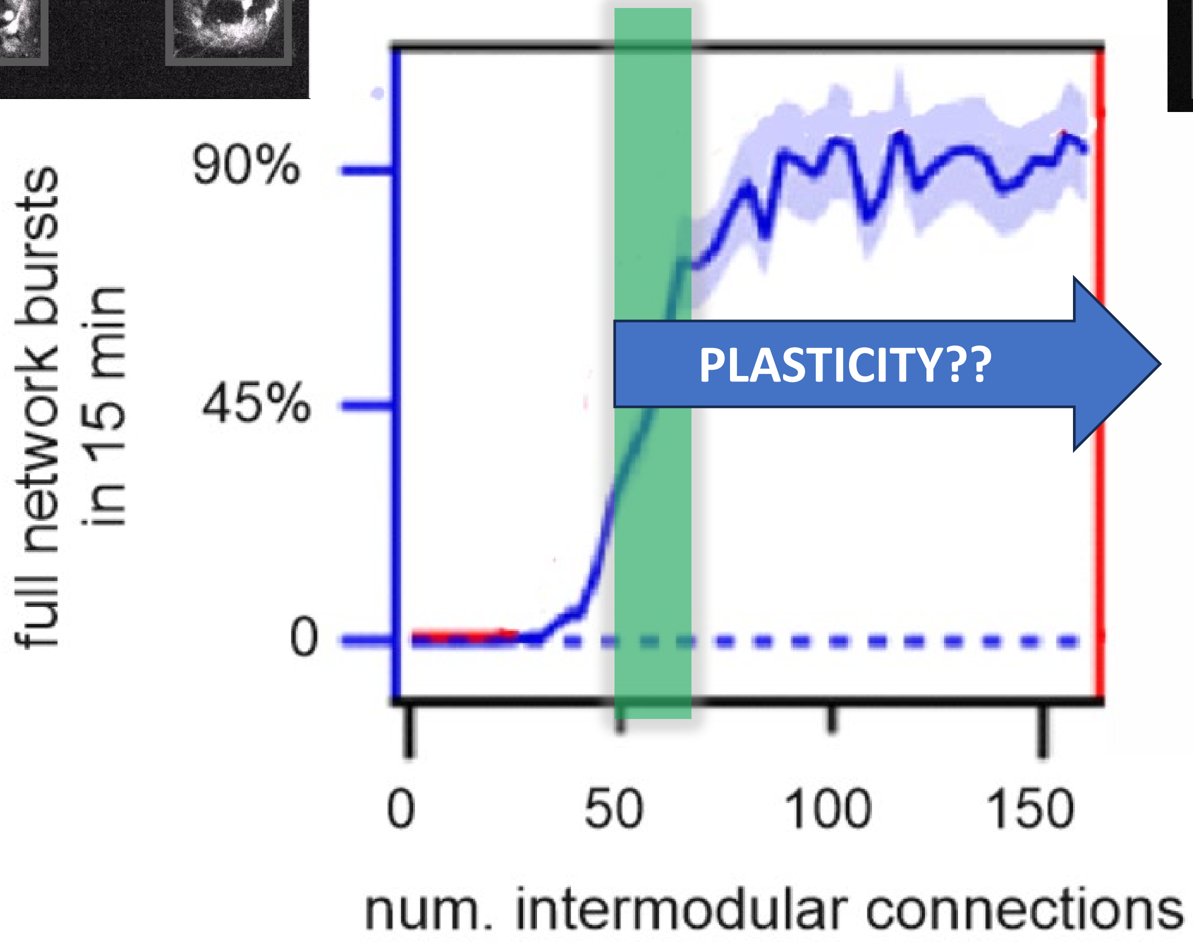



t = 140.00 s


full network bursts
in 15 min
90%
45%
0
0
50
100
150
num. intermodular connections
External stimulation

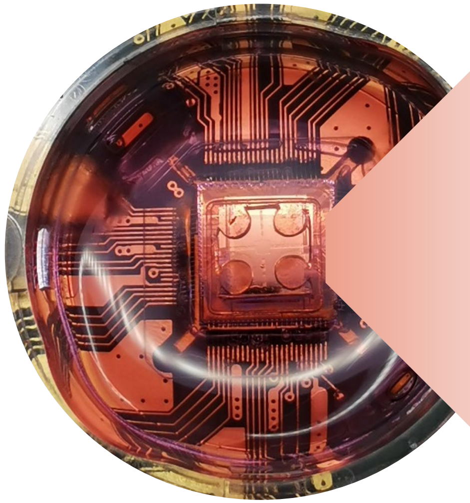


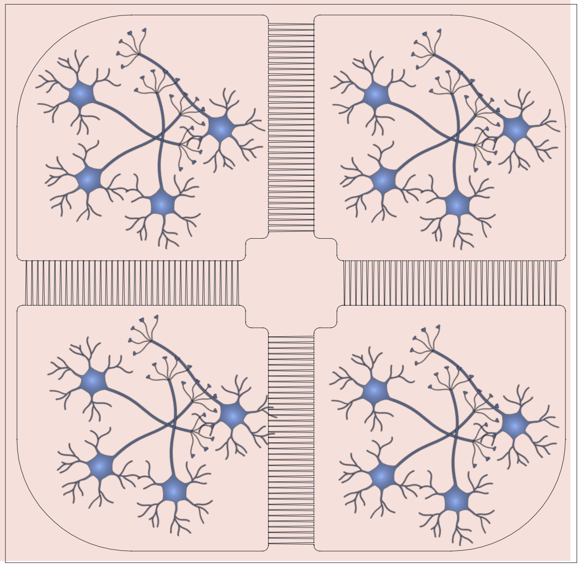

t = 140.00 s


full network bursts
in 15 min
90%
45%
0
0
50
100
150
num. intermodular connections
PLASTICITY??

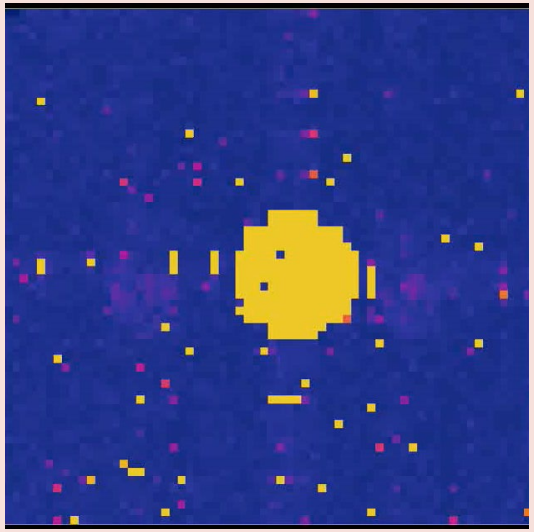
Plasticity on modular networks (with **MEAs**)

4 networks connected by channels

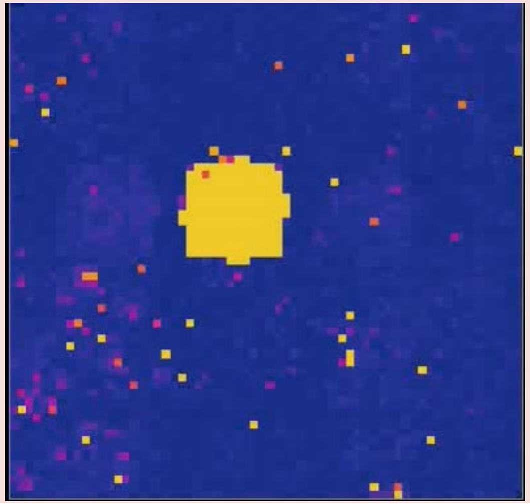




Integration



Segregation



Anna-Christina Haeb

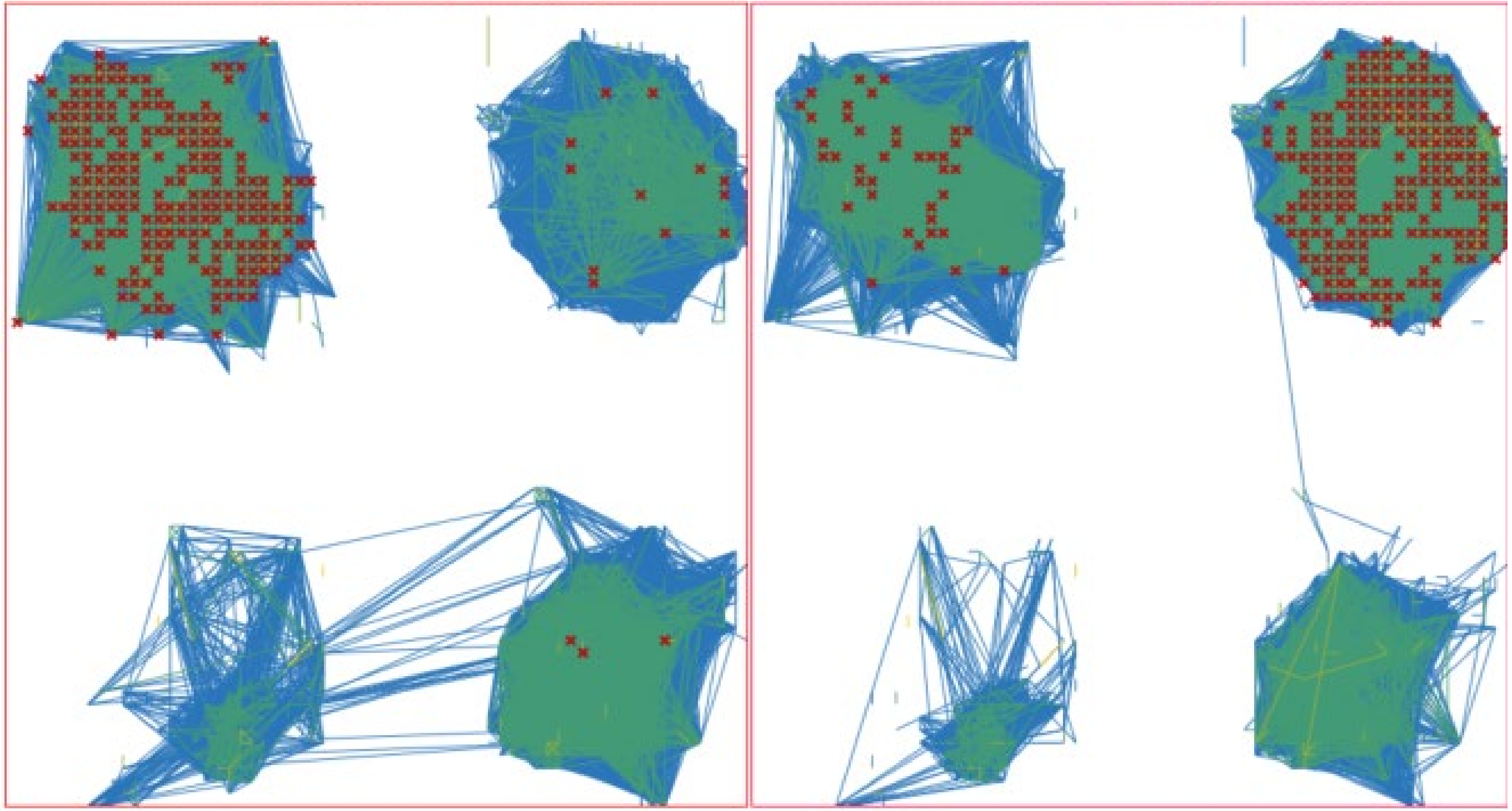
Akke Houben

Mikel Ocio

Plasticity on modular networks (with **MEAs**)

We quantify **funcional connectivity** before and after stimulation

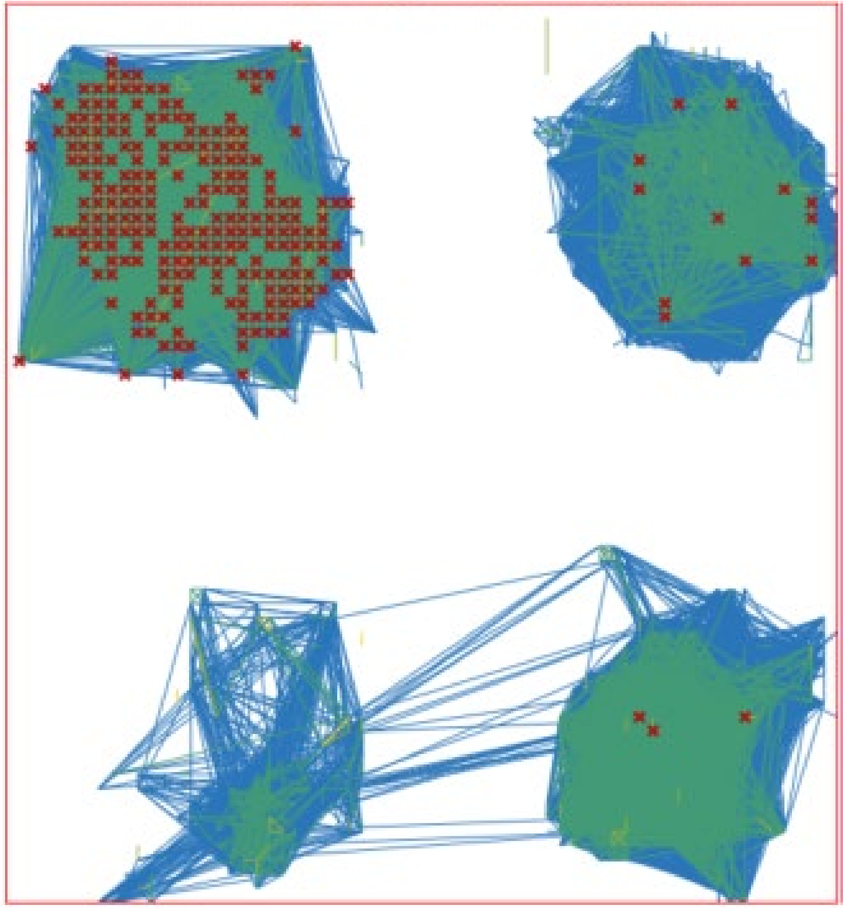
Before | After **random stimulation** (NO CHANGE)



Plasticity on modular networks (with **MEAs**)

We quantify **funcional connectivity** before and after stimulation

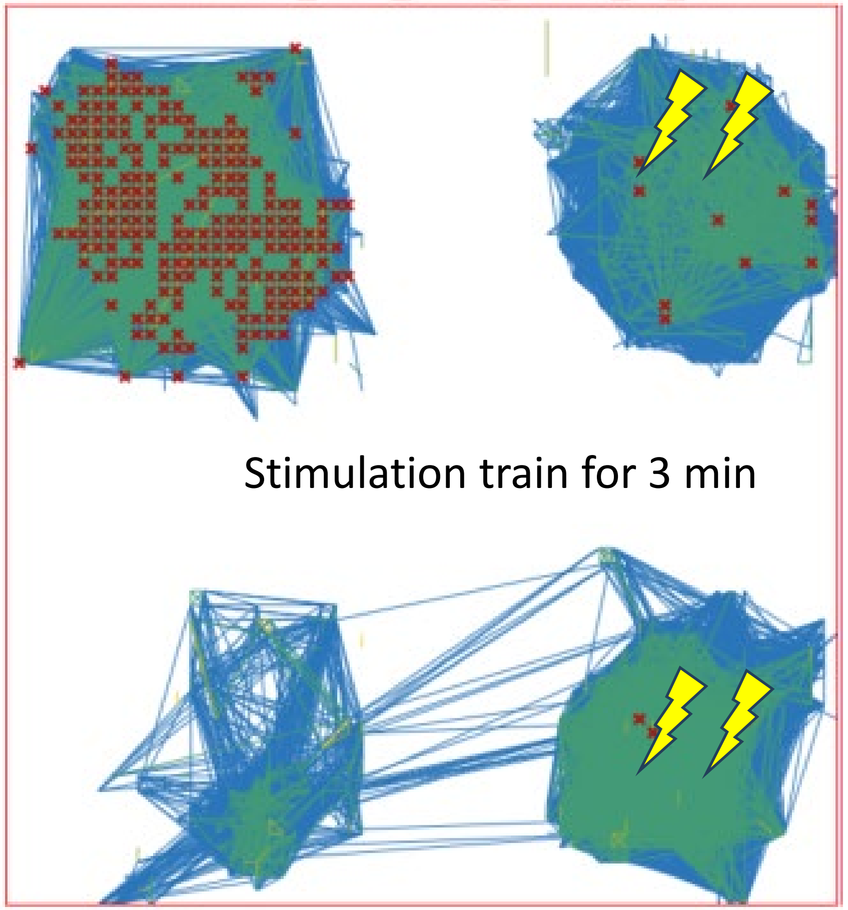
Before



Plasticity on modular networks (with **MEAs**)

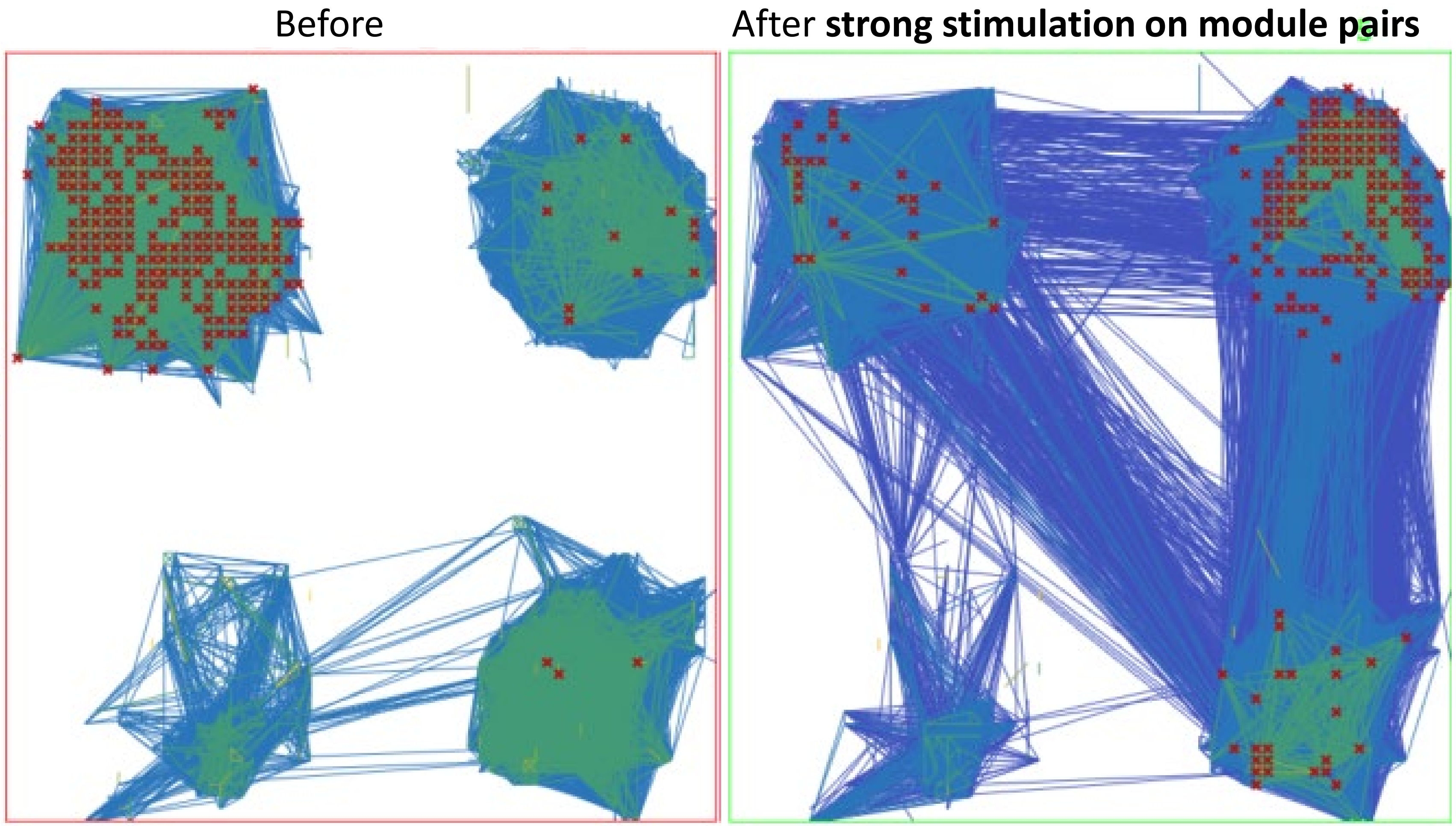
We quantify **funcional connectivity** before and after stimulation

Before



Plasticity on modular networks (with **MEAs**)

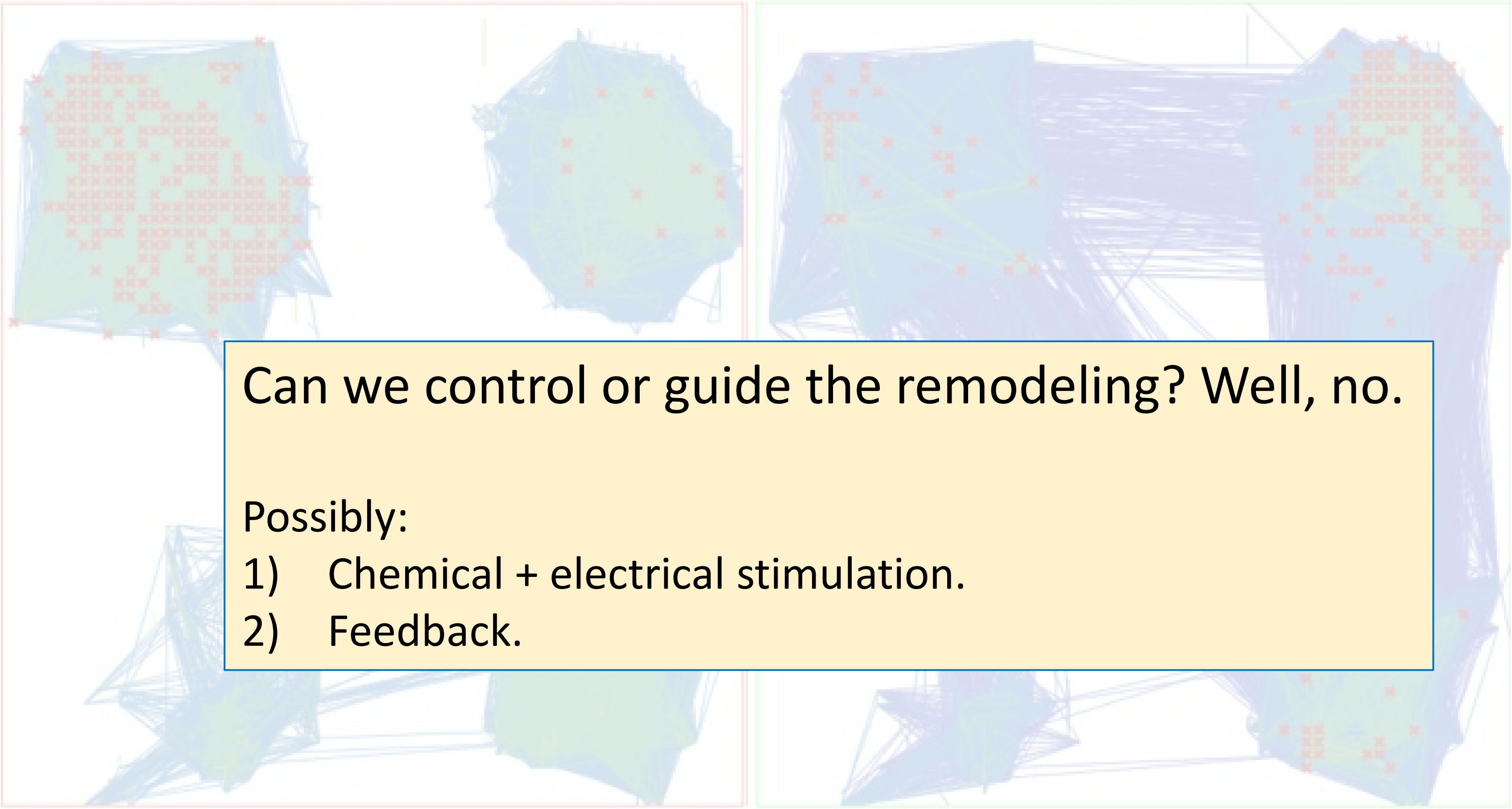
We quantify **funcional connectivity** before and after stimulation



Plasticity on modular networks (with **MEAs**)

We quantify **funcional connectivity** before and after stimulation

Before | After **strong stimulation on module pairs**



Can we control or guide the remodeling? Well, no.

Possibly:

1) Chemical + electrical stimulation.
2) Feedback.

$\boxed{3}$

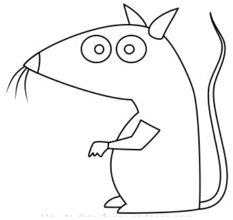
Neuronal cultures as complexity labs

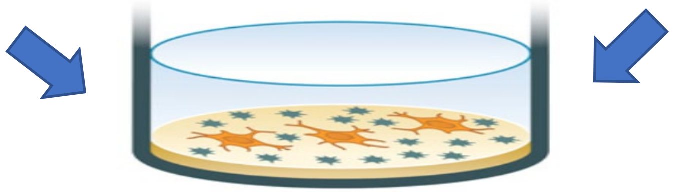
Neuronal cultures and engineering

Yamanaka 2012

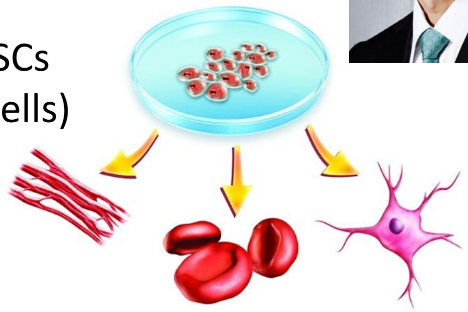
CELL SOURCE

Rat primary cultures

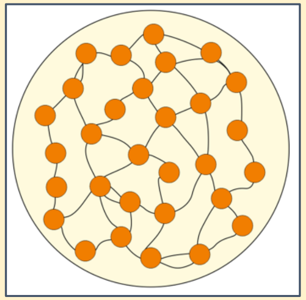




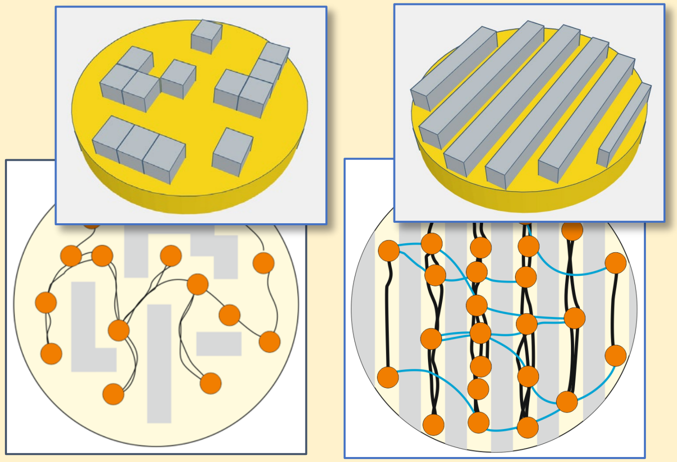
Human iPSCs
(induced stem cells)



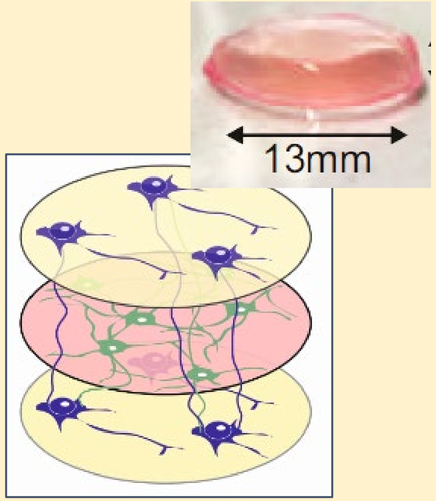
2D, homogeneous



2D, topographical design
(**mesoscale patterning**)



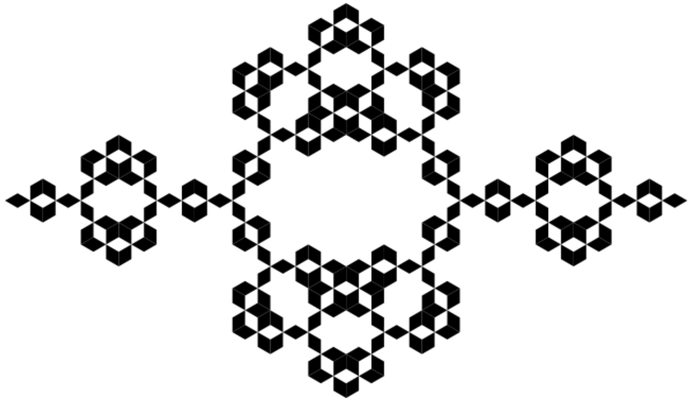
3D (hydrogels & organoids)

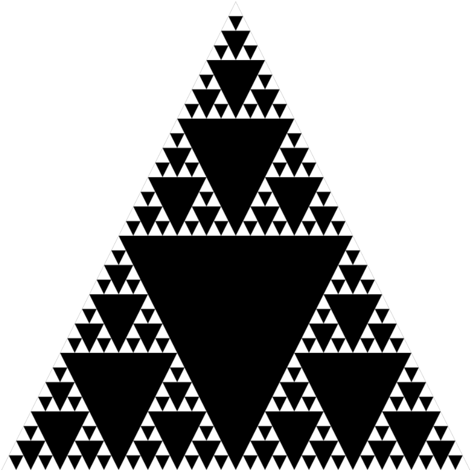


Network structure shapes dynamics

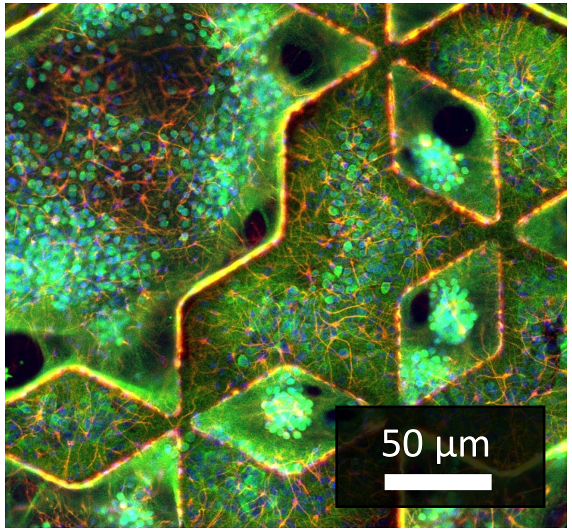
Mireia Olives

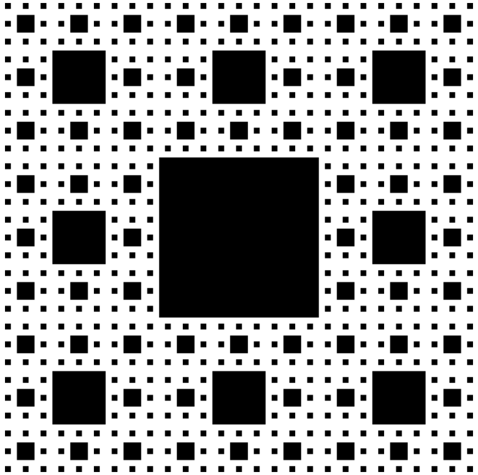
Concept: place neurons in areas of a broad range of sizes





Sierpinski triangle

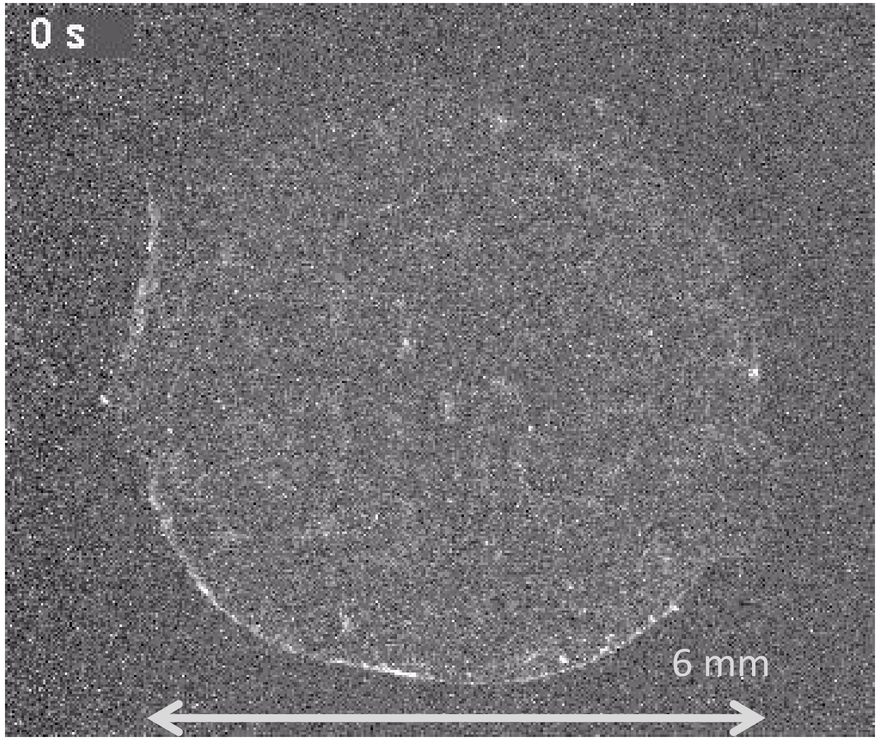


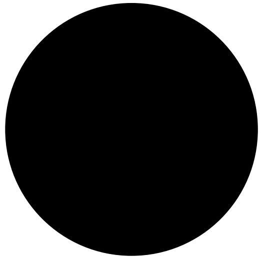


Sierpinski square

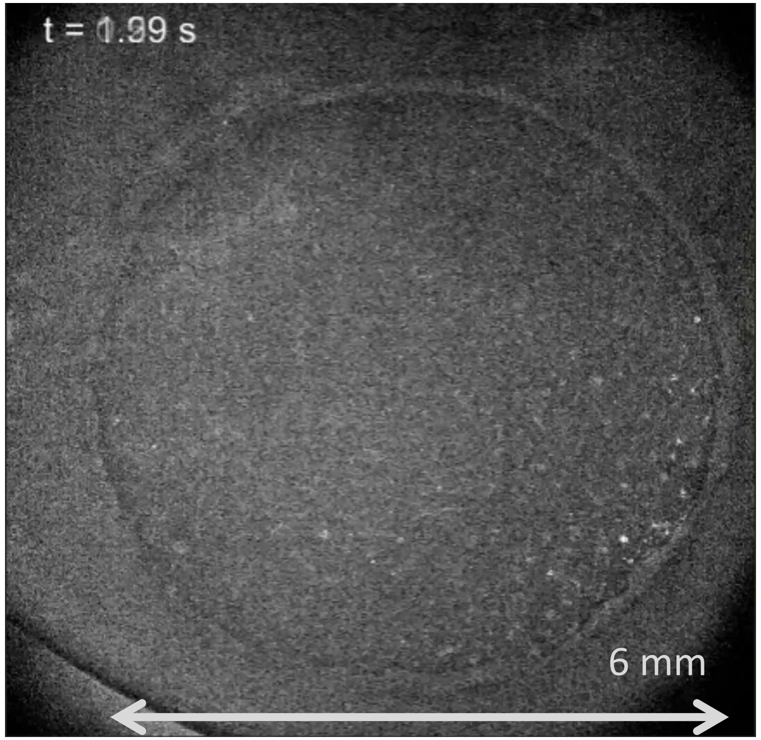
Network structure shapes dynamics

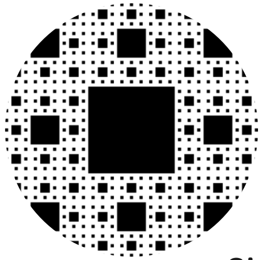
HOMOGENEOUS





FRACTAL

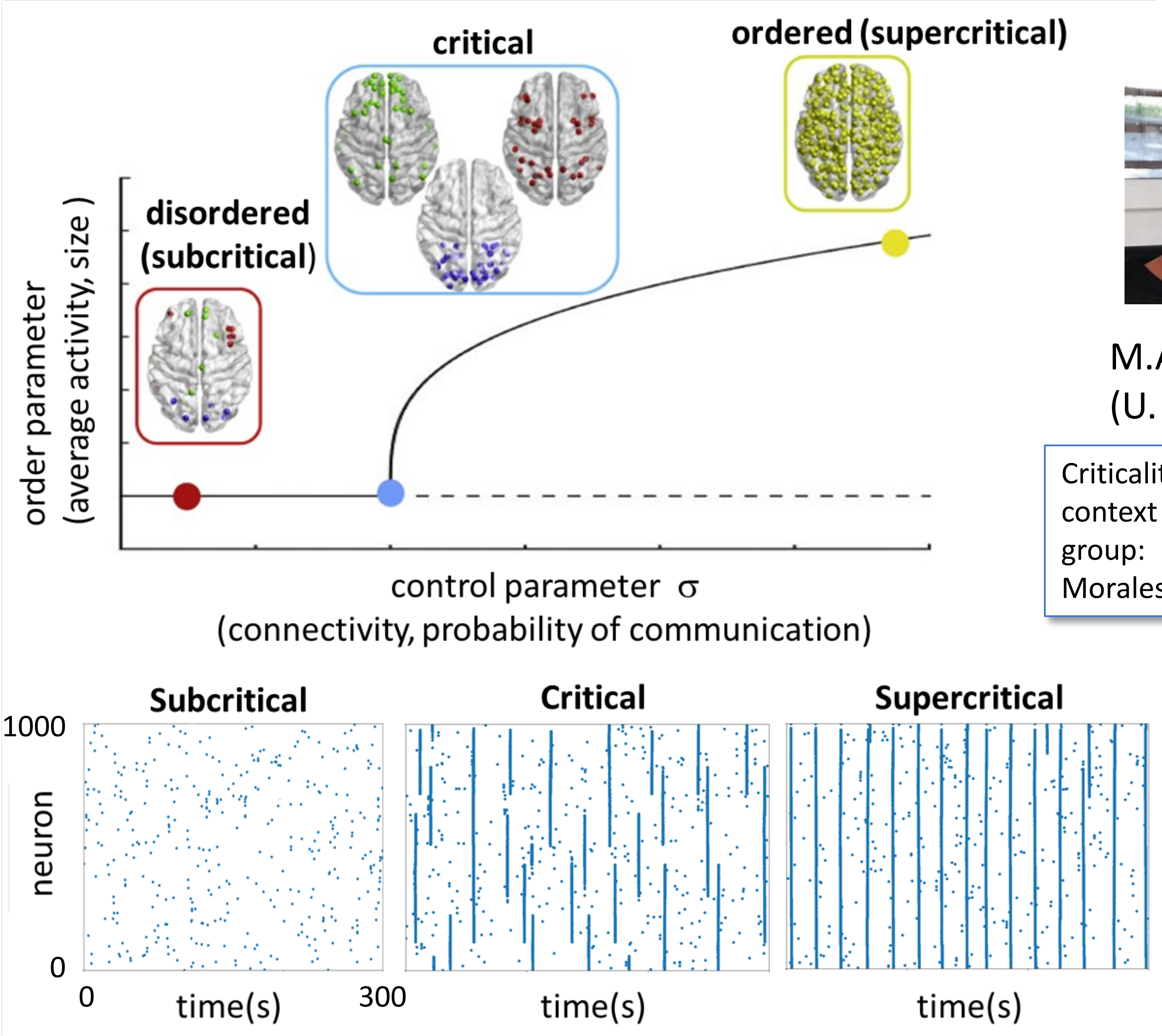




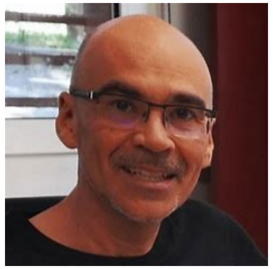
Sierpinski square

Olives at al., in preparation

Is it brain-like? Compare in the context of critcality



(numerical simulations)

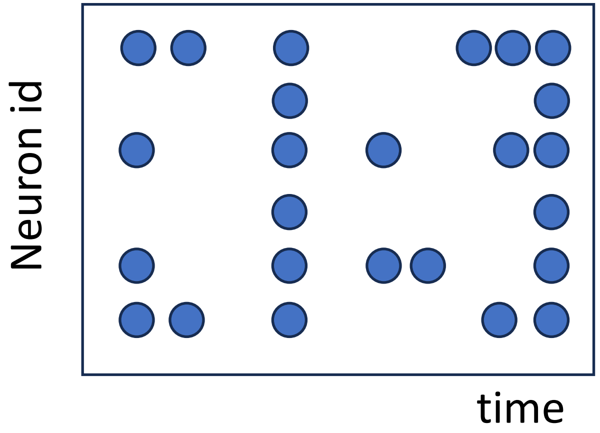


M.A. Muñoz
(U. Granada, Spain)

Criticality analysis in the context of renormalization group:
Morales et al., *PNAS* 2023

Renormalization group approach

1 Consider your original raster plot. $\mathbf{K_0 = 6}$



Renormalization group approach

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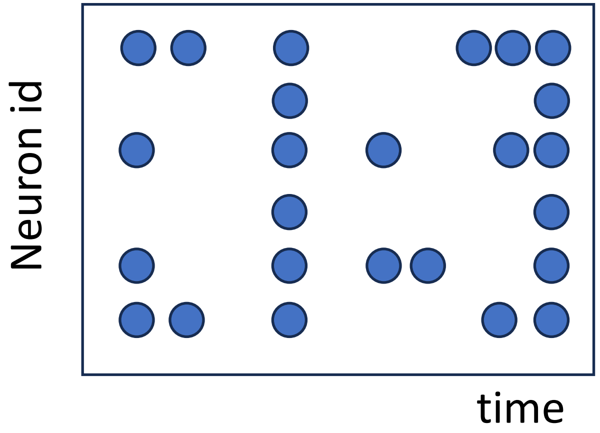
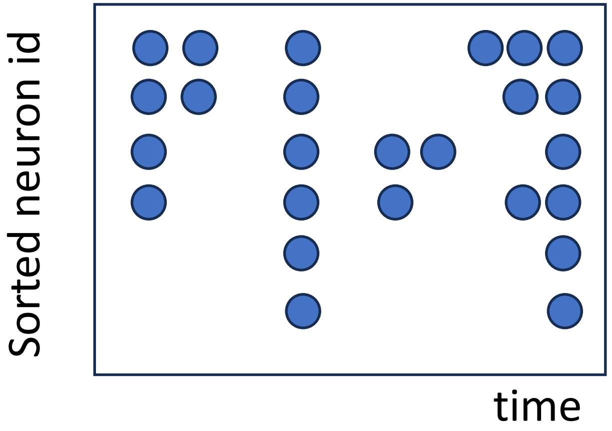


2 Sort neurons by similarity (cross correlation)



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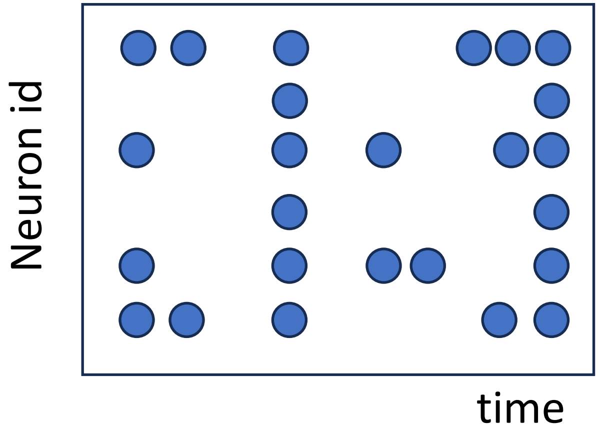

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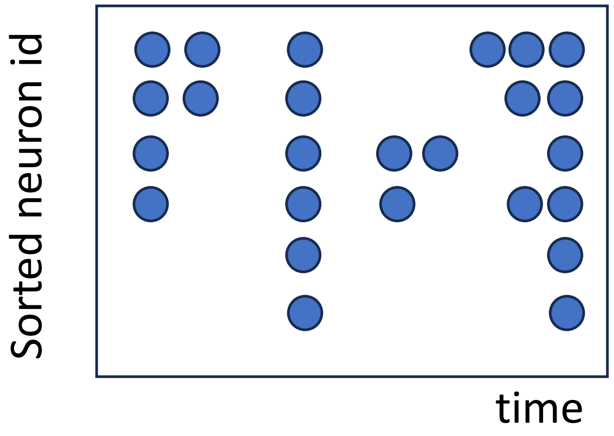
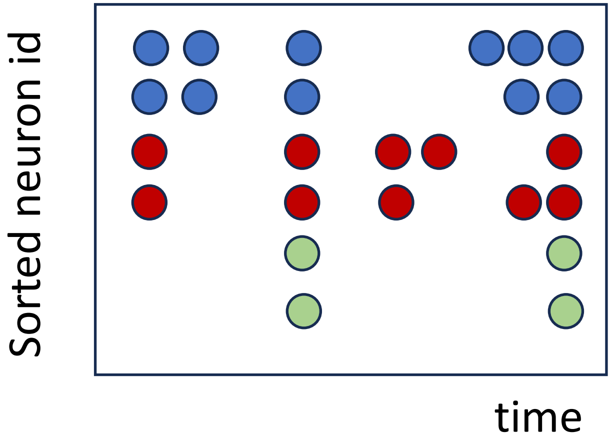


3 Sum up activity **in pairs** (k=2) of similar neurons. $K_1 = 3$



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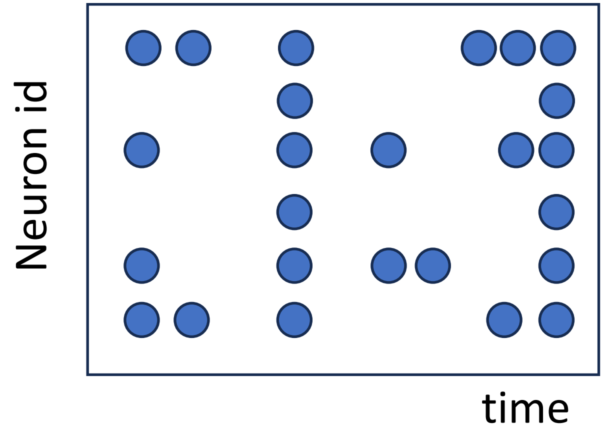

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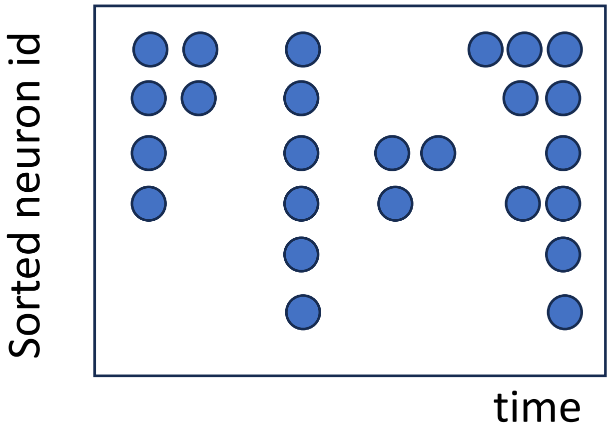

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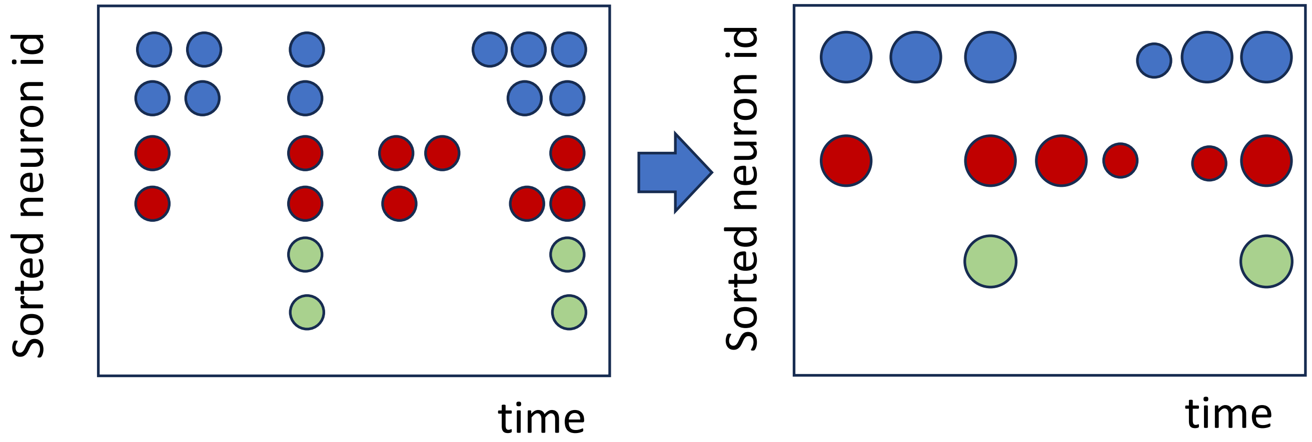

Renormalization group approach

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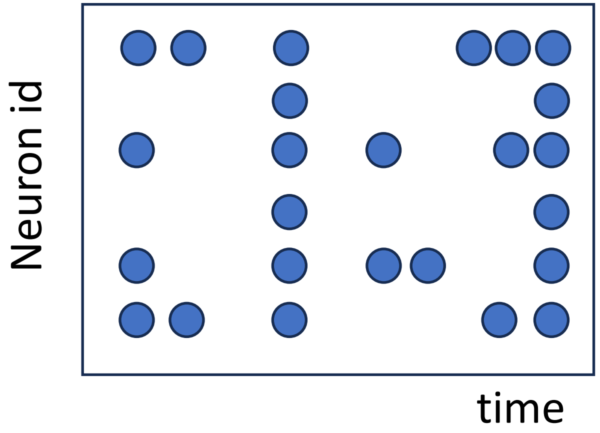

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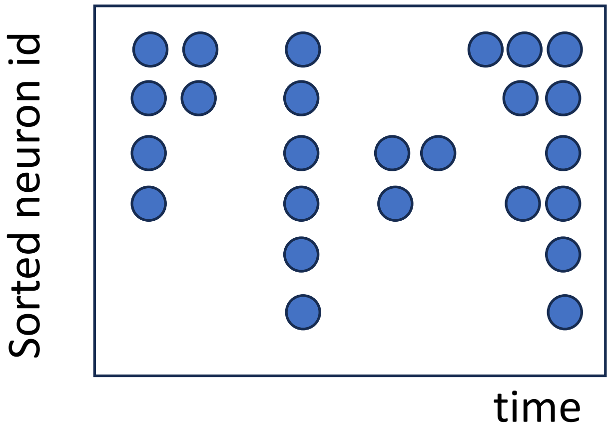

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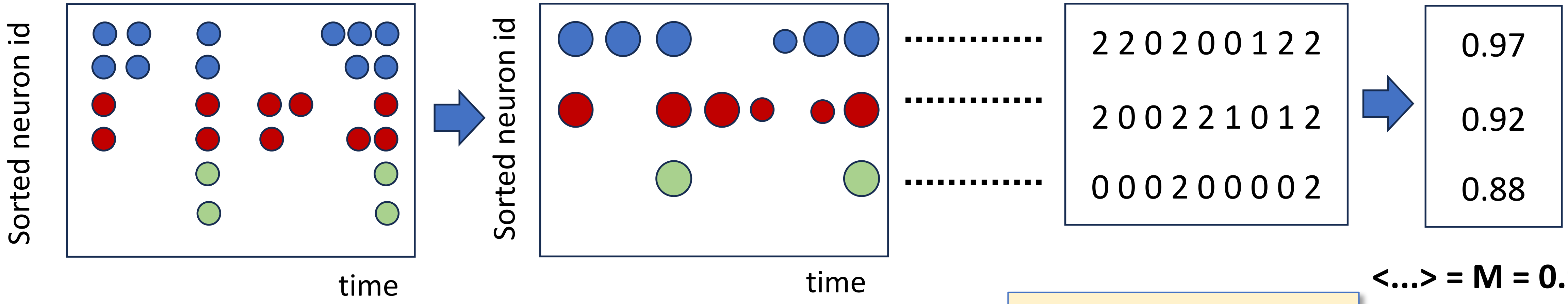
4 Compute variance of spike trains & average

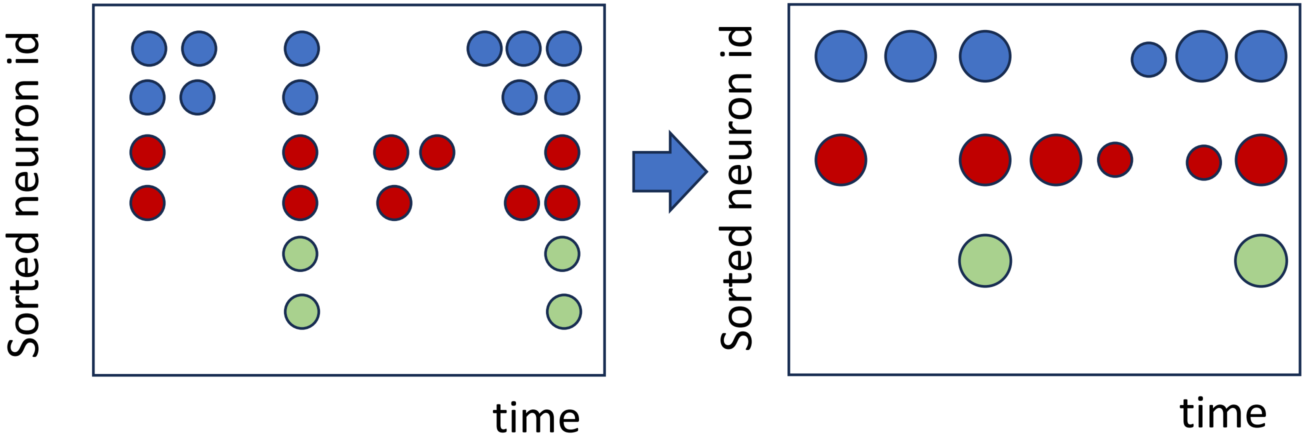


<...> = M = 0.92

Repeat to next K_n

Renormalization group approach

3 Sum up activity **in pairs** (k=2) of similar neurons. $K_1 = 3$



4 Compute variance of spikes trains & average

2 2 0 2 0 0 1 2 2	0.97
2 0 0 2 2 1 0 1 2	0.92
0 0 0 2 0 0 0 0 2	0.88

<...> = M = 0.92

Repeat to next K_n

Extreme cases:

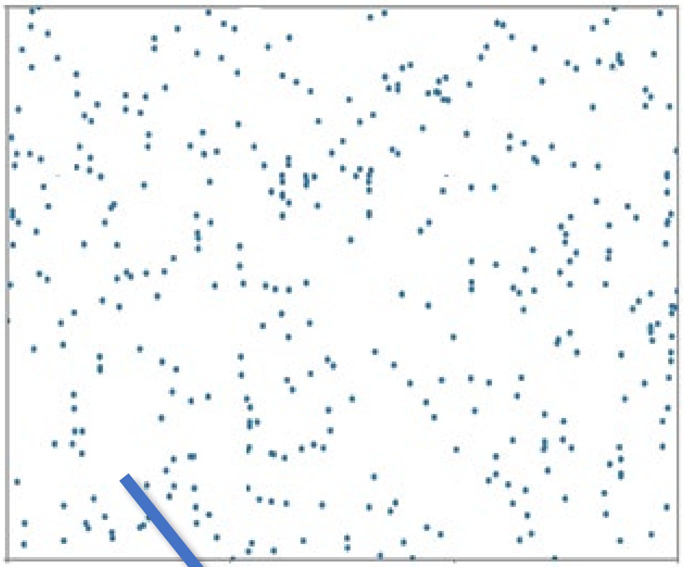
Independent:

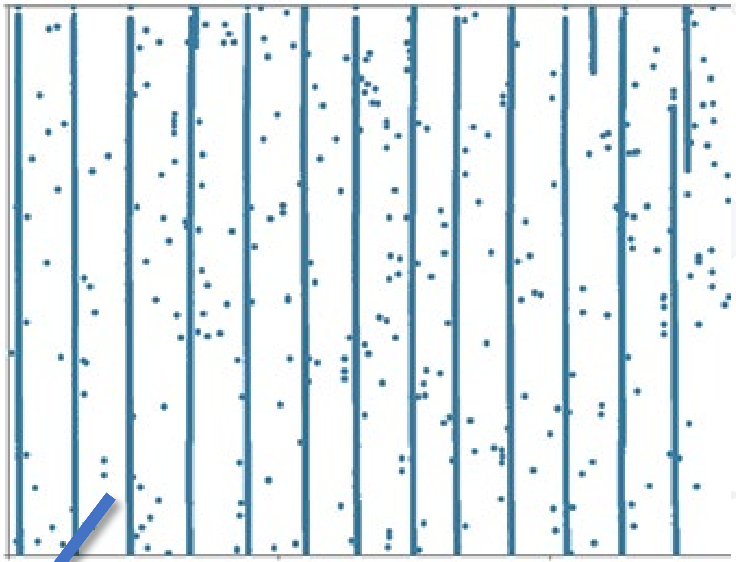
$$Var\left(\sum_{j=1}^{K} x_j\right) = \sum_{j=1}^{K} Var\left(x_j\right) \rightarrow M\ (K) = \frac{1}{N_k}\sum_{i=1}^{N_k}\sum_{j=1}^{K} Var\left(\sum_{j=1}^{K} x_j\right) \sim K^{1}$$

Dependent:

$$Var\left(\sum_{j=1}^{K} x_j\right) \sim K^{2}$$

Renormalization group approach





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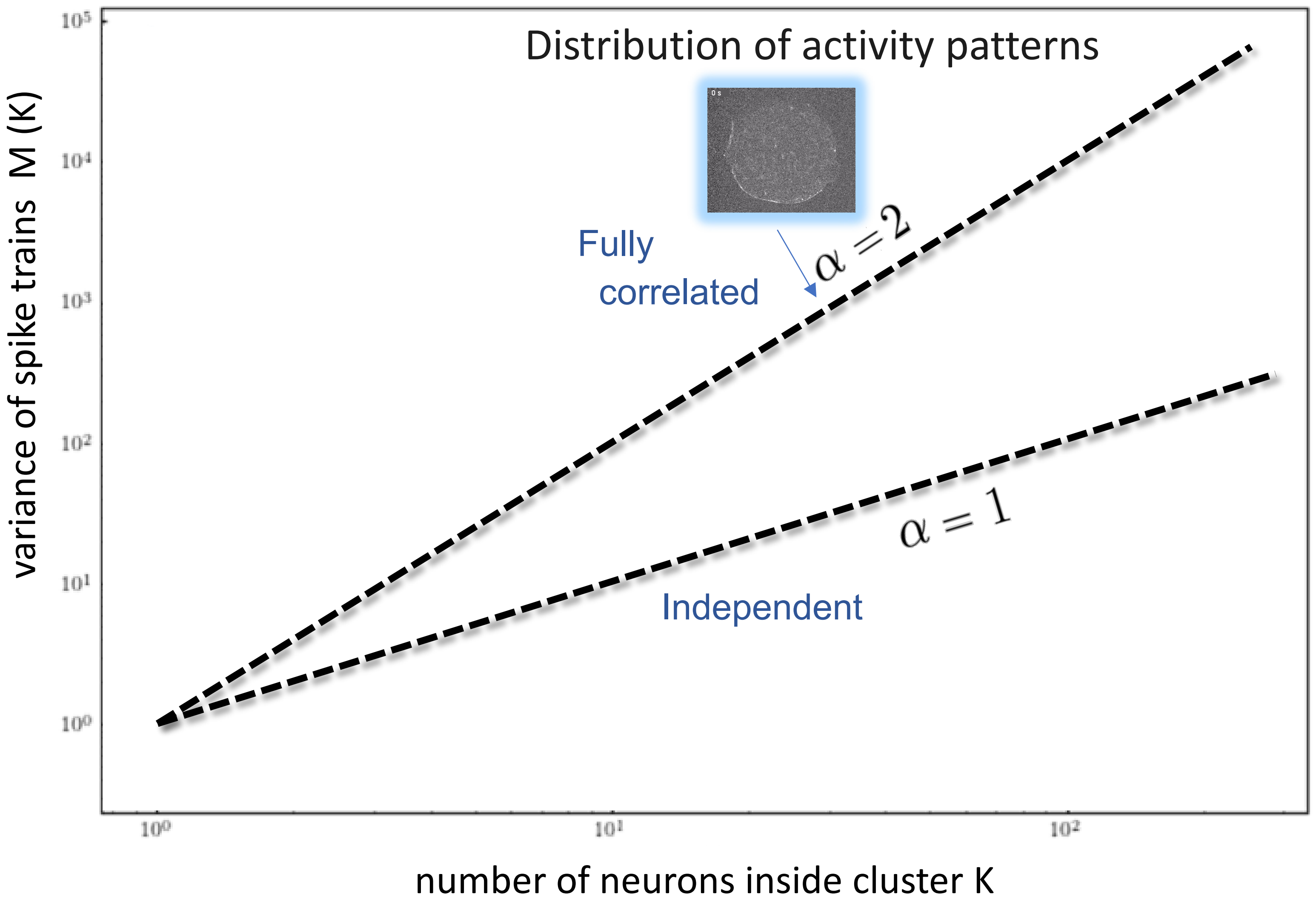
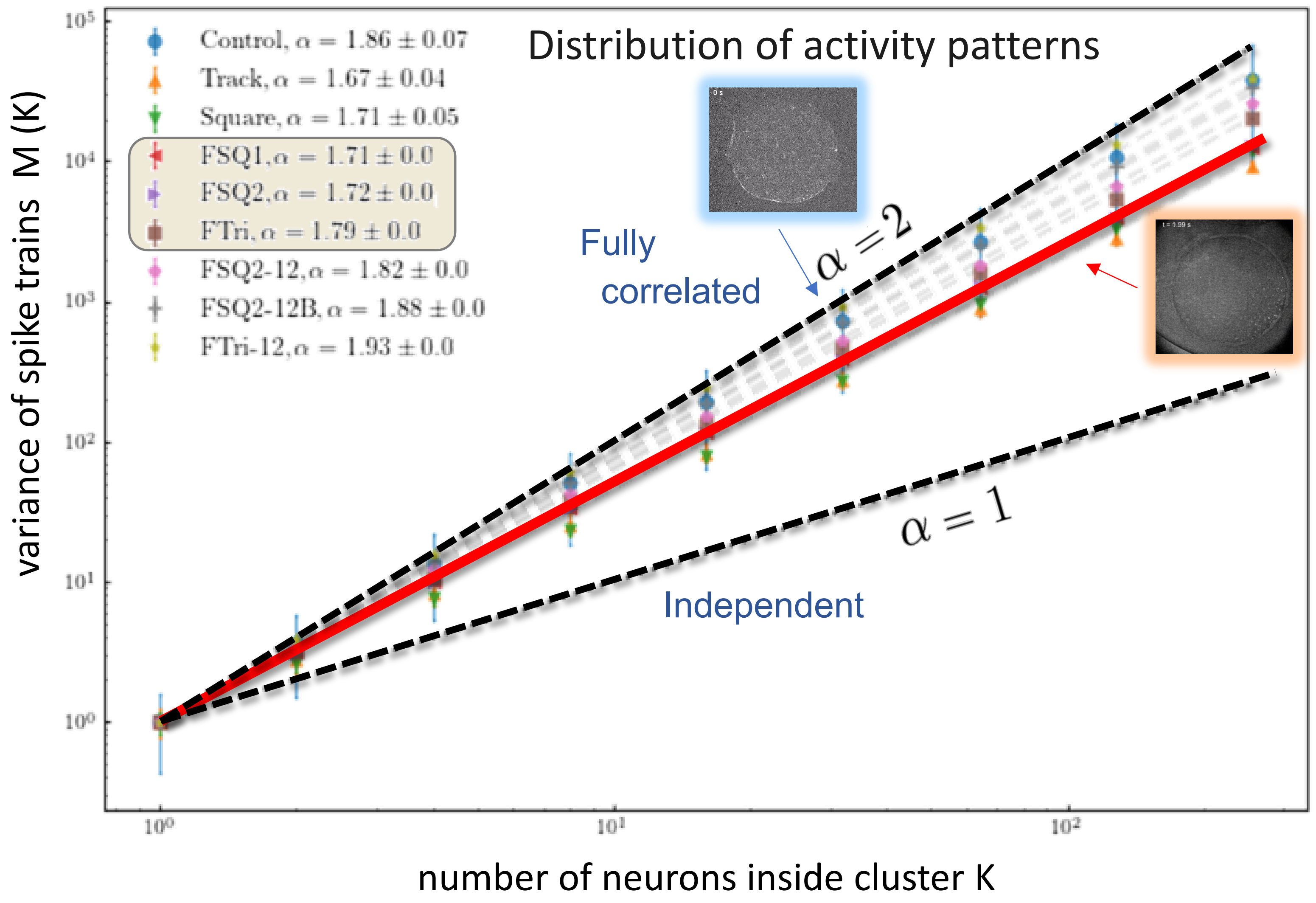
Dependent:

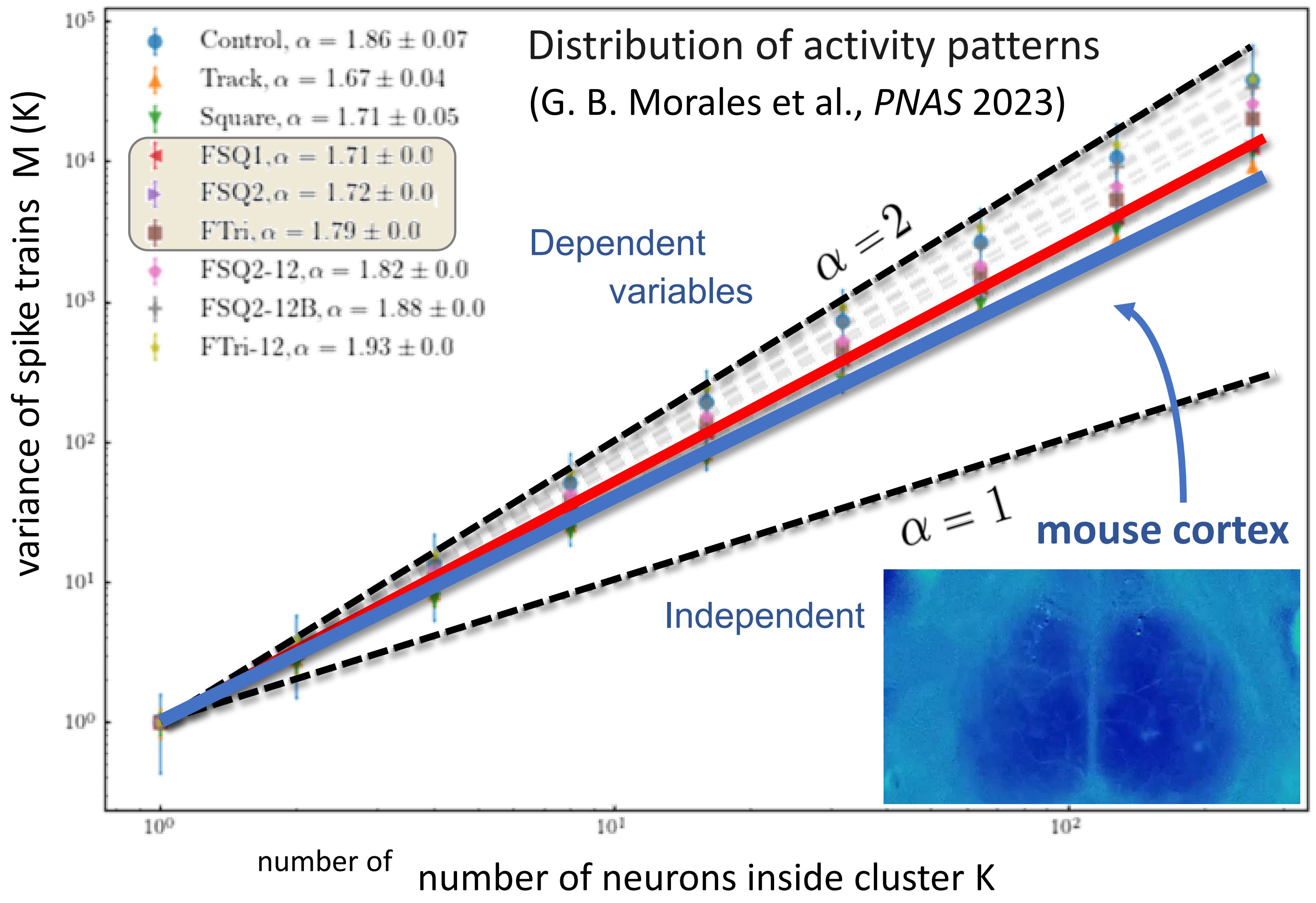
$$Var\left(\sum_{j=1}^{K} x_j\right) \sim K^{2}$$

Is it brain-like? Compare in the context of critcality





Distribution of activity patterns
Control, α = 1.86 ± 0.07
Track, α = 1.67 ± 0.04
Square, α = 1.71 ± 0.05
FSQ1, α = 1.71 ± 0.0
FSQ2, α = 1.72 ± 0.0
FTri, α = 1.79 ± 0.0
FSQ2-12, α = 1.82 ± 0.0
FSQ2-12B, α = 1.88 ± 0.0
FTri-12, α = 1.93 ± 0.0
Fully correlated
α = 2
α = 1
Independent
variance of spike trains M (K)
number of neurons inside cluster K



Distribution of activity patterns
(G. B. Morales et al., *PNAS* 2023)
Control, $\alpha = 1.86 \pm 0.07$
Track, $\alpha = 1.67 \pm 0.04$
Square, $\alpha = 1.71 \pm 0.05$
FSQ1, $\alpha = 1.71 \pm 0.0$
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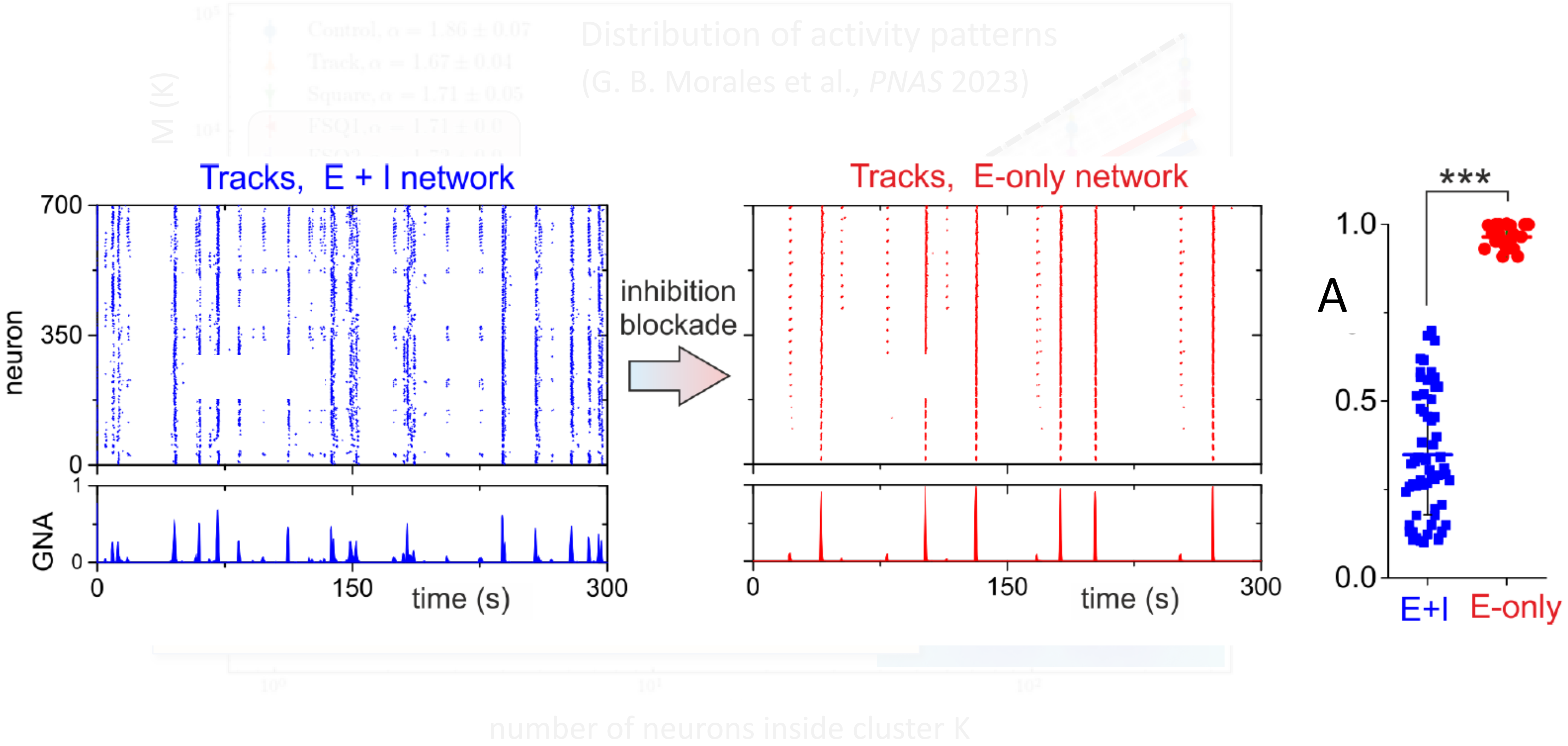
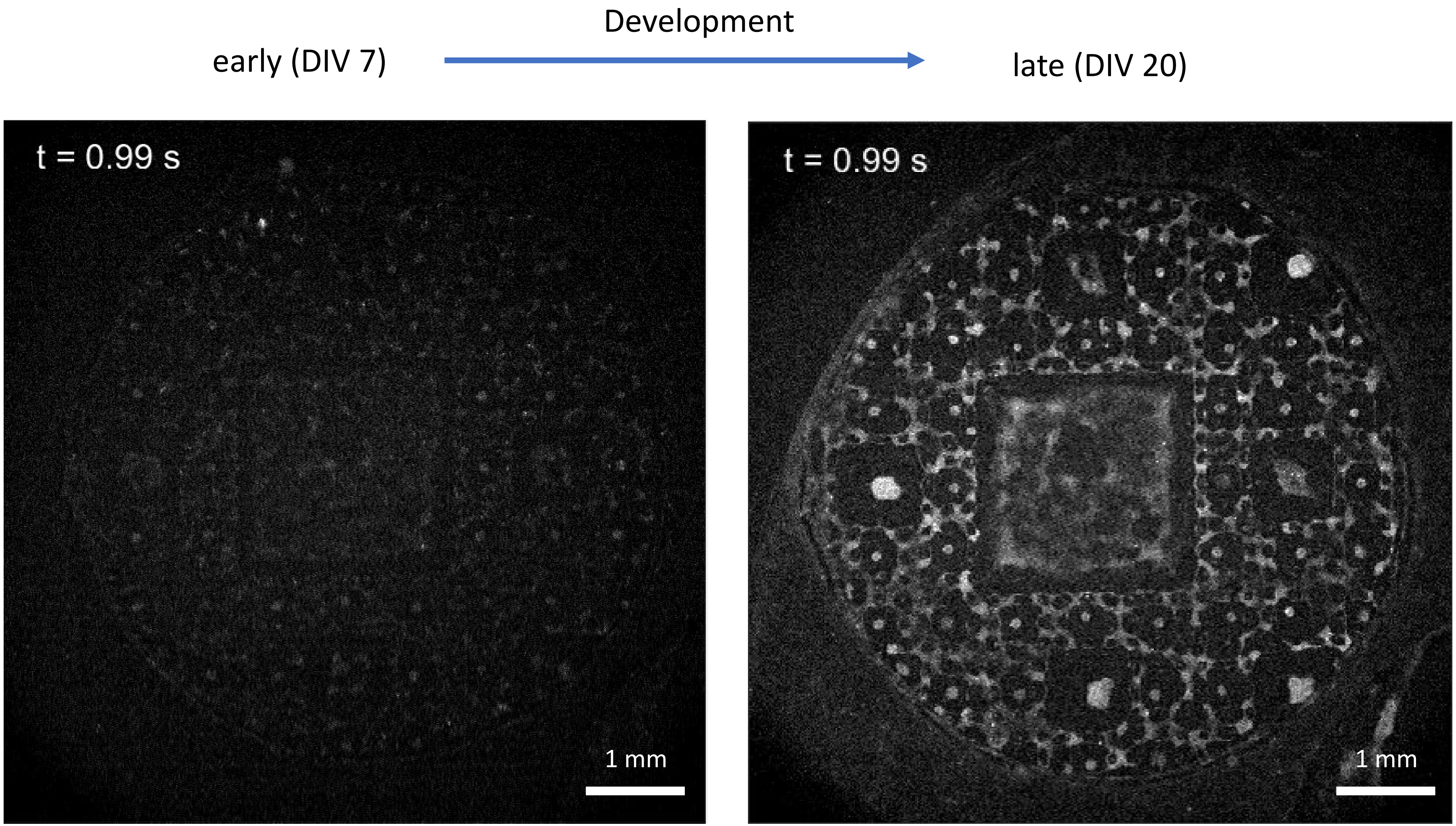
FSQ2-12, $\alpha = 1.82 \pm 0.0$
FSQ2-12B, $\alpha = 1.88 \pm 0.0$
FTri-12, $\alpha = 1.93 \pm 0.0$
Dependent variables
$\alpha = 2$
$\alpha = 1$
Independent
mouse cortex
variance of spike trains M (K)
number of
number of neurons inside cluster K
10^0
10^1
10^2
10^3
10^4
10^5

E/I balance is fundamental to keep the system dynamically rich

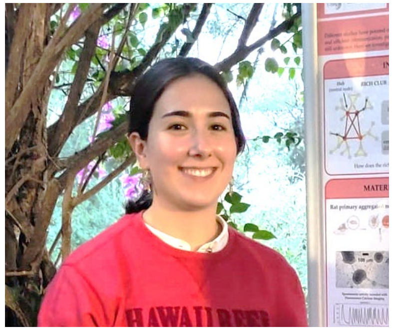



Development
early (DIV 7)
late (DIV 20)
t = 0.99 s
1 mm
t = 0.99 s
1 mm

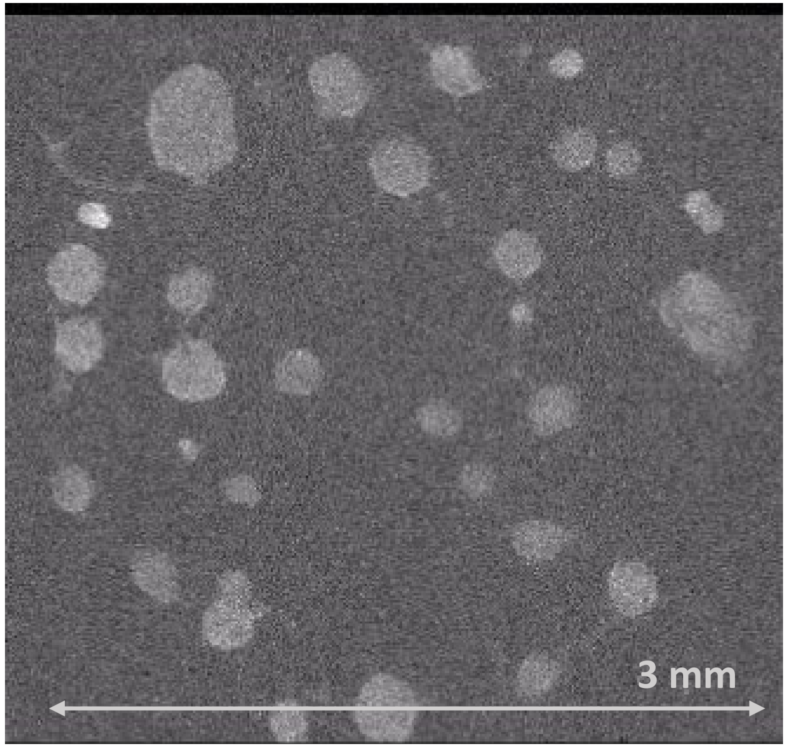
4

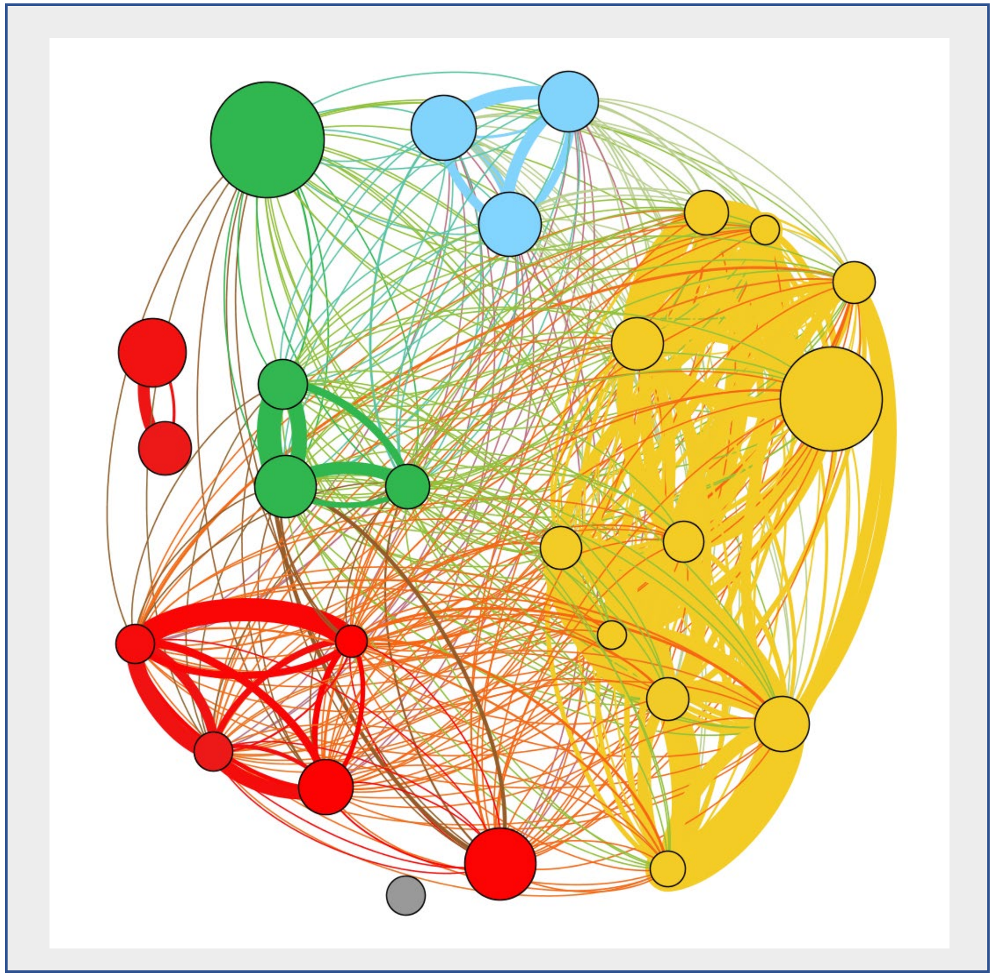
Plasticity in damage experiments

Effective networks and their change



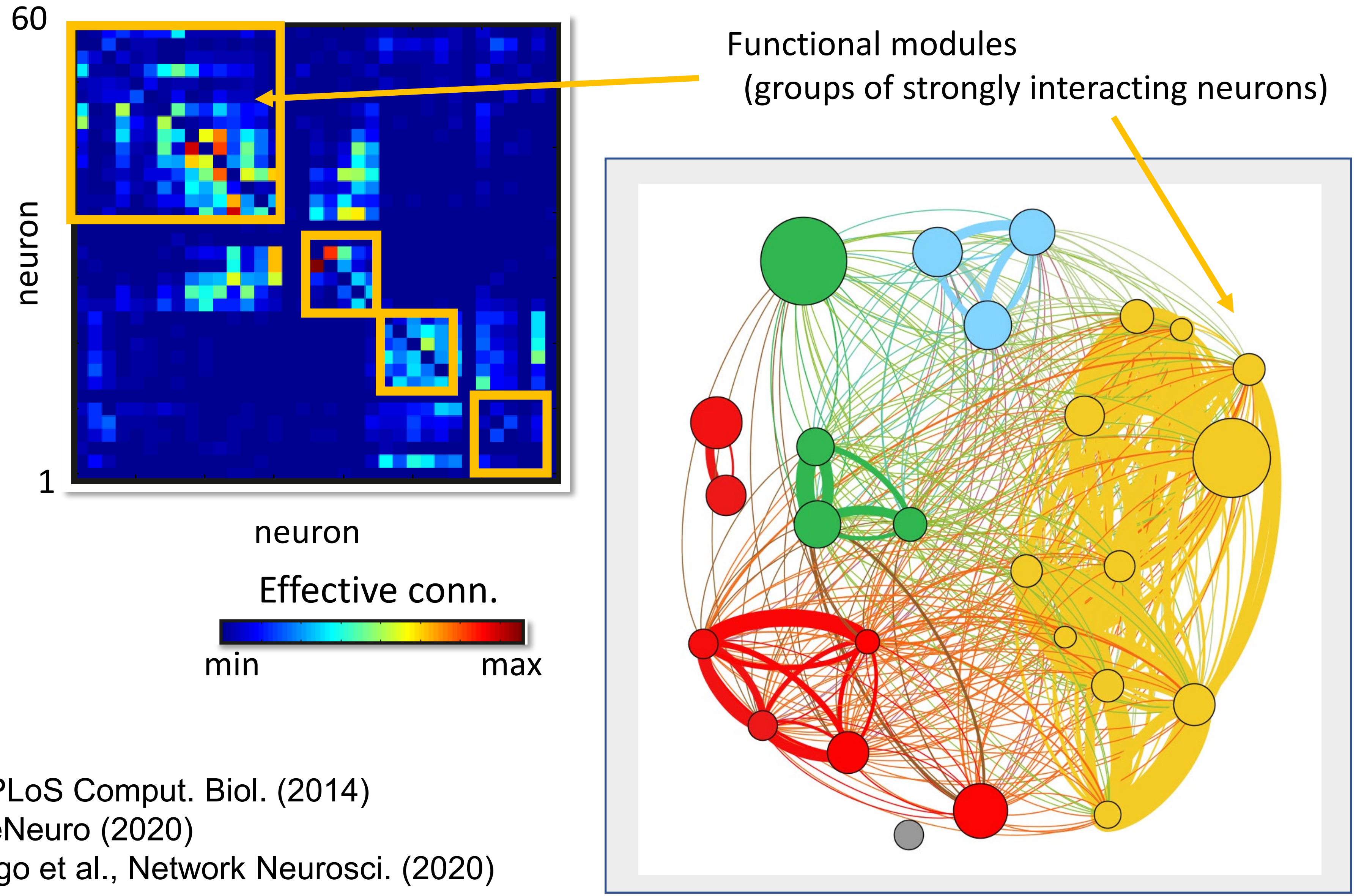
Belén
Montenegro





S. Teller et al., PLoS Comput. Biol. (2014)
S. Teller et al., eNeuro (2020)
E. Estévez-Priego et al., Network Neurosci. (2020)

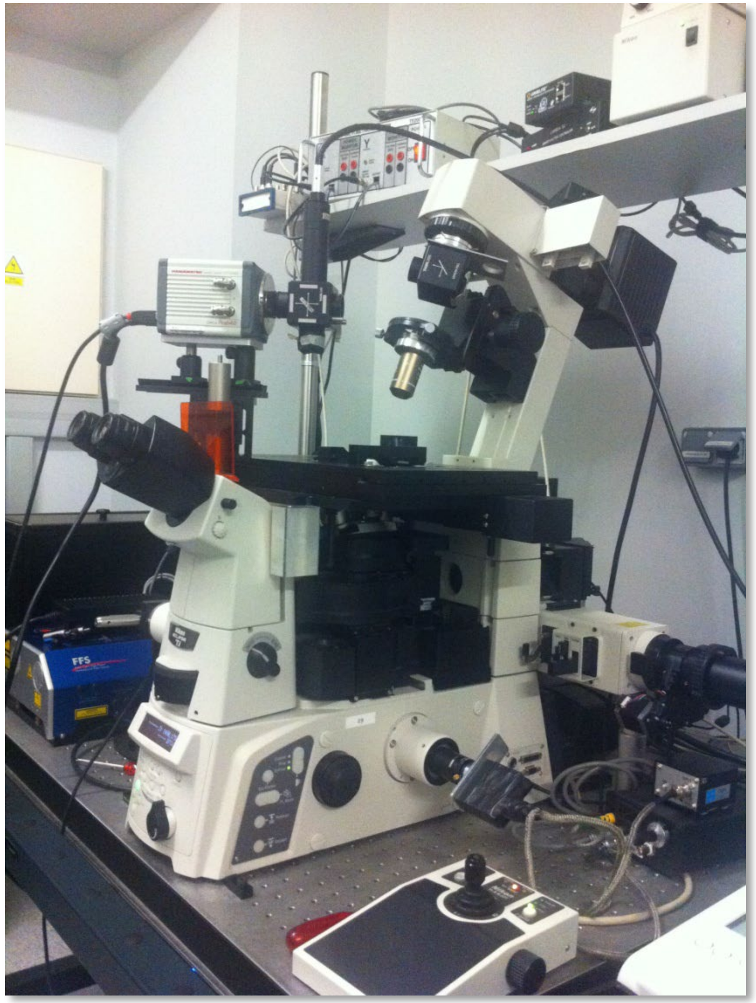
Effective networks and their change



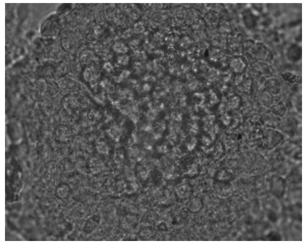
S. Teller et al., PLoS Comput. Biol. (2014)
S. Teller et al., eNeuro (2020)
E. Estévez-Priego et al., Network Neurosci. (2020)

Medicine 1. Stroke model

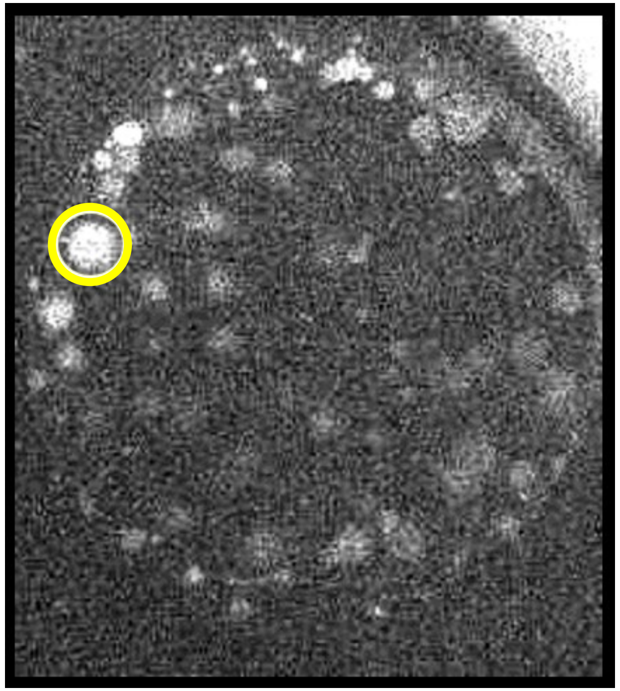
- A node of the network was destroyed through a UV laser.

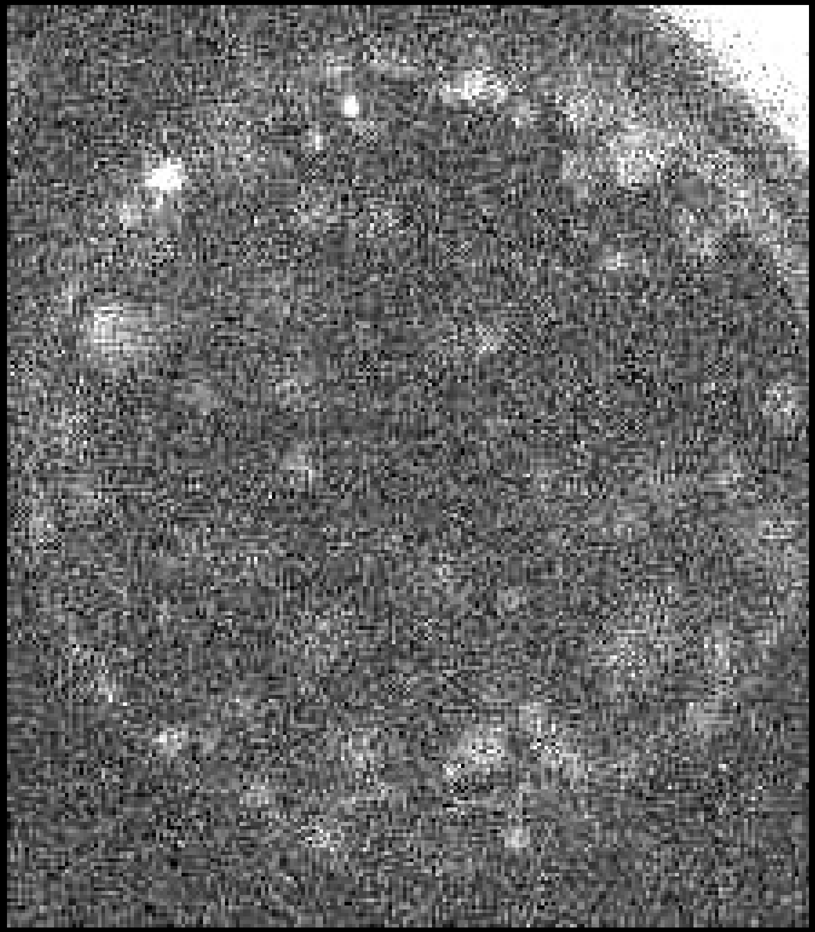


Multimodal microscope (fluorescence + ablation)

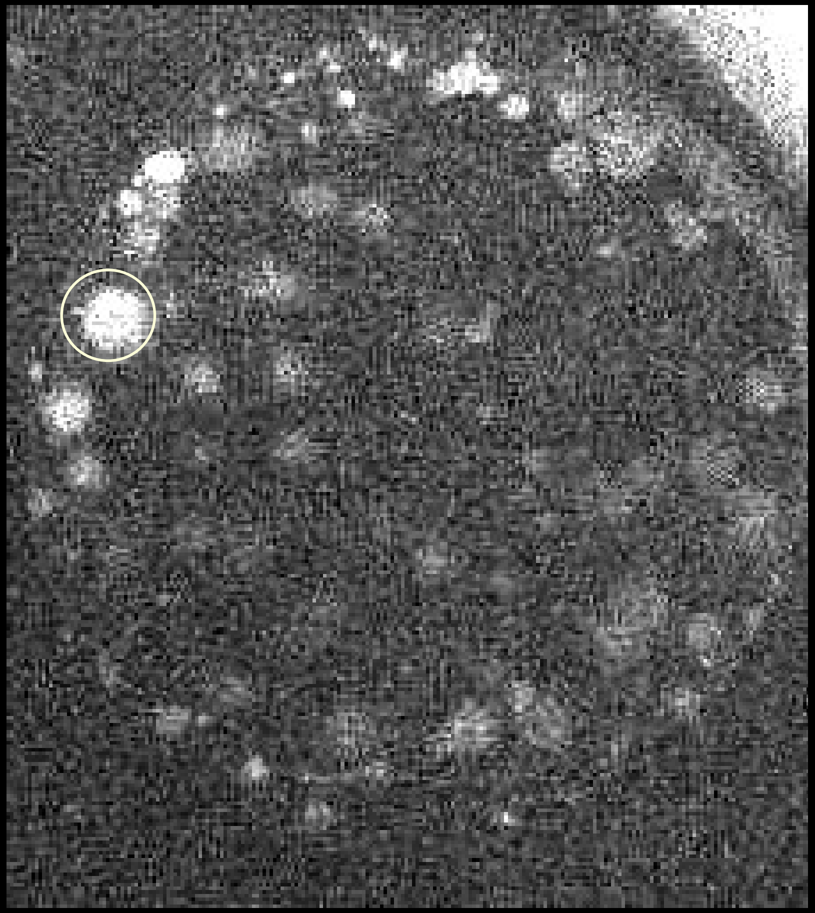


Destruction of a node

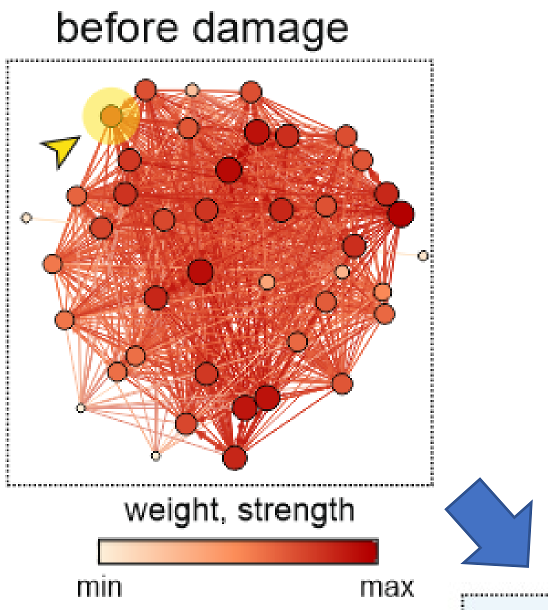




BEFORE DAMAGE



AFTER DAMAGE



eNeuro
Methods/New Tools

Novel Tools and Methods

Spontaneous Functional Recovery after Focal Damage in Neuronal Cultures

Sara Teller,[1,2] Estefanía Estévez-Priego,[1,2] Clara Granell,[2,3,4] Daniel Tornero,[1,5,6] Jordi Andilla,[7] Omar E. Olarte,[7,8] Pablo Loza-Alvarez,[7] Alex Arenas,[9] and Jordi Soriano[1,2]

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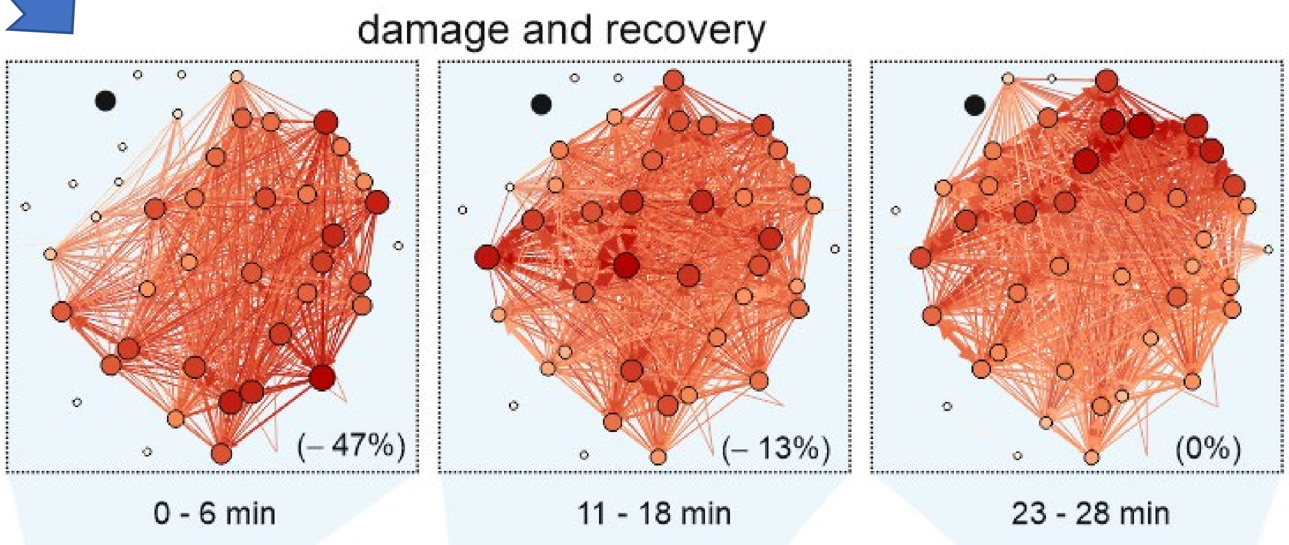
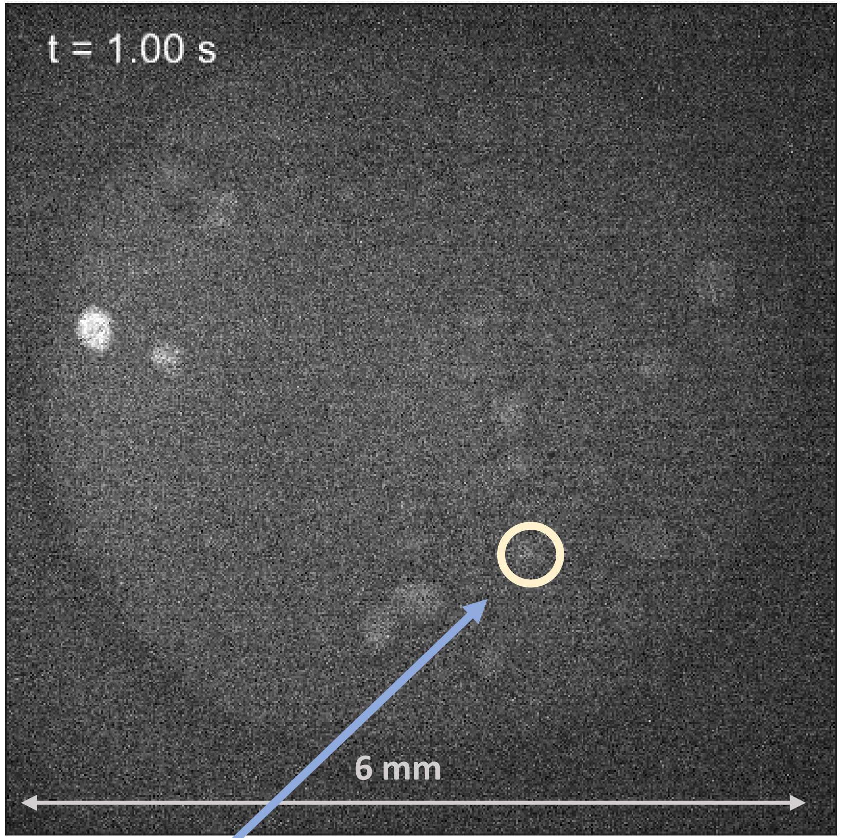
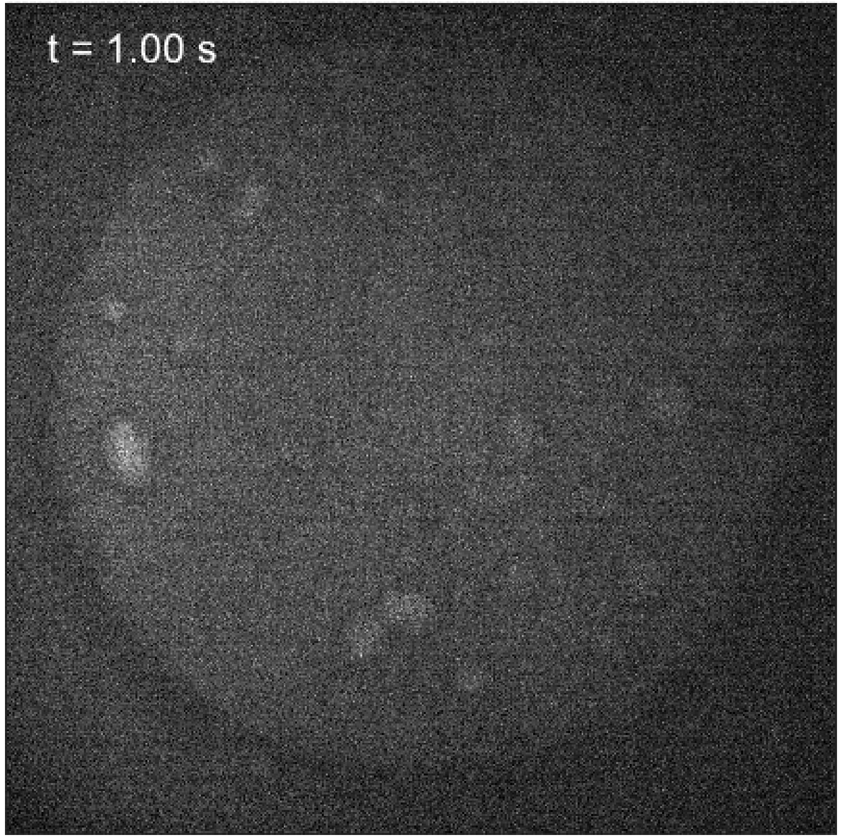


S. Teller et al., eNeuro (2020)

How about malicious damage (**targeted attack**)?.

Aggregated:



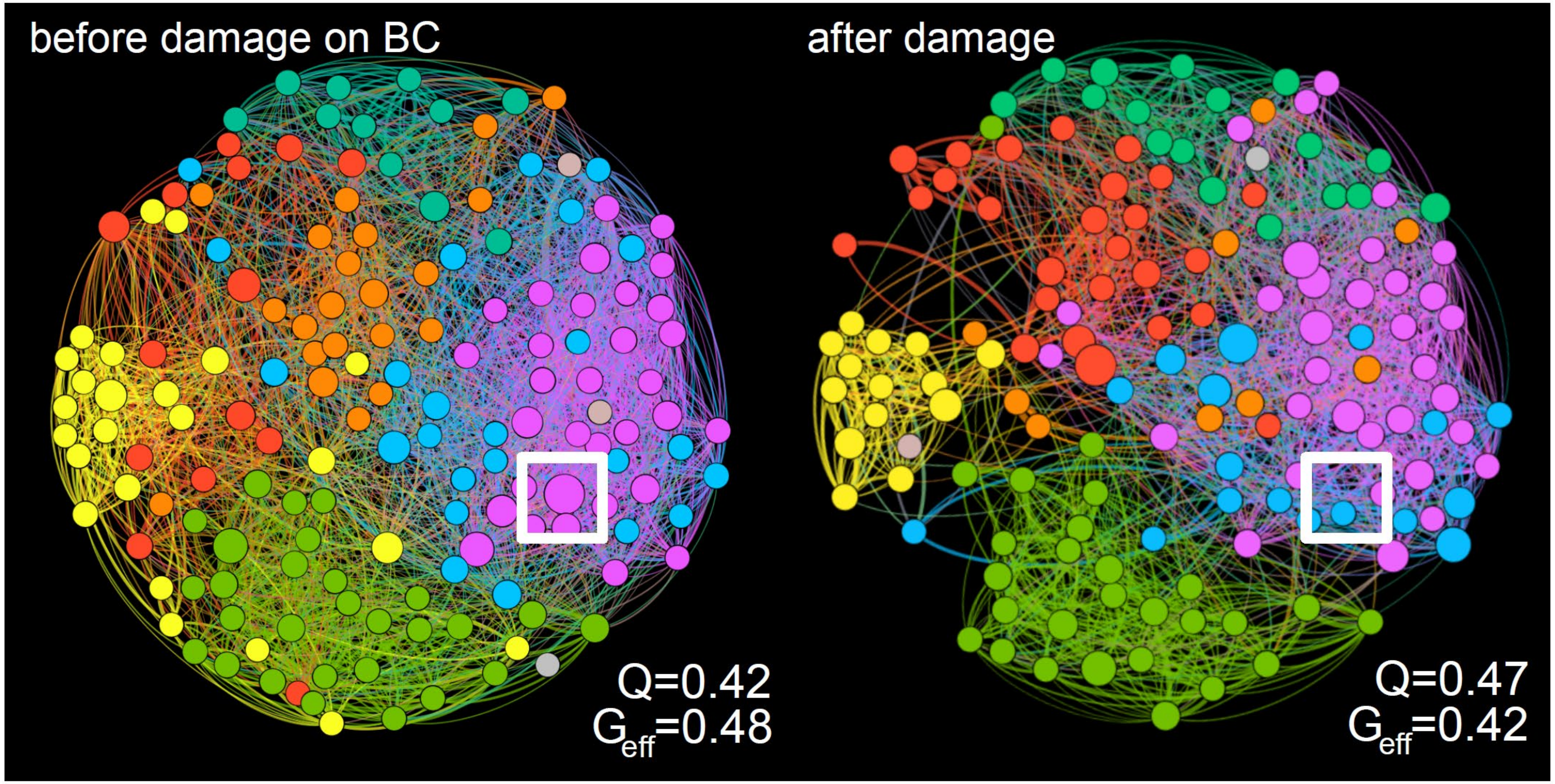


BC: Betweenness centrality (importance of node in information flow)

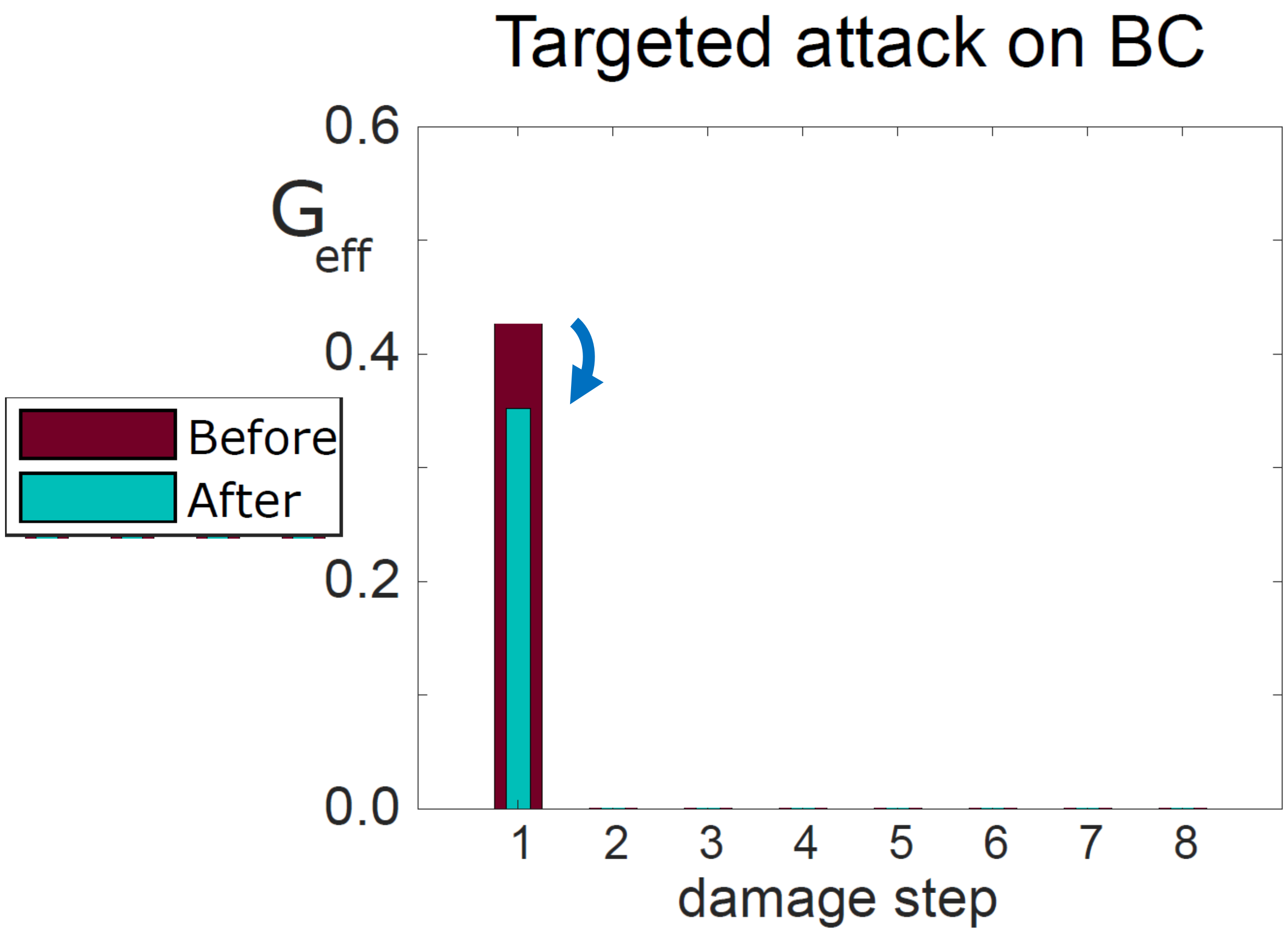
S. Ayasreh et al., Micromachines (2022)

How about malicious damage (**targeted attack**)?.

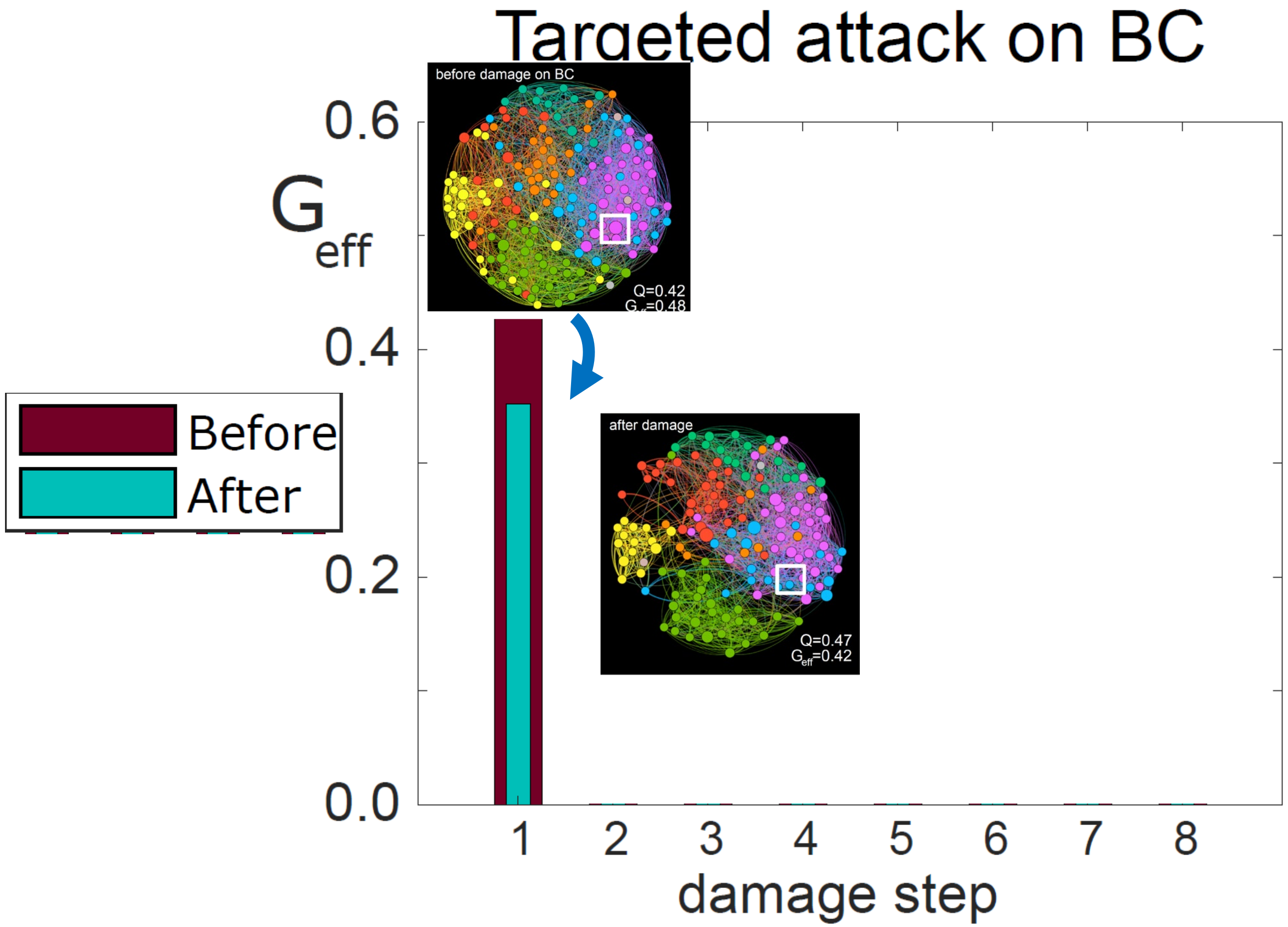
BC: Betweenness centrality (importance of node in information flow)



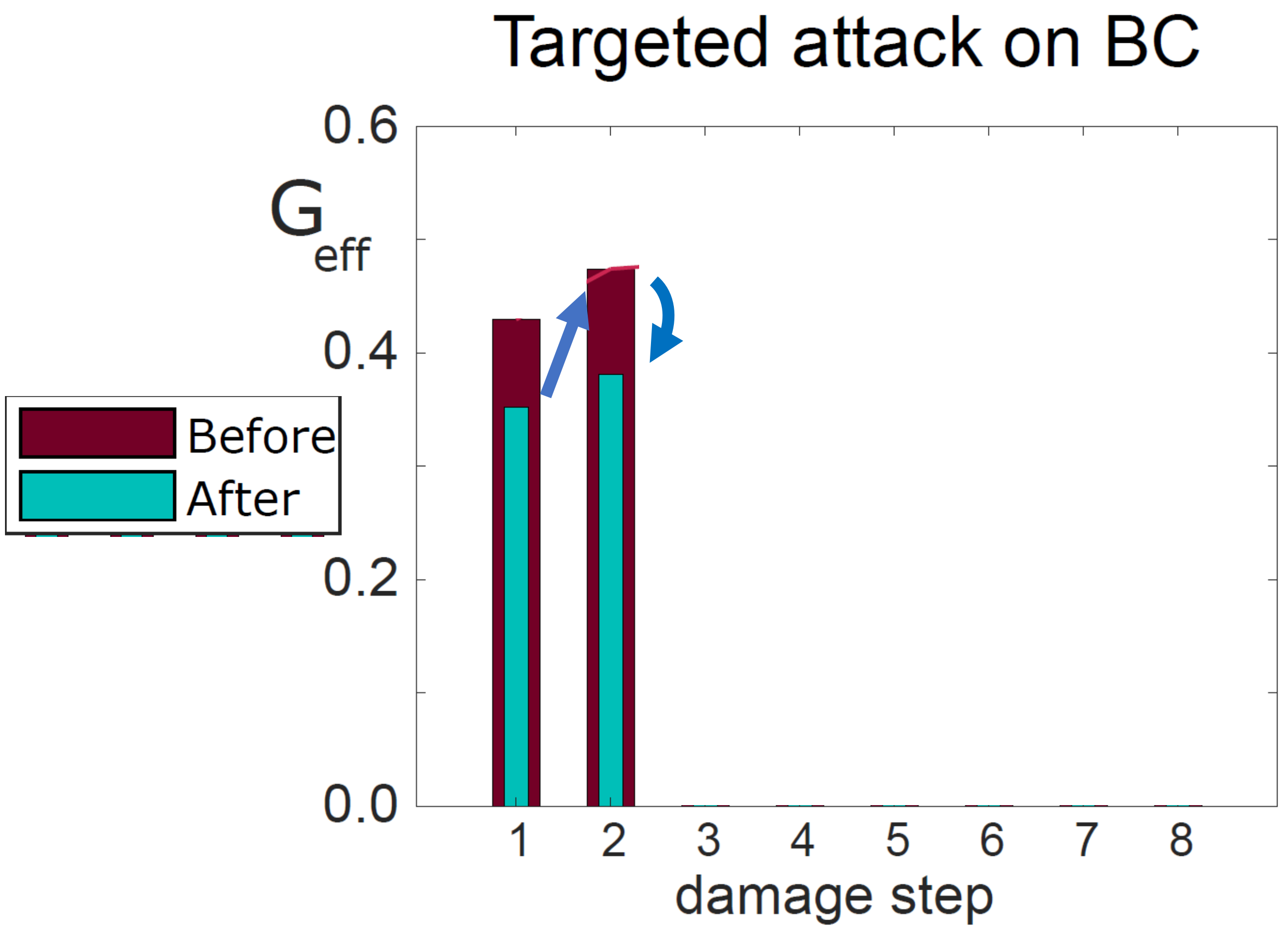
S. Ayasreh et al., *Micromachines* (2022)



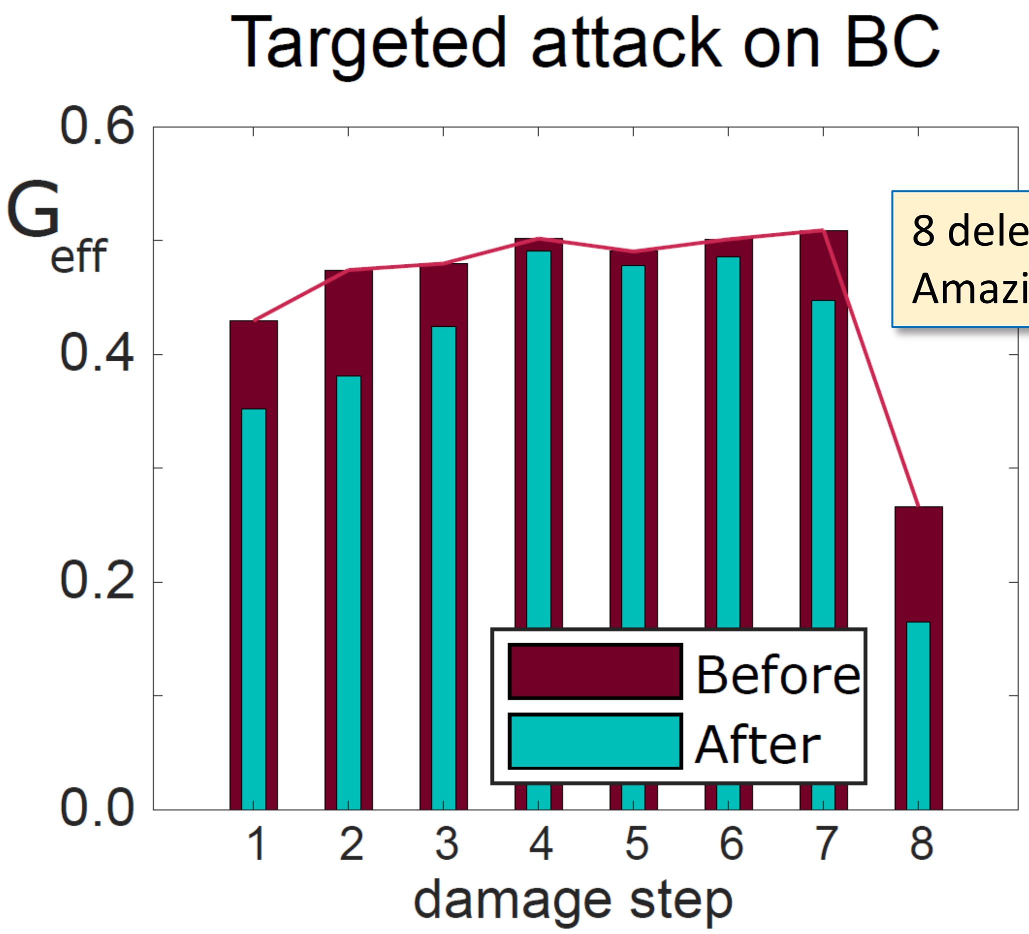
S. Ayasreh et al., *Micromachines* (2022)



S. Ayasreh et al., *Micromachines* (2022)



S. Ayasreh et al., *Micromachines* (2022)



8 deletions to stop activity
Amazing resilience!

S. Ayasreh et al., *Micromachines* (2022)

Conclusions:

- Neuronal cultures offer an environment to design circuits whose spontaneous activity exhibits brain-like dynamics. However, one has to be aware of developmental *tempos.*

- Plasticity mechanisms activate upon physical or chemical damage, but the underlying mechanism (synaptic scaling, STDP, homeostastis,….) is not clear. It may be a combination of different mechanisms.

- Electrical stimulation can be used to train in vitro networks, but it is obscure so far how specific training and plasticity can be induced.

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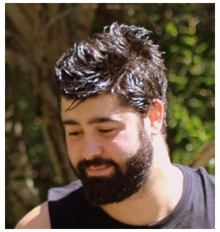

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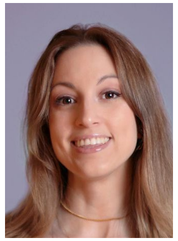

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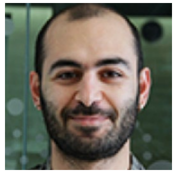

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