Tampereen yliopisto
Tampere University

# Part 1:
# Electrical Assessment and Analysis of Neuronal Activity

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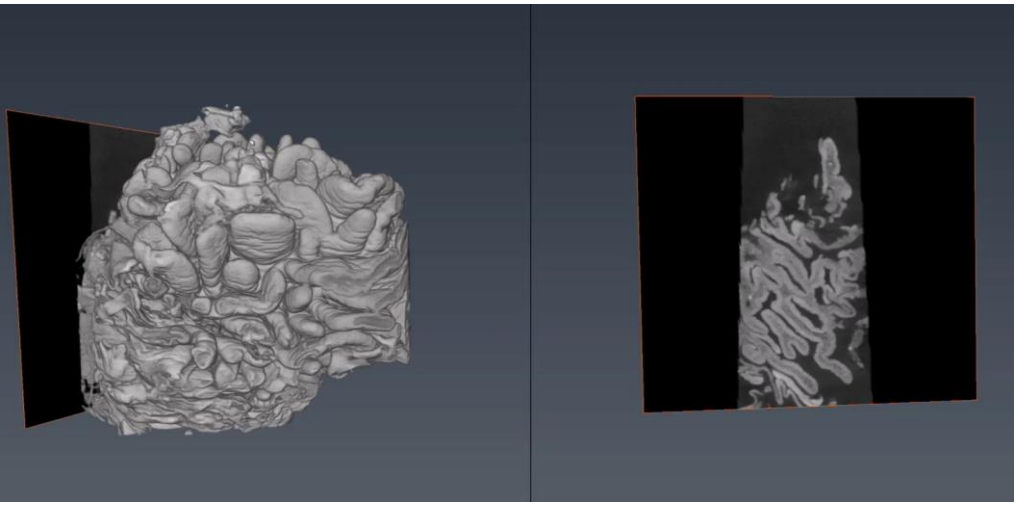
# Main research topics and expertise in CBIG

- **Biophysics:** From electrophysiology to mechanobiology
- **Bioimaging:** Tomographic 3D imaging and analysis
- **Computational Modelling:** Simulations of cardiac and epithelial cells as well as neural networks

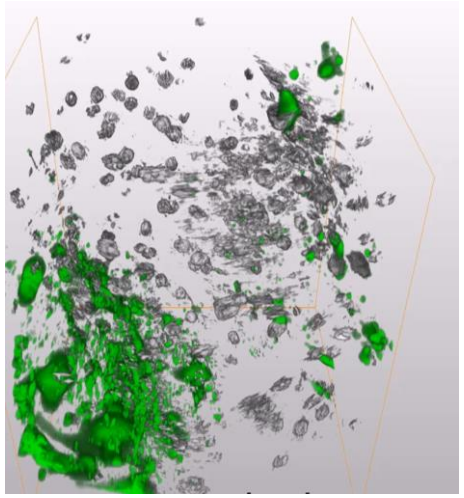
Image: Jarno Tanskanen

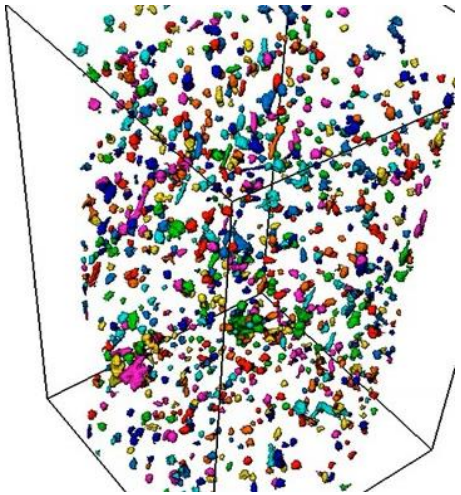
# 3D hybrid bioimaging in CBIG - From visualization to quantification

## 3D X-ray micro CT

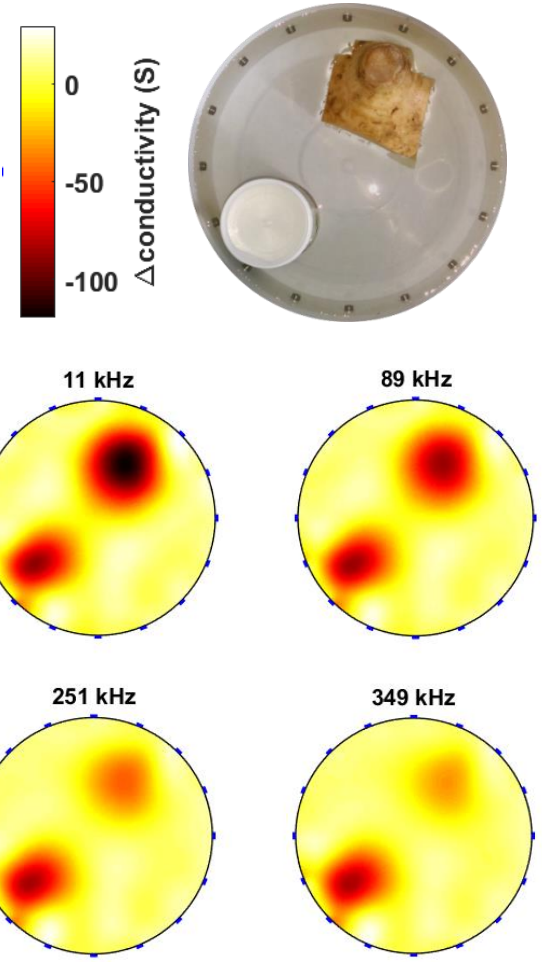


## Optical: combining OPT and SPIM





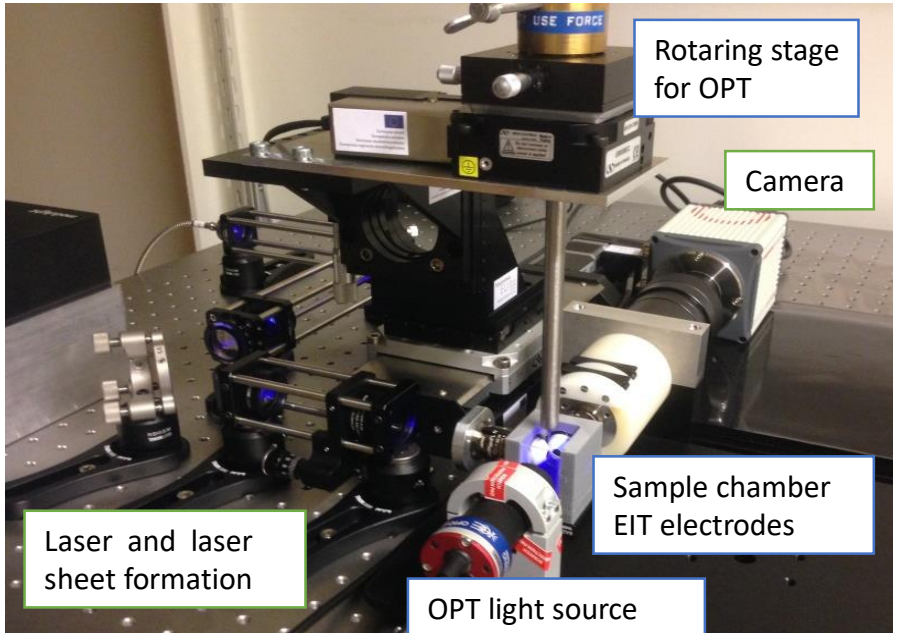
## Electric imaging




**Development of instrumentation, imaging procedures, tomographic reconstruction methods**

**Target:**

- Biomaterial 3D microstructure analysis (X-ray, optical, electric)
- Soft tissue X-ray microtomography enabling 3D histology
- Optical and hybrid 3D bioimaging for quantifying cells in 3D BoC




Slide courtesy to Jari Hyttinen

# *In silico* modelling in CBIG

- ***In silico* hIPSC derived cardiomyocytes:**

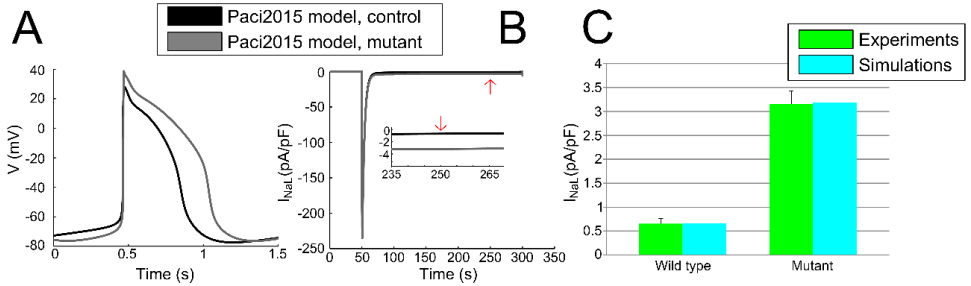
  **First population models of hIPSC CMs with *in silico* drug studies**: Dr. Michelangelo Paci, collaboration with Universites Bologna, Oxford and Washington

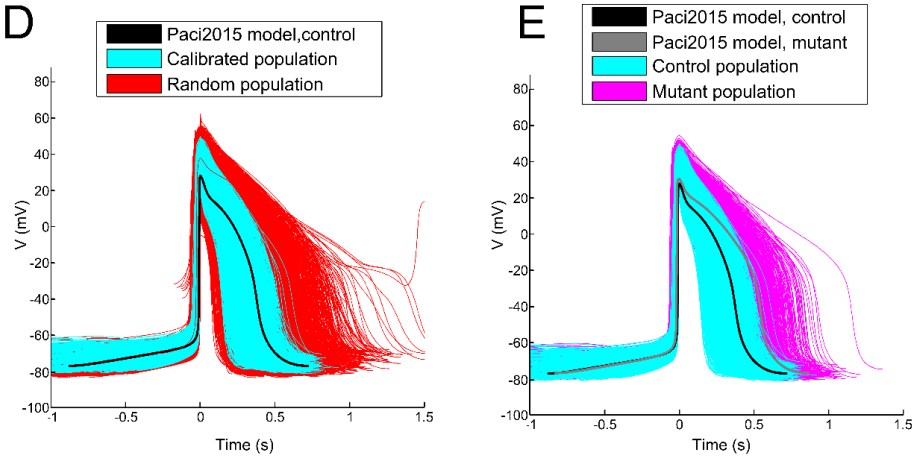
- ***In silico* astrocyte-neuronal interaction:**

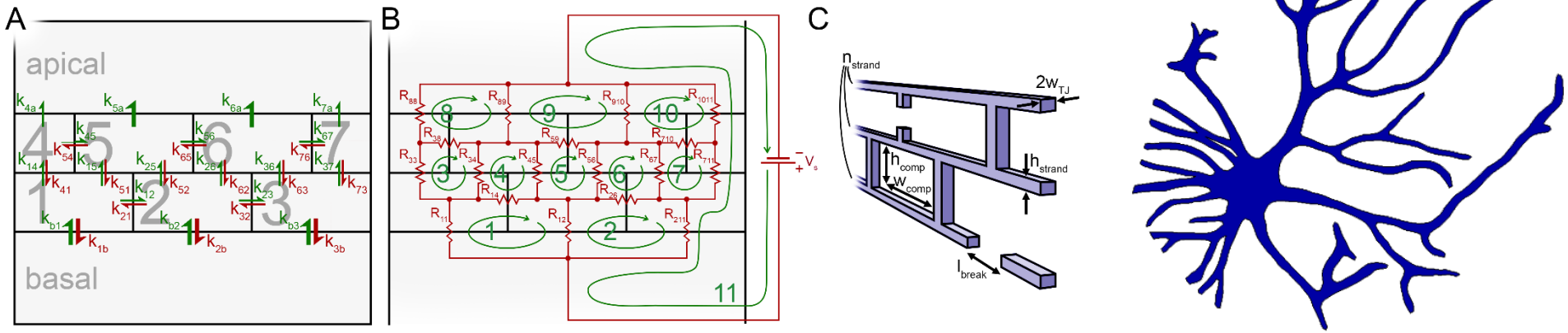
  **One of the first astrocyte-neuronal network models**: Dr. Kerstin Lenk, in collaboration with INRIA and University of Marburg

- ***In silico* epithelia barrier development:**

  **First epithelia tight junction dynamics modelling** combining electric and molecular diffusion barriers: Aapo Tervonen




Slide courtesy to
Jari Hyttinen

# Contents

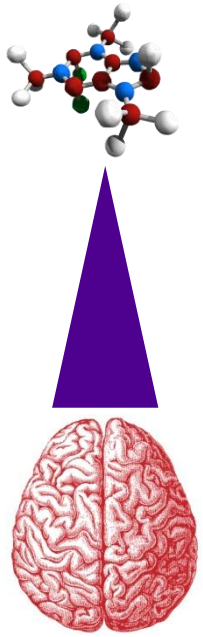
1. Bioelectric assessment of neural networks in vitro
2. Spike and burst analysis methods/ tools
3. Network connectivity/ synchronization analysis
4. Calcium imaging

# Levels of brain organization

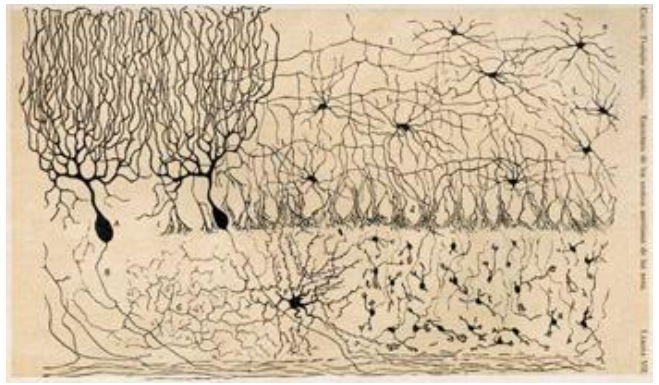
The brain contains both large scale and small scale anatomical structures and different functions take place at higher and lower levels.

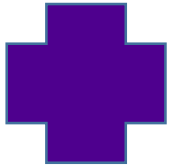
Hierarchy of interwoven levels of organization:

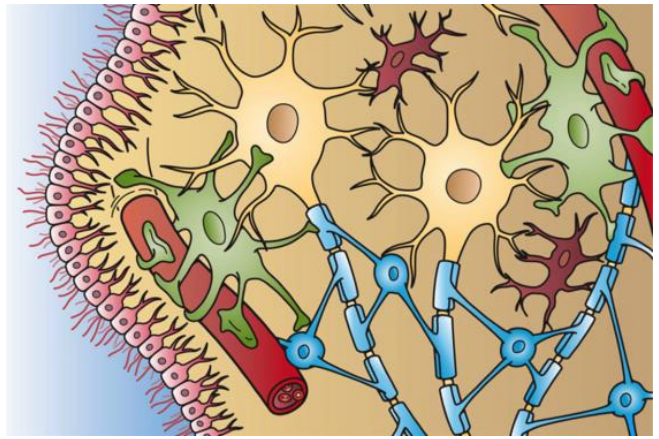
1. Molecules and Ions
2. Synapses
3. Neuronal microcircuits
4. Dendritic trees
5. **Neurons**
6. **Local circuits**
7. Inter-regional circuits
8. Central nervous system



**Neuron doctrine**







Images: https://en.wikipedia.org/wiki/Neuron_doctrine, https://en.wikipedia.org/wiki/Glia

# Neuron

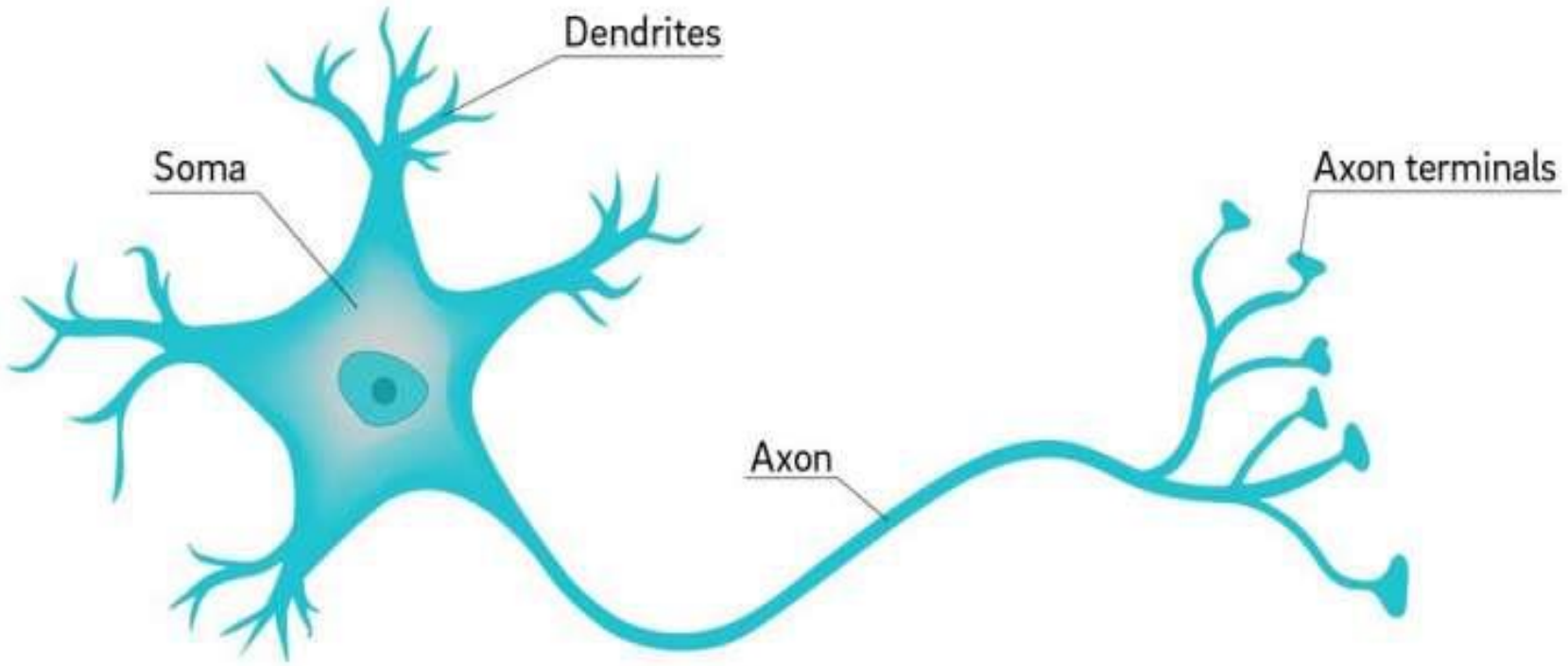
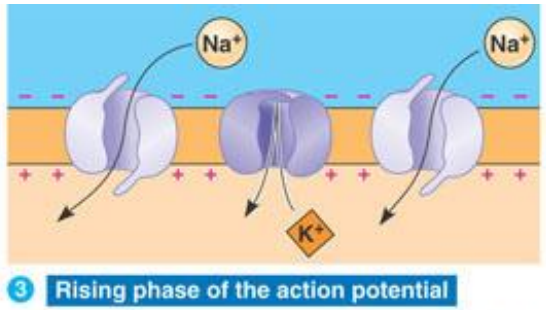
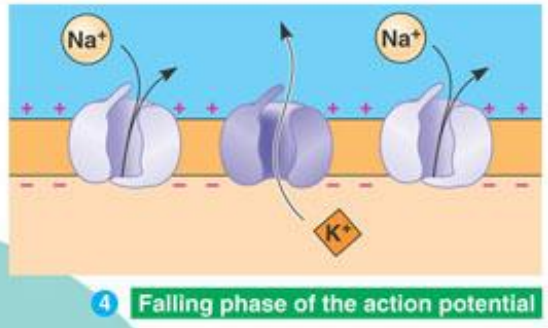



Image: https://medicalxpress.com/news/2018-07-neuron-axons-spindly-theyre-optimizing.html

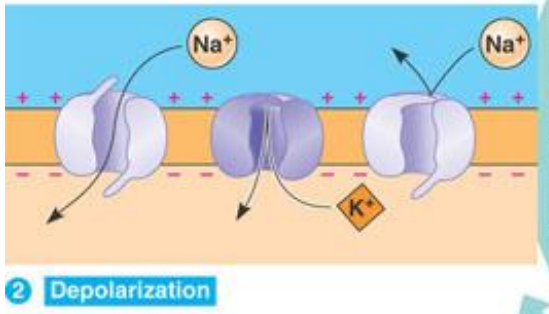
# Action potentials (AP)



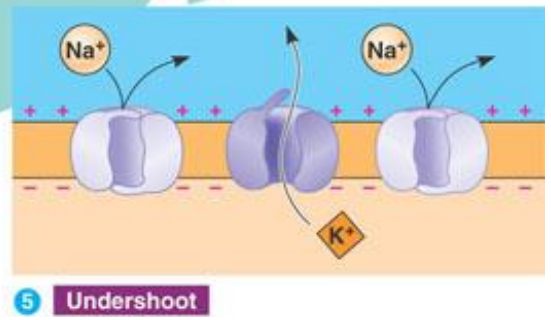

***All Na+ channels open***
***=> $V_m$ → $E_{Na}$~ 60mV***




***Na+ channels inactivate;***
***K+ channels open***
***=> $V_m$ → $E_K$~ -80mV***




***Depolarization causes***
***some Na+ channels to open***


***K+ channels open;***
***Undershoot closes the K+ channels***

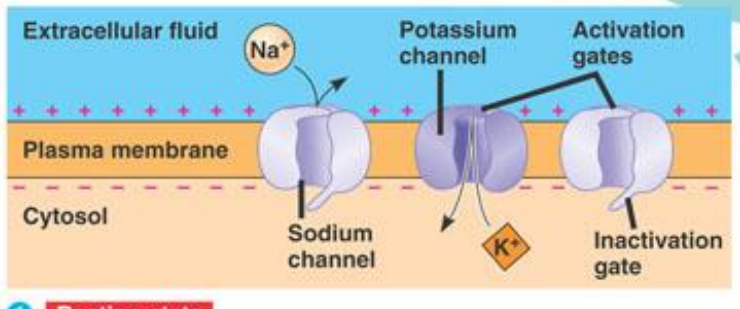
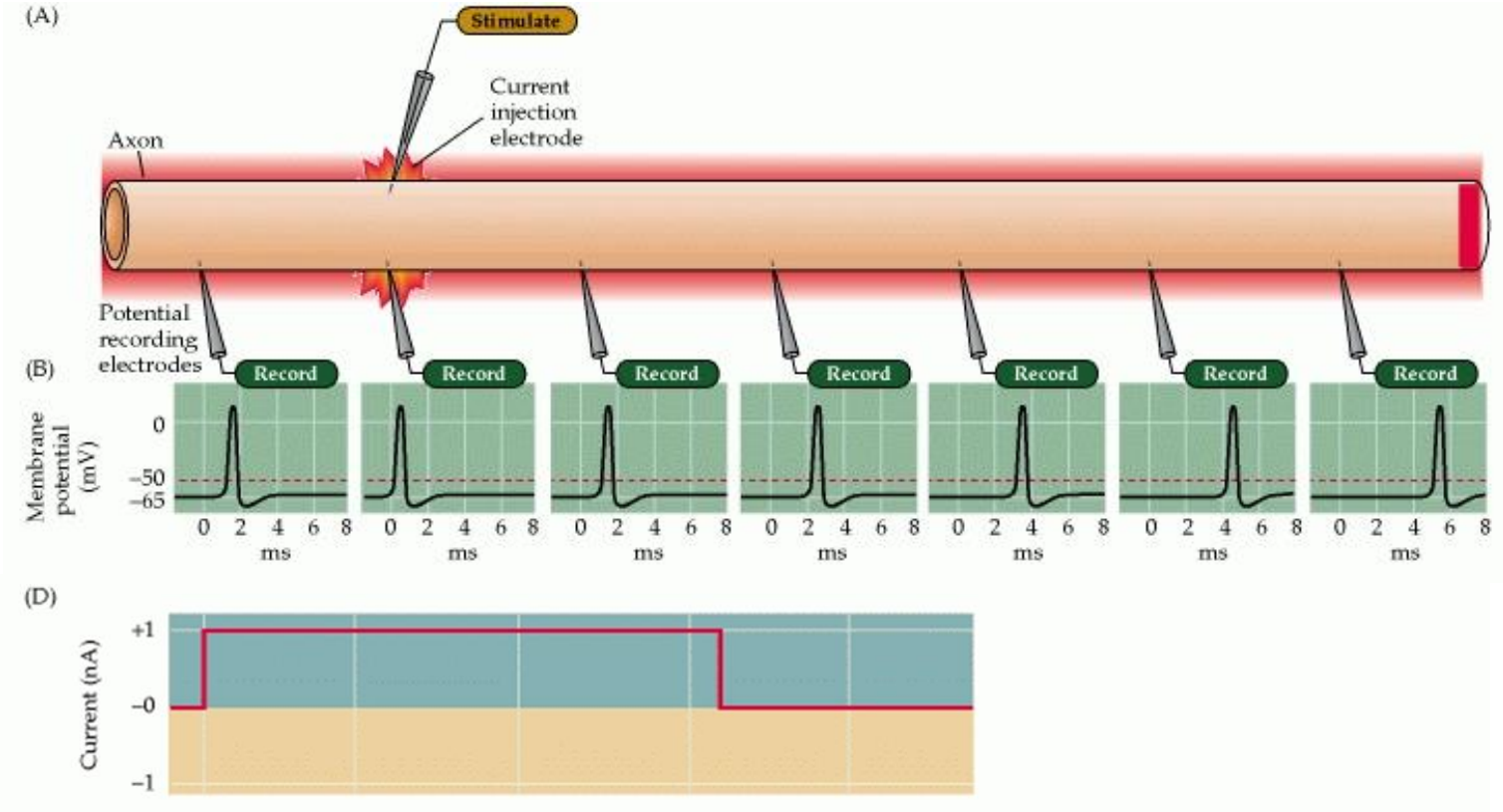



Image: http://uoitbiology12u2014.weebly.com/nerve-impulse--action-potential.html

Slide courtesy to Soile Nymark

# Active flow of current (=AP propagation)




- Amplitude constant
- Signal can travel long distances unchanged

Slide courtesy to
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# Intracellular and extracellular recordings

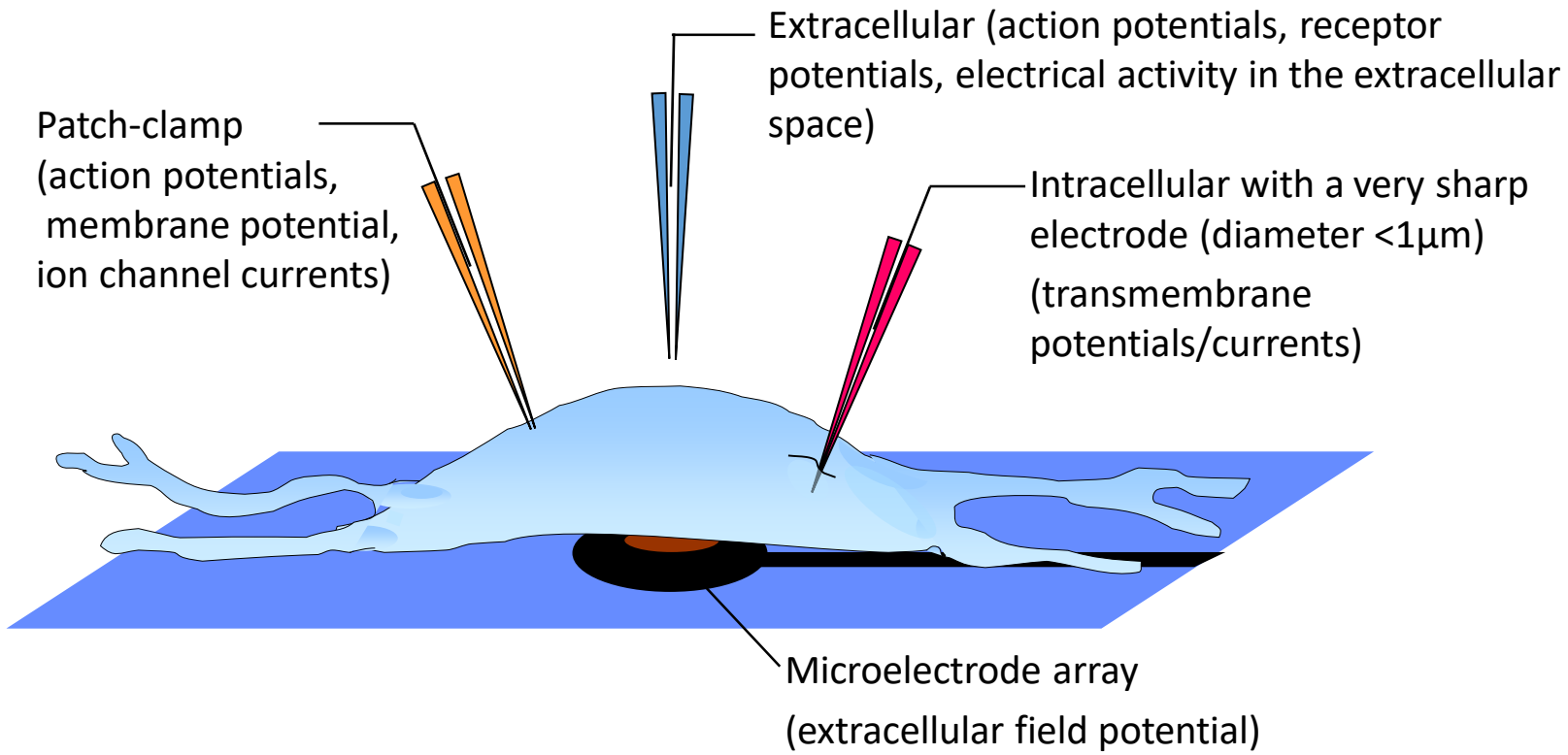


*Image: Modified from* Lohmann Research Equipment, http://www.lohres.de/products/05_qt-screen.htm

Slide courtesy to
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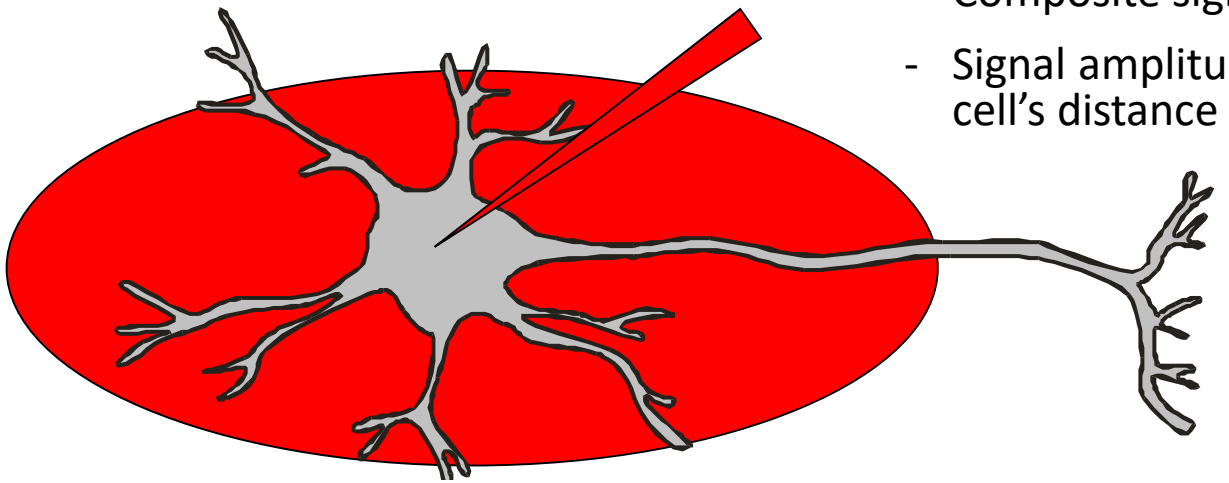
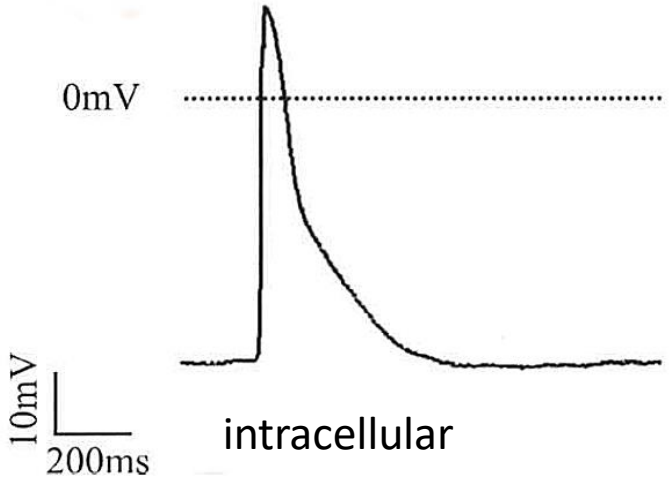
# Intracellular and extracellular recordings

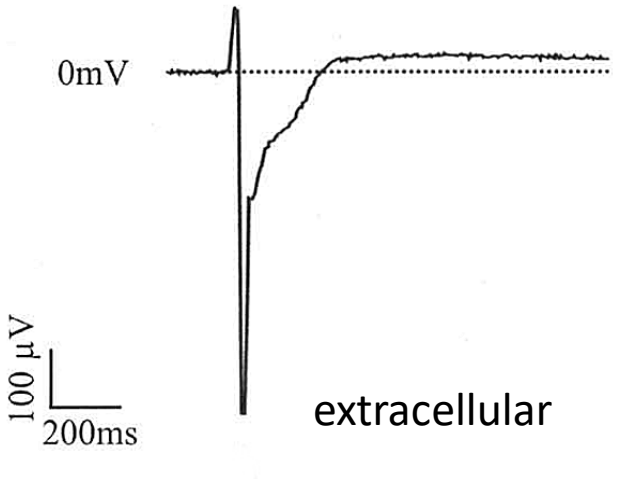
**Extracellular recordings:**

- Composite signals
- Signal amplitude depends on cell's distance to the electrode






intracellular




extracellular

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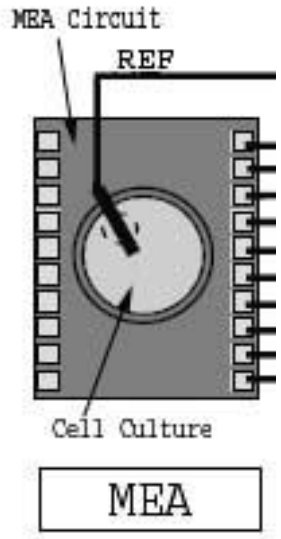
# Field potential recordings – *in vitro* MEA

Microelectrode array technique (MEA) for recording field potentials from excitable cells

- Neuronal tissue / cell cultures
- Cardiac tissue / cell cultures




Slide courtesy to
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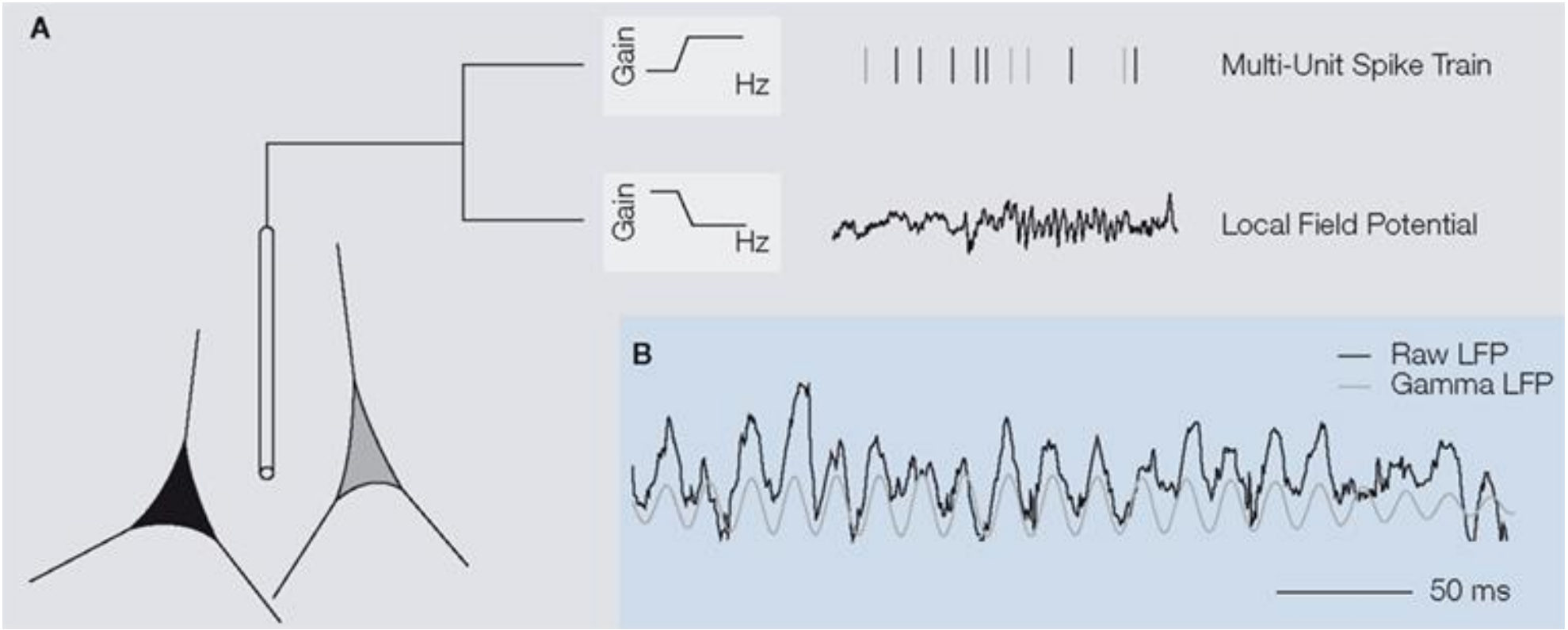
# Field potential recordings




Berens, et al., Front Neurosc 2008

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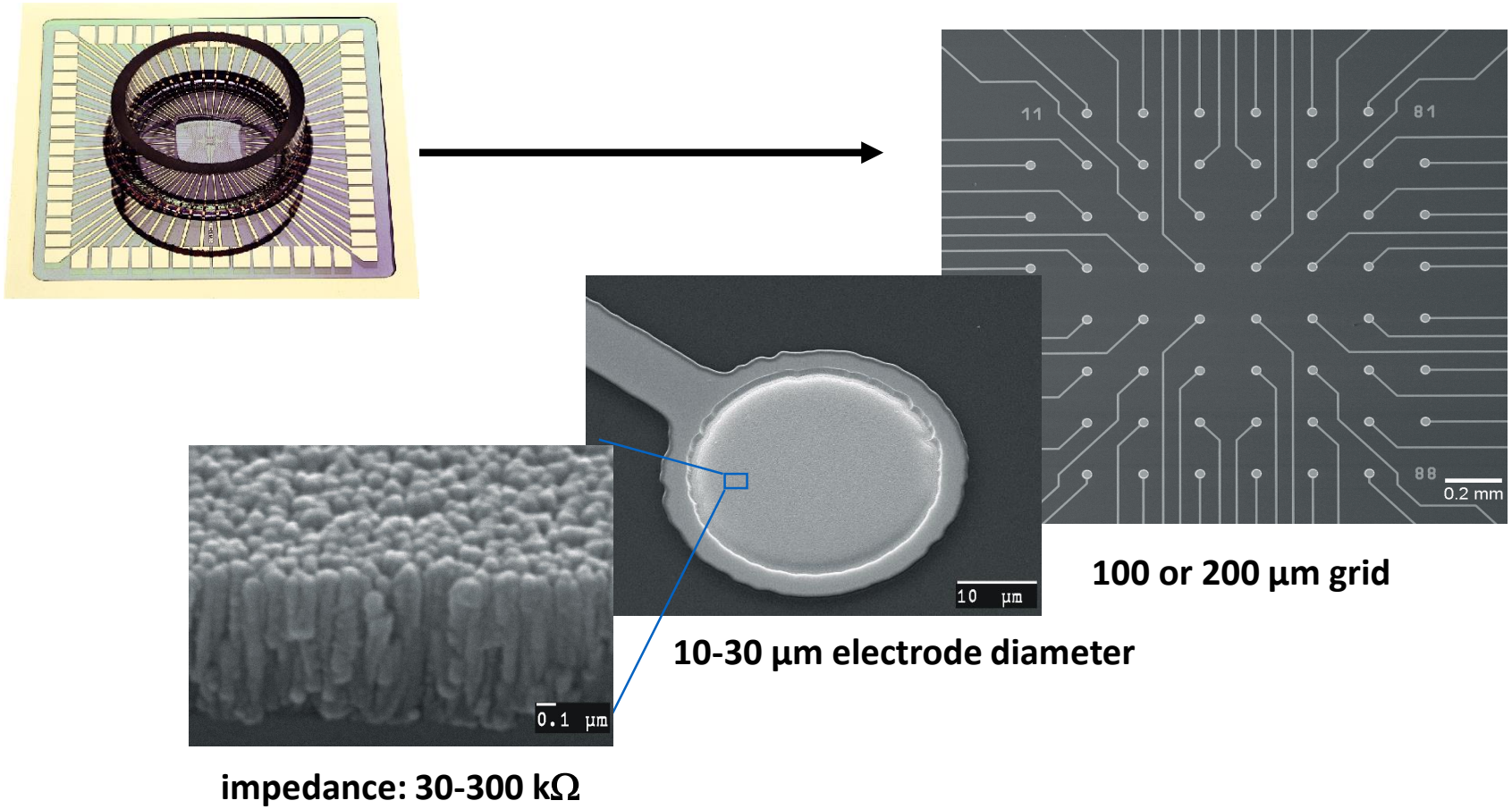
# MEA electrode plates




100 or 200 µm grid

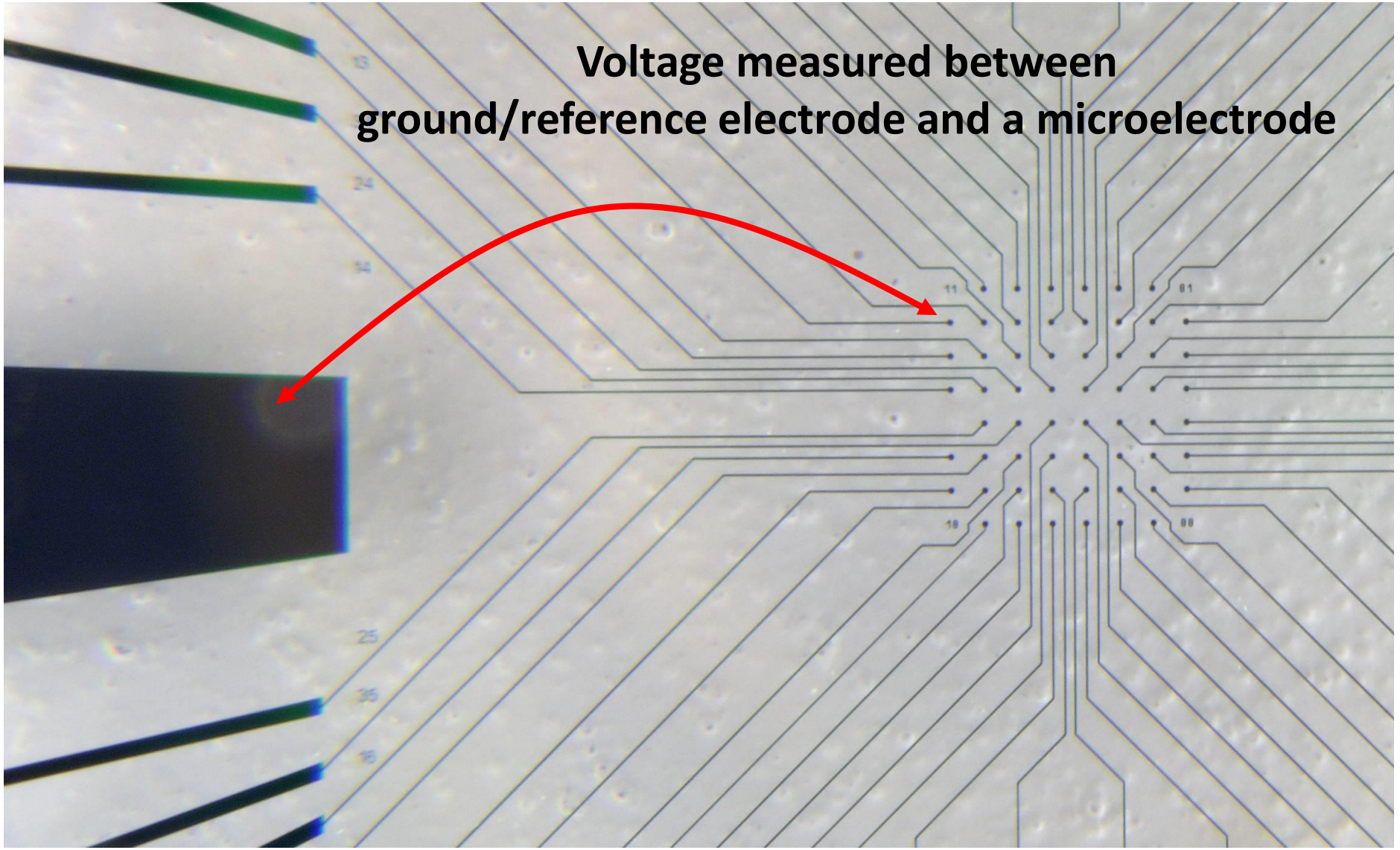
10-30 µm electrode diameter

impedance: 30-300 kΩ

Egert et al. 1998

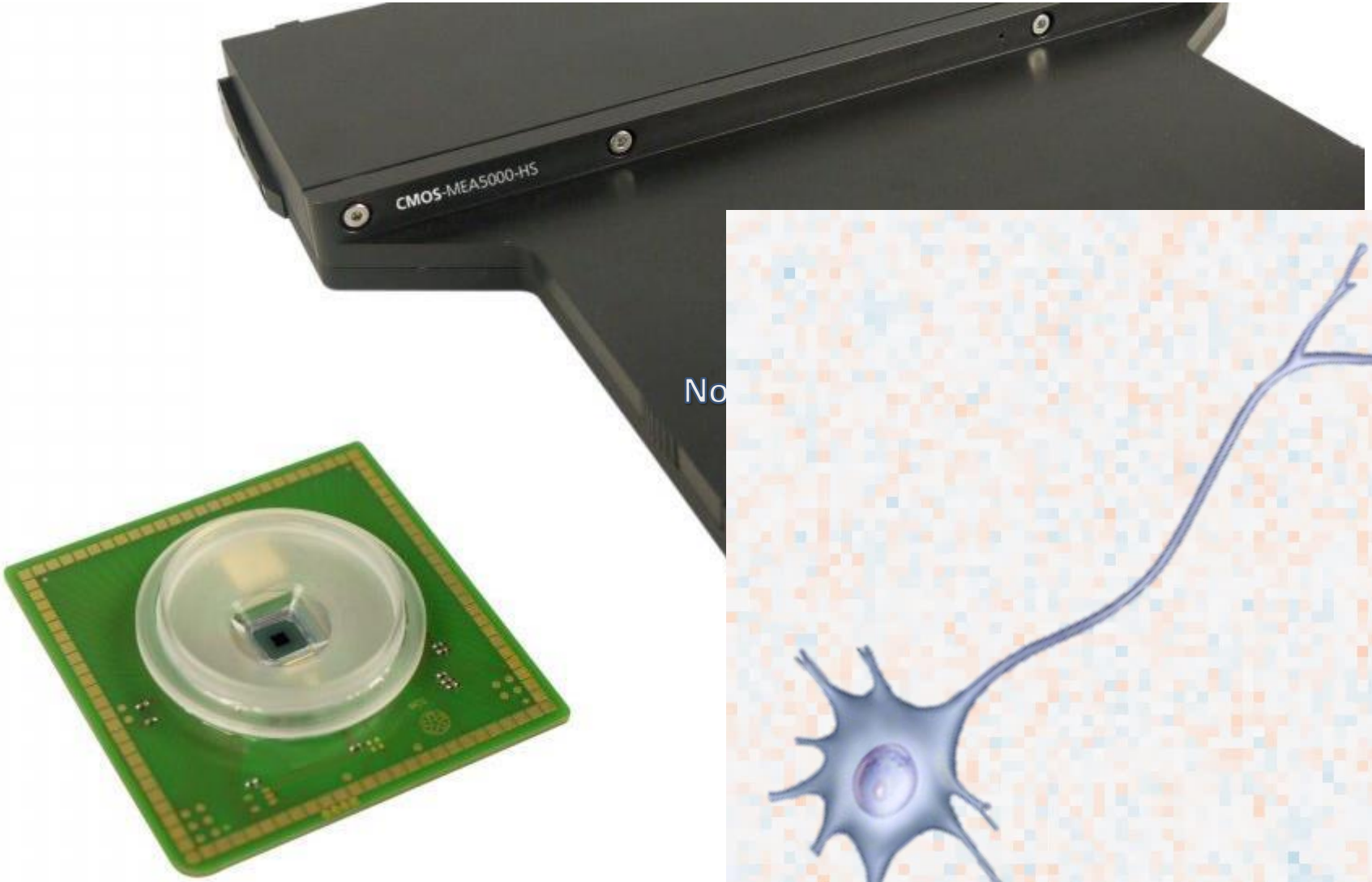
Slide courtesy to
Soile Nymark

# MEA measurement




Slide courtesy to
Jarno Tanskanen

# High density CMOS MEA






Images: Multi Channel Systems

Slide courtesy to
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# *In vitro* preparations: Ex vivo slices

**Advantages:**

- High-throughput
- Original connectivity
- More controlled environment

**Disadvantages:**

- Cells can be cut at the edge
- Outside their natural environment

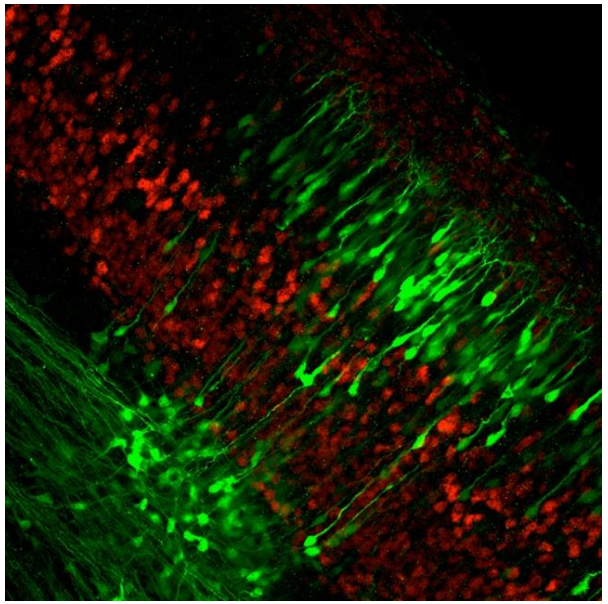


Image: http://www.scripps.edu/polleux/Polleux_lab_website/Polleux_lab_Pics%26Movies.html

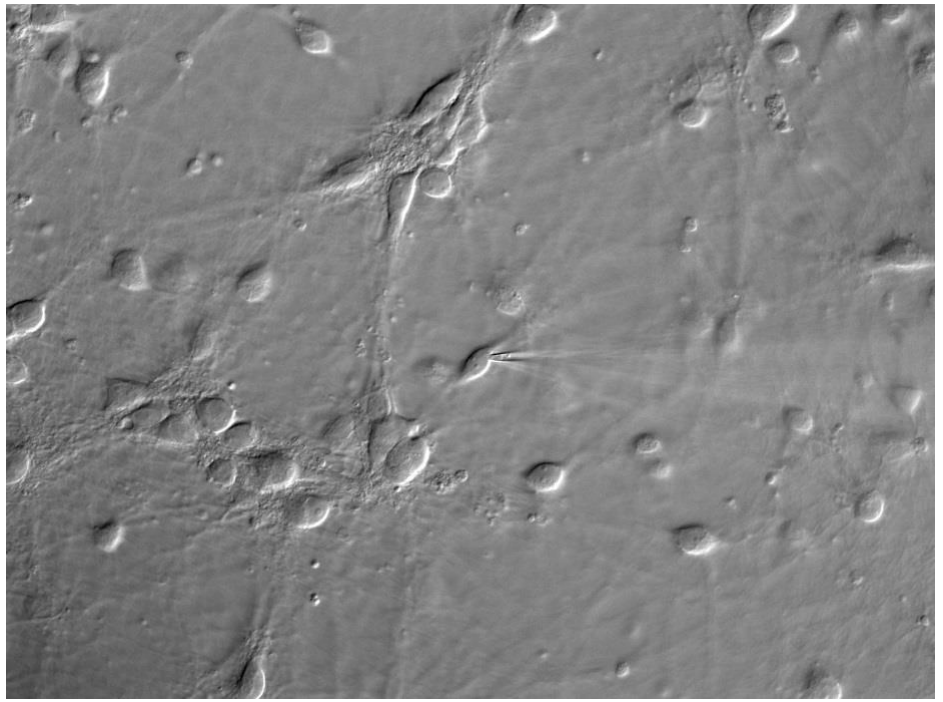
# *In vitro* preparations: Dissociated cultures

**Advantages:**

- Whole cells
- More controlled environment
- High-throughput → drug screening

**Disadvantages:**

- Outside their natural environment
- Original connections destroyed (but new ones) → maturation process
- 2D (but we are working on functional 3D neuronal networks)

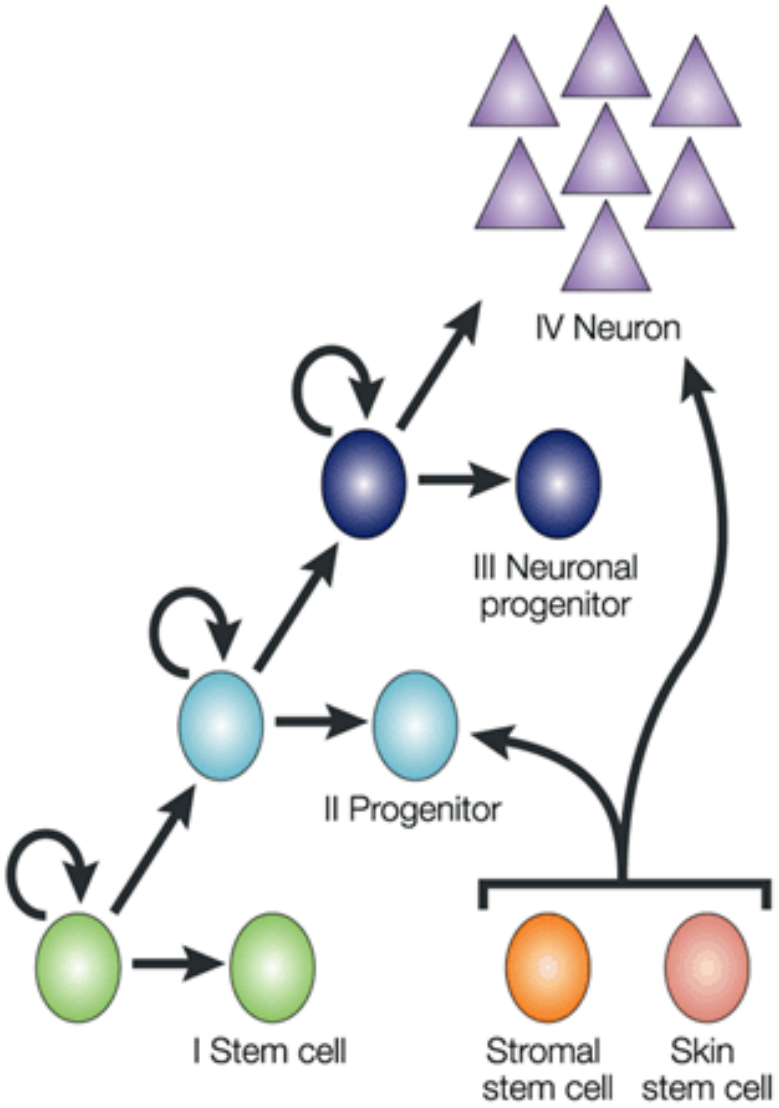


**Used tissue (examples):**

- Cortical, hippocampal, and spinal neurons
- Rat, mouse, and chicken
- Human embryonic stem cells (hESC) and human-induced pluripotent stem cells (hiPSCs)

Image: http://www.nbio.uni-heidelberg.de/Groups/Bengtson/Home.html

# Neuronal stem cell maturation




A schematic showing how a neuron ***might*** develop. After genesis of a new progenitor from a stem cell, continual restriction occurs as neuronal maturation proceeds.

**The final maturation of the neuron is associated with specific anatomical, biochemical and electrophysiological changes.**

Svendsen, et al. Nature Rev Neurosci 2001

Nature Reviews | Neuroscience

Kerstin Lenk

# iPS cells - derivation and applications

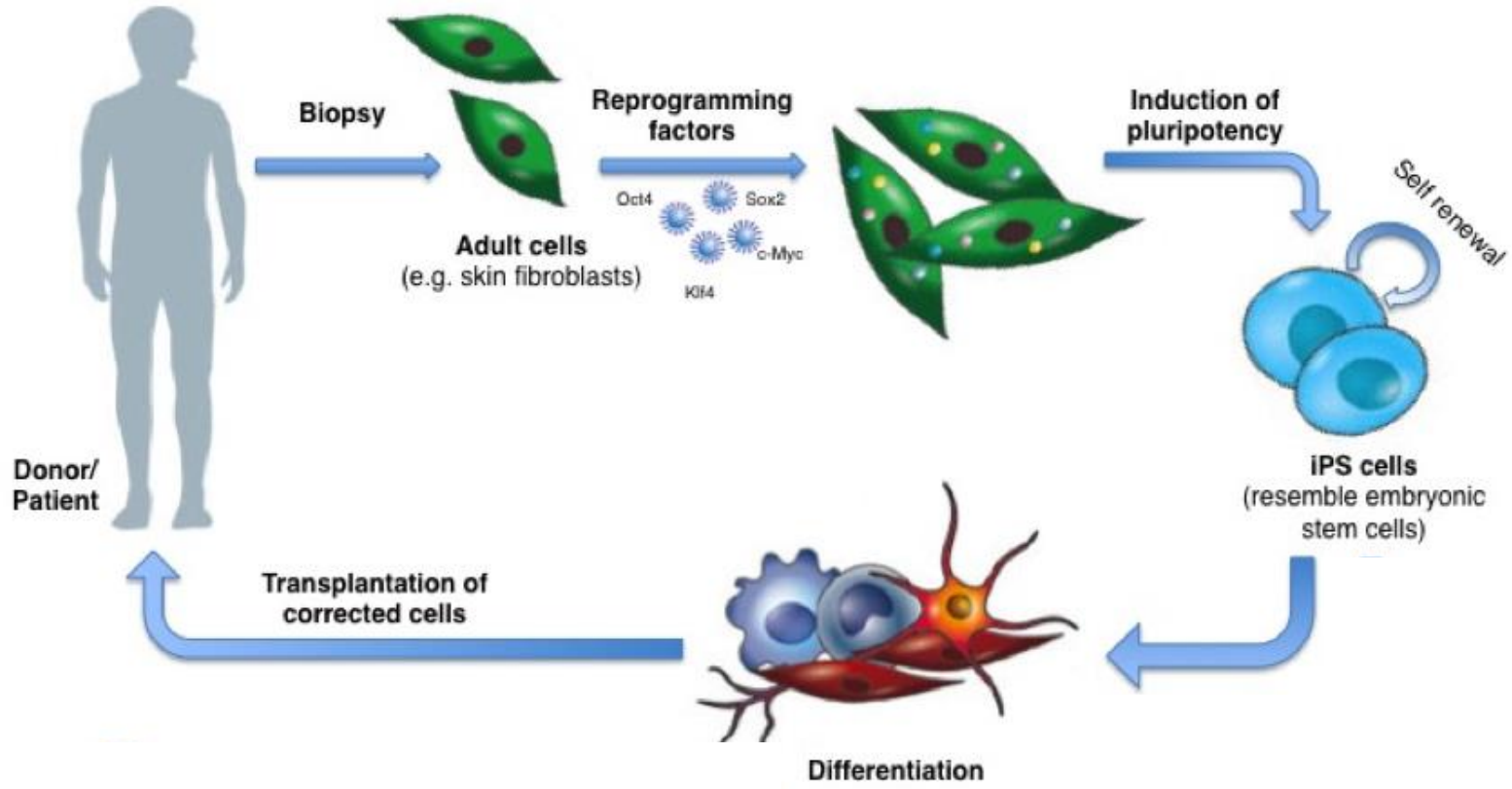



Image: https://www.eurostemcell.org/ips-cells-and-reprogramming-turn-any-cell-body-stem-cell

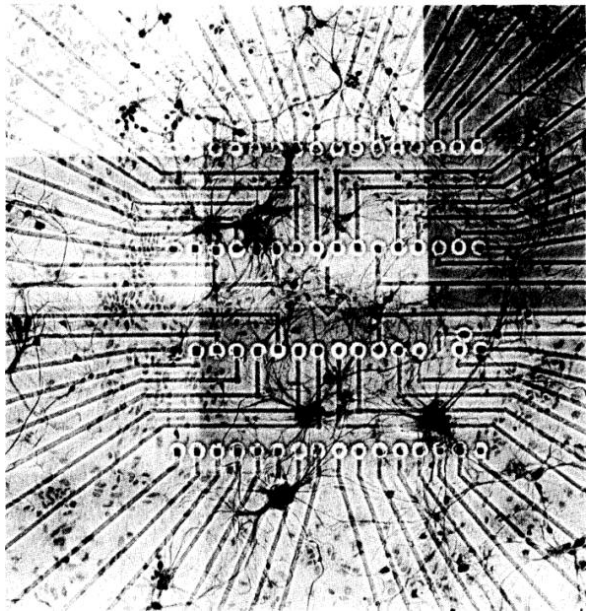
M. Rossbach

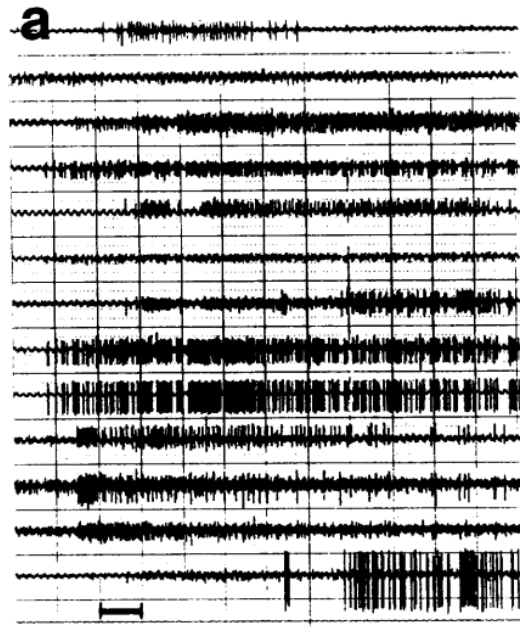
Kerstin Lenk

# Planar microelectrode array measurements

**Example 1 (Gross et. al., 1995)**

- Mammalian spinal cord
- Simultaneous monitoring of spike activity from many neurons
- Maturation process can be recorded
- Reaction of the network to different neuro-active substances $\rightarrow$ concentration dependence of oscillatory states



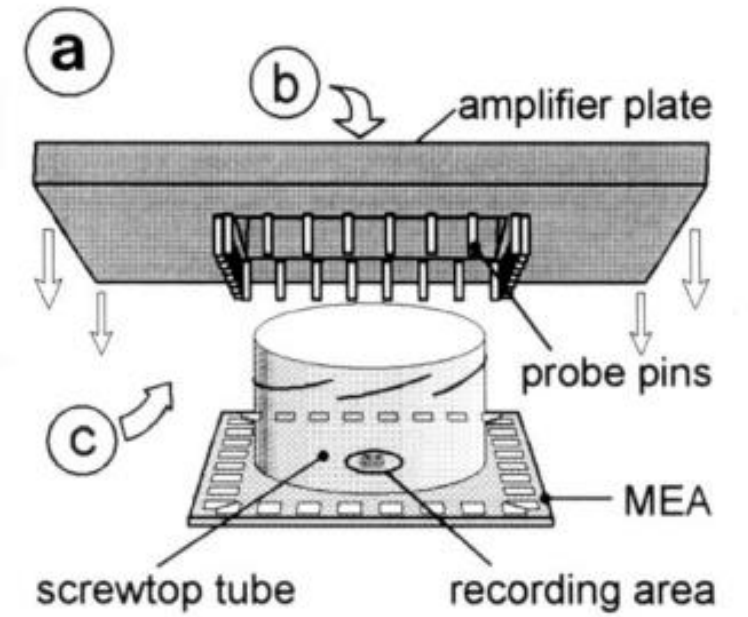


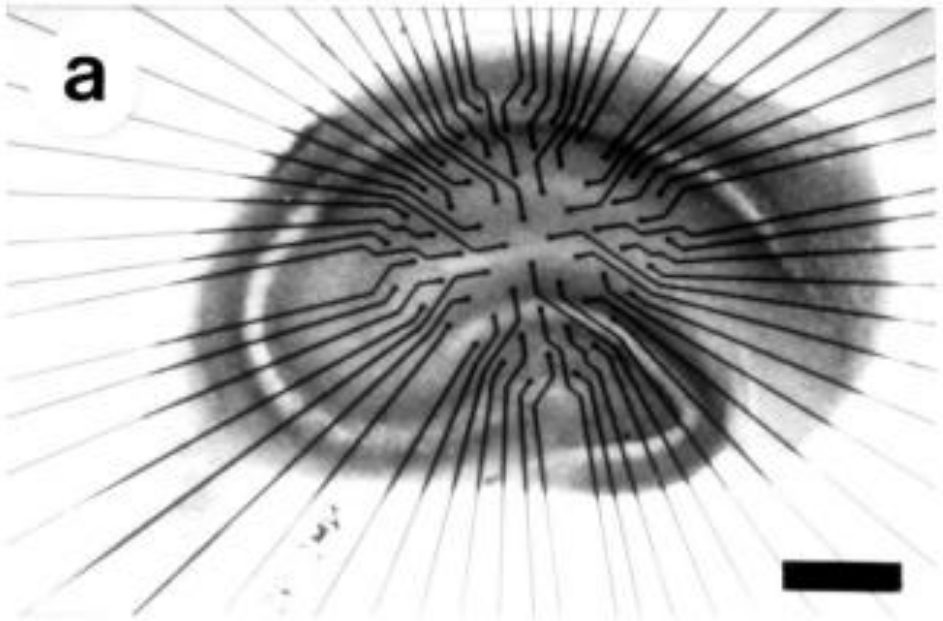

Gross, et al. Biosensors & Bioelectronics 1995

# Planar microelectrode array measurements

**Example 2 (Egert et. al., 1998)**

- Cultured rat hippocampal slices
- Multiple single-unit spontaneous spike activity and LFPs
- Possibility to correlate local spike patterns to the overall states of activity



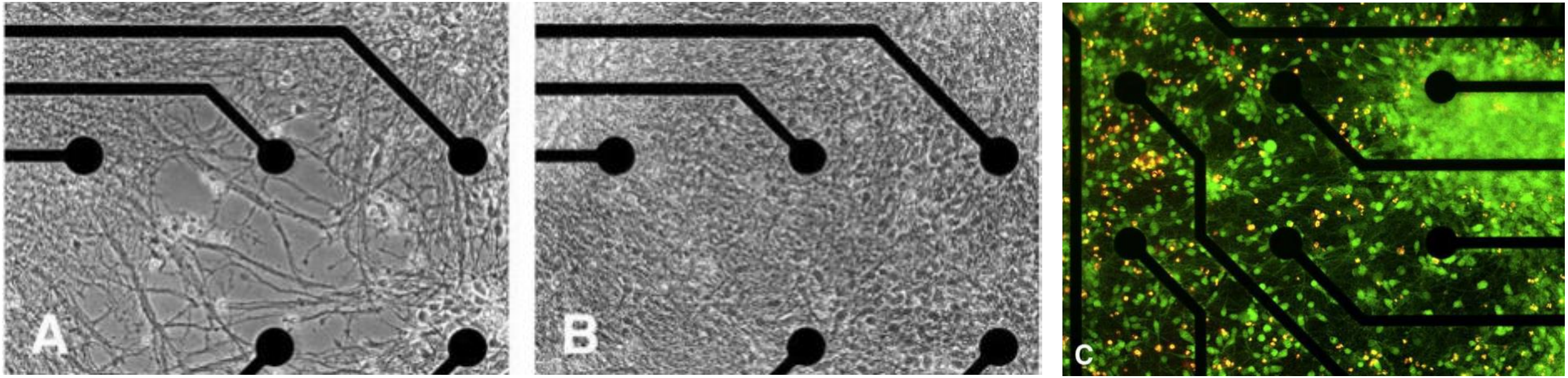




Egert, et al. Brain Research Protocols 1998

# Planar microelectrode array measurements

**Example 3 (Heikkilä et al., 2009)**

- Cultured hESC derived neurons
- Spontaneous and chemical induced spike activity
- Maturation process

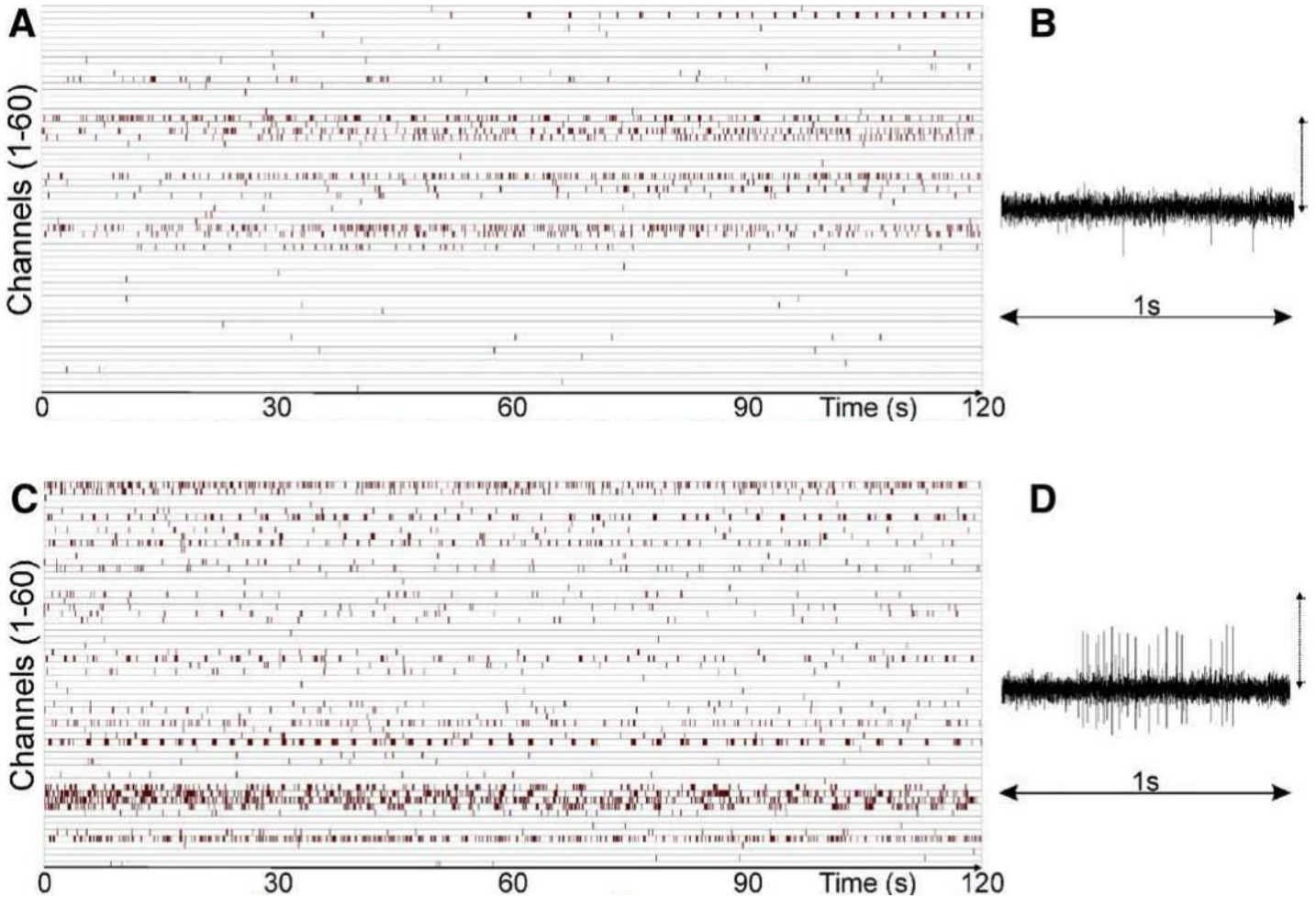



Maturation of a neuronal network during the 2nd (A) and 3rd (B) weeks of culturing on MEA dish. Neuronal cells were mostly viable (green color) after 6 weeks of culturing (C).

Heikkilä et al., Experimental Neurology 2009

# Planar microelectrode array measurements

**Example 3 (Heikkilä et al., 2009) - continued**



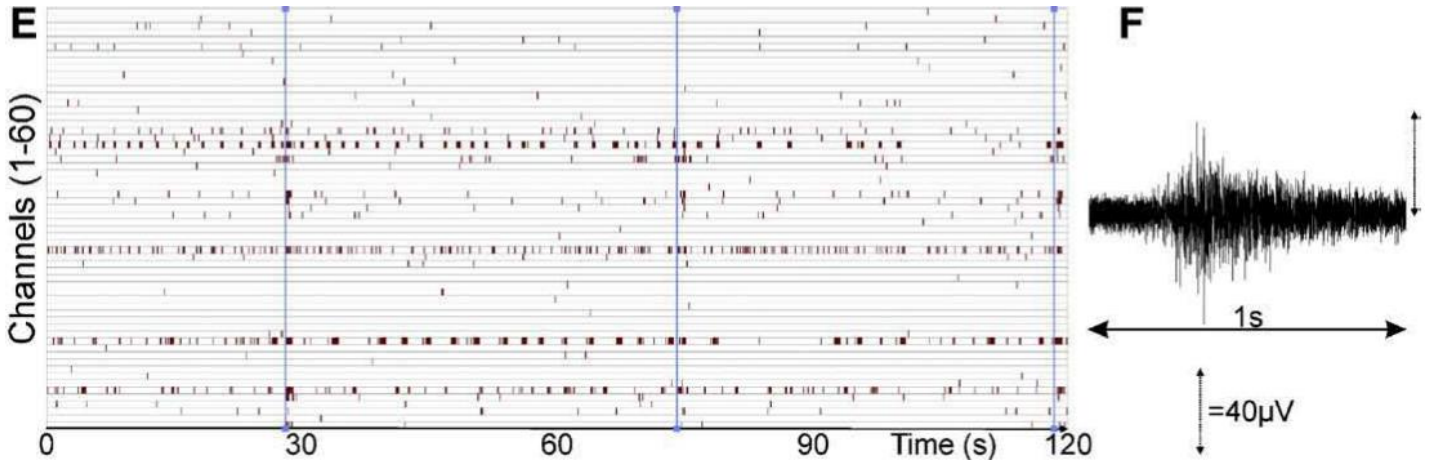

Development of neuronal signalling over 4 weeks of culturing. At the first stage (1st week on MEA), **single spike activity** (B) was recorded only by some electrodes (A).

At the second stage (weeks 2 to 3), the activity developed to **spike trains** (D) that were detected at multiple electrodes (C).

Heikkilä et al., Experimental Neurology 2009

# Planar microelectrode array measurements

**Example 3 (Heikkilä et al., 2009) - continued**




At the third stage (from 4 weeks onwards), **synchronous bursts** (F) took over as the dominant kind of activity.

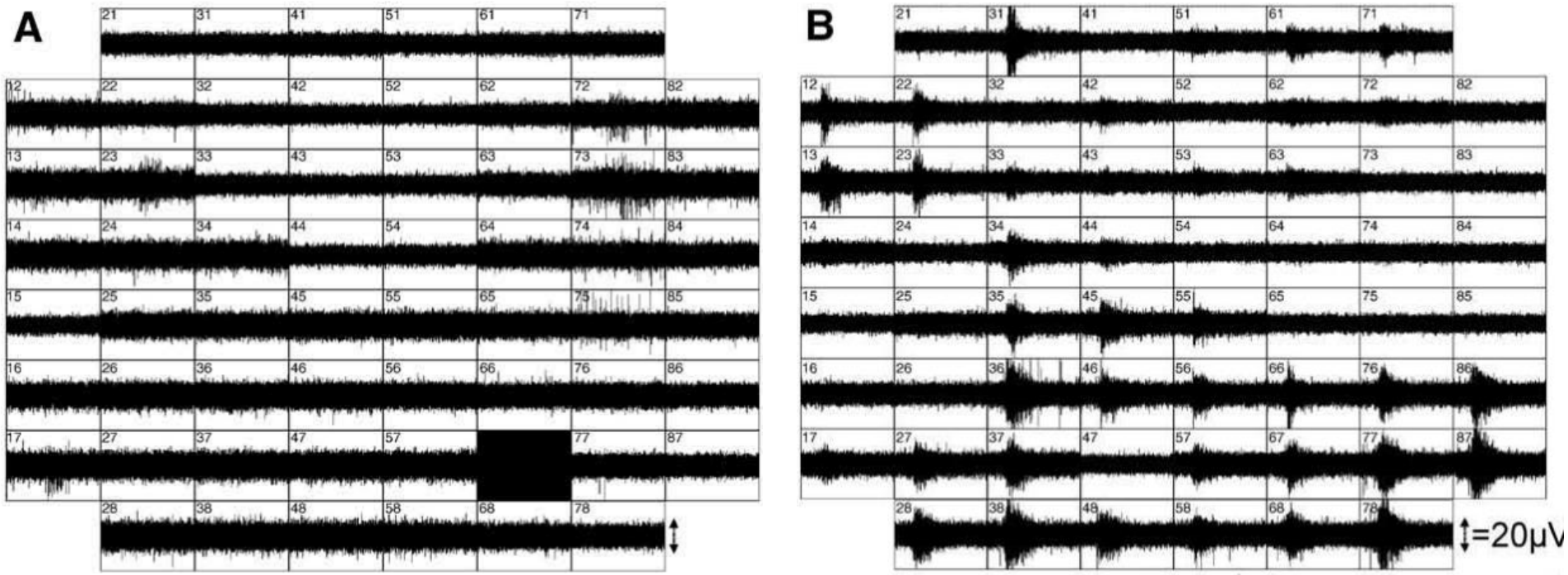
Heikkilä et al., Experimental Neurology 2009

# Planar microelectrode array measurements

**Example 3 (Heikkilä et al., 2009) - continued**

3-4 weeks

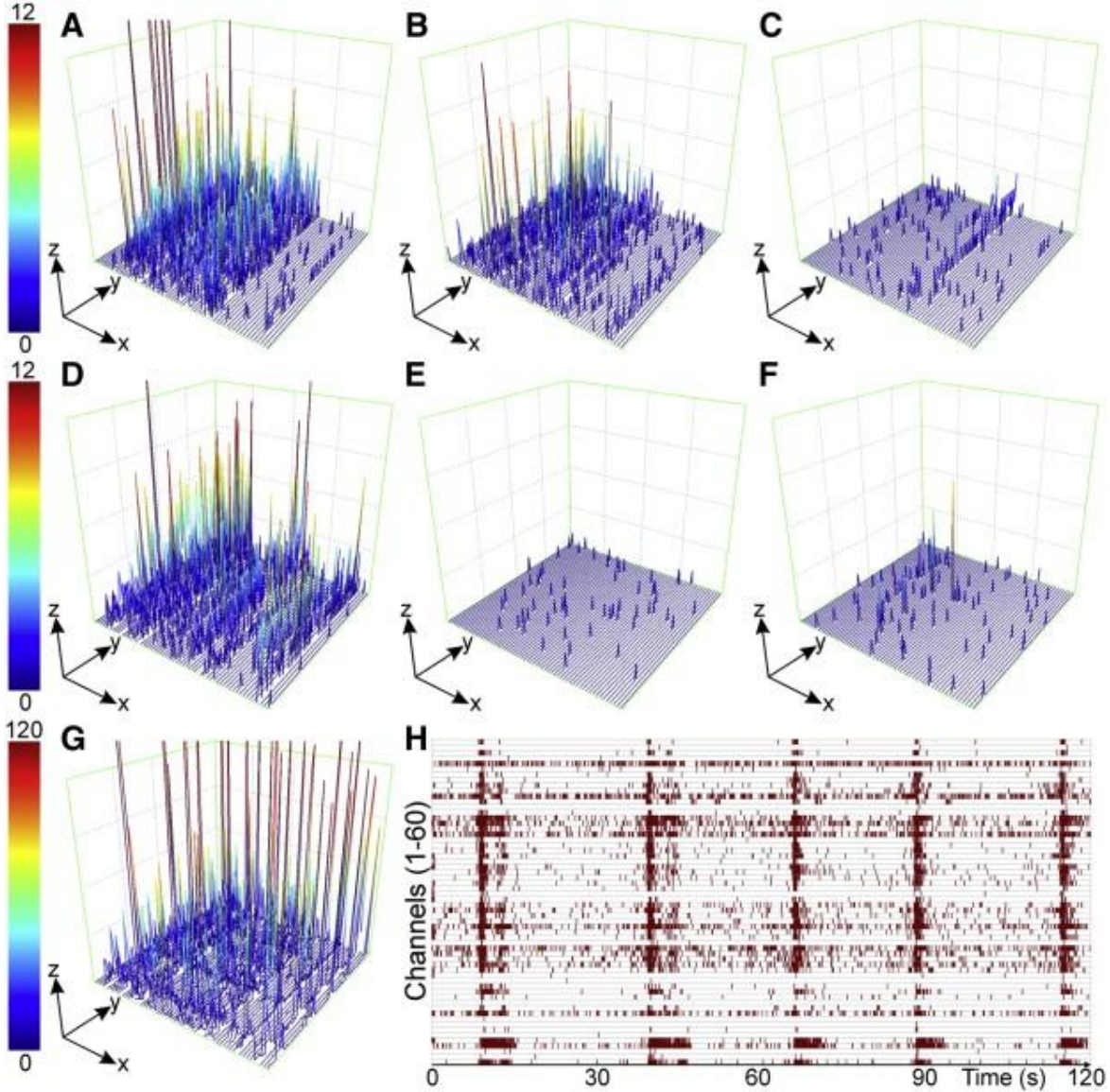
4-6 weeks




Heikkilä et al., Experimental Neurology 2009

# Planar microelectrode array measurements

**Example 3 (Heikkilä et al., 2009) - continued**




**Baseline activity (A).**

**The activity was partly suppressed by CNQX (B).**

**CNQX and D-AP5 together blocked all activity (C).**

**After a washout, activity reappeared (D).**

**GABA inhibited all activity (E),**

**and the activity did not return after a washout (F).**

**The addition of bicuculline restored the activity (G) to a higher level than at the baseline (A).**

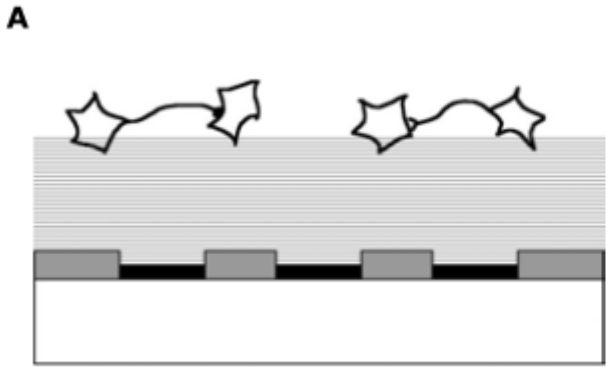
Raster plot of the bicuculline-induced synchronous activity (H).

Heikkilä et al.

# 3D microelectrode array measurements

**Example (Heuschkel et. al., 2006)**

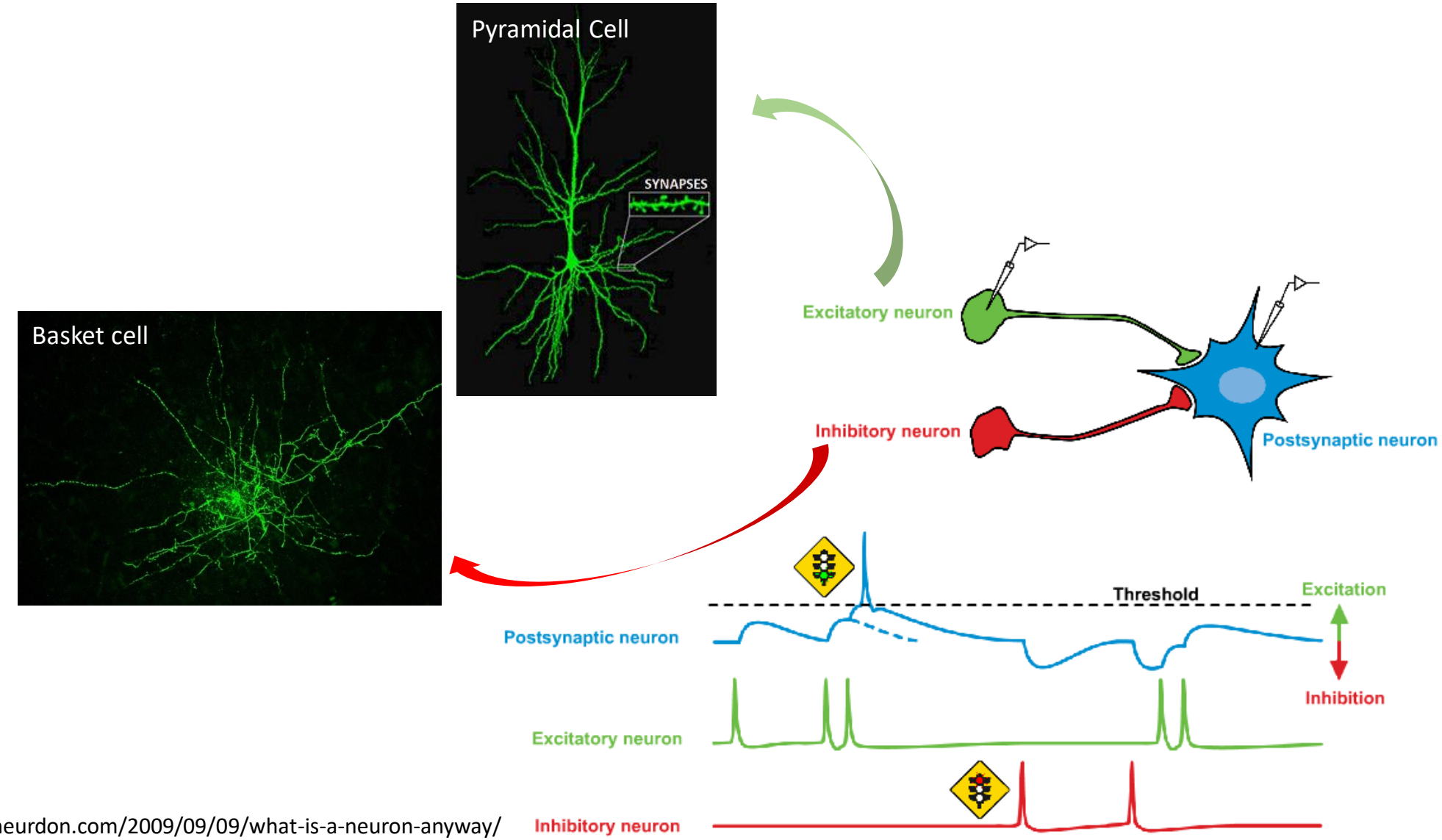
- Acute rat hippocampal slices → dead-cell layer problem




- Reduction of distance between the electrodes and active living neurons
- Geometrical advantage with an increased surface → reduces electrode impedance → increases recorded signal amplitudes

Heuschkel et al., In: Advances in Network Electrophysiology Using Multi-Electrode Arrays; Springer, 2006

# Excitation and inhibition




Images: http://www.neurdon.com/2009/09/09/what-is-a-neuron-anyway/
http://www.people.vcu.edu/~kmjacobs/res/basket.jpg
http://neuroscience.uth.tmc.edu/s1/introduction.html

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# Single neuron oscillators

Different types of *ion channels* can result in neurons exhibiting different firing patterns of action potentials in response to a constant driving input

- Regular firing
- Bursting: this is an *oscillating* pattern → train of spikes followed by quiescence
- Irregular firing
- Examples of recordings from neurons in the sea snail, Aplysia

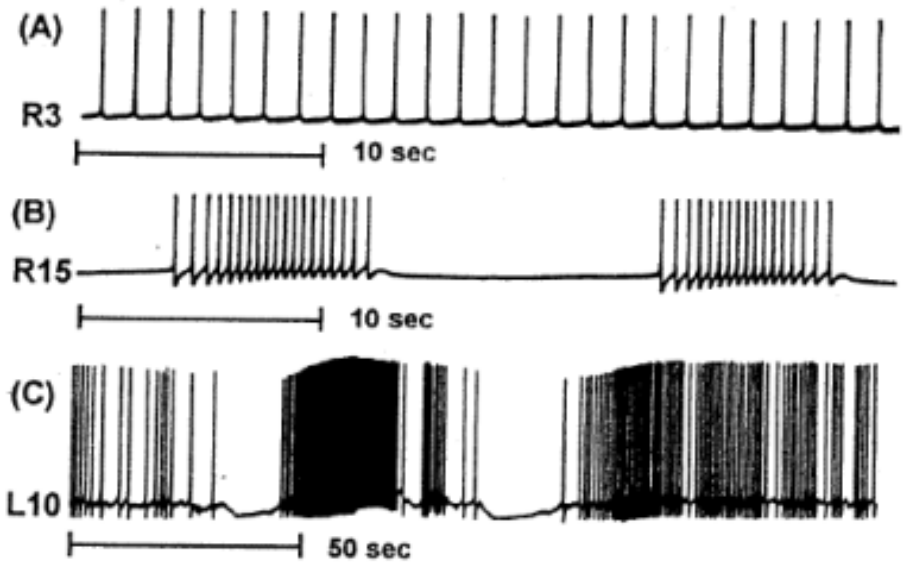



Image: http://www.cs.stir.ac.uk/courses/31YF/lectures/BNN/BNN-4-networks.pdf

# Simple circuit oscillators

- Simplest oscillating circuit consists of one excitatory (E) neuron reciprocally coupled with one inhibitory (I) neuron
  - E-I oscillator
- Excitatory neuron receives constant drive
- Delay around loop leads to alternating firing between the E and I neurons

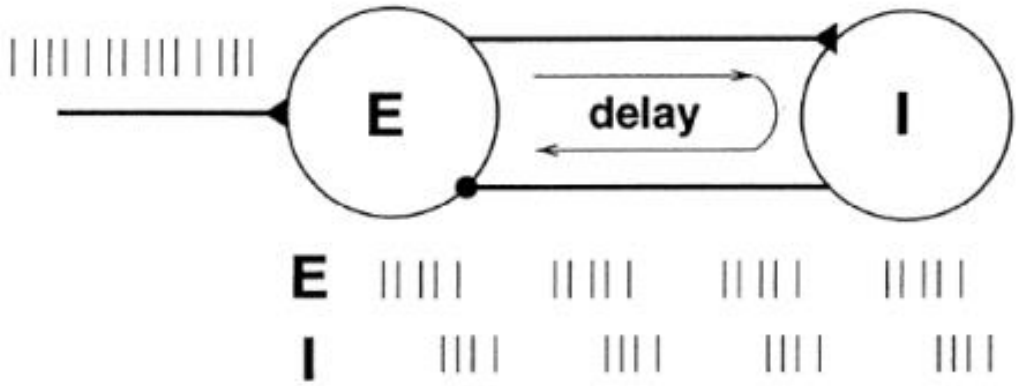
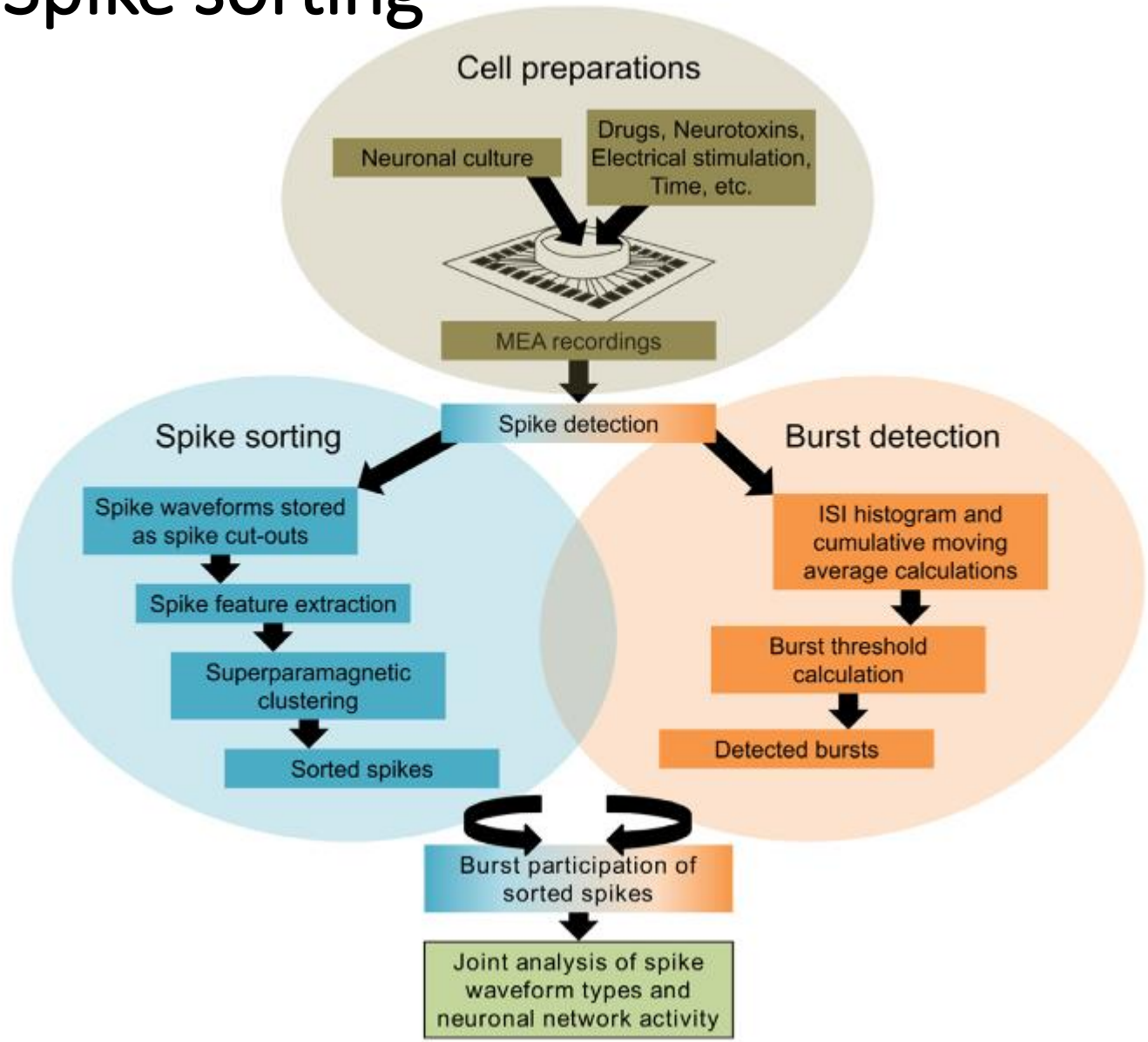


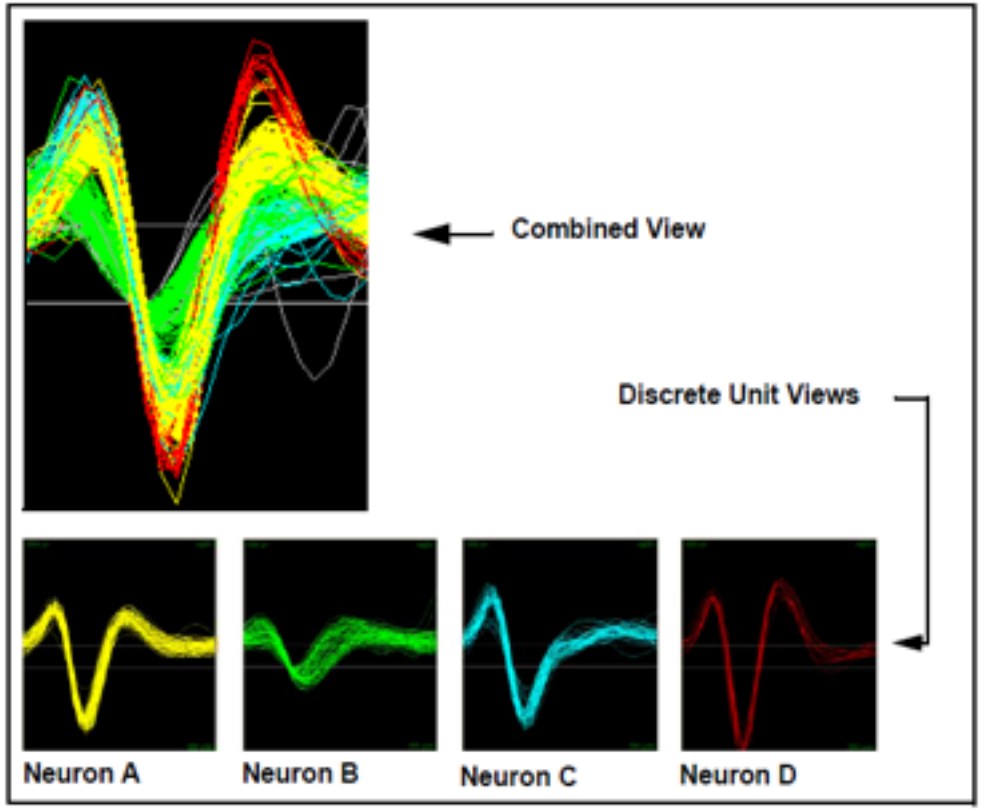

Image: http://www.cs.stir.ac.uk/courses/31YF/lectures/BNN/BNN-4-networks.pdf

# Contents

1. Bioelectric assessment of neural networks in vitro
2. Spike and burst analysis methods/ tools
3. Network connectivity/ synchronization analysis
4. Calcium imaging

# Spike sorting




Software tools, e.g.:

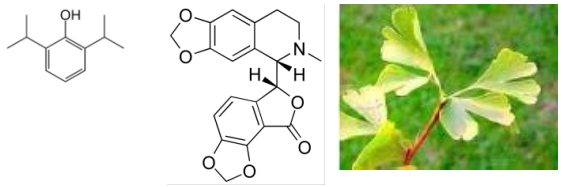
- NeuroExplorer
- Wave Clus

Kapucu, et al., J Neurosci Meth 2016

# How can different activity patterns be quantified?

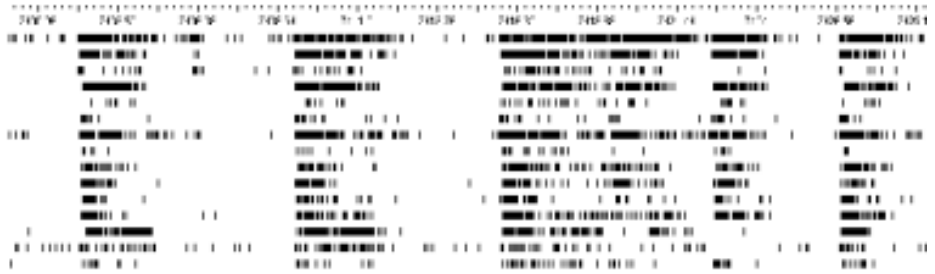
Native activity

Adding a neuro-active substance






Blockade of $GABA_A$ Receptor

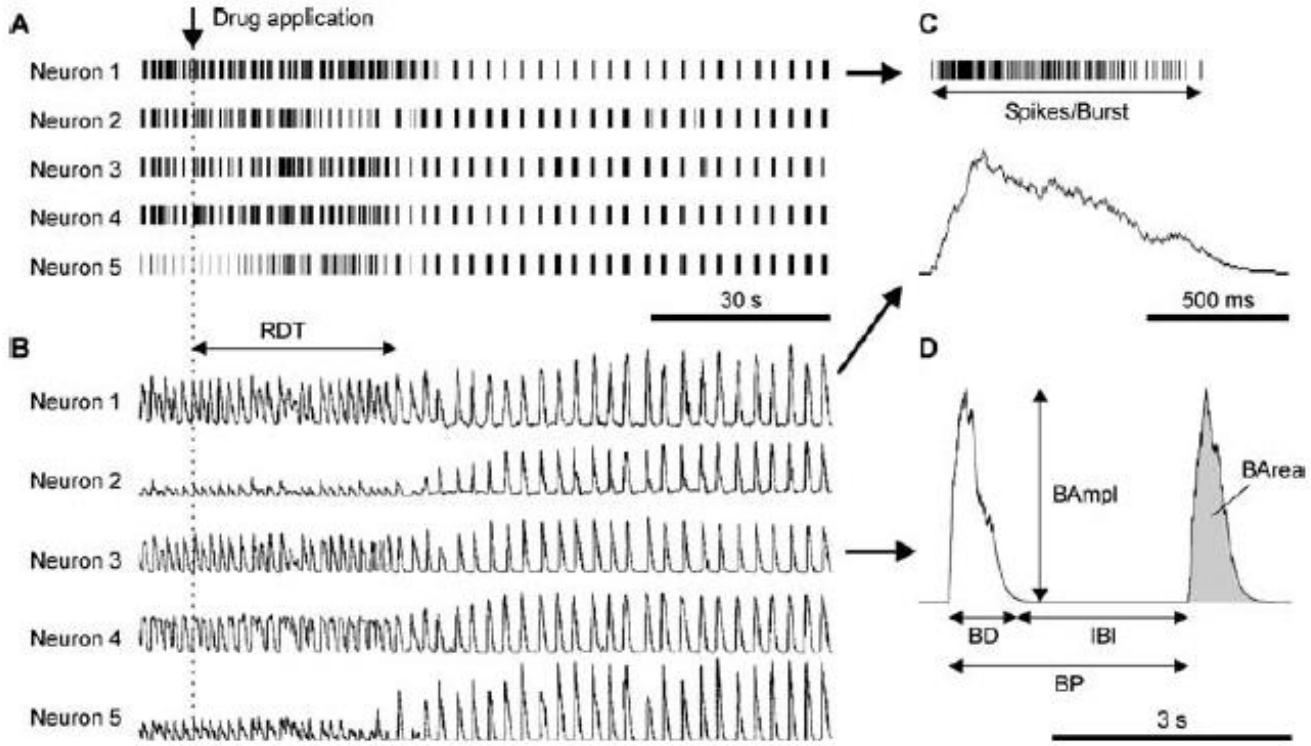


Activity mediated by NMDA receptor only

10 s

Gross et al. (1995). Biosensors & Bioelectronics;
Schroeder et al. (2008). 6th Int. Meeting on Substrate-Integrated Microelectrodes;
Motulsky et al. (2004). Oxford University Press
Images: NeuroProof GmbH

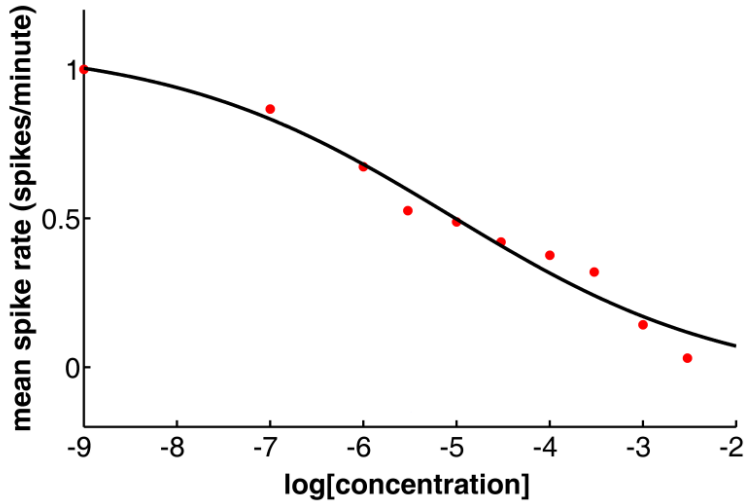
# Spike train features




**Calculation of spike train features:**

Spike rate
Burst rate
Burst duration
Spikes in burst
Burst amplitude
Burst area
Interspike intervals (ISI)
etc.

**Concentration-response curve**




Gramowski et al. (2004). Eur J Neurosci

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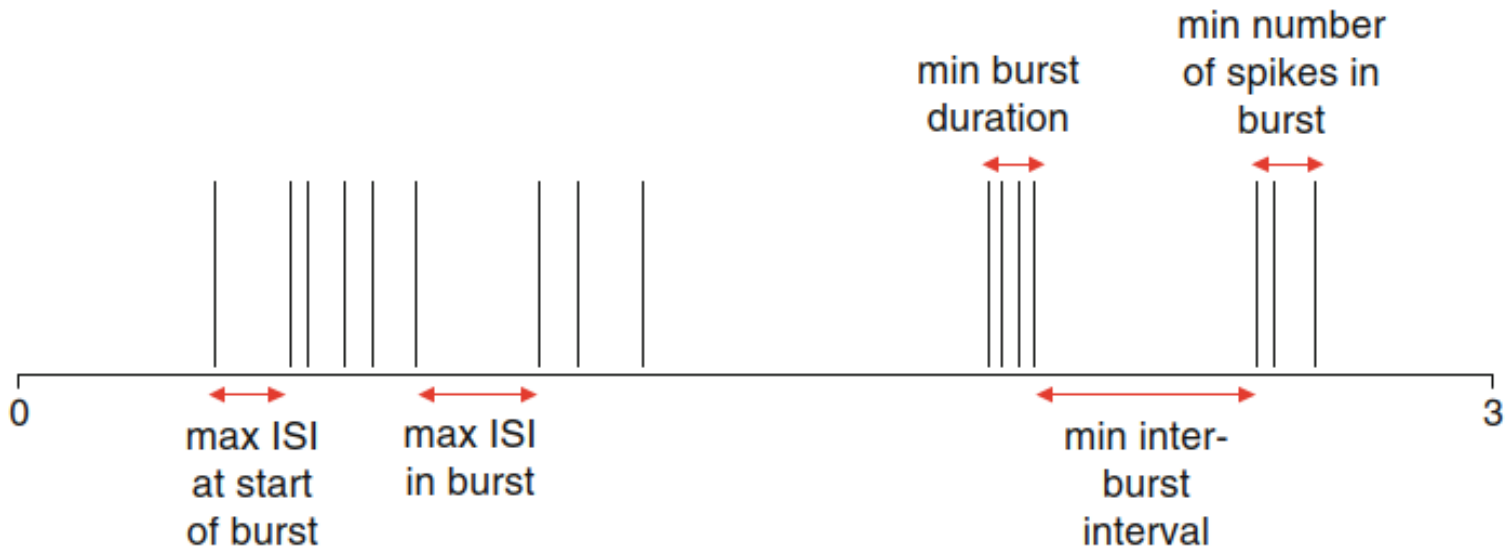
# What defines a burst?

**Table 1** Burst detectors classified by their approach to burst detection

| Abbreviation | Method | Reference |
|---|---|---|
| *Fixed threshold-based methods* | | |
| MI | MaxInterval | Nex Technologies (2014) |
| *Adaptive threshold-based methods* | | |
| logISI | LogISI | Pasquale et al. (2010) |
| CMA | Cumulative Moving Average | Kapucu et al. (2012) |
| IRT | ISI Rank Threshold | Hennig et al. (2011) |
| *Surprise-based methods* | | |
| PS | Poisson Surprise | Legéndy and Salcman (1985) |
| RS | Rank Surprise | Gourévitch and Eggermont (2007) |
| RGS | Robust Gaussian Surprise | Ko et al. (2012) |
| *Other methods* | | |
| HSMM | Hidden Semi-Markov Model | Tokdar et al. (2010) |

Cotterill & Eglen. Chapter in: In Vitro Neuronal Networks, Advances in Neurobiology 2019

# Example 1: MaxInterval



# Example 2: logISI

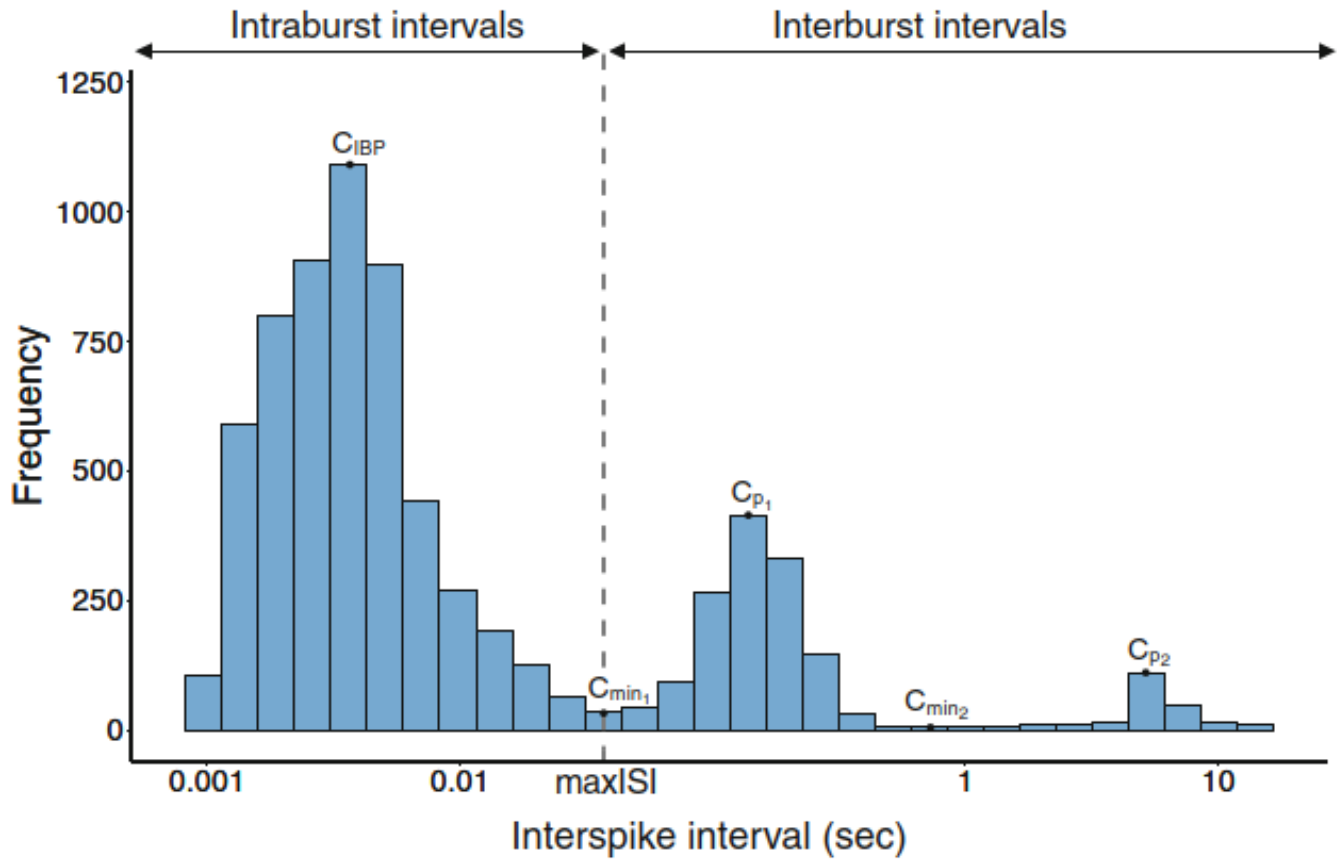



**Fig. 4** Example of log-adjusted ISI histogram with the threshold for intra- and interburst intervals found using the logISI method

For each minimum:

$$void(i) = 1 - \frac{C_{min_i}}{\sqrt{C_{IBP} \cdot C_{p_i}}}$$

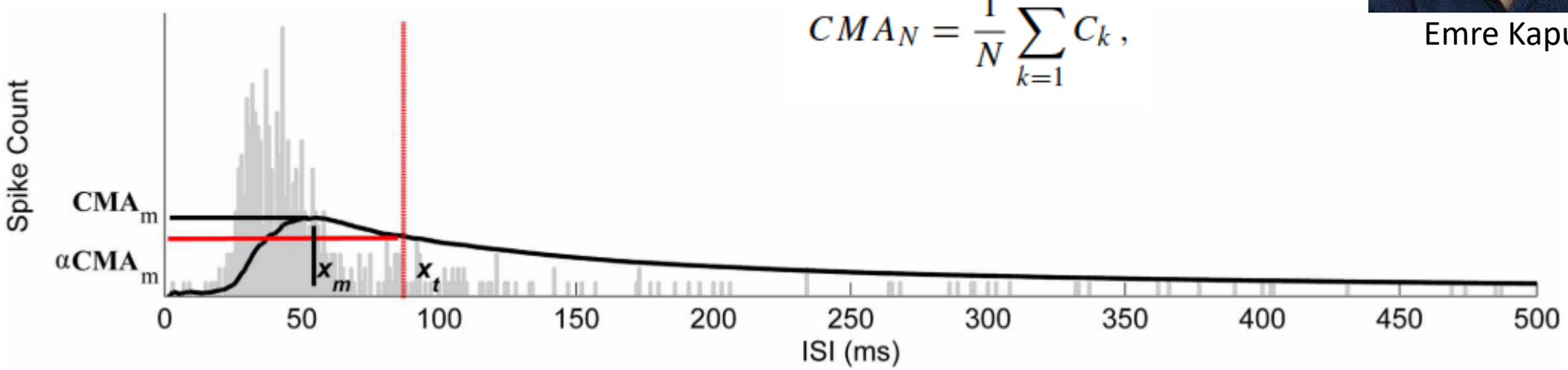
The smallest $ISI_{min_i}$ for which void(i) > 0.7 is set as the threshold for the maximum ISI in a burst, maxISI.

Cotterill & Eglen. In: In Vitro Neuronal Networks, Advances in Neurobiology 2019

# Example 3: Cumulative moving average (CMA)

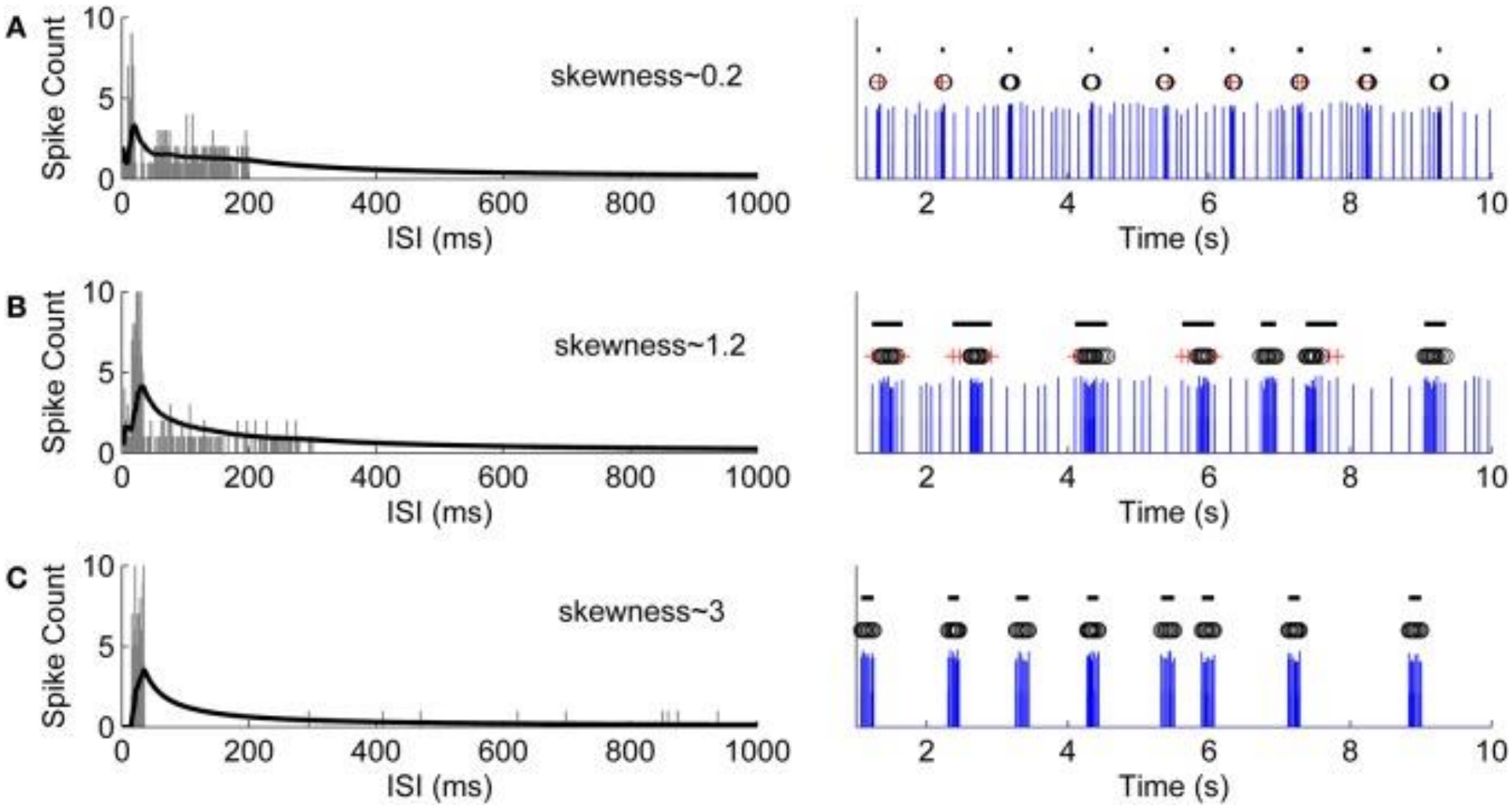
Emre Kapucu

$$CMA_N = \frac{1}{N}\sum_{k=1}^{N} C_k \,,$$




Kapucu, et al., Front Comput Neurosci 2012

# Example 3: Cumulative moving average (CMA)




Kapucu, et al., Front Comput Neurosci 2012

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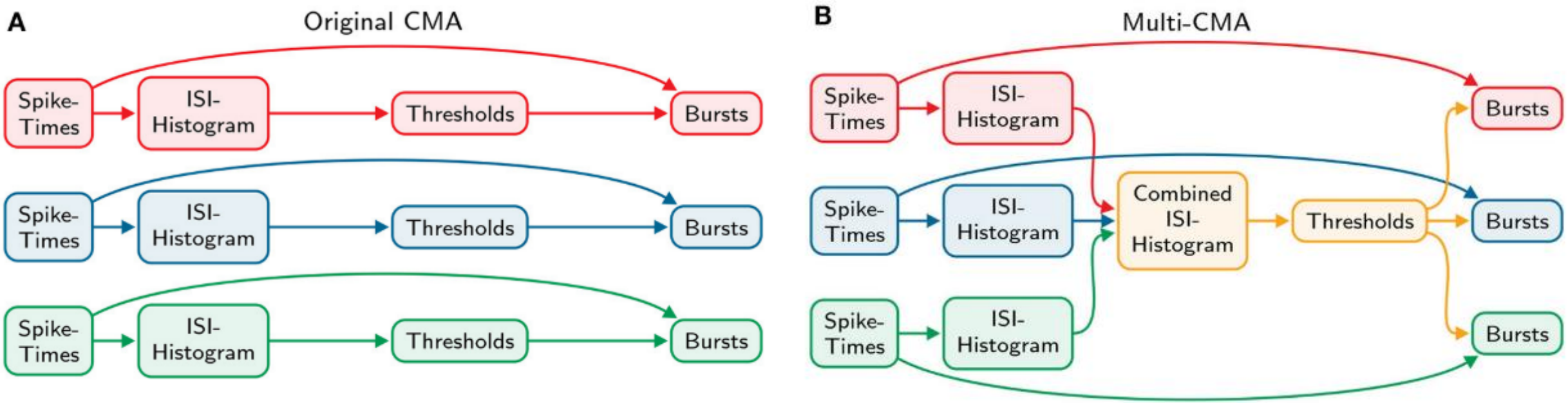
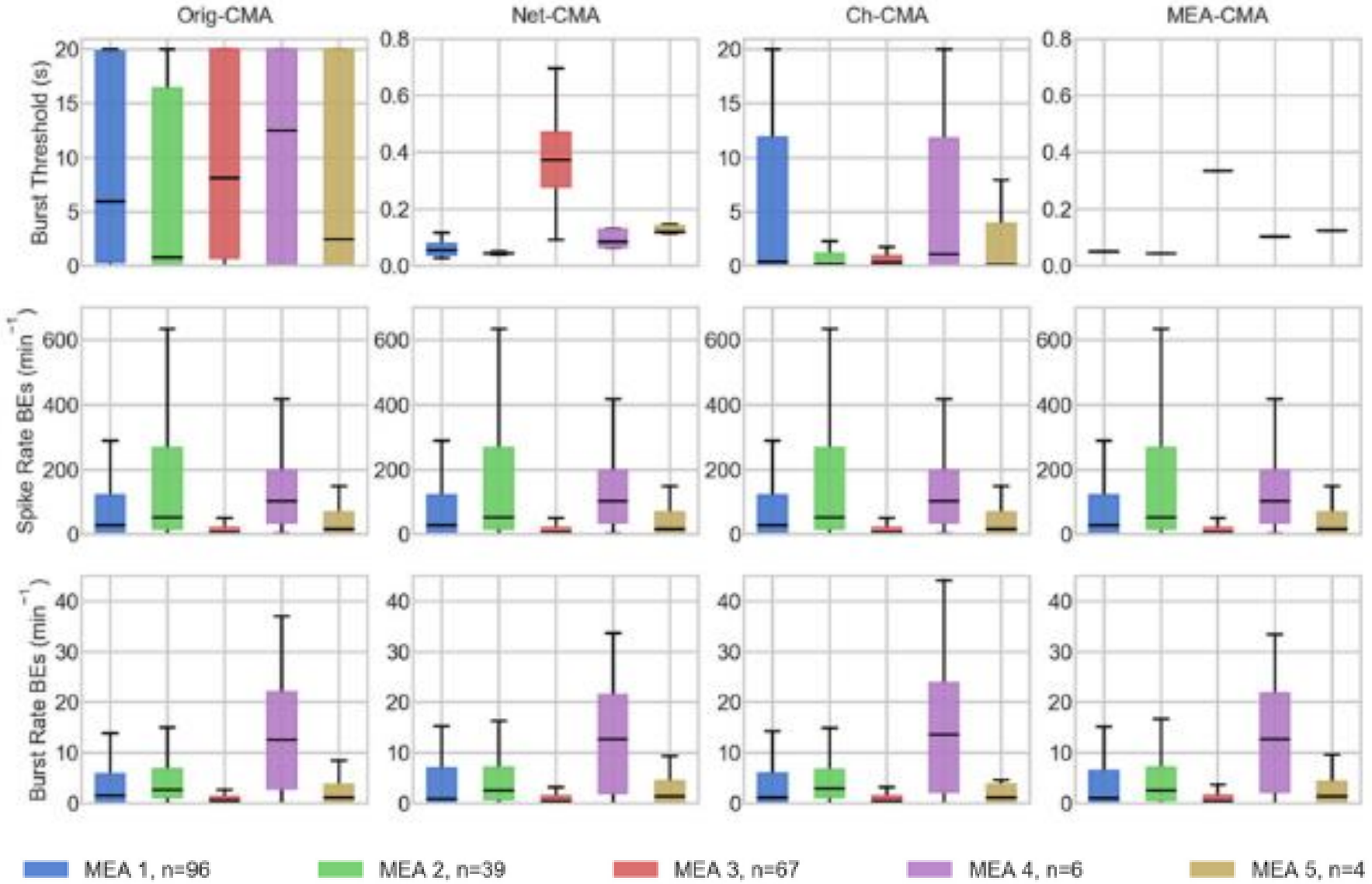
# Example 3: Network-wide cumulative moving average

Inkeri Välkki




Välkki, et al., Front Comput Neurosci 2017

# Example 3: Network-wide cumulative moving average




Välkki, et al., Front Comput Neurosci 2017

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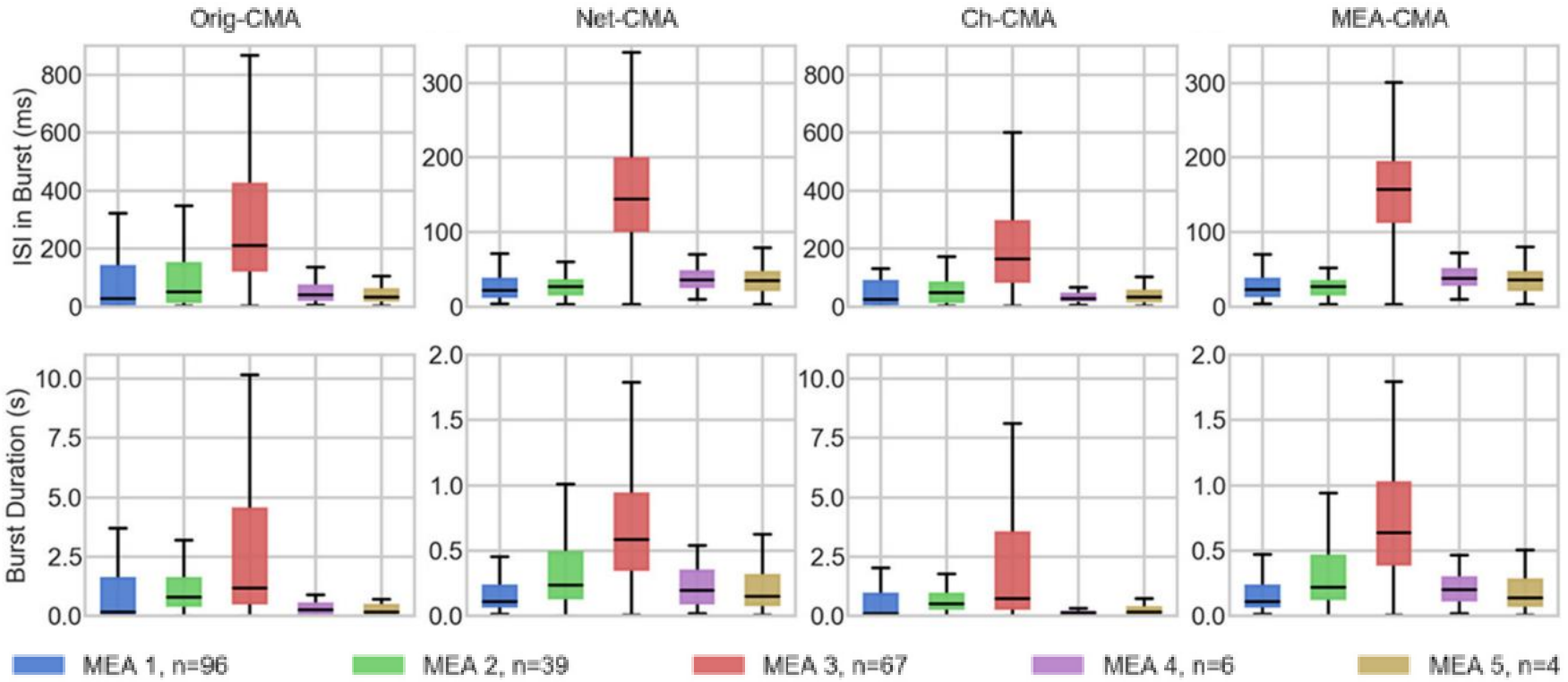
# Example 3: Network-wide cumulative moving average




Välkki, et al., Front Comput Neurosci 2017

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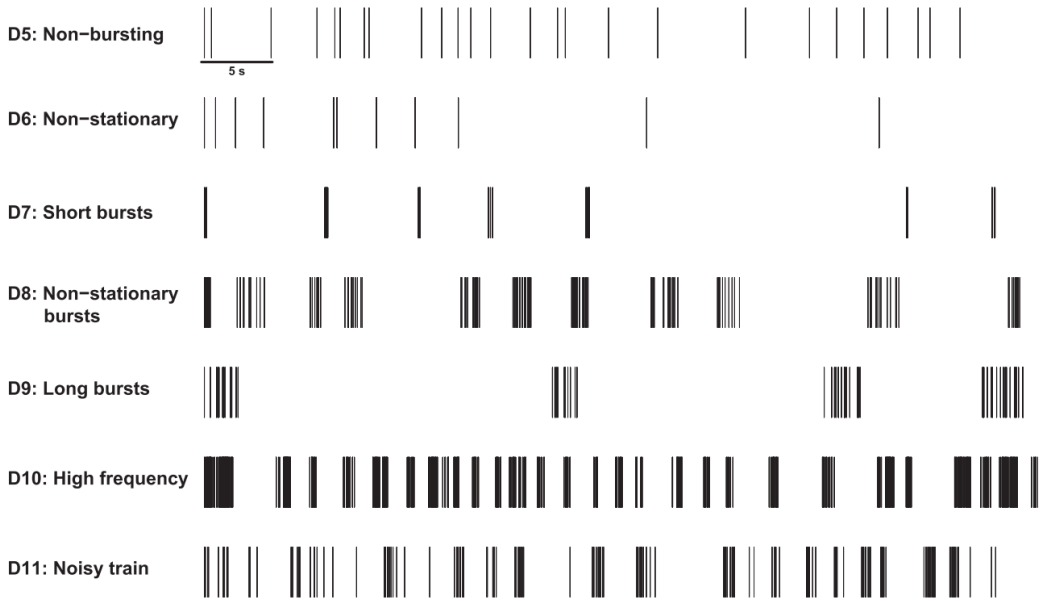
*J Neurophysiol* 116: 306–321, 2016.
First published April 20, 2016; doi:10.1152/jn.00093.2016.

# A comparison of computational methods for detecting bursts in neuronal spike trains and their application to human stem cell-derived neuronal networks

**Ellese Cotterill,[1] Paul Charlesworth,[2] Christopher W. Thomas,[2] Ole Paulsen,[2] and Stephen J. Eglen[1]**




| | PS | MI | CMA | RS | IRT | RGS | LogISI | HSMM |
|---|---|---|---|---|---|---|---|---|
| *D5*: nonbursting | 4 | 1 | 7 | 5 | 6 | 3 | 1 | 8 |
| *D6*: nonstationary | 6 | 2 | 7 | 4 | 5 | 3 | 1 | 8 |
| *D7*: regular bursting | 4 | 1 | 2 | 7 | 6 | 7 | 5 | 3 |
| *D8*: nonstationary bursts | 4 | 3 | 5 | 7 | 6 | 8 | 2 | 1 |
| *D9*: long bursts | 2 | 4 | 3 | 8 | 5 | 7 | 6 | 1 |
| *D10*: high frequency | 5 | 1 | 4 | 7 | 6 | 8 | 2 | 3 |
| *D11*: noisy bursts | 5 | 1 | 2 | 7 | 6 | 8 | 4 | 2 |
| Total (relative rank) | 30(4) | 13 (1) | 30 (4) | 45 (8) | 40 (6) | 44 (7) | 21 (2) | 26 (3) |

1 = best, 8 = worst.

Cotterill et al., J Neurophysiol 2016

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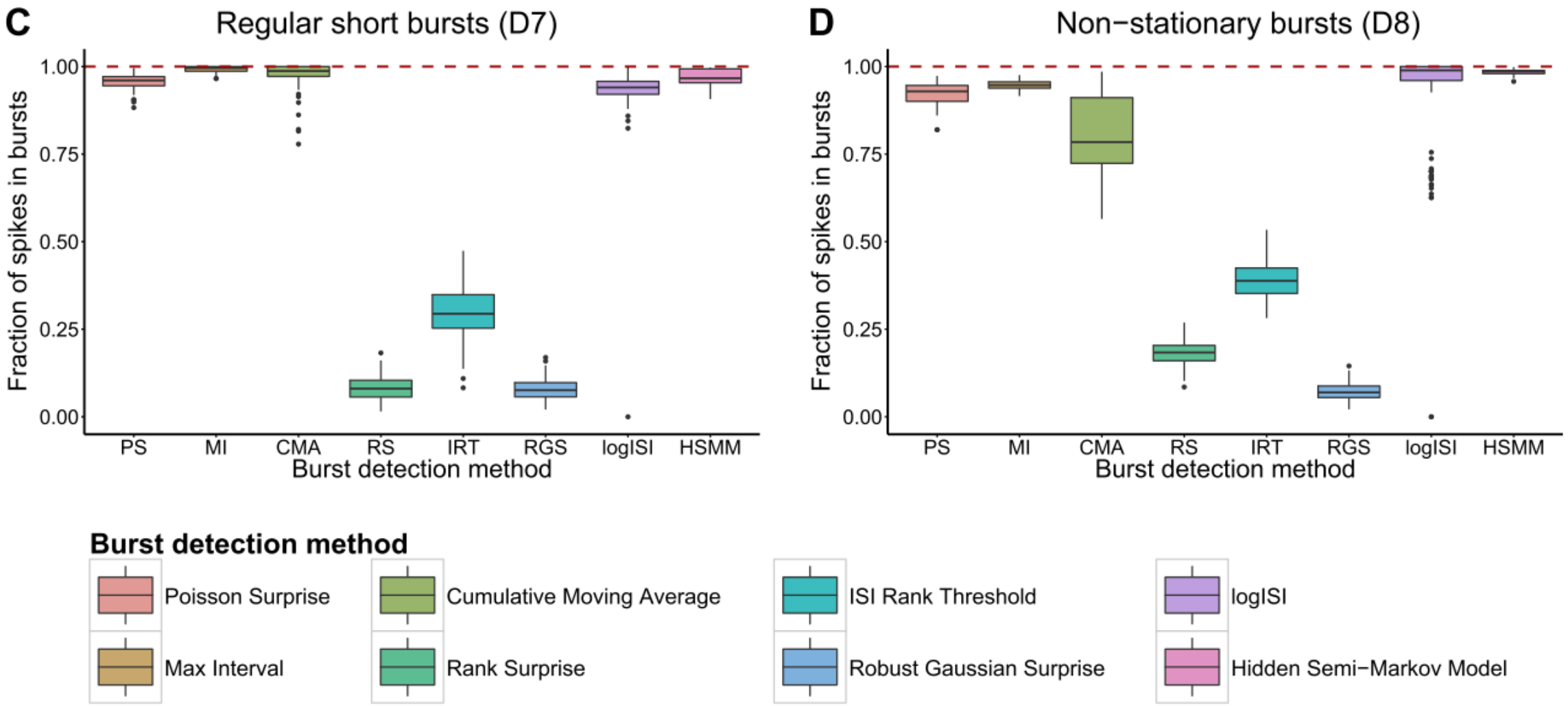
# Fraction of spikes in bursts found by each burst detector




Cotterill et al., J Neurophysiol 2016

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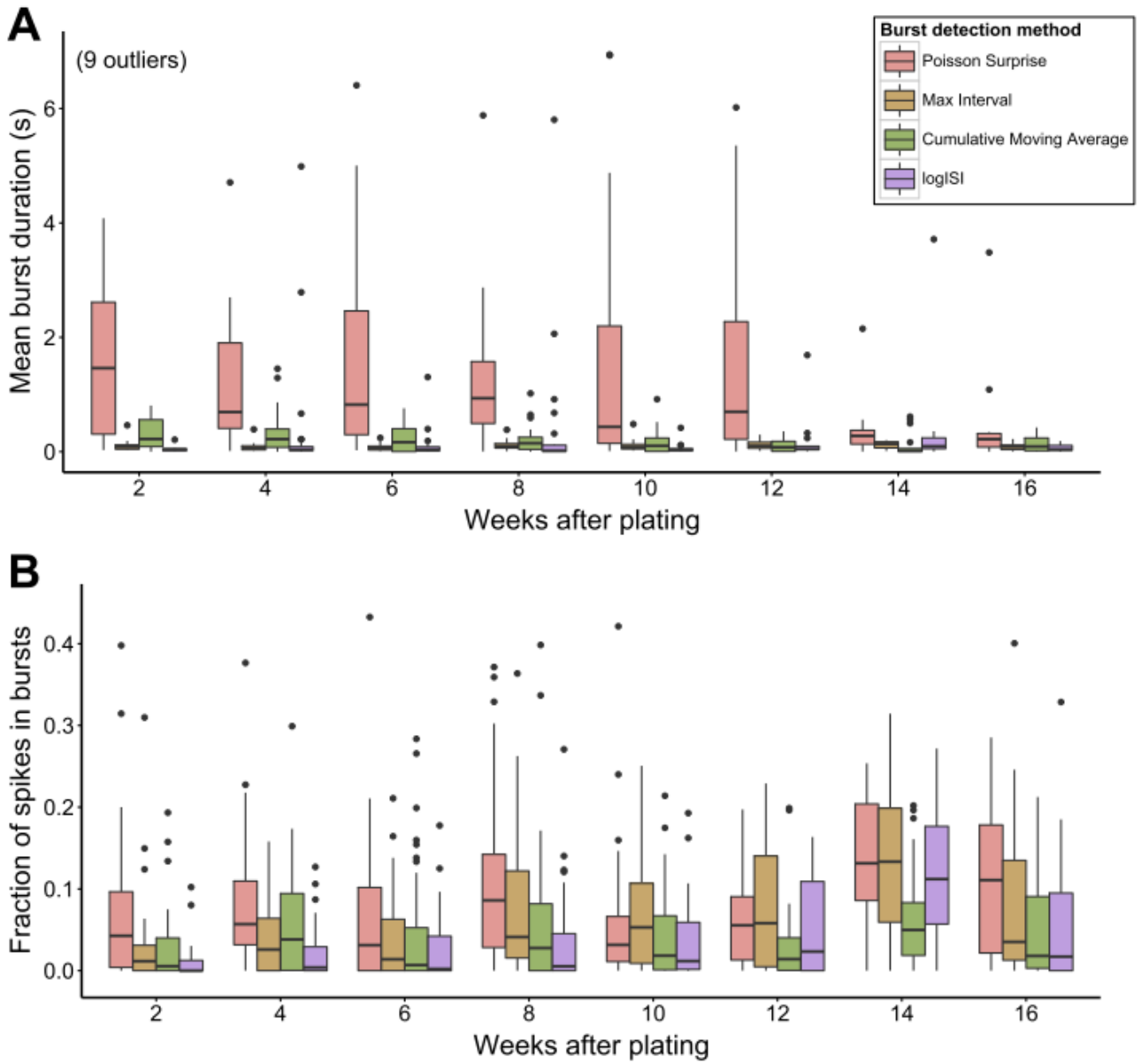
# Burst statistics for hiPSC neurons




Cotterill et al., J Neurophysiol 2016

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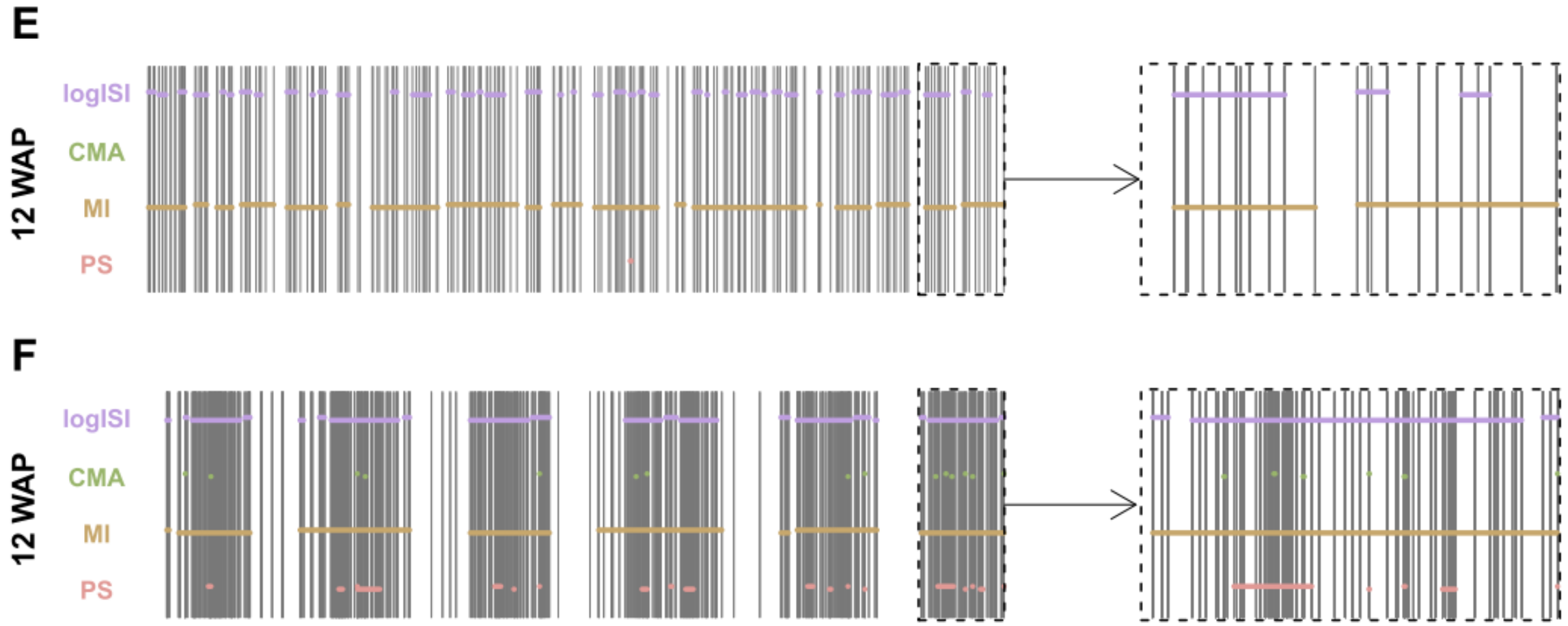
# Burst statistics for hiPSC neurons




Cotterill et al., J Neurophysiol 2016

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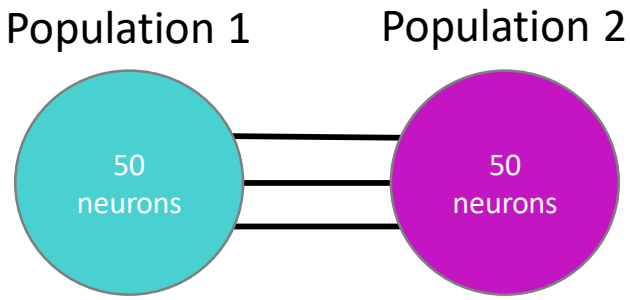
frontiers in Computational Neuroscience

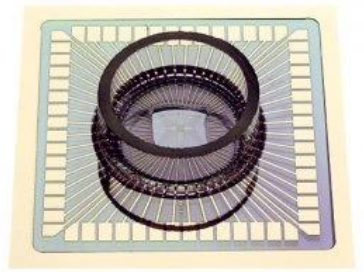
METHODS
published: 18 October 2016
doi: 10.3389/fncom.2016.00112

Check for updates

# Spectral Entropy Based Neuronal Network Synchronization Analysis Based on Microelectrode Array Measurements

*Fikret E. Kapucu[1,2]*, Inkeri Välkki[2], Jarno E. Mikkonen[3], Chiara Leone[4], Kerstin Lenk[2], Jarno M. A. Tanskanen[2] and Jari A. K. Hyttinen[2]*



Kapucu et al., Front Comput Neurosci 2016; Image: www.multichannelsystems.com

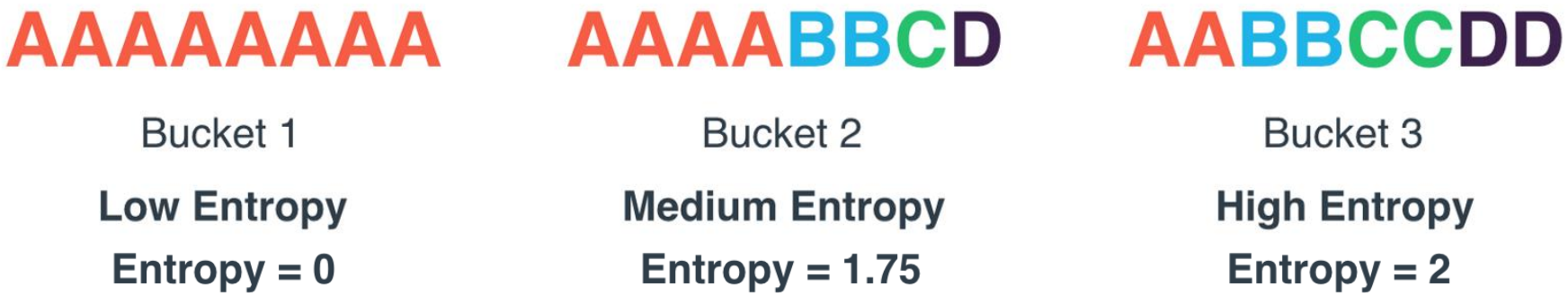
Kerstin Lenk

# CorSE, Shannon Entropy (SE) Based Synchronization Analysis

SE in general:

$$H = -\sum_i p_i \log p_i,$$

$p_i$ = probability that an amplitude value occurs in the ith amplitude bin, given by the probability density function of the time series




Kapucu et al., Front Comput Neurosci 2016; Image: https://medium.com/udacity/shannon-entropy-information-gain-and-picking-balls-from-buckets-5810d35d54b4

# CorSE, Shannon Entropy (SE) Based Synchronization Analysis

$$\boldsymbol{X}(f) = \sum_{n} \mathbf{x}(n) e^{-i2\pi fn}$$

frequency spectrum of the time series x(n), sampled at discrete time points n, by fast Fourier transform X(f) at frequency points f

$$\boldsymbol{P}(f) = \boldsymbol{X}(f)\,\boldsymbol{X}^{*}(f)$$

$X^{*}(f)$ is the complex conjugate of X(f)

$$\sum_{f_k=f_1}^{f_K} \boldsymbol{P_{norm}}(f_k) = C \sum_{f_k=f_1}^{f_K} \boldsymbol{P}(f_k) = 1$$

Power spectrum was normalized with a constant C at K frequency points $[f_1, \ldots, f_k, \ldots, f_K]$

$$S = \sum_{f_k=f_1}^{f_K} \boldsymbol{P_{norm}}(f_k)\ \log\left(\frac{1}{\boldsymbol{P_{norm}}(f_k)}\right)$$

SE S was calculated from the normalized power spectrum

$$S_{norm} = \frac{S}{\log(K)}$$

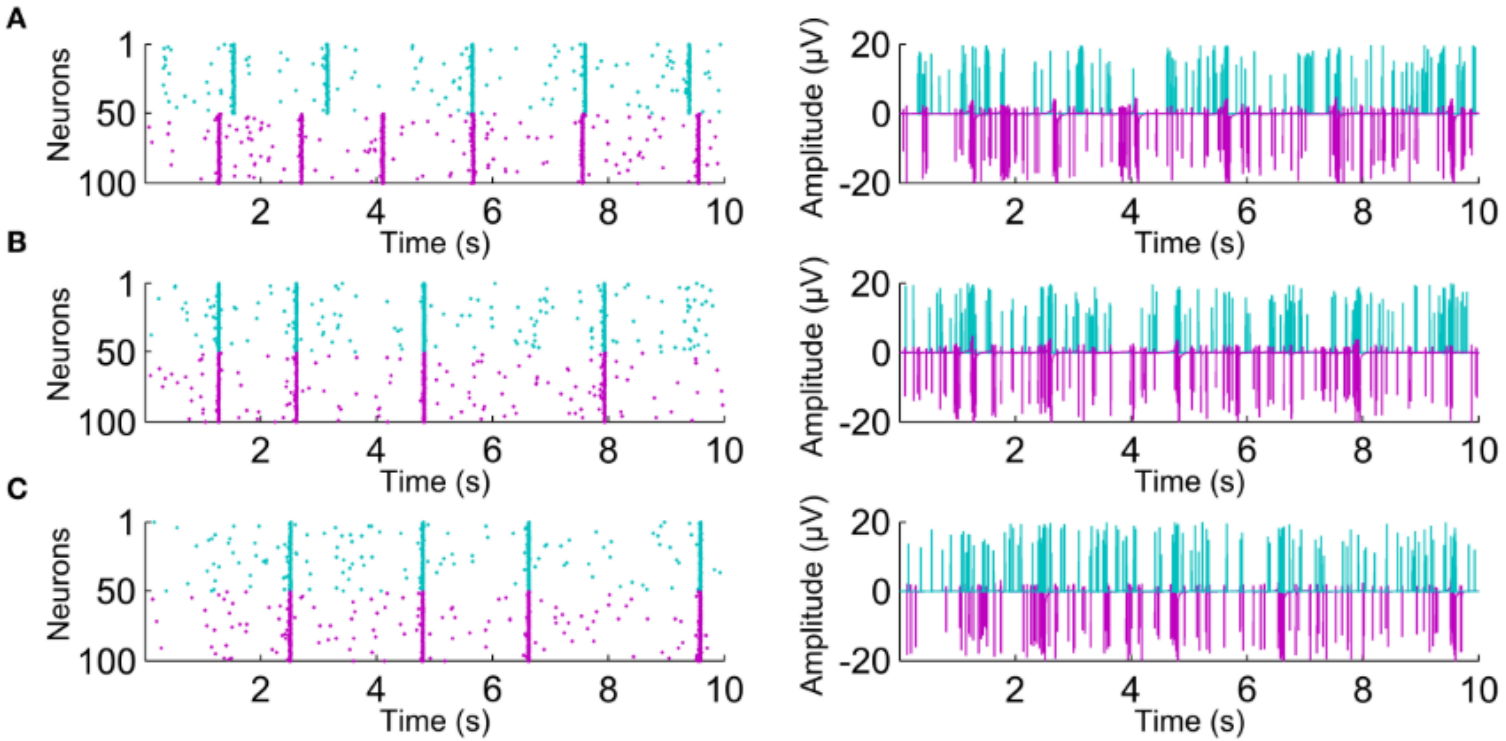
S was normalized to reside between 1 and 0

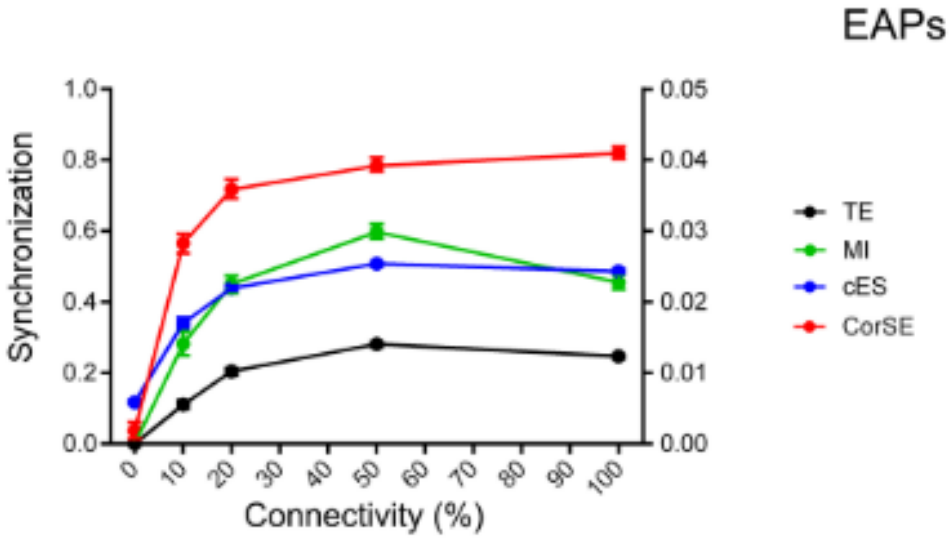
$$C_{S_x S_y} = \frac{1}{O} \sum_{i=1}^{O} \left(\left(\boldsymbol{S_{x,i}} - \overline{S_x}\right)\left(\boldsymbol{S_{y,i}} - \overline{S_y}\right)\right)$$

Cross covariance of the SEs $S_x$ and $S_y$ of the signals x and y

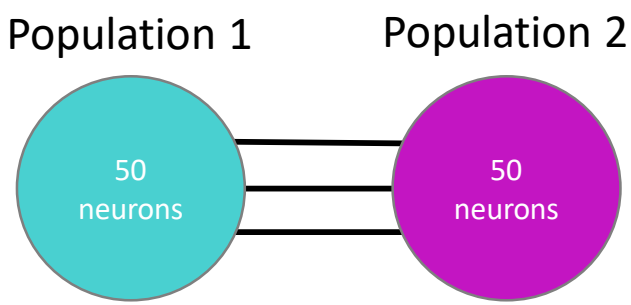
Kapucu et al., Front Comput Neurosci 2016

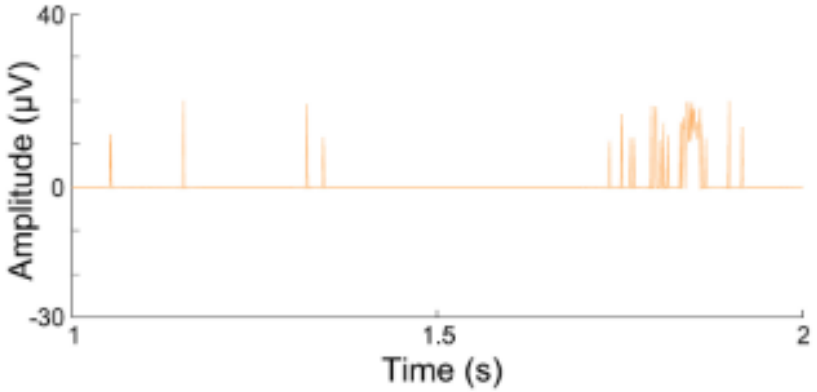
# Raster plots with the increasing connectivity



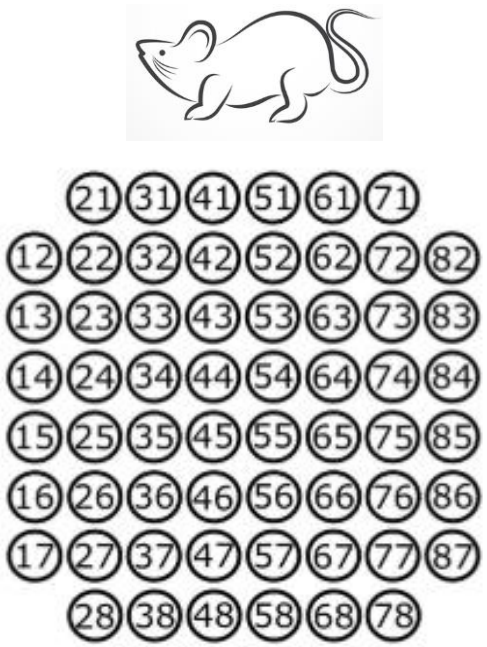



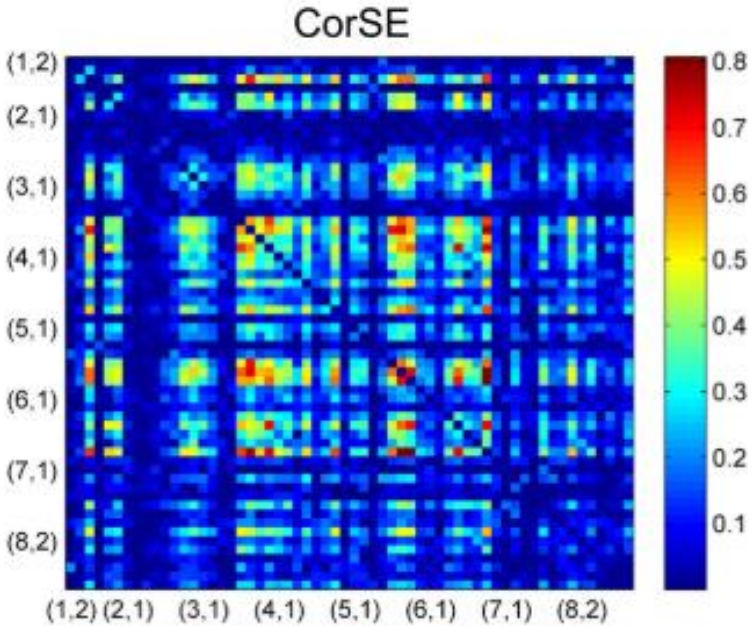

CorSE and cES on left vertical axis
TE and MI on right vertical axis



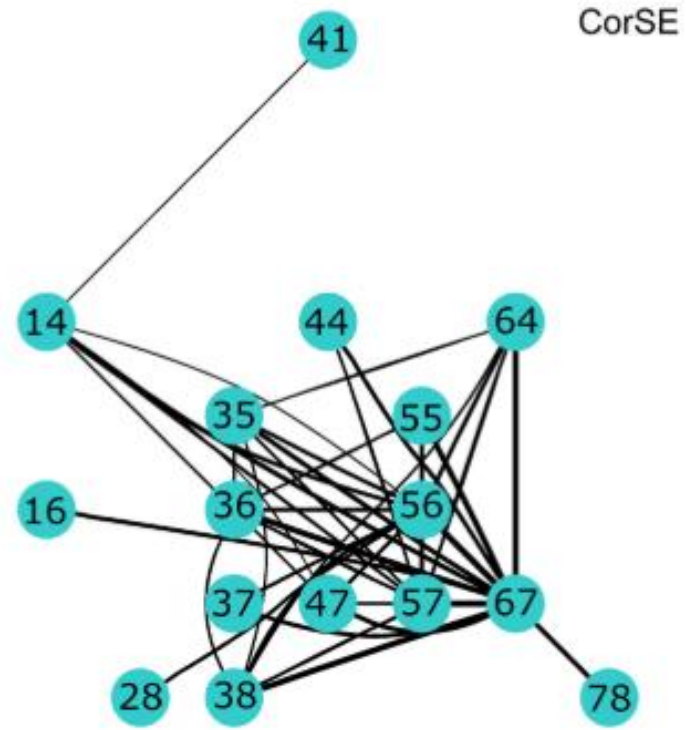



# Rat cortical neurons on MEA 1




Highest synchronization in channels 56 and 67




Kapucu et al., Front Comput Neurosci 2016

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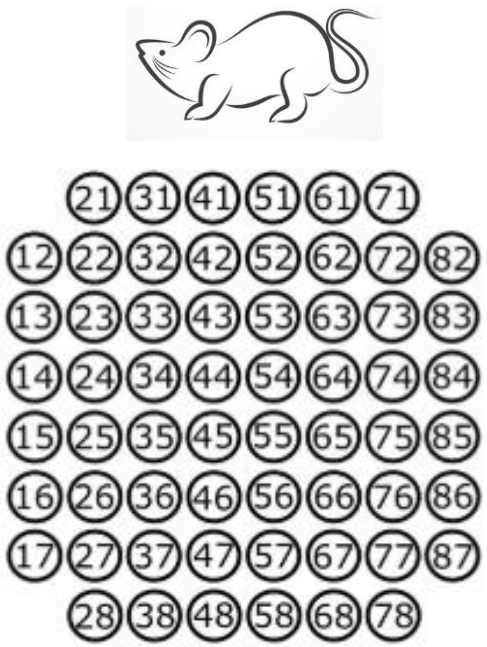
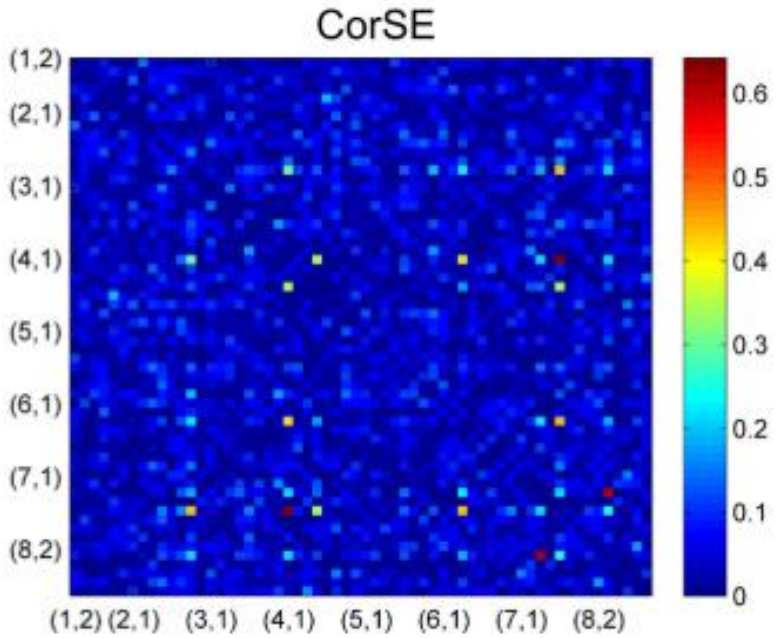
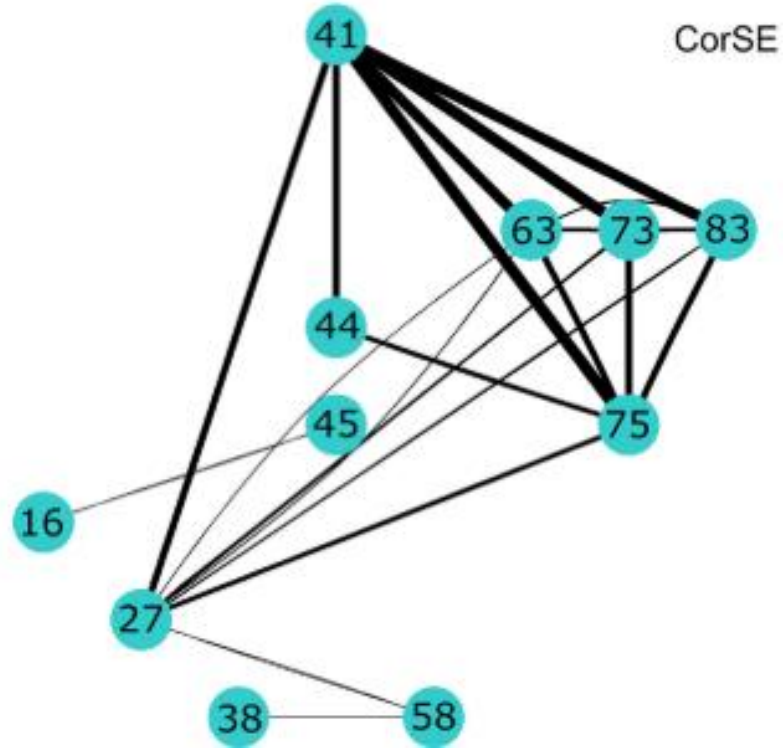
# Rat cortical neurons on MEA 2




Highest synchronization in channels 73 and 83




Kapucu et al., Front Comput Neurosci 2016

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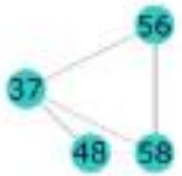
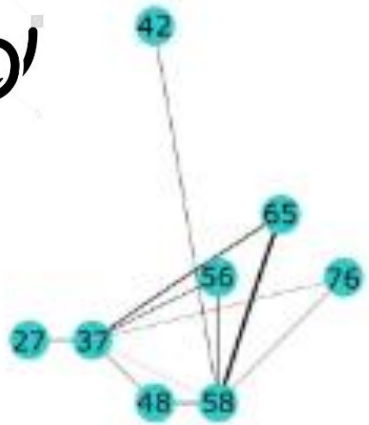
# Development of a functional mouse cortical neuronal network




DIV 13

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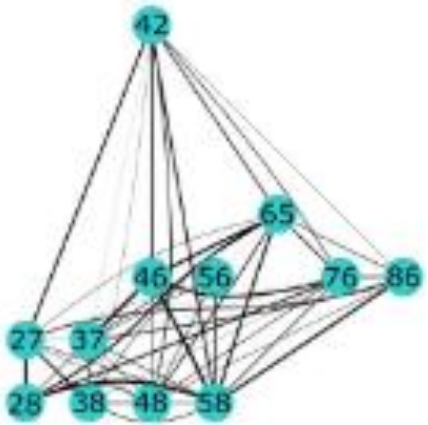


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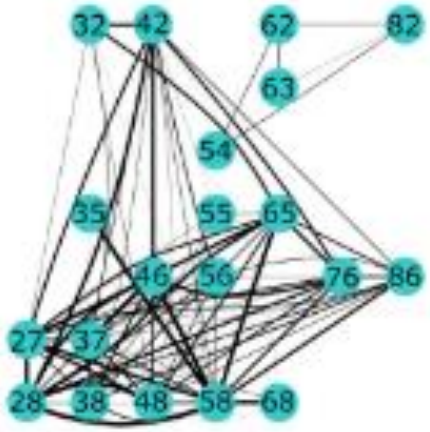


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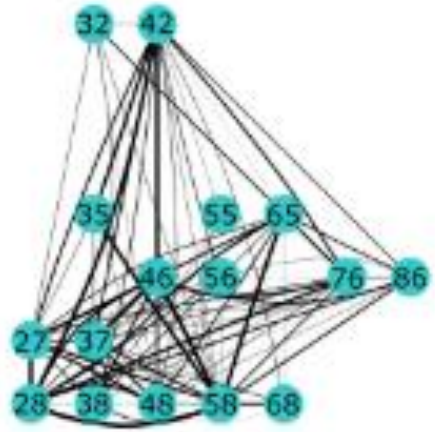
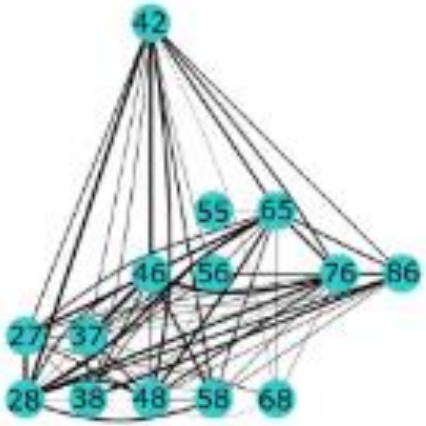
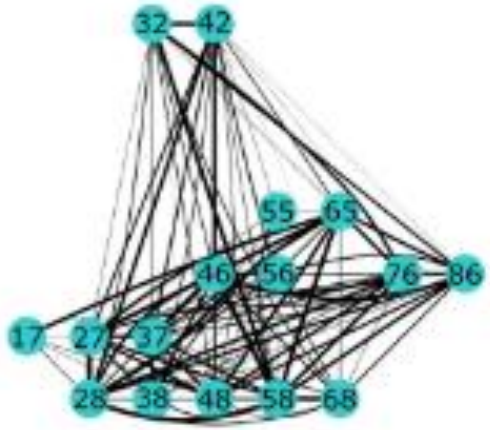


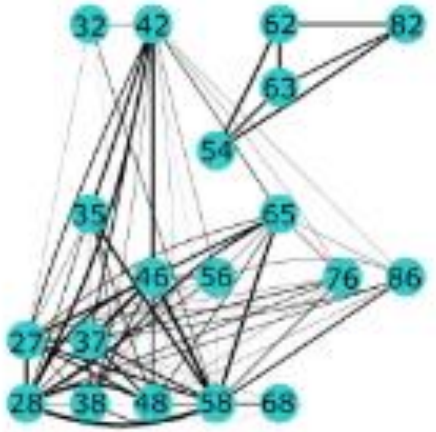

DIV 27




DIV 20




DIV 22




DIV 29

Kapucu et al., Front Comput Neurosci 2016

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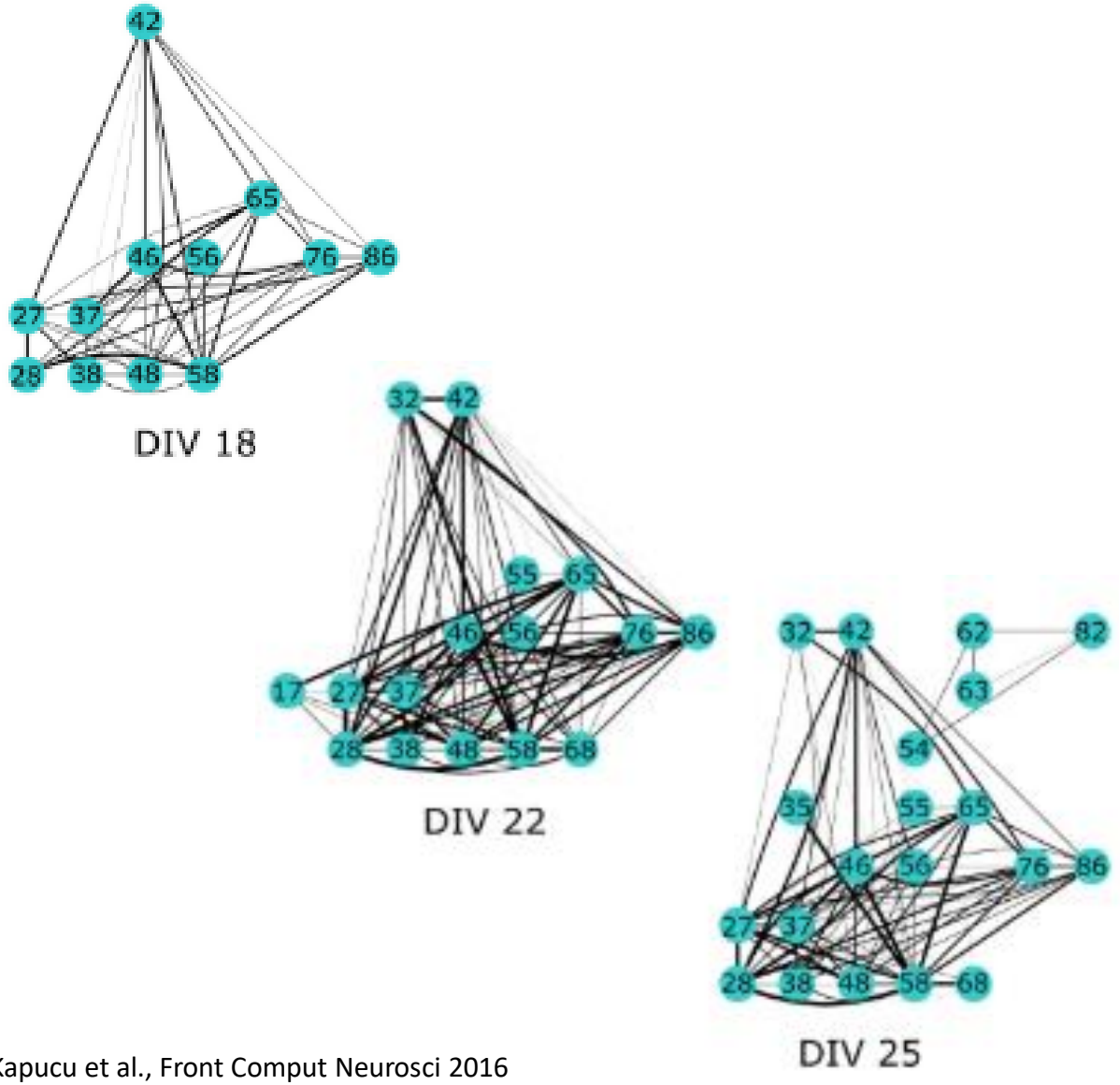
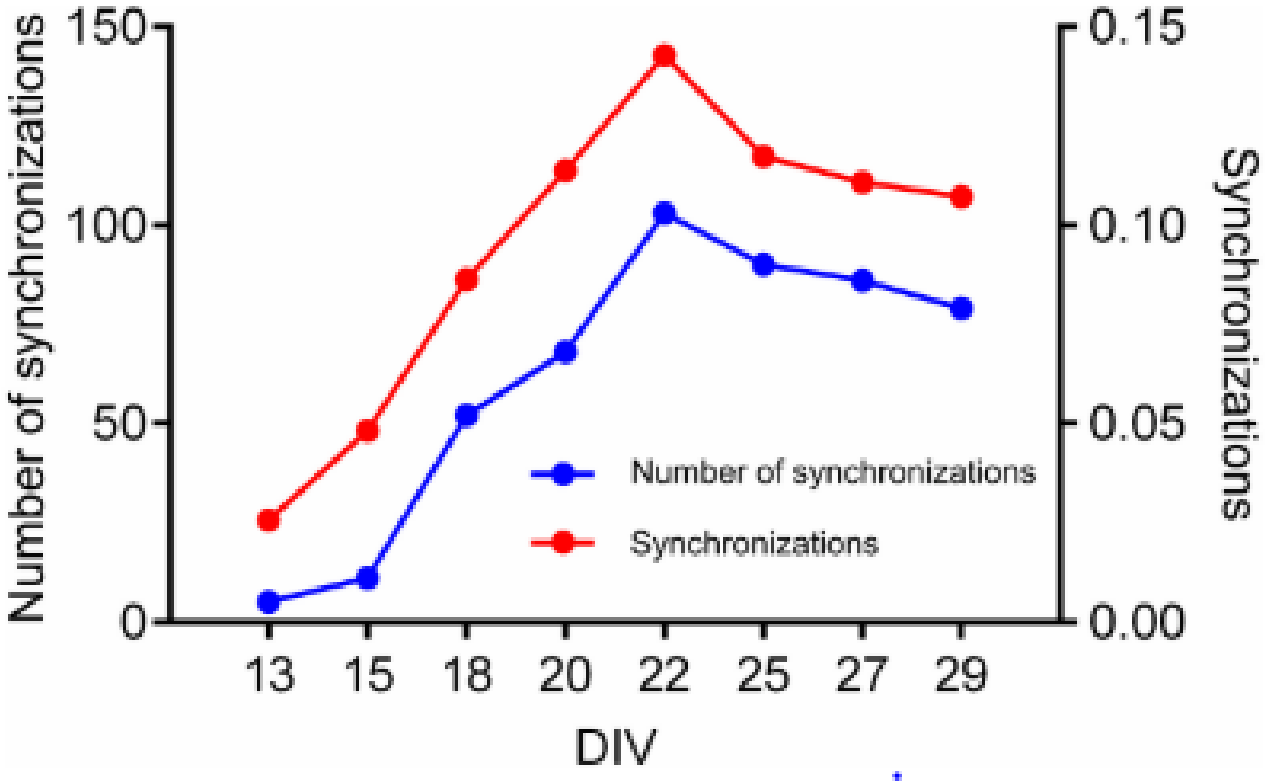
# Number of strong synchronizations and mean overall synchronization

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Kerstin Lenk

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1. Bioelectric assessment of neural networks in vitro
2. Spike and burst analysis methods/ tools
3. Network connectivity/ synchronization analysis
4. Calcium imaging

# $Ca^{2+}$ is extremely important in cell signalling

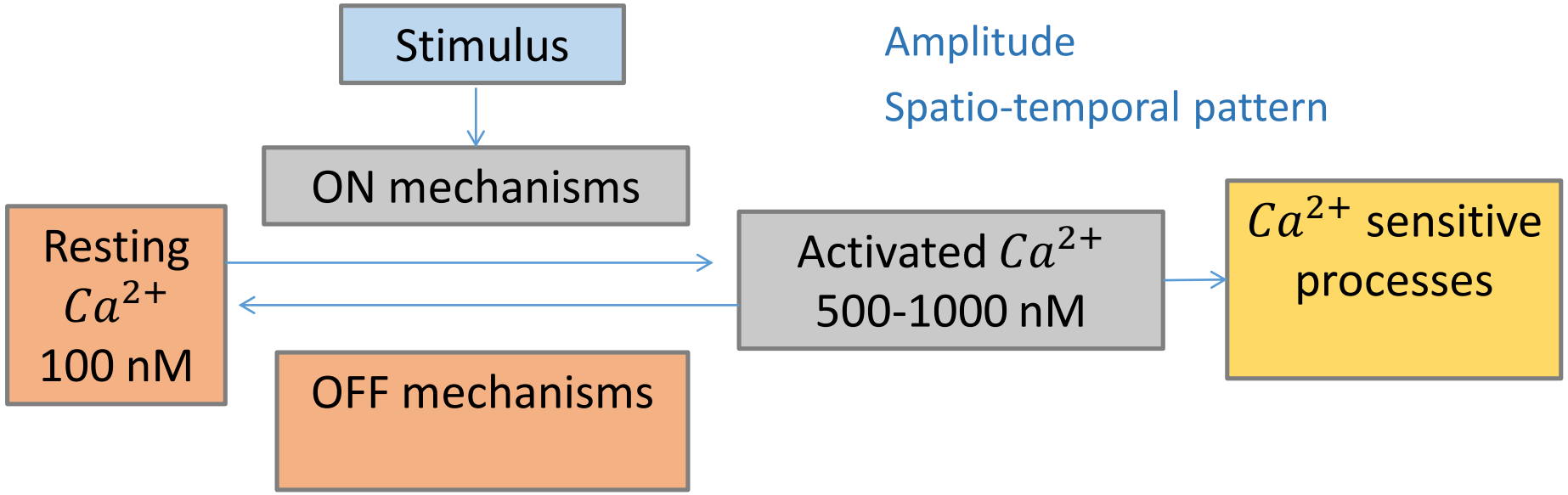
Many cellular processes involve changes in calcium concentration

Important concepts:

Speed

Amplitude

Spatio-temporal pattern




Nature Rev Mol Cell Biol 2000

Slide courtesy to
Soile Nymark

# Calcium indicators

- Calcium imaging shows the $Ca^{2+}$ status of a tissue or medium.
- It uses calcium indicators, molecules that can respond to the binding of $Ca^{2+}$ ions by changing their spectral properties.
- Two main classes of calcium indicators: chemical indicators and genetically encoded indicators.

Calcium wave
in ARPE-19 cells

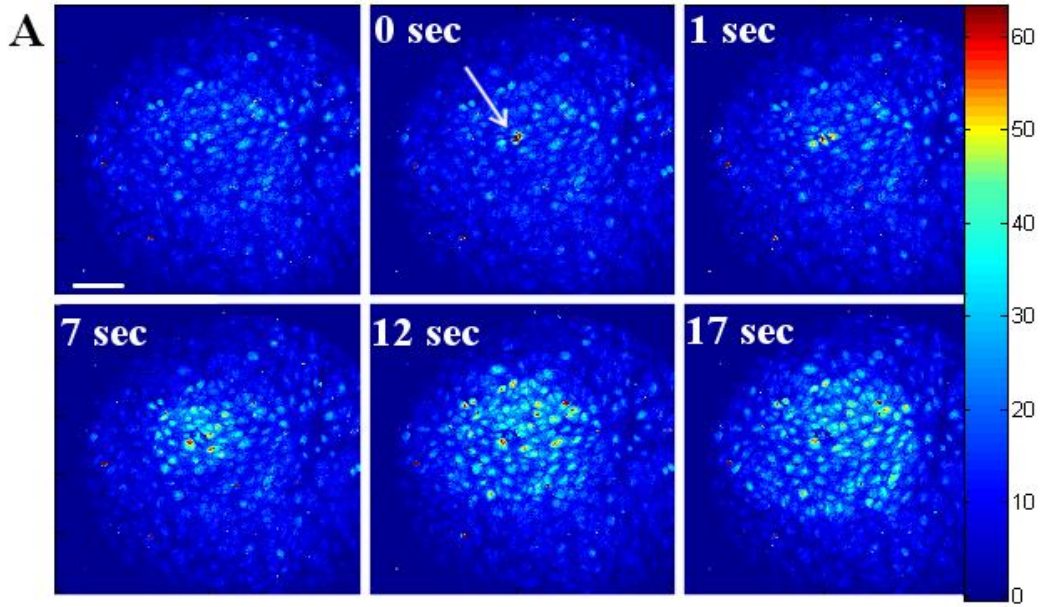



Image: AbuKhamidakh et al., 2013

Slide courtesy to
Soile Nymark

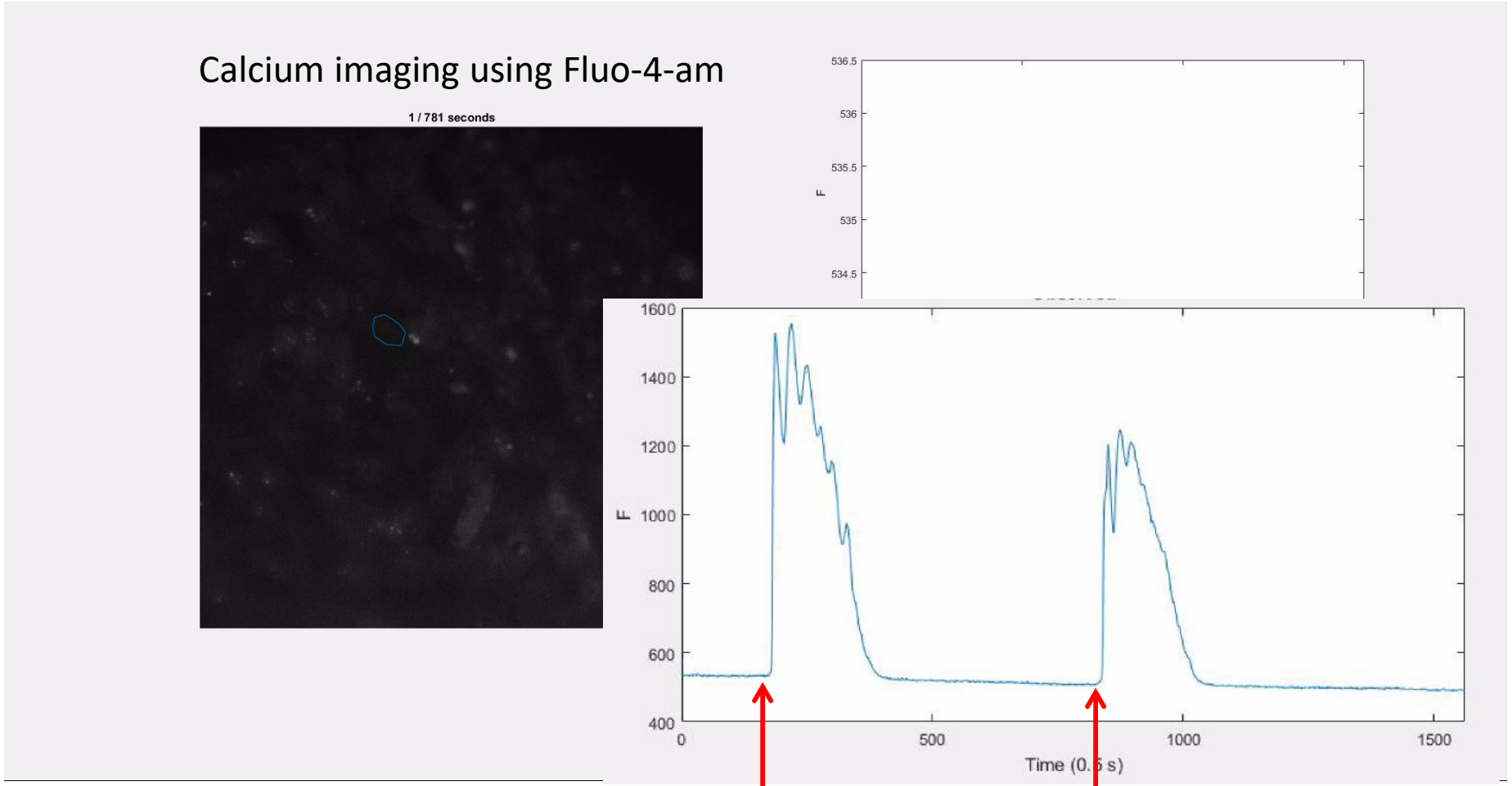
# Calcium indicators

- Chemical indicators:
  - Small molecules that can chelate (=bind) calcium ions.
  - Need to be loaded into the cells
  - Examples: fura-2, indo-1, fluo-3, fluo-4, Calcium Green-1.
- Genetically encoded indicators
  - Fluorescent proteins derived from green fluorescent protein (GFP) or its variants, fused with calmodulin (CaM).
  - Do not need to be loaded into the cells
  - Genes encoding for these proteins can be transfected to cell lines.
  - Transgenic animals expressing the dye in all cells or selectively in certain cellular subtypes, can be created.

Slide courtesy to
Soile Nymark

# Response of RPE to ATP stimulation

Calcium imaging using Fluo-4-am

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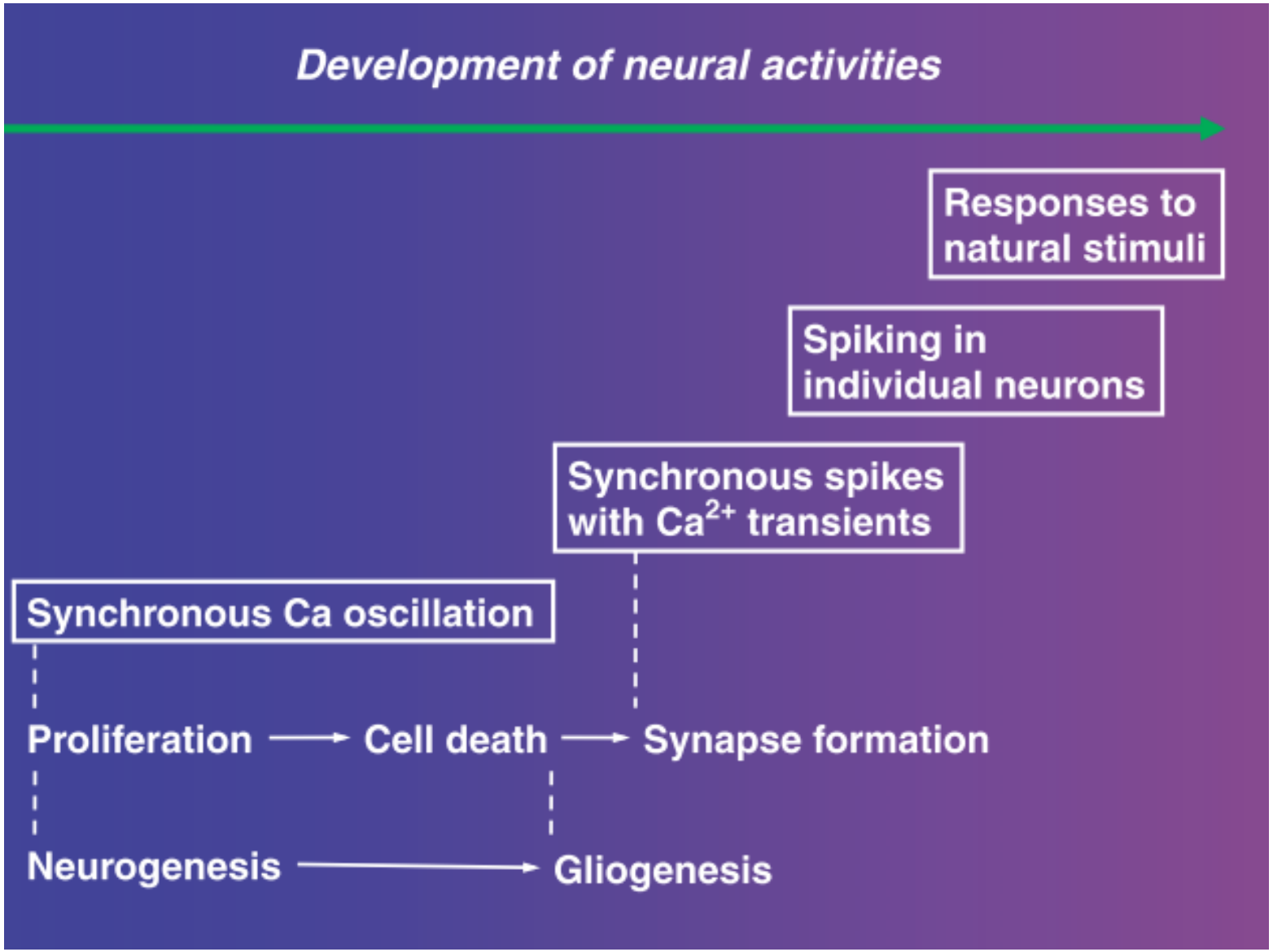
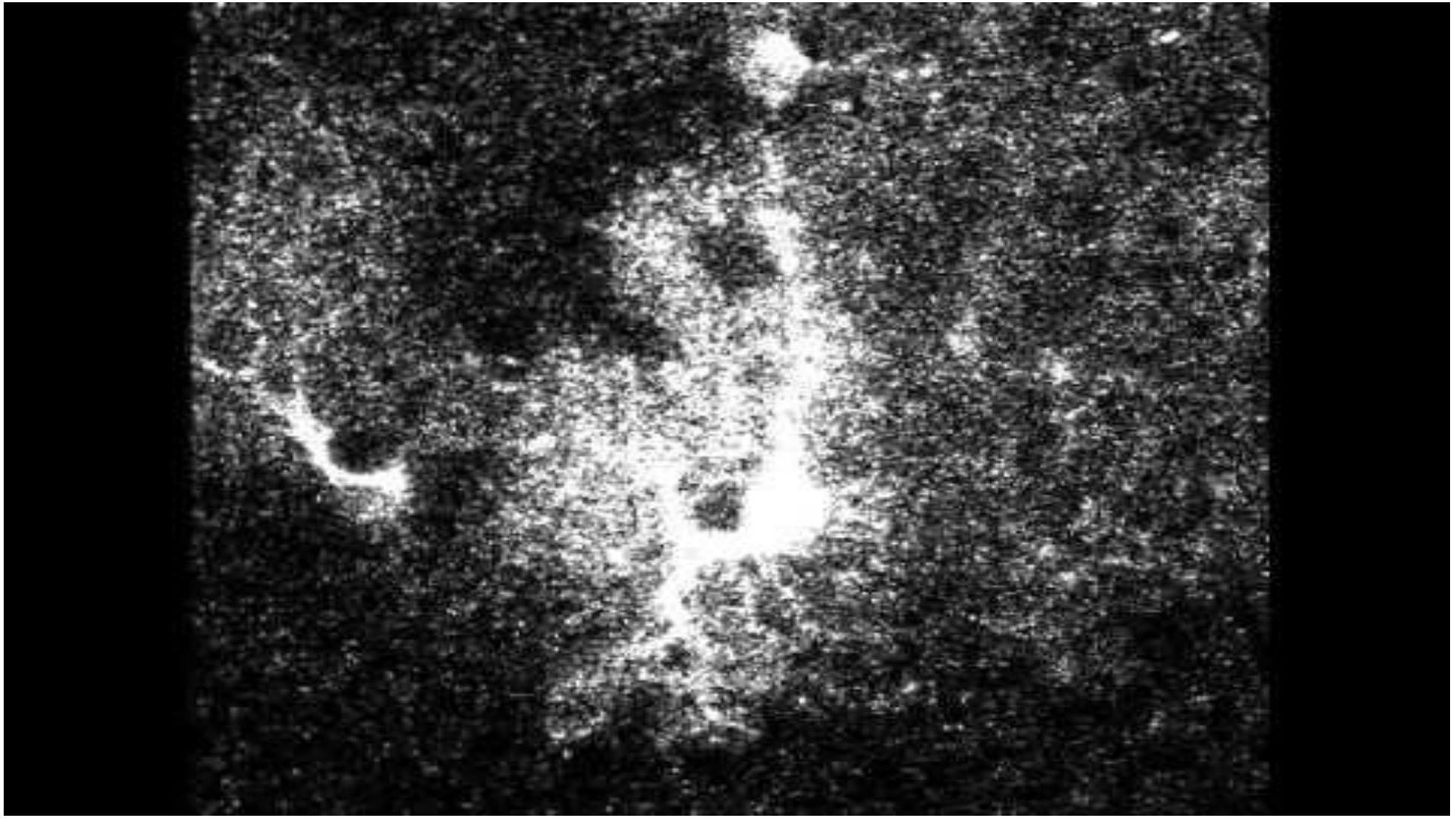
# Changes in cellular activities during CNS development




Yamashita, FEBS Journal 2010

# Astrocyte listening to neurons



Video: https://www.youtube.com/watch?v=y7Yg1mlvLlc

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Image: https://bit.ly/2Ig4uQj