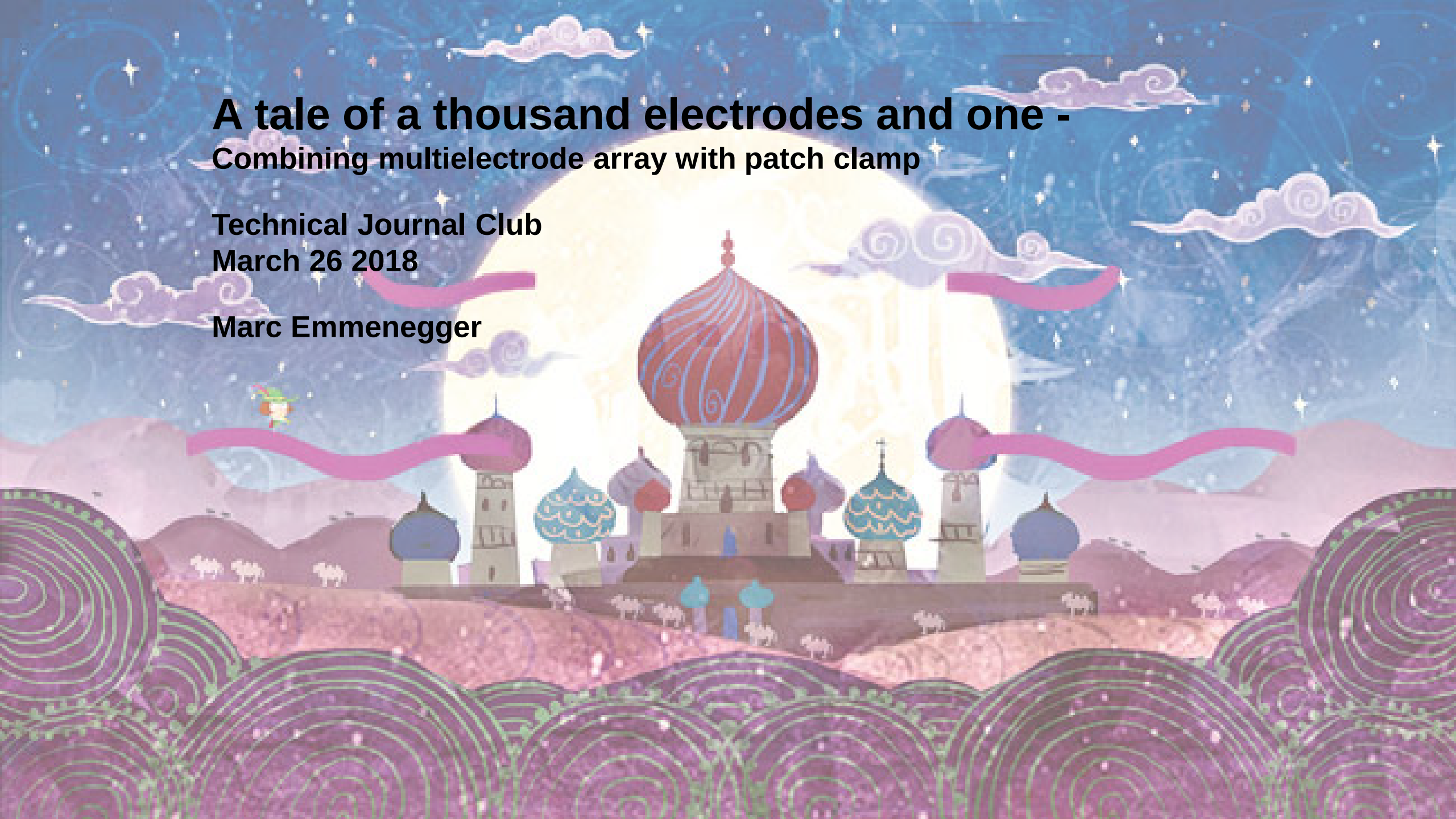


A tale of a thousand electrodes and one -

Combining multielectrode array with patch clamp

Technical Journal Club
March 26 2018

Marc Emmenegger

The brain

- Regulation of involuntary and voluntary internal processes
- Perception and processing of internal and external stimuli
- Cognition, encoding and decoding of information
- Alterations in the brain are associated with physical and mental pathologies

http://sites.psu.edu/siowfa14/2014/12/03/do-we-truly-use-10-of-our-brain/

Understanding the brain – neuroscience in a nutshell

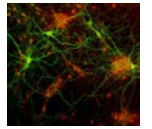
1000 AD: Al-Zahrawi of Iberia and Avicenna of Bukhara: Description of neurological patients, surgical treatments of head and spinal injuries, epilepsy and migraine.

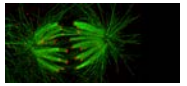
1500 AD: Andreas Vesalius: De humani corporis fabrica – detailed anatomy.

1600 AD: René Descartes (brain mind dualism) and Thomas Willis, Oxford, the father of modern neurology.

1700 AD: Luigi Galvani: Electric excitability of brain and muscles.

1850: Camillo Golgi with neuron stain; Ramón y Cajal coin **neuron as functional unit of brain (neuron doctrine**).





1950+: Molecular biology, electrophysiology, computational neuroscience

2000 BC 1000 BC 500 BC 1000 AD 1500 AD 1900 AD 2000 AD

100 AD: Galen: Brain controls muscles via specific spinal nerves.

300 BC: Erisastratos and Herophilos: Insights into brain anatomy and physiology, e.g. distinction of cerebrum and cerebellum, ventricles.

500 BC: Alcmaeon of Croton: Brain is seat of mind.

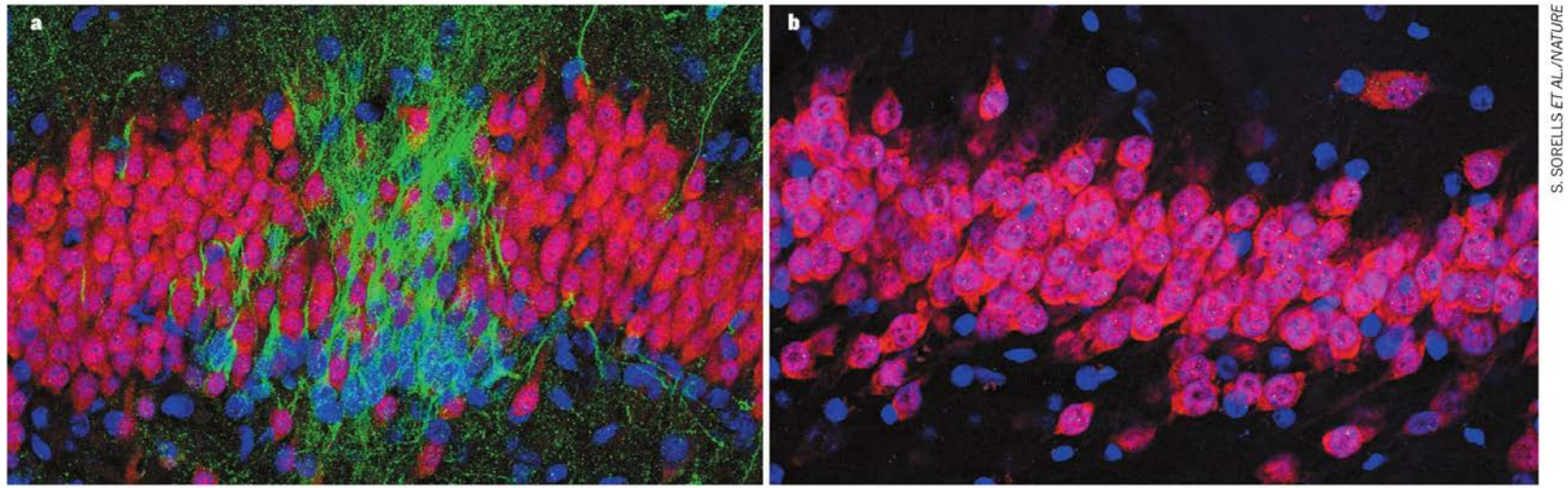
Homeric period: The diaphragm (φρενός) as the seat of the mind.

1700 BC: Earliest record of the word brain in the Edwin Smith Surgical Papyrus
Aphasia and seizure after head injury

Understanding the brain – many key processes remain obscure

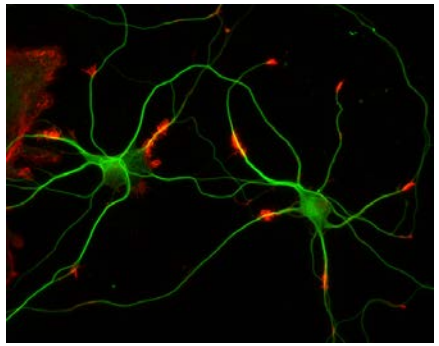
Questioning human neurogenesis

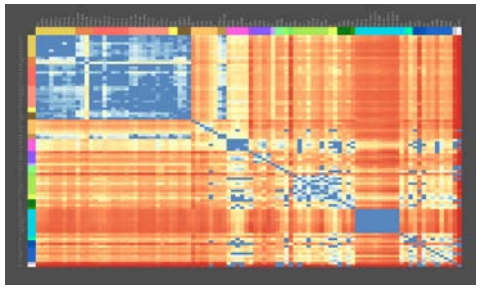
Neurons are born in the brain's hippocampus throughout adulthood in mammals, contributing to the region's functions in memory and mood. But a study now questions whether this phenomenon really extends to humans. SEE LETTER P.377



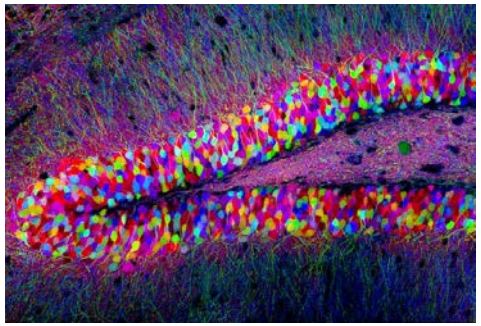
S. SORELLS *ET AL./NATURE*

Methods to study brain functions on the cellular level

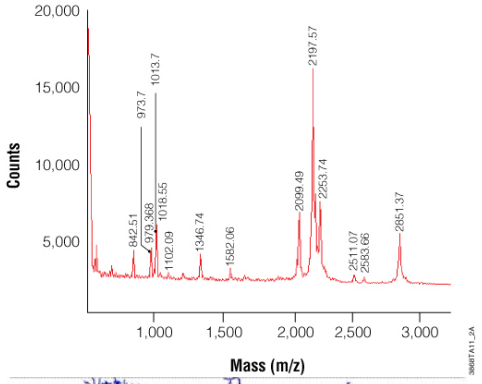


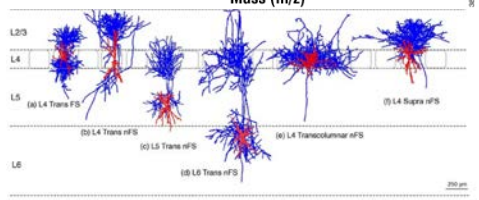


On level of transcript



On level of protein



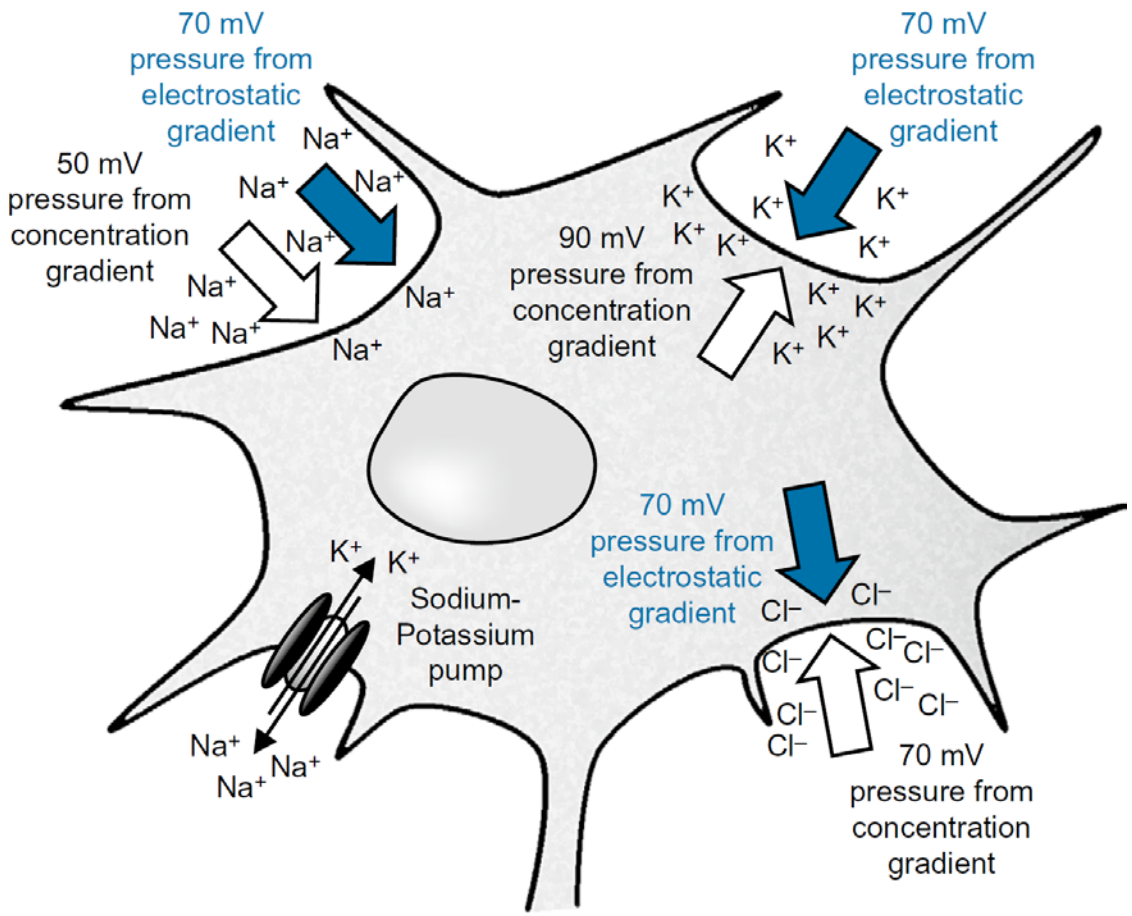


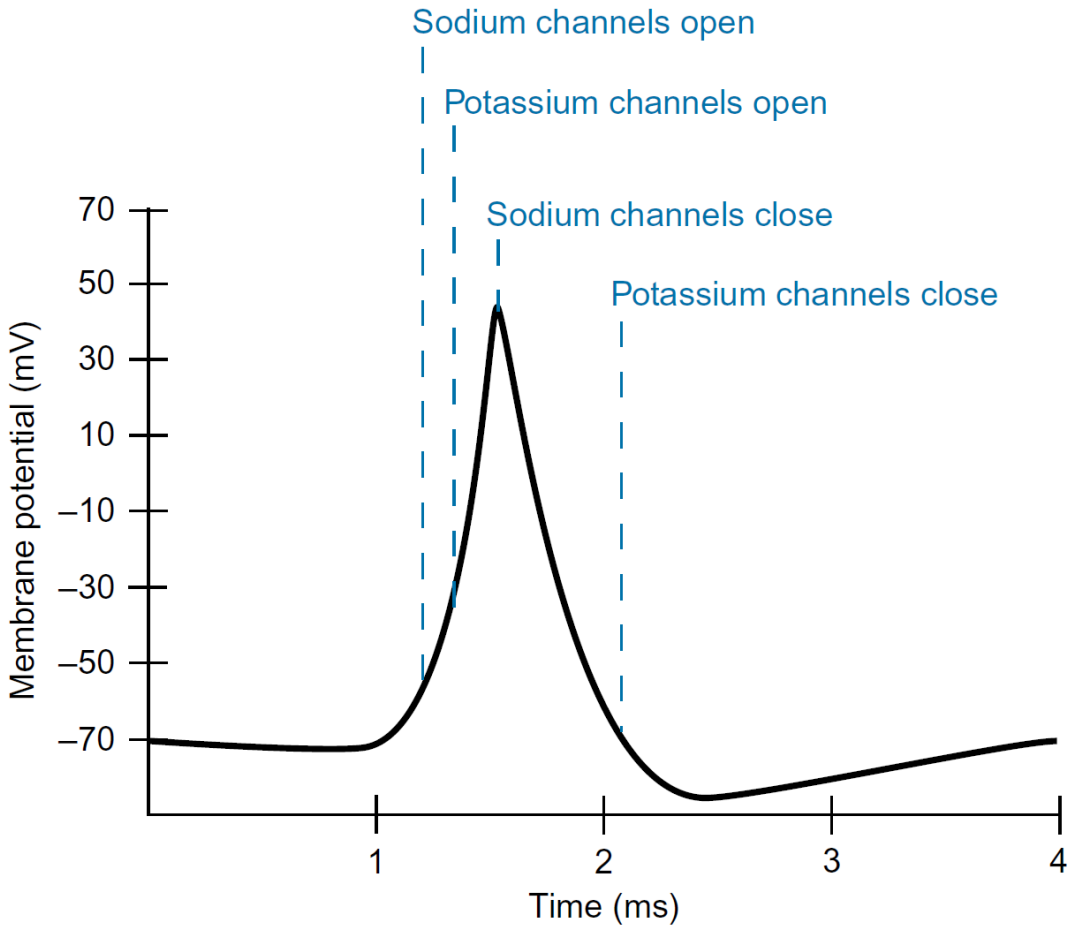
Morphology

Surrogates to infer function

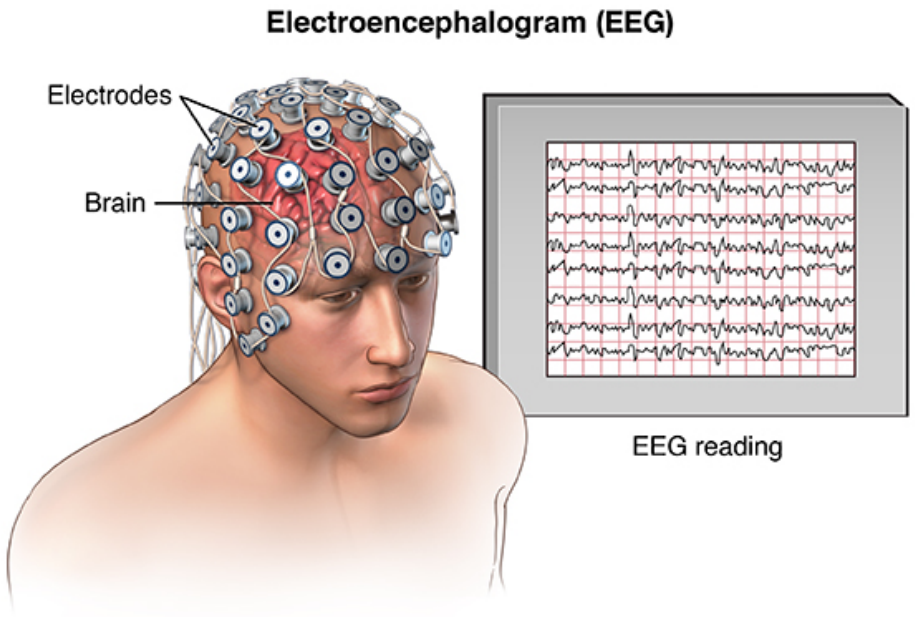
http://casestudies.brain-map.org/ggb
http://www.cell.com/pictureshow/brainbow
Feldmeyer et al. 2017.

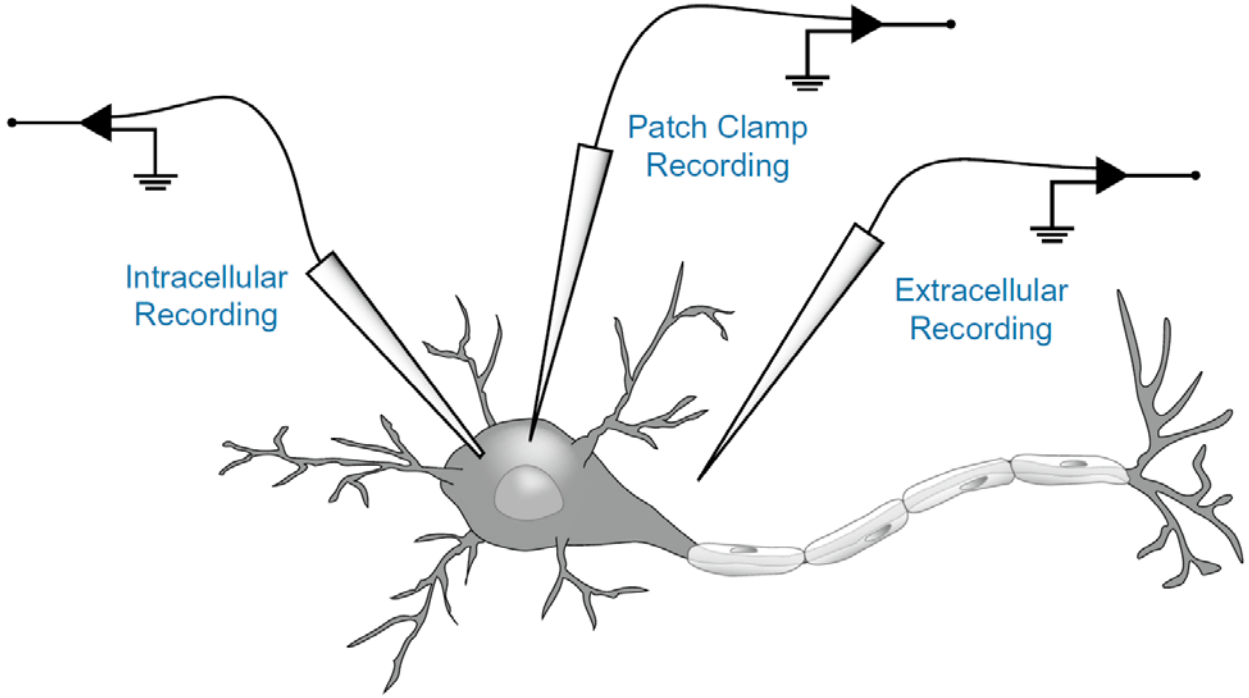
Neurons are electrically excitable cells – measure function



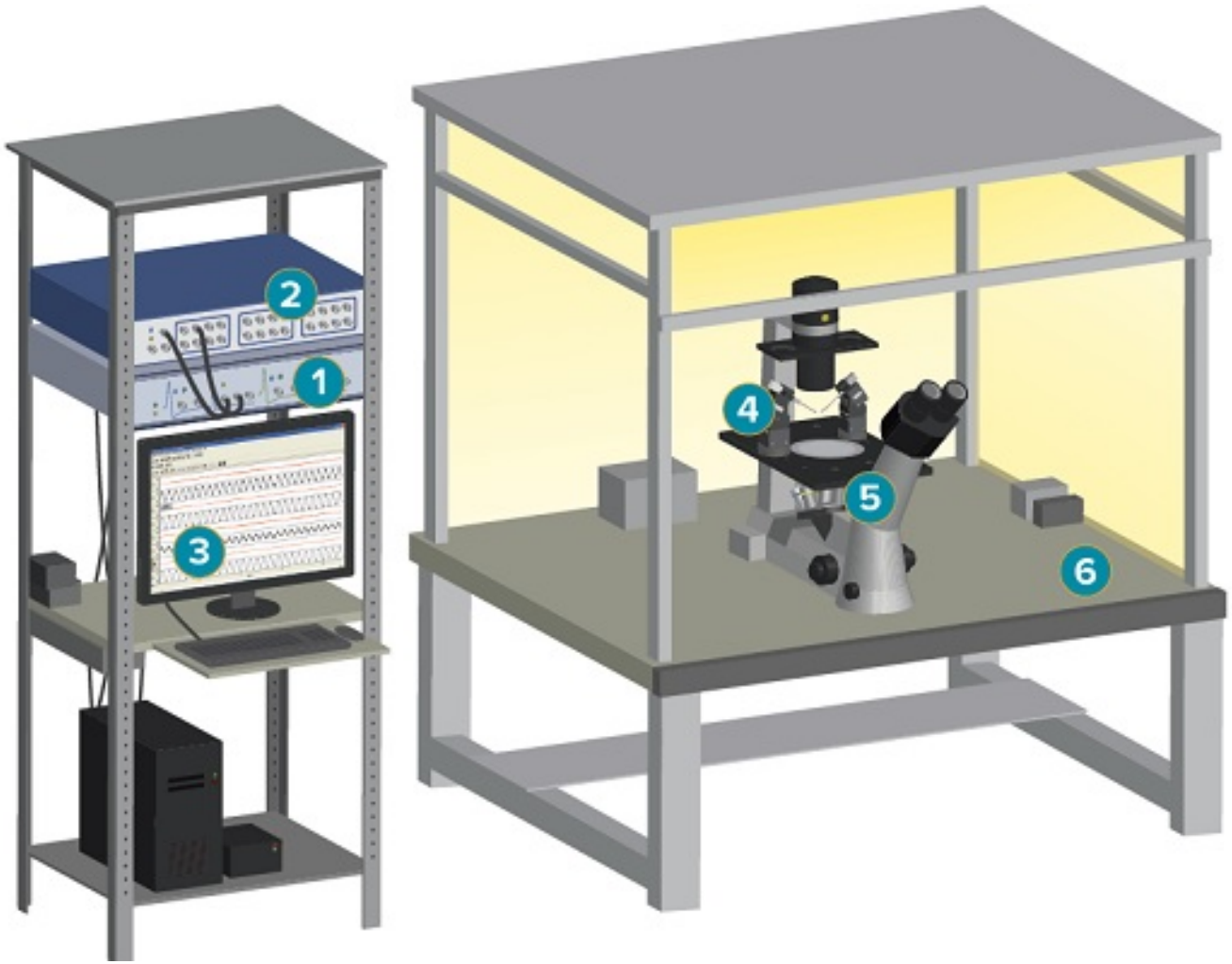


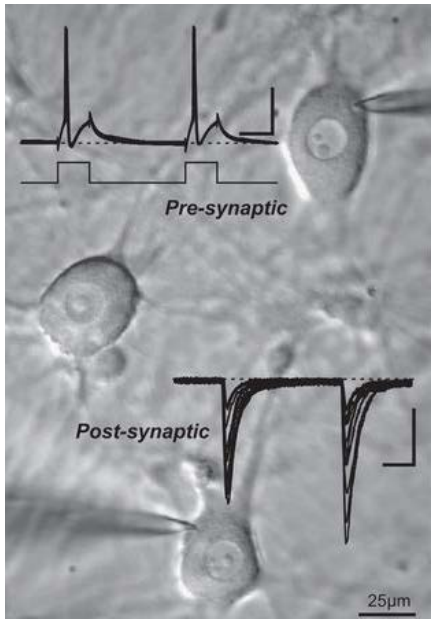
How to measure the electrical signatures of neurons?





Patch clamp rig

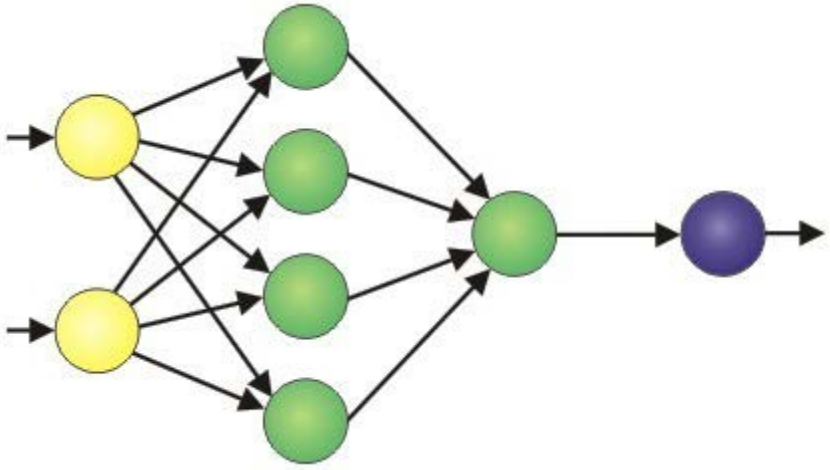




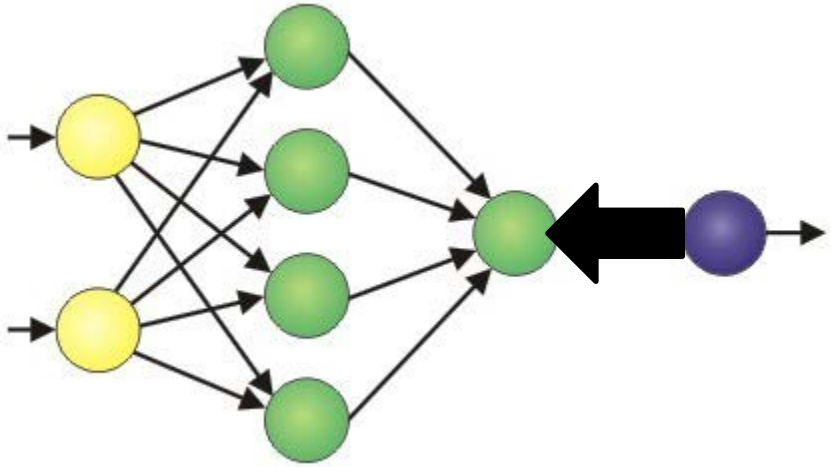
https://www.moleculardevices.com/systems/axon-conventional-patch-clamp

Limitations of traditional whole-cell patch clamp

- Single cell (and even single-molecule) resolution.
- Low-throughput.
- Recordings are tedious, even more so for infrequent neuron types that are hard to discover.
- Patch clamp is invasive.
- Measure of neuronal dynamics is restricted to minutes to few hours.
- Sparse information on relevance for network.

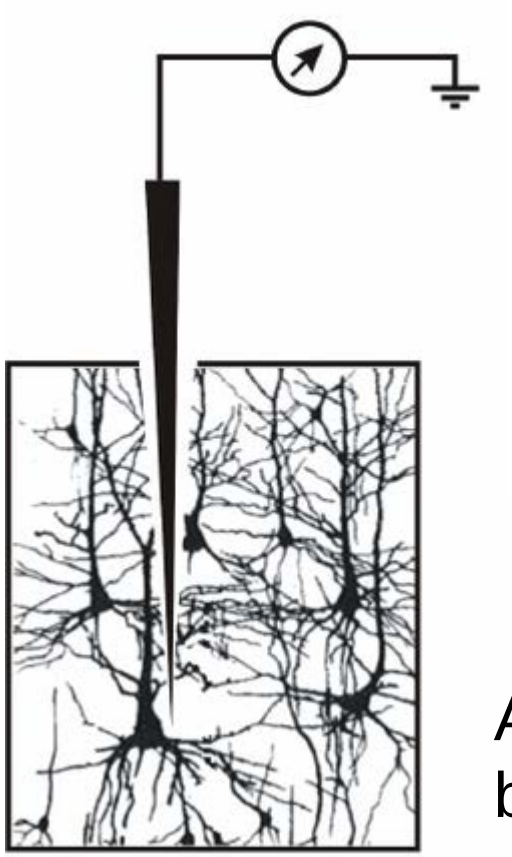


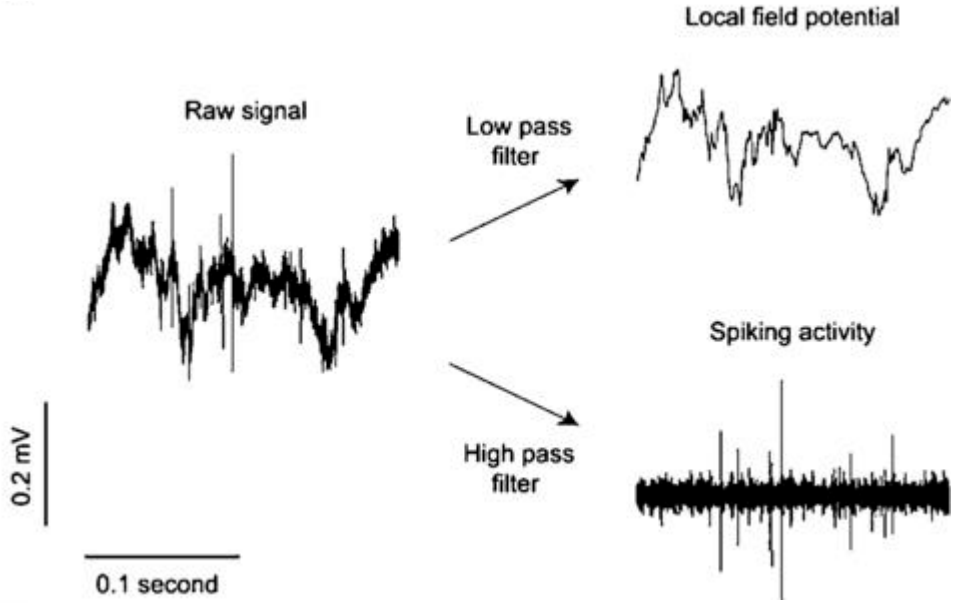
Network → single cell



Single cell → network

Extracellular recording – single electrode





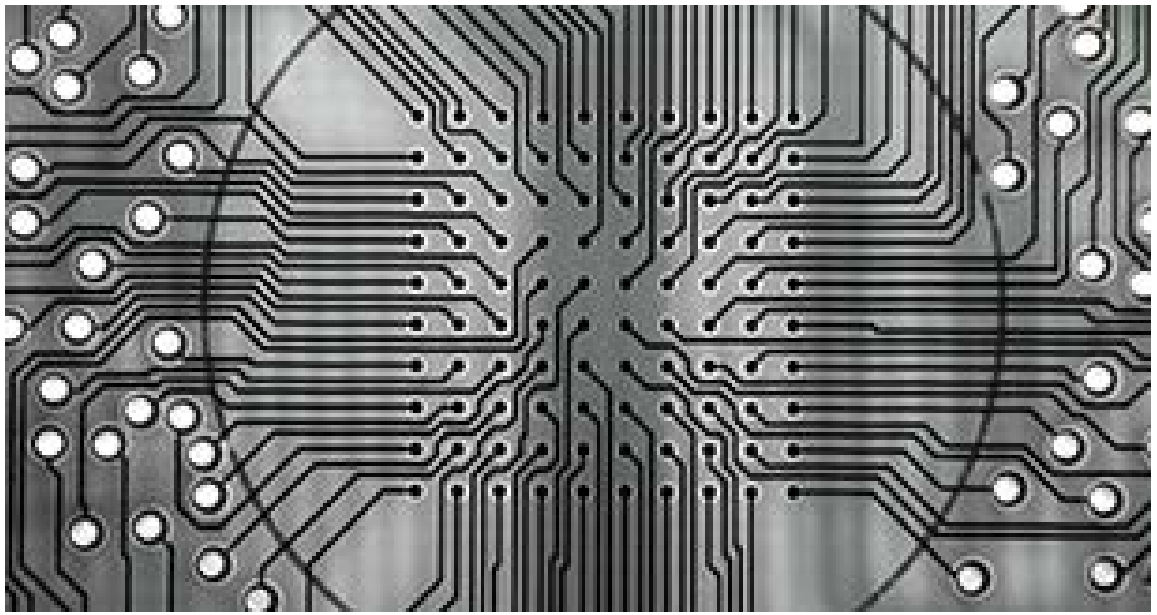
slower network oscillations of small populations (local field potentials; bandwidth: 1-300 Hz)

spiking activity of individual neurons (extracellular action potentials; bandwidth: 300-3000 Hz)

An electrode placed inside a brain slice *in vitro* or inserted in the brain *in vivo* detects electrical signals produced by the surrounding cells.

Multielectrode arrays (MEAs) – whole network

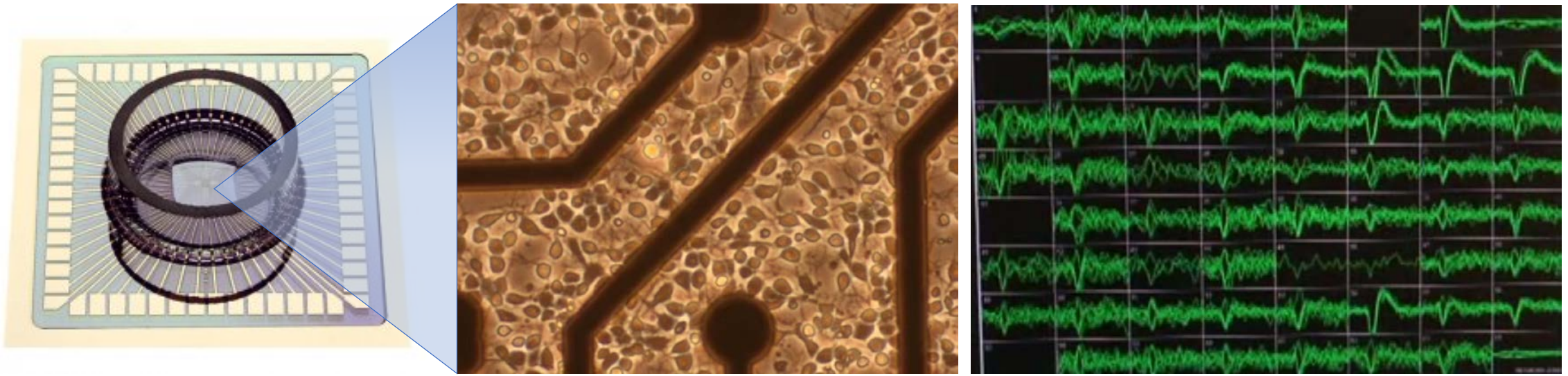
To achieve high-resolution activity mapping of neuronal networks, multiple electrical sensors tightly spaced in an array can be utilized.



MEAs allow simultaneous long-term recording of LFPs and EAPs from a population of neurons at sub-millisecond time scale.

Hierlemann et al. 2011
Radivojevic et al. 2017

Standard MEAs – 64 electrodes



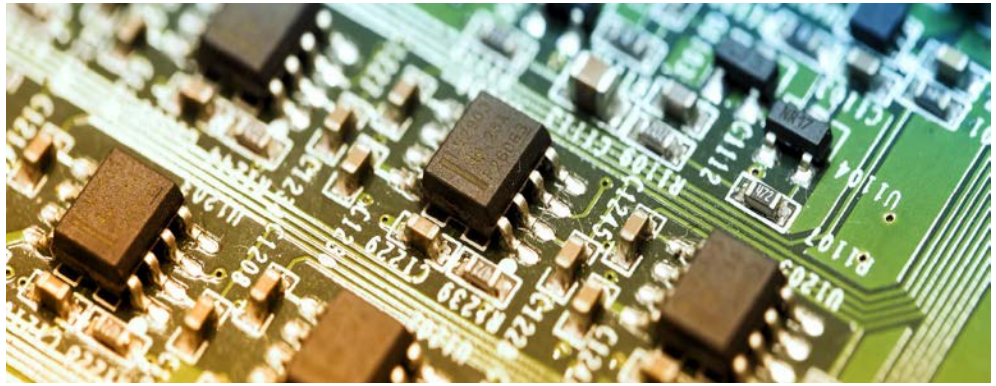
Typical MEAs measure the average of population activity, while missing signals from the majority of cells in the preparation

Hierlemann et al. 2011
Radivojevic et al. 2017

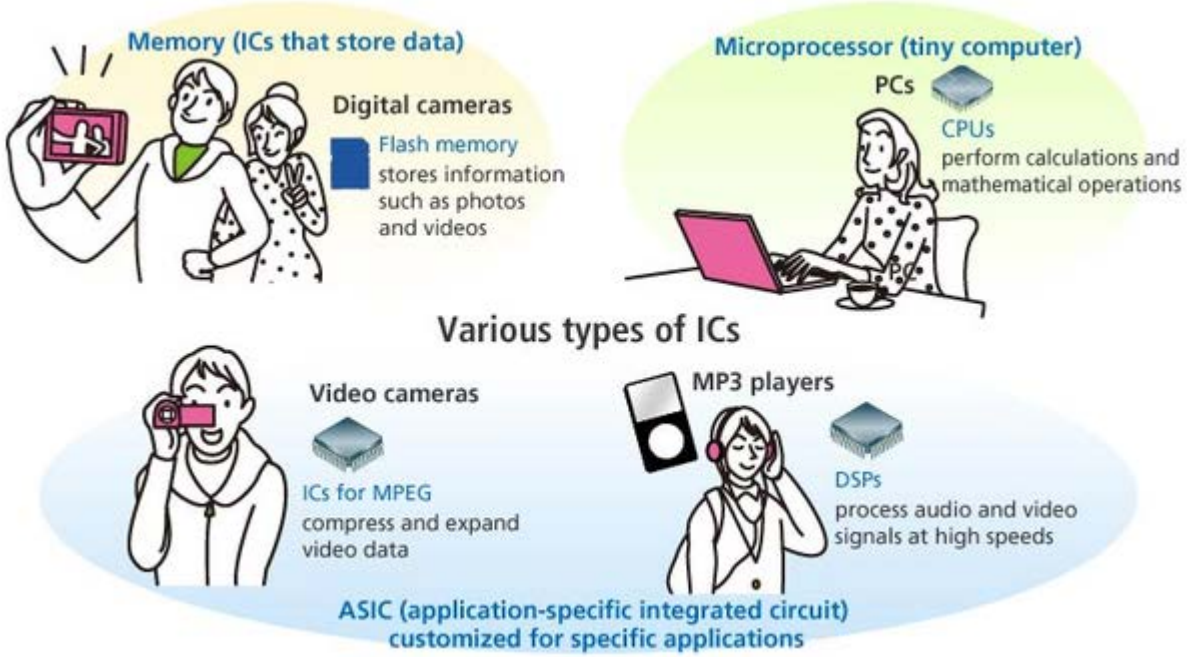
Next generation MEAs

- Even in 2018, the standard MEAs comprise around 64 electrodes and are commonly used.
- A technically advanced MEA using CMOS technology is developed in few labs which compasses up to 60,000 electrodes in an area of 4x2 mm^2.
- Description of technological challenges and method development. However, almost total lack of clear cut proven biomedical relevance.

Integrated circuits or CMOS technology – Complementary metal-oxide semiconductor

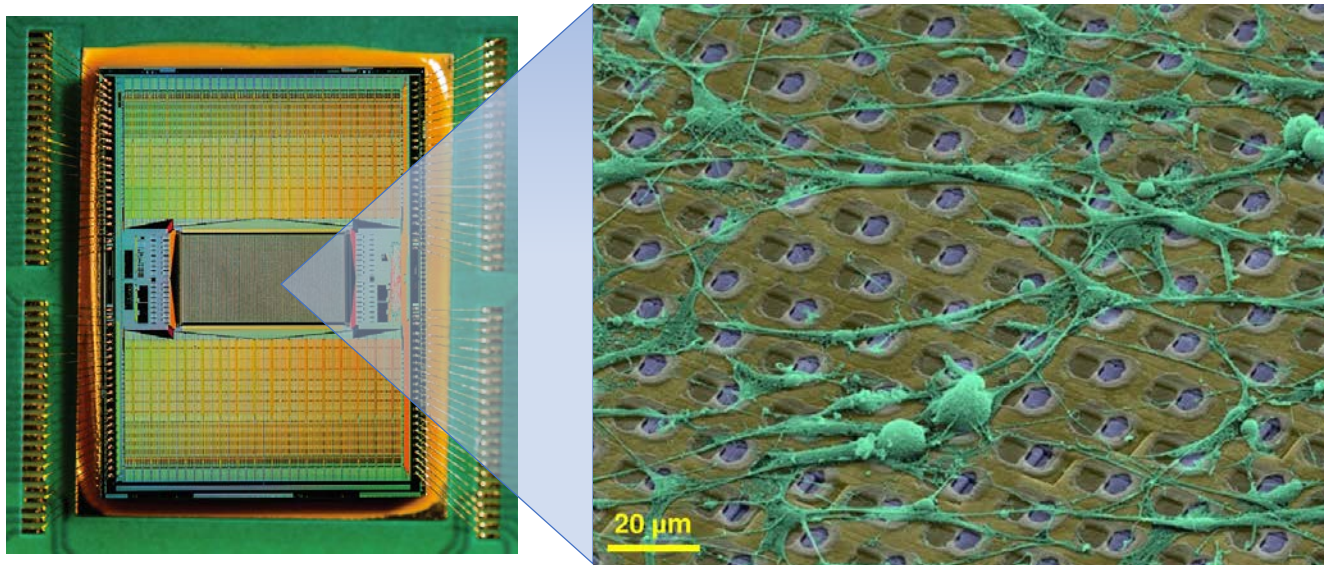


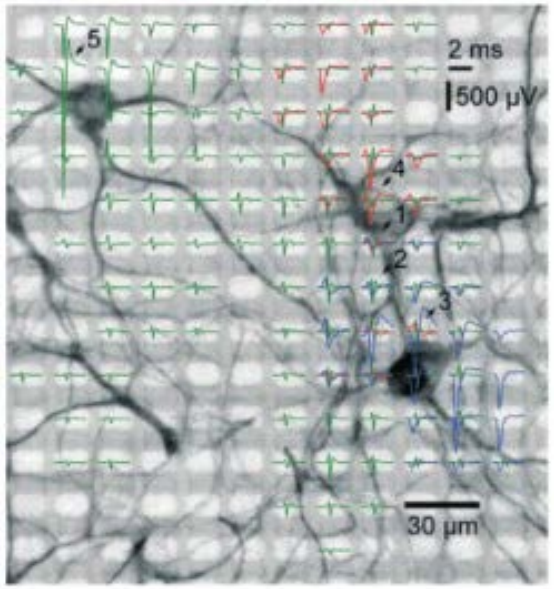
- Connectivity – large number of electrodes
- Signal quality – integrated filters and amplifiers
- Ease of handling and use – devices and signals are robust



Hierlemann et al. 2011
Radivojevic et al. 2017

High-density MEAs > 10,000 electrodes





Extracellular APs

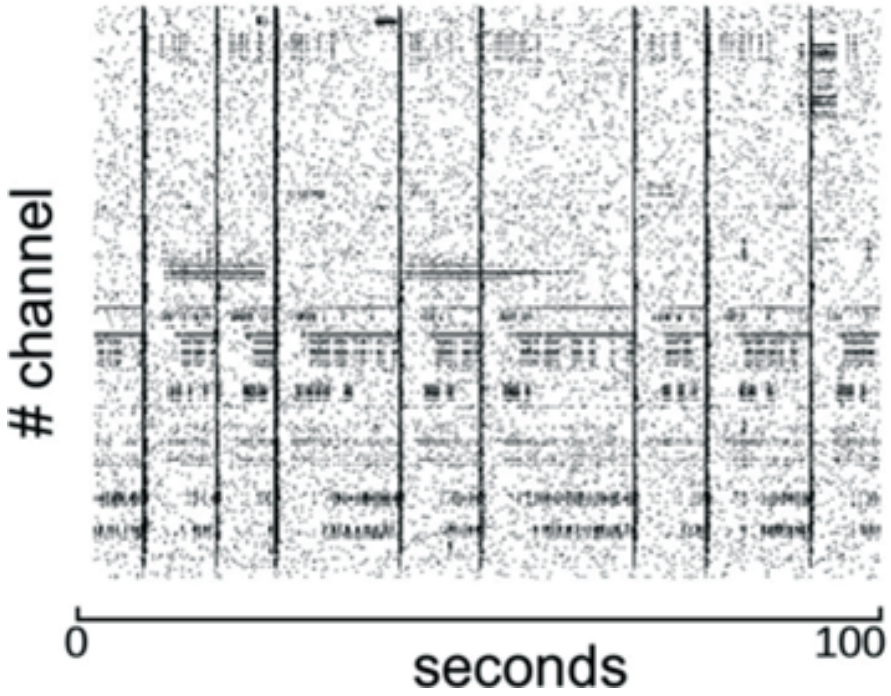
HD-MEAs allow access to the activity of individual cells at sub-cellular resolution

26,400 microelectrodes arranged at low pitch (17.5 µm) within a large overall sensing area (3.85 × 2.10 mm^2)

Hierlemann et al. 2011
Radivojevic et al. 2017

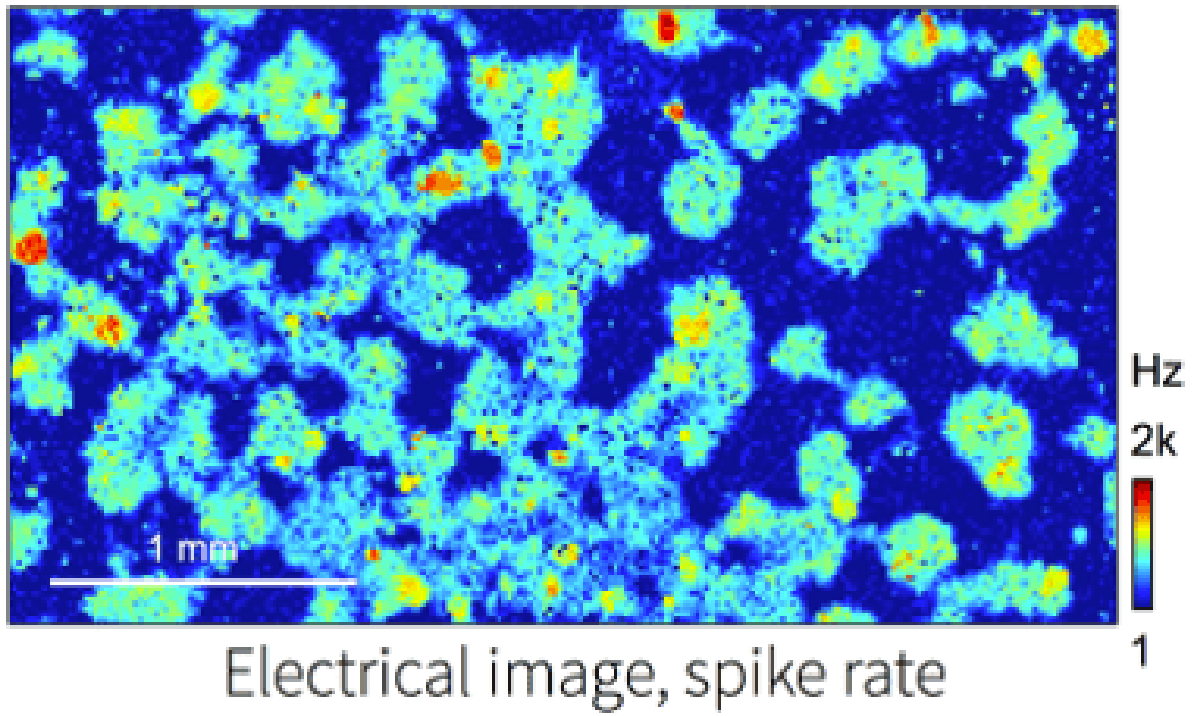
1) Population recording

Raster plot shows the dynamics of the network activity



Each dot represents the spontaneously active neuron showing spiking activity

Spiking frequency of the neurons plated on 4x2 mm array

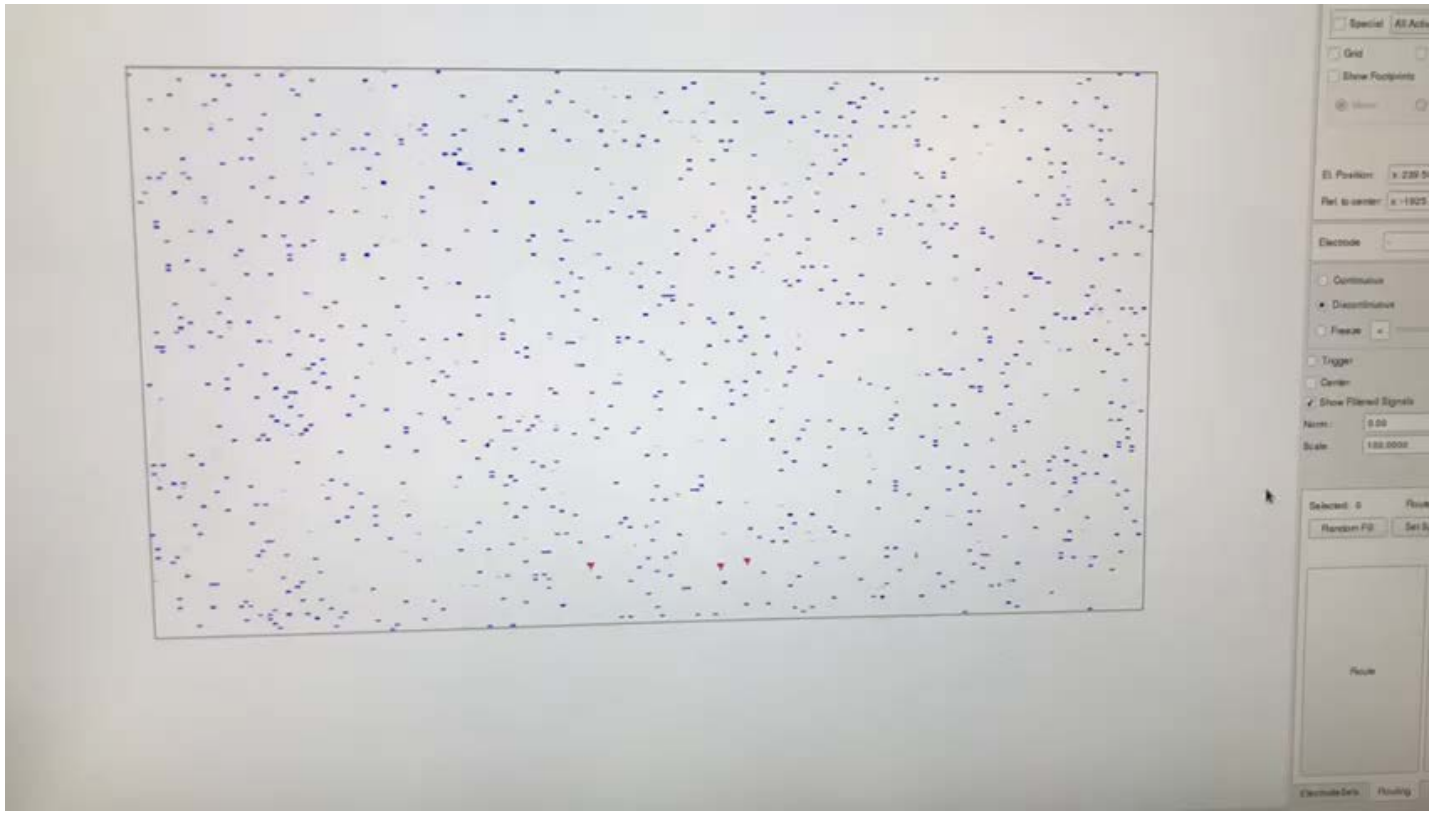


Red colour denotes high frequency firing and blue denotes low firing

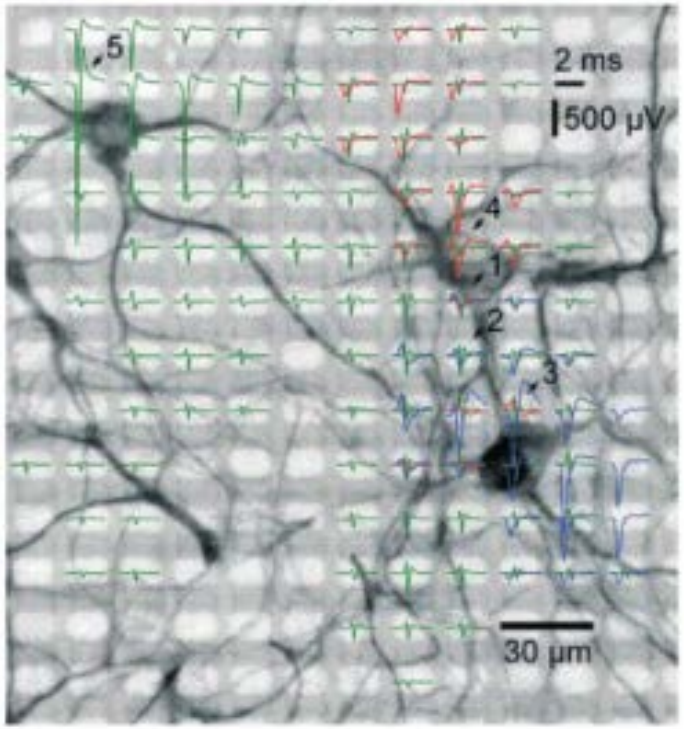
Hierlemann et al. 2011
Radivojevic et al. 2017

1) Population recording

Activity scan of 26,000 electrode chip with 1024 channels active in parallel.



2) Single Cell Level



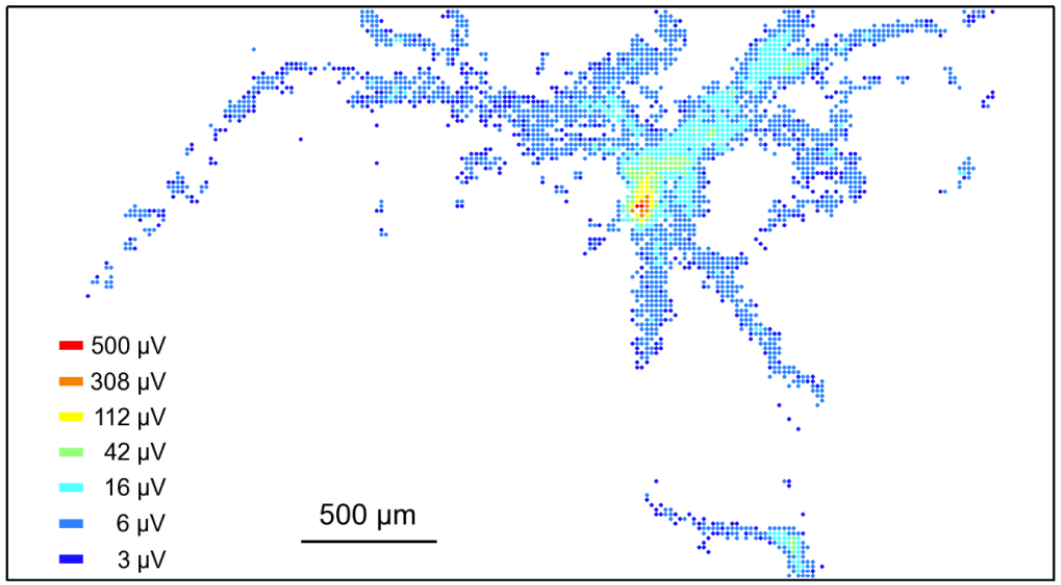
Extracellular APs

Electrical footprints can be generated by which individual neurons can be isolated based on the extracellular firing pattern.

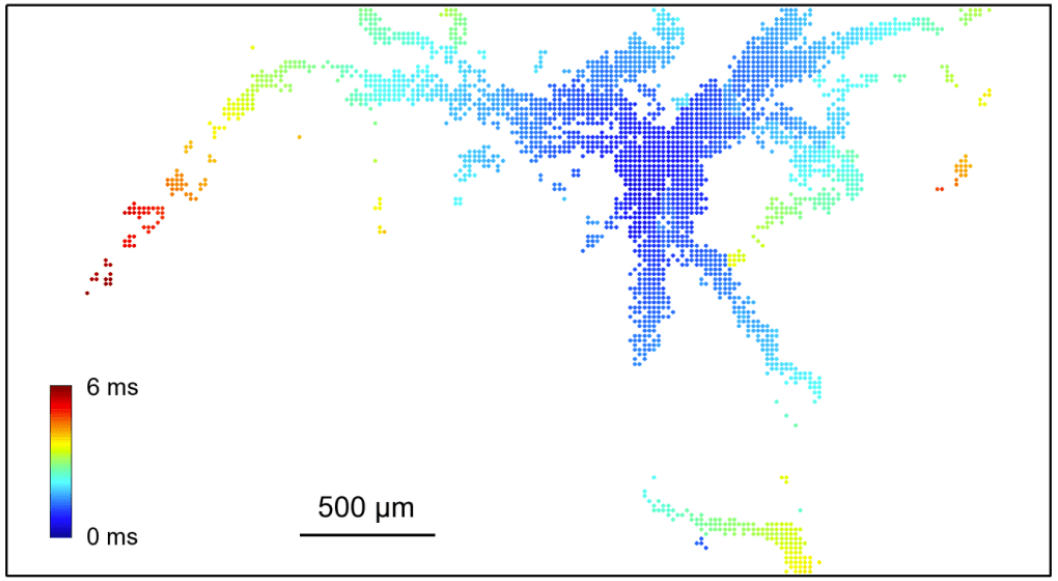
Here 3 individual neurons (green, red and blue) can be distinguished based on their electrical footprint

Hierlemann et al. 2011
Radivojevic et al. 2017

3) Subcellular level



Amplitude map



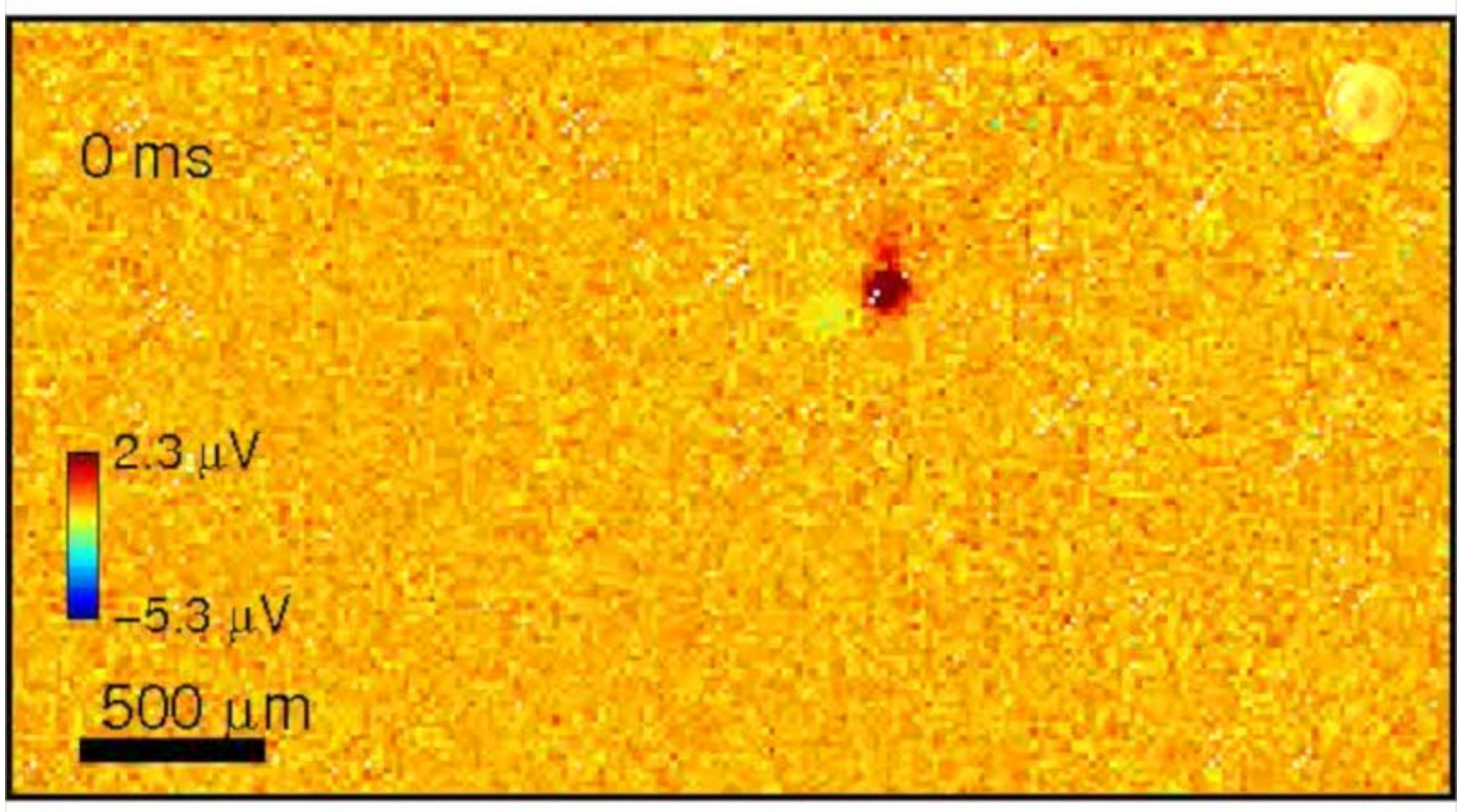
Delay map

Single neuronal axon can be traced through out the array which enables to track the action potential amplitude and latency.

The soma and AIS shows the largest amplitude whereas towards the end of axonal arbors, the amplitude is much smaller. Similarly the delay of the action potential propagation is increased with the distance from the soma.

Hierlemann et al. 2011
Radivojevic et al. 2017

Action potential propagation



High-resolution tracking of axonal action potential propagation allows for investigating changes in axonal conduction velocity.

Hierlemann et al. 2011
Radivojevic et al. 2017

frontiers in Neuroscience | Neural Technology

METHODS ARTICLE

Front. Neurosci., 22 November 2016

Multiple Single-Unit Long-Term Tracking on Organotypic Hippocampal Slices Using High-Density Microelectrode Arrays

 Wei Gong[1*],  Jure Senčar[2],  Douglas J. Bakkum[1],  David Jäckel[1],  Marie Engelene J. Obien[1],  Milos Radivojevic[1] and  Andreas R. Hierlemann[1]

Aims of the study

- How do individual neuronal activities contribute to the overall network activity?
- Assess the dynamics and details of the activities of multiple individual neurons within a network environment, over extended times of days or weeks.

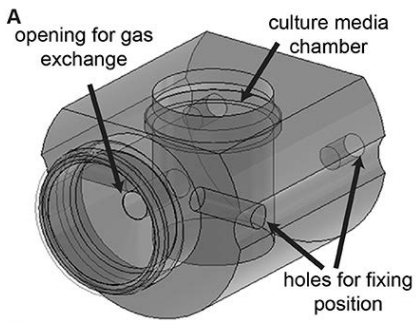
Possible methods

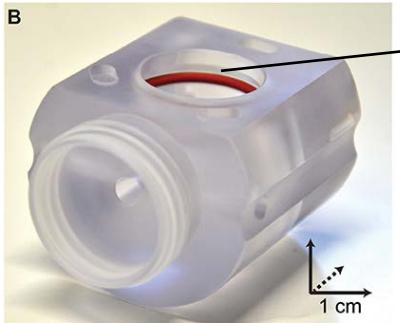
- **Patch clamp** – invasive approach which reduces the recording duration only to hours
- **Optical imaging** – relatively low temporal resolution of fluorescence imaging methods may become a problem when an identification of single units is desired
- **Extracellular recordings using traditional MEAs** - only population activities have been typically observed, no single-cell or even subcellular resolution.

Methods employed

- Organotypic brain slice cultures
  - *ex vivo* system,
  - partially preserving the cytoarchitecture, synaptic circuits, and chemical signalling,
  - bridge the gap between dissociated cell cultures and *in vivo* animal experiments,
  - large time window to conduct study on same neuronal population.
- Requirements: Long-term recording, single cell and network activity recording → CMOS-based HD-MEA.

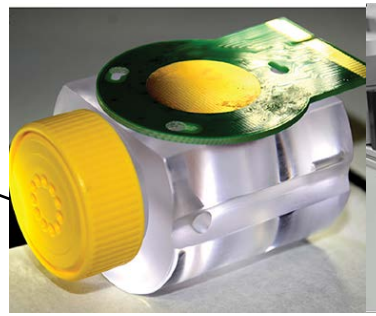
Culturing organotypic slices directly on HD-MEAs





form a seal with the plastic ring around the HD-MEA

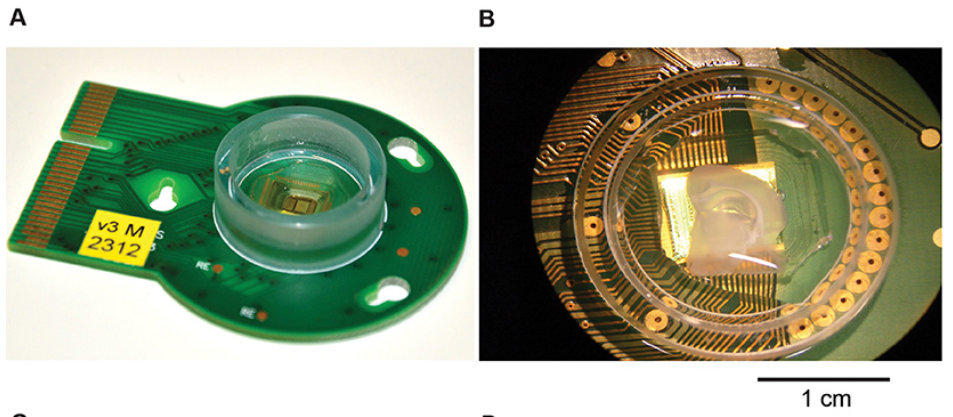
A yellow cap with a sterile filter allows gas exchange and preserves sterile conditions inside the chamber



The rotation rack can hold multiple slice cultivation chambers at the same time

Hippocampal slice recorded with a HD-MEA

HD-MEA chip with a plastic ring



A brain slice on top of the HD-MEA

Different sub-structures of the hippocampus are labelled

Amplitude map of the slice culture shows the sub-structures of the hippocampus

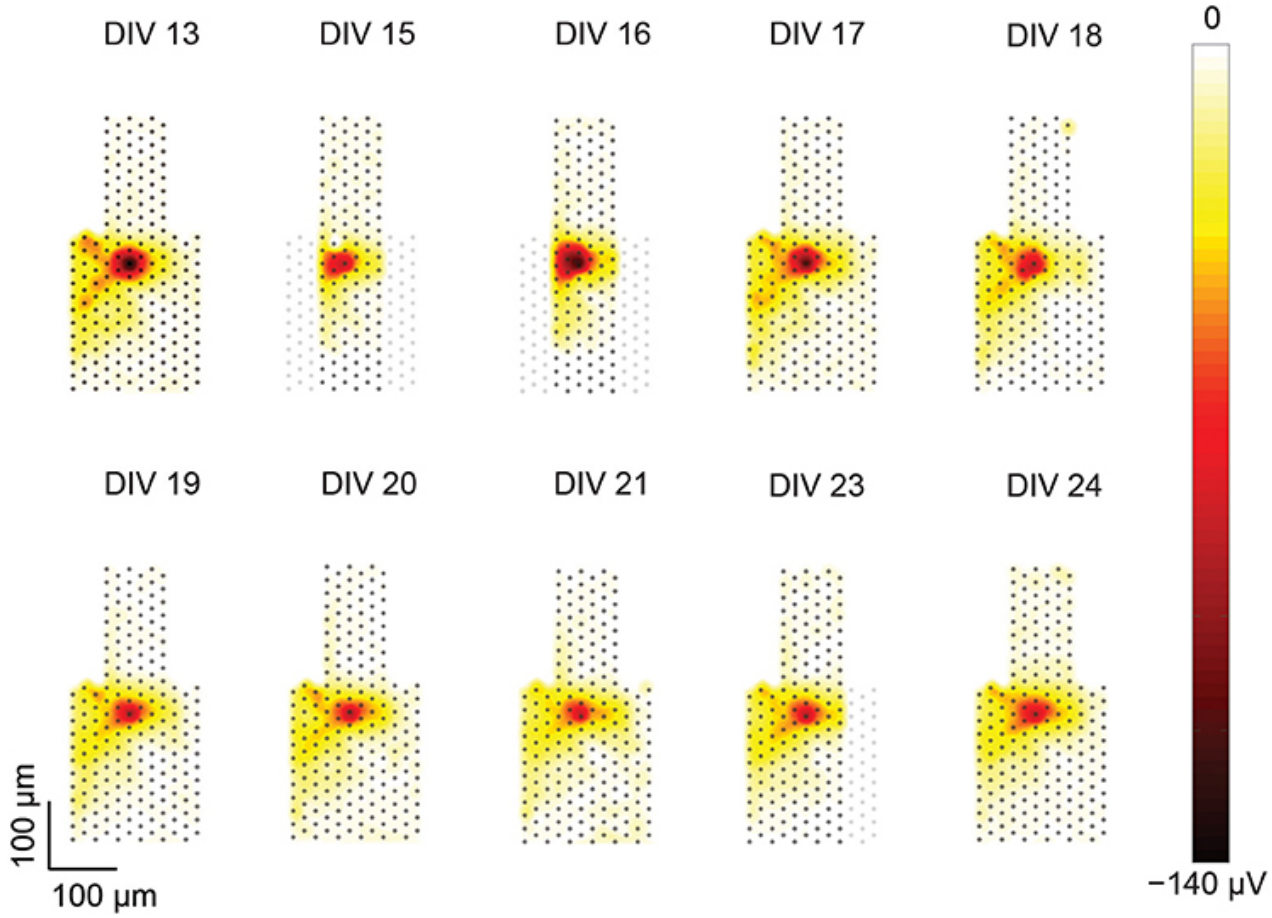
Can a single neuron be traced over several days?

Identify sites of electrodes, e.g. 16,403 – 17.000 → automatically check region.

The extracellular *electrical* signature of one single *neuron* on all electrodes is denoted as *electric* '*footprint*'.

Grey dots = positions of the electrodes
Black = largest negative amplitude (probably from the AIS)
Red = Amplitudes larger than 50% of the largest negative amplitude.

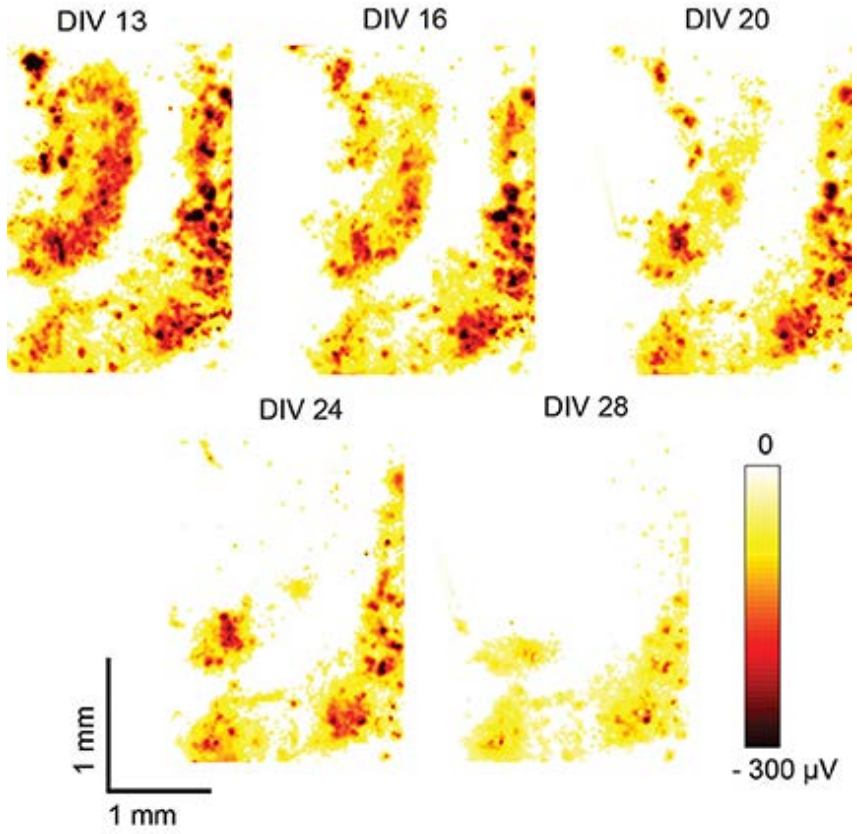
'Electrical activity of a single neuron in a organotypic slice can be reliably monitored over several days.'



Spontaneous activity of a hippocampal slice recorded with an HD-MEA during several weeks

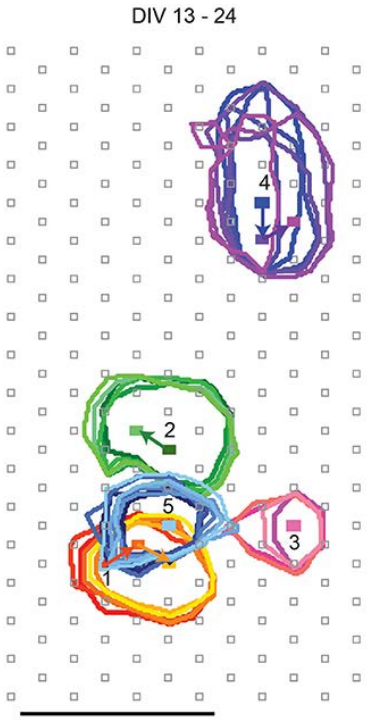
The largest negative spike amplitudes produced by spontaneous activity are displayed as a heat map across the whole array, dark colour indicates the largest negative spike amplitude

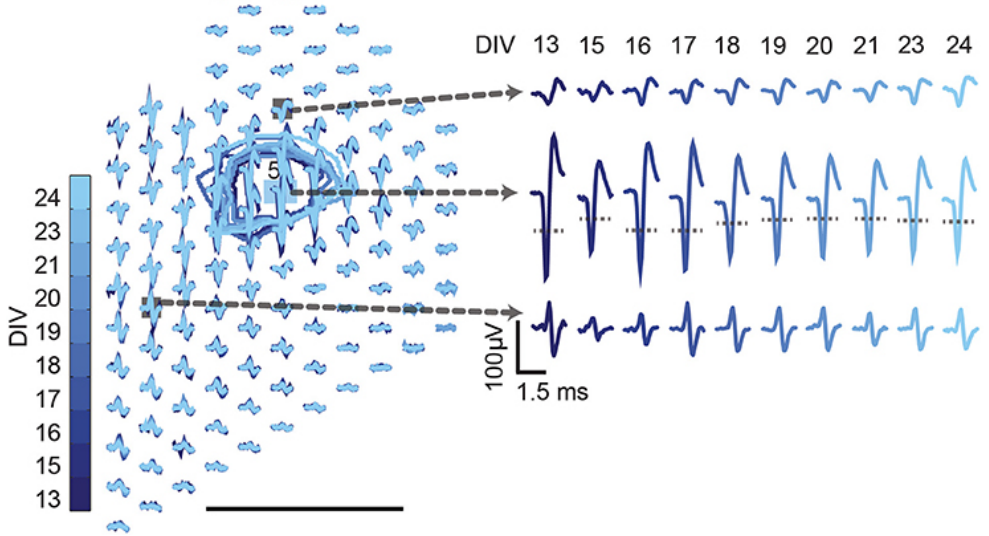
'Global network activities of the slice cultures decreased with increase in days'



Neuron activity patterns changed over time

Cells move with time
MEA can trace them.





Multiple single neuron activities were distinguished and tracked over days

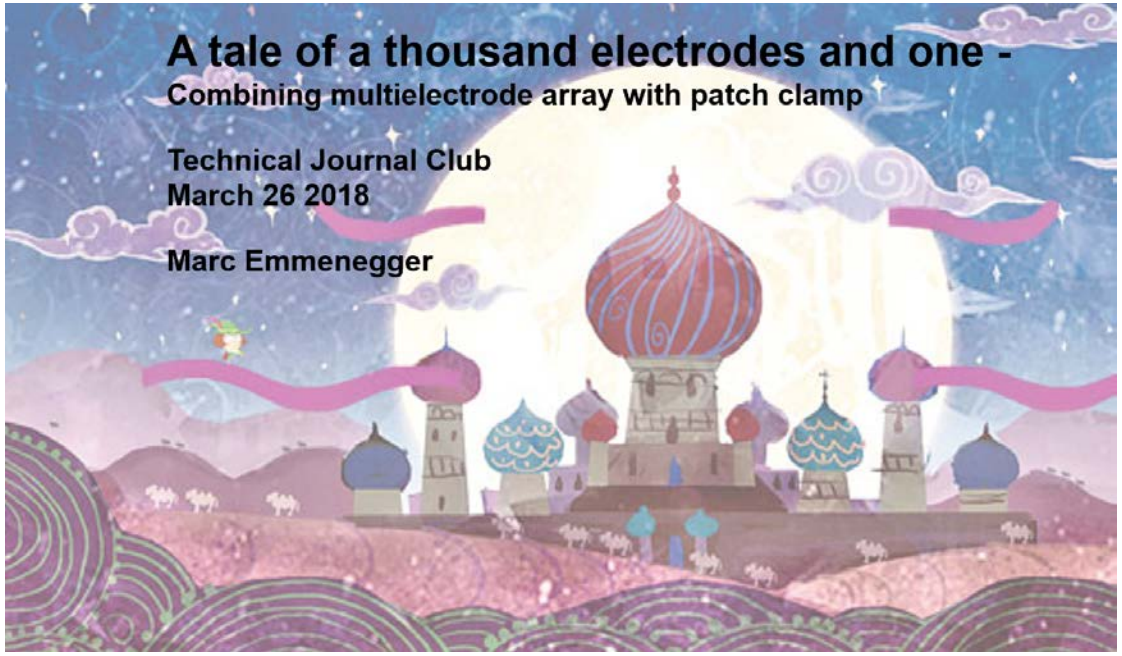
Darker colours indicate early DIVs, lighter colours indicate later DIVs

'Neuron activity patterns changed over time, but the magnitudes of the changes between every two consecutive recording days were relatively small'

Outlook

- Organotypic slices can be used as model in HD-MEA.
- Potential to study chronic impacts of pharmacophores or genetic modifications on individual neurons and neuronal populations in parallel.
- Electrical stimulation experiments can be performed to identify and possibly activate silent neurons and to investigate axonal signal propagations and neural network plasticity.

Combination of MEA and patch clamp



Combination of High-density Microelectrode Array and Patch Clamp Recordings to Enable Studies of Multisynaptic Integration

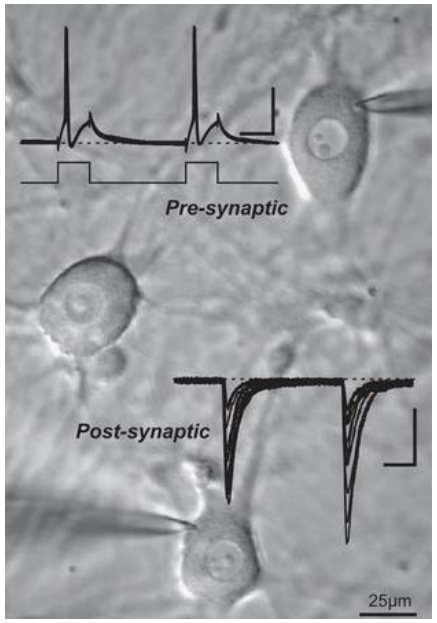
David Jäckel, Douglas J. Bakkum, Thomas L. Russell, Jan Müller, Milos Radivojevic, Urs Frey, Felix Franke & Andreas Hierlemann

SCIENTIFIC REPORTS
April 2017

Introduction

To understand the signal transmission and information processing in the neurons, it is essential to do simultaneous recordings of pre- and postsynaptic neurons.

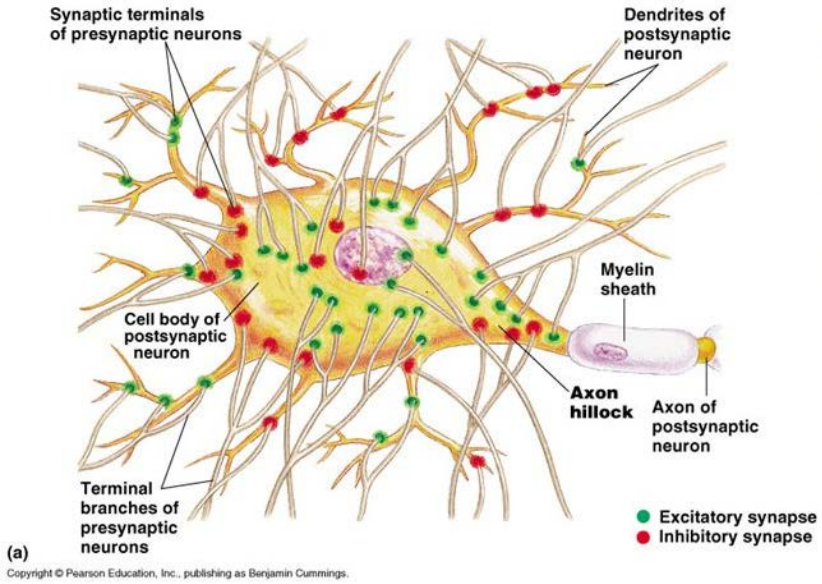
Simultaneous patching of two neurons

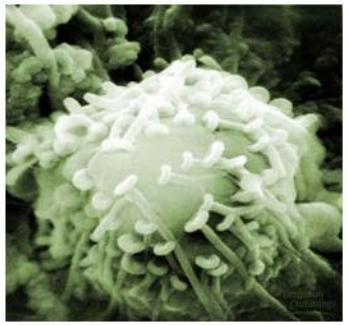


If two neurons are connected, APs evoked in the presynaptic neuron will trigger a response in the postsynaptic neuron. It could be excitatory or inhibitory postsynaptic potential.

Paired recordings are traditionally used to study short-term plasticity and long-term plasticity effects, such as long-term potentiation (LTP), or long-term depression (LTD)

Synaptic integration





'How multiple synaptic inputs integrate and interact within the postsynaptic cell?'

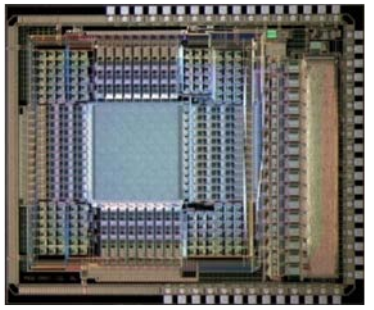
- Nonlinear summation properties of multiple inputs through dendrites,
- Temporal precision and reliability of combined synaptic inputs,
- Heterosynaptic long-term and short-term plasticity effects.

Usually neurons receive not just one input; typically up to thousands of inputs!

Task: To be able to find multiple synaptic connections

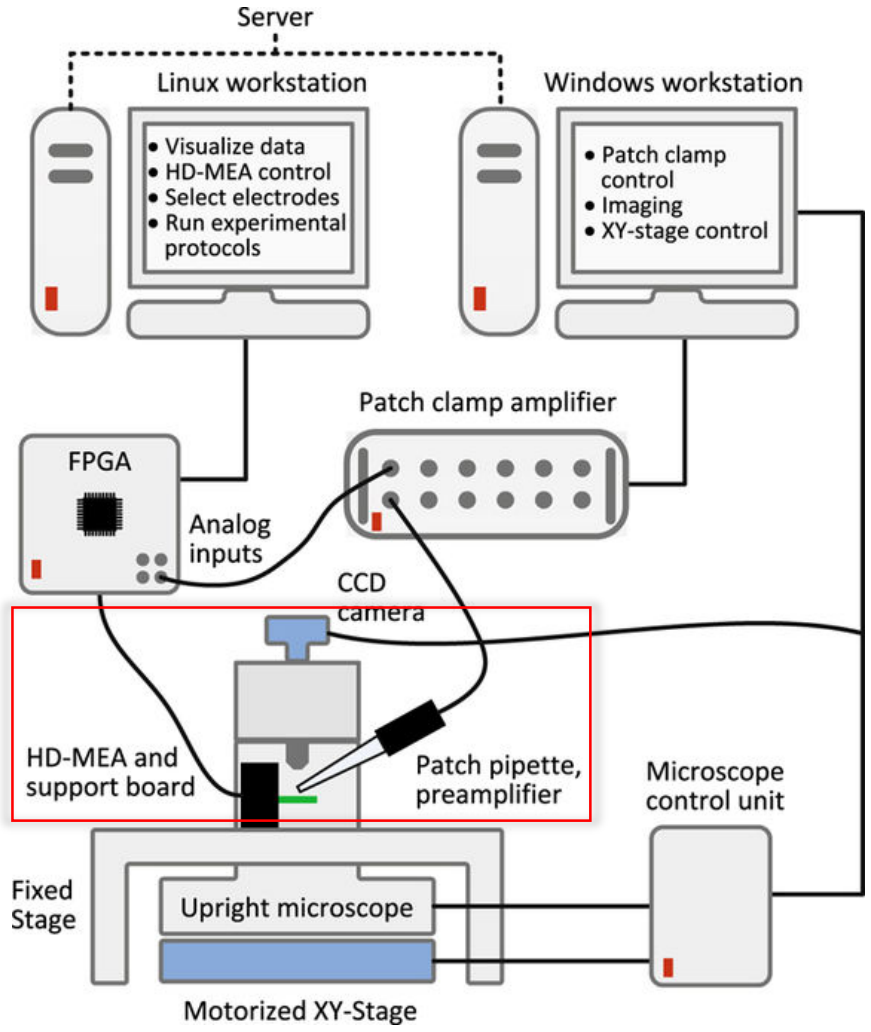
Advantages of HD-MEA

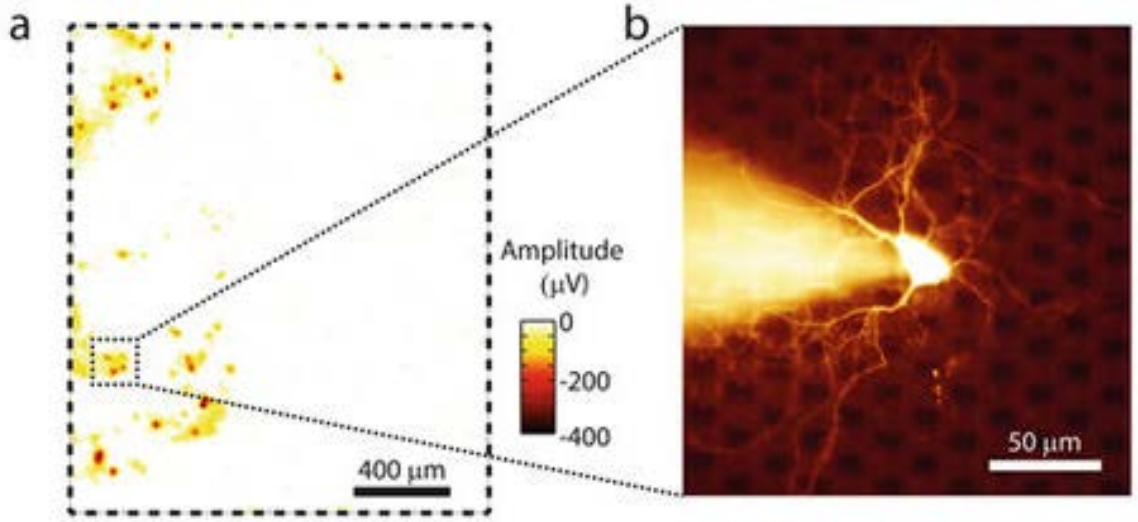
- 11,011 densely packed electrodes in an area of 1.99 × 1.75 mm^2, which could be used for electrical stimulation and recording of neuronal activity.
- Using HD-MEA, spontaneous extracellular activity can be recorded from hundreds of neurons, and they can also be stimulated to evoke APs.
- Combining whole-cell patch clamp and HD-MEA enhances the possibility to find multiple synaptic connections while patching just a single neuron.



- 11k electrodes
- 126 channels

Experimental setup



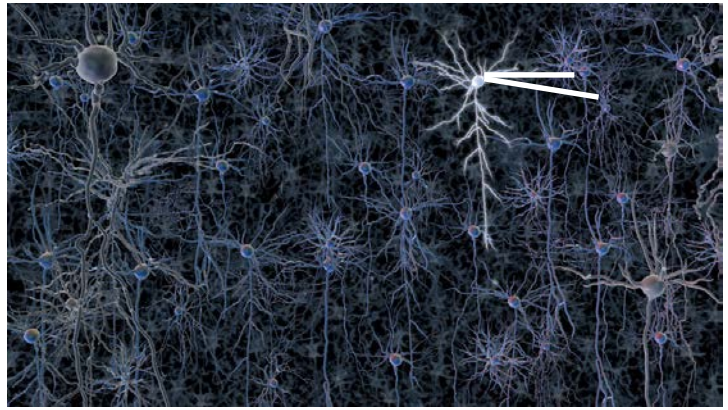


(Left) Amplitude map of the spontaneously active neurons in the array.
(Right) Fluorescent image of the patched neuron with pipette loaded with fluorescent dye.

Experimental procedure – Method 1

Intracellular recording from the patched neuron and extracellular recording from the MEA electrodes based on spontaneous activity

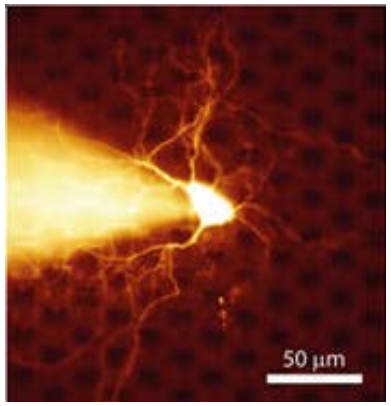
- A neuron is patched => MEA electrodes in the vicinity of the patched neuron were identified



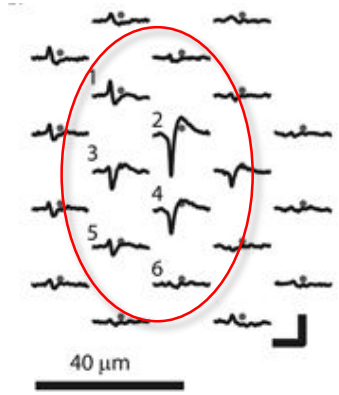
  - Spontaneous spikes of the patched neuron and spontaneous extracellular spikes were compared (Experiment 1)
  - Sometimes, if the patched neurons did not fire spontaneously, APs were evoked by injecting current pulses and the spontaneous extracellular spikes were compared (Experiment 2)
- A neuron is patched => spontaneous extracellular activity of the entire array were recorded to compare the network activity (Experiment 3)
- A neuron is patched => identification of single pre-neurons that are synaptically connected (Experiment 4).

'Strongly depends on spontaneous extracellular activity'

Experiment 1: Simultaneous intracellular and extracellular recordings

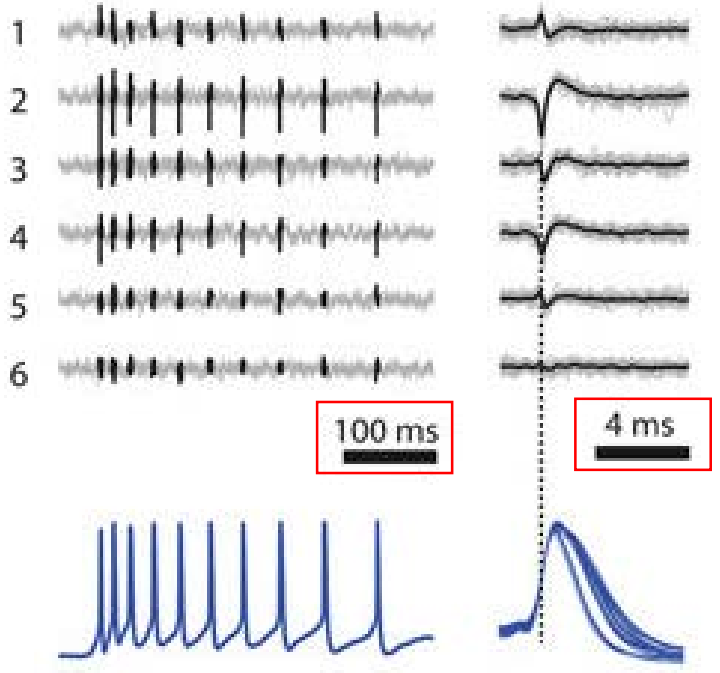


A neuron is patched



MEA electrodes in the vicinity of the patched neuron were identified

Superimposition of all detected waveforms

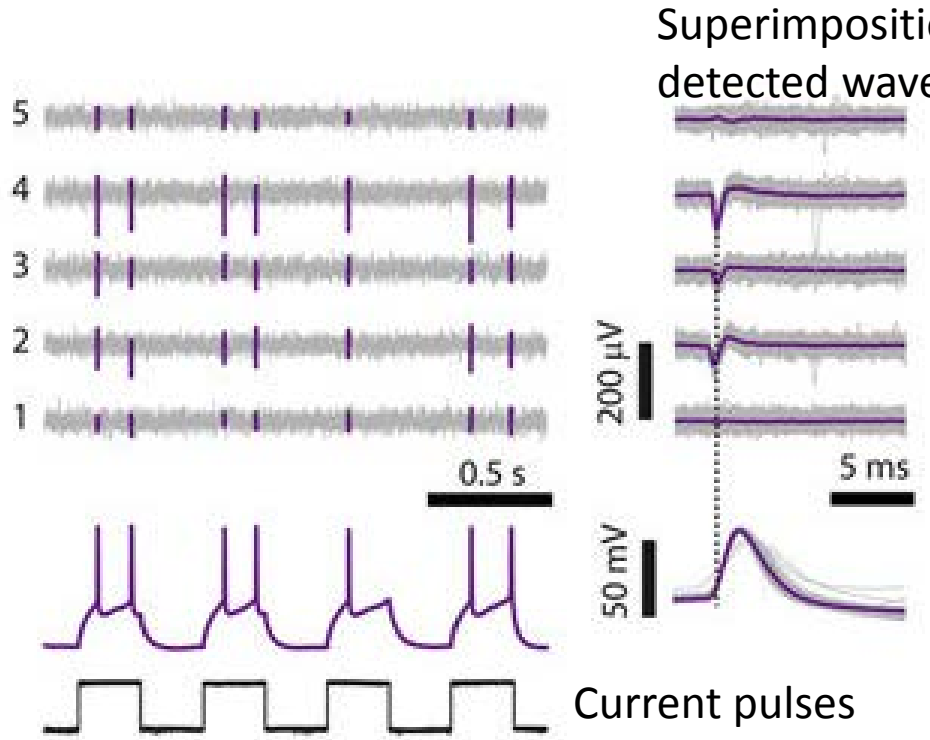


Spontaneous activity from six selected MEA electrodes => extracellular recordings

Spontaneous activity from a patched neuron => intracellular recordings

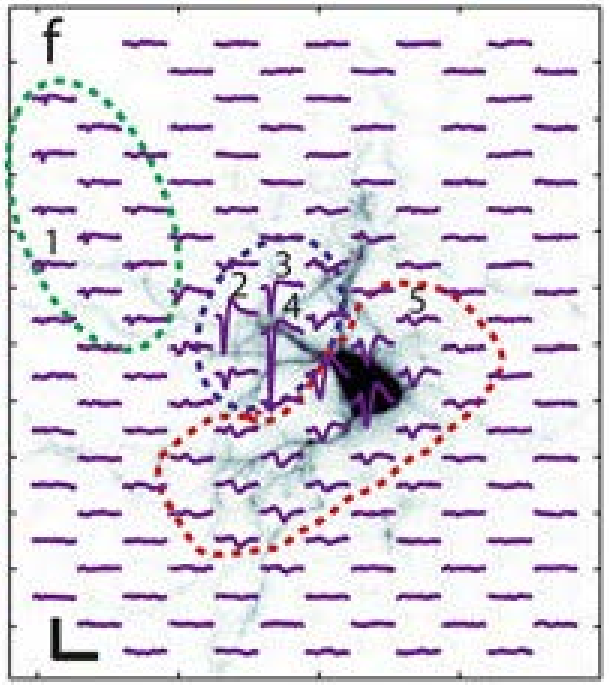
Intracellular recording from the patched neuron and extracellular recording from the MEA electrodes

Experiment 2: Simultaneous intracellular and extracellular recordings

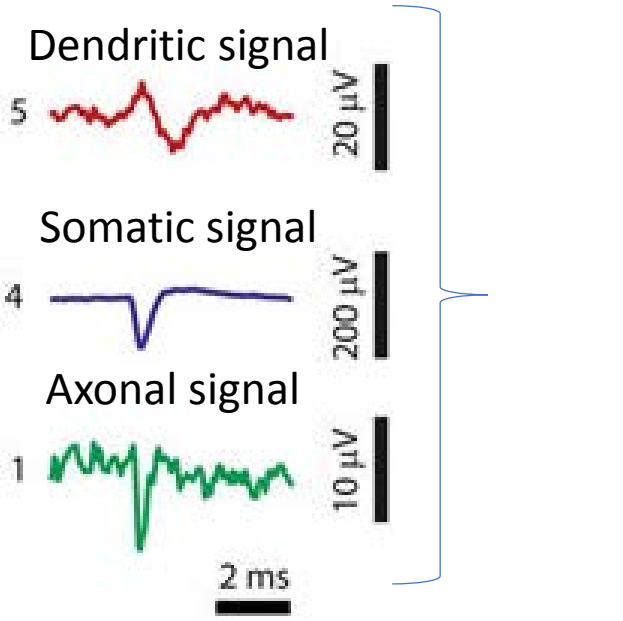


Current pulses of same amplitude

Sometimes, if the patched neurons did not fire spontaneously, APs were evoked by injecting current pulses

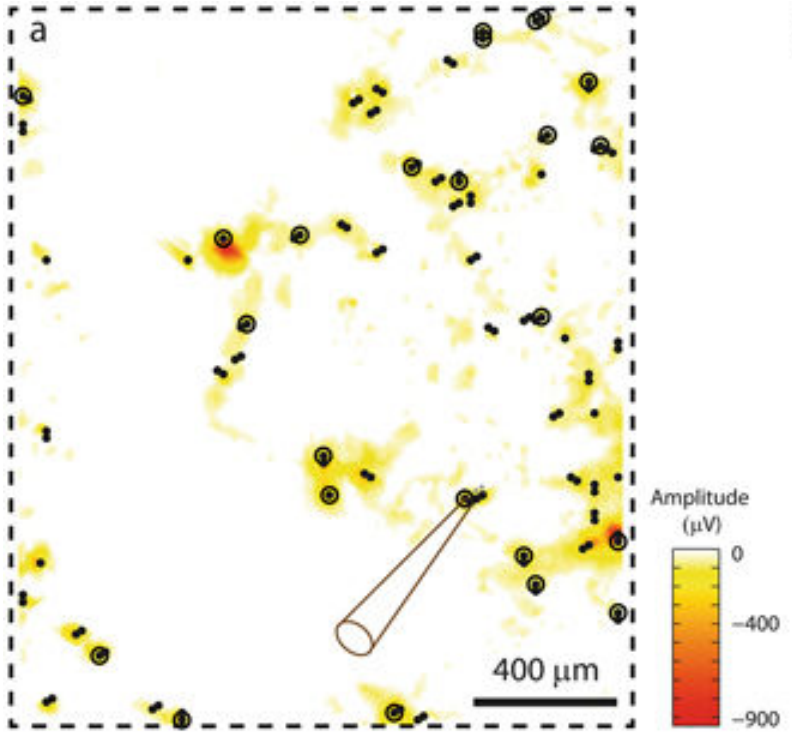


Electrical footprint superimposed to a fluorescent image of the patched neuron

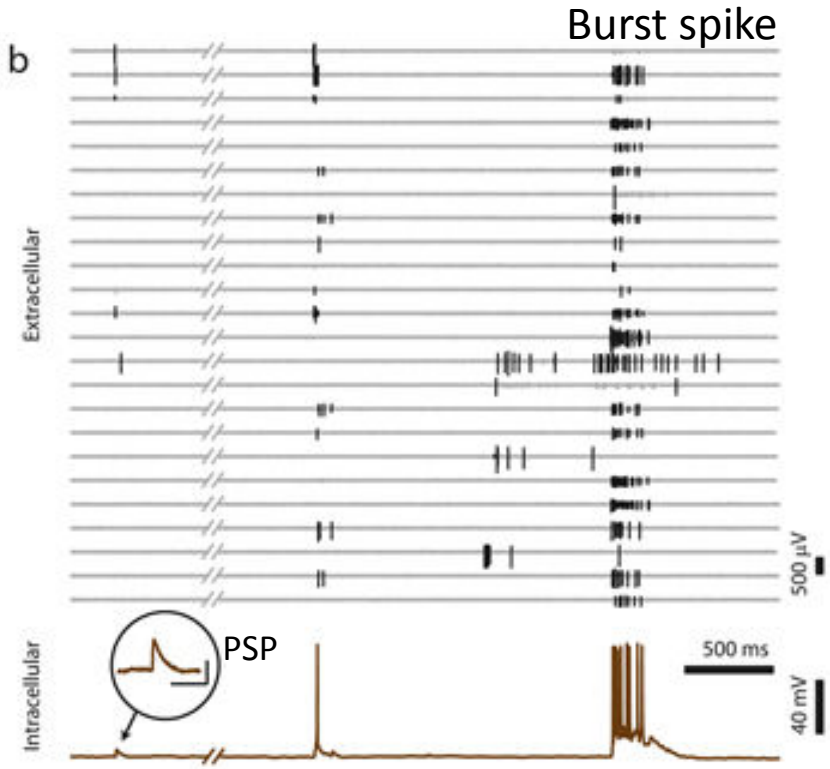


Differences in amplitude

Experiment 3: Simultaneous recording of network activity and intracellular activity



Amplitude map of the entire array



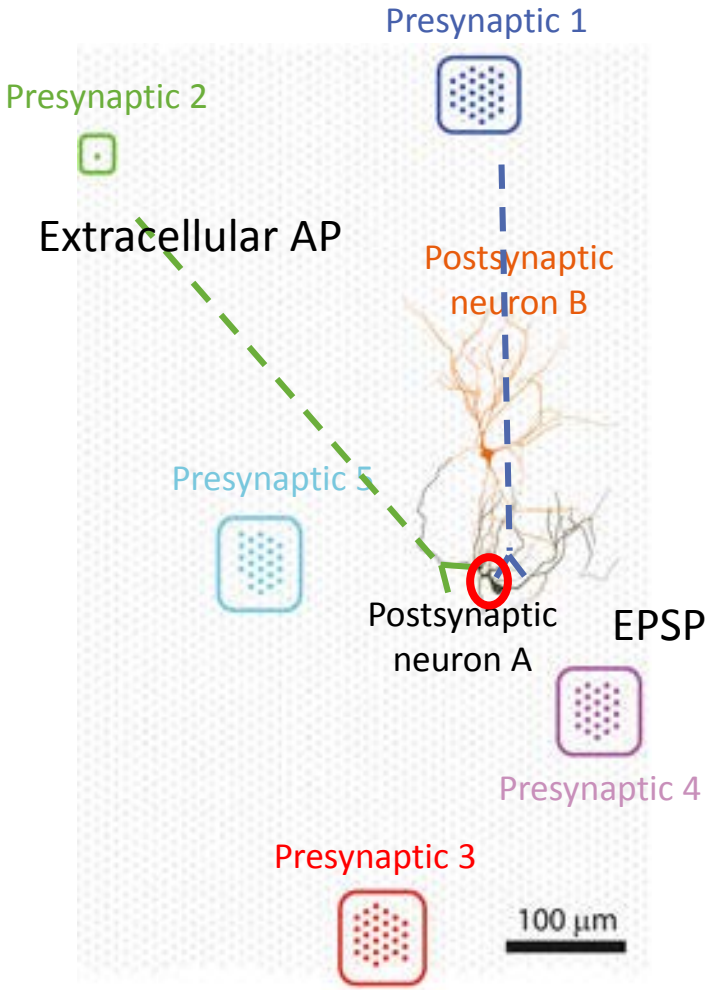
Correlations between the intracellular and the network activity can be observed

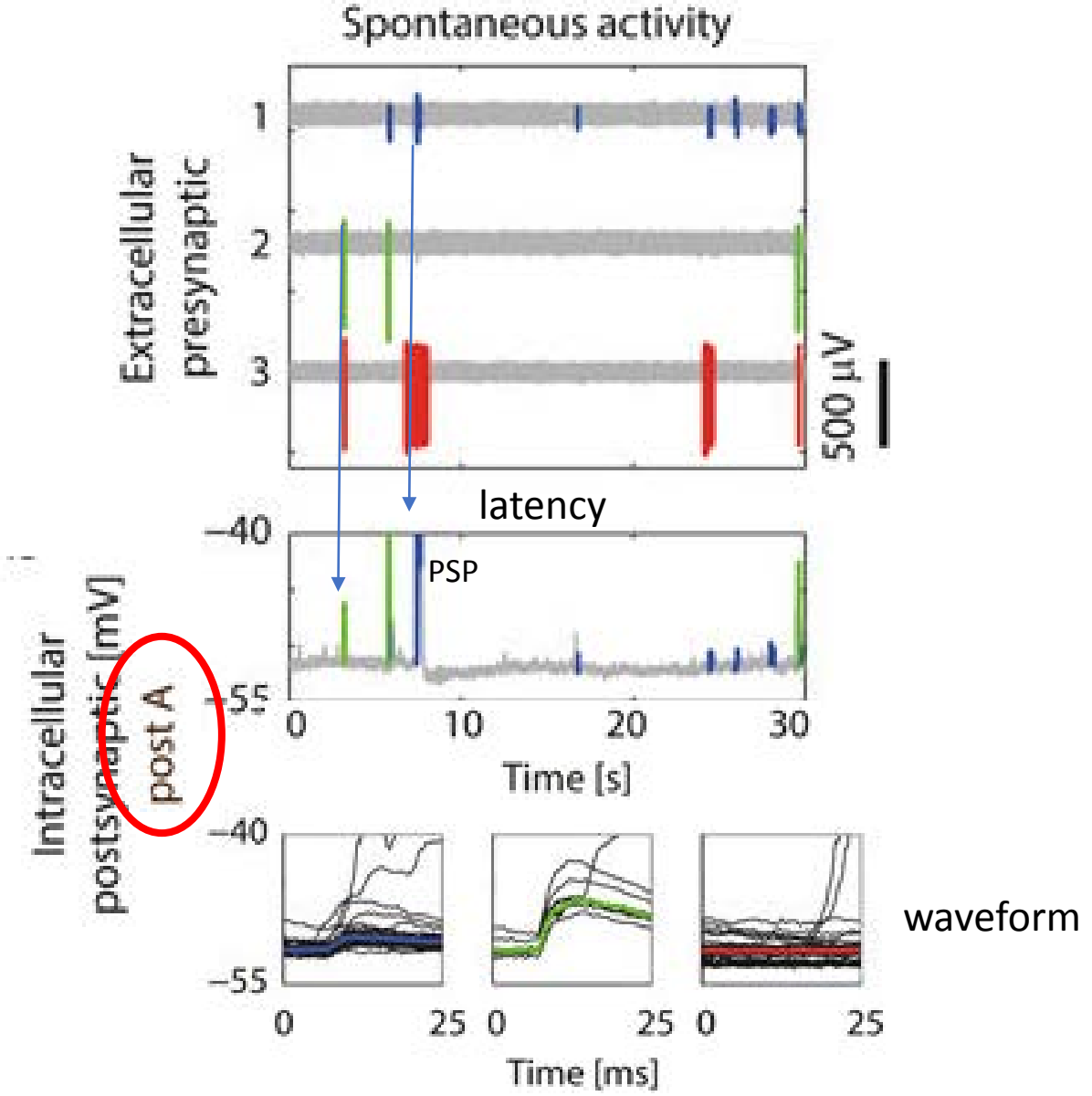
A single neuron is patched

- Sparsely distributed electrodes

⦿ Subset of electrodes that show synchronised activity with patched neuron

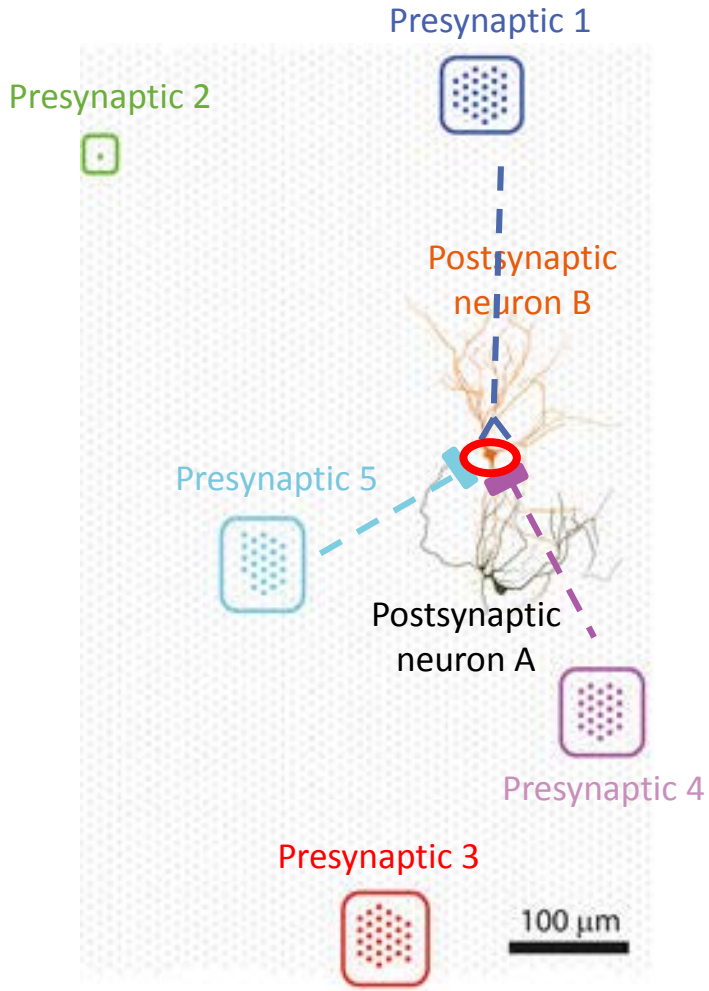
Experiment 4: Include latency and spike sorting

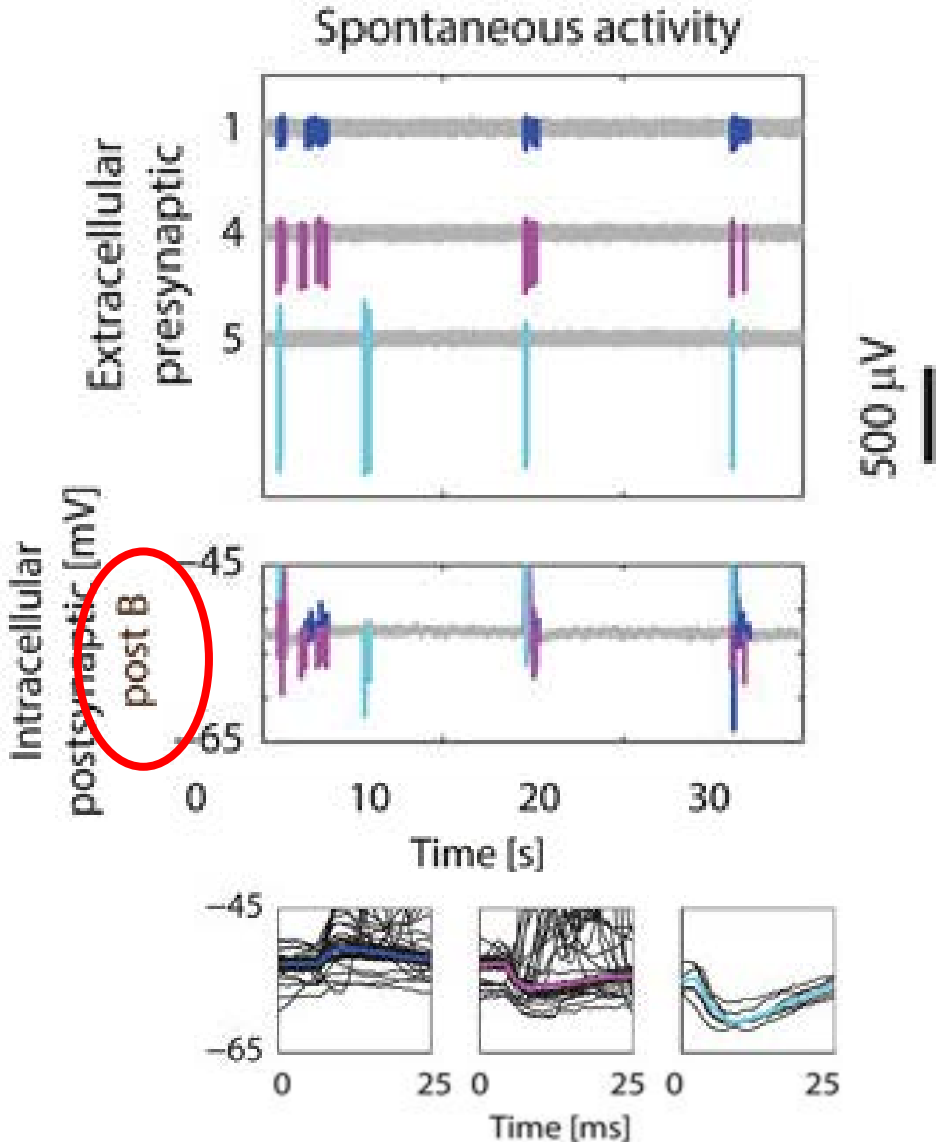




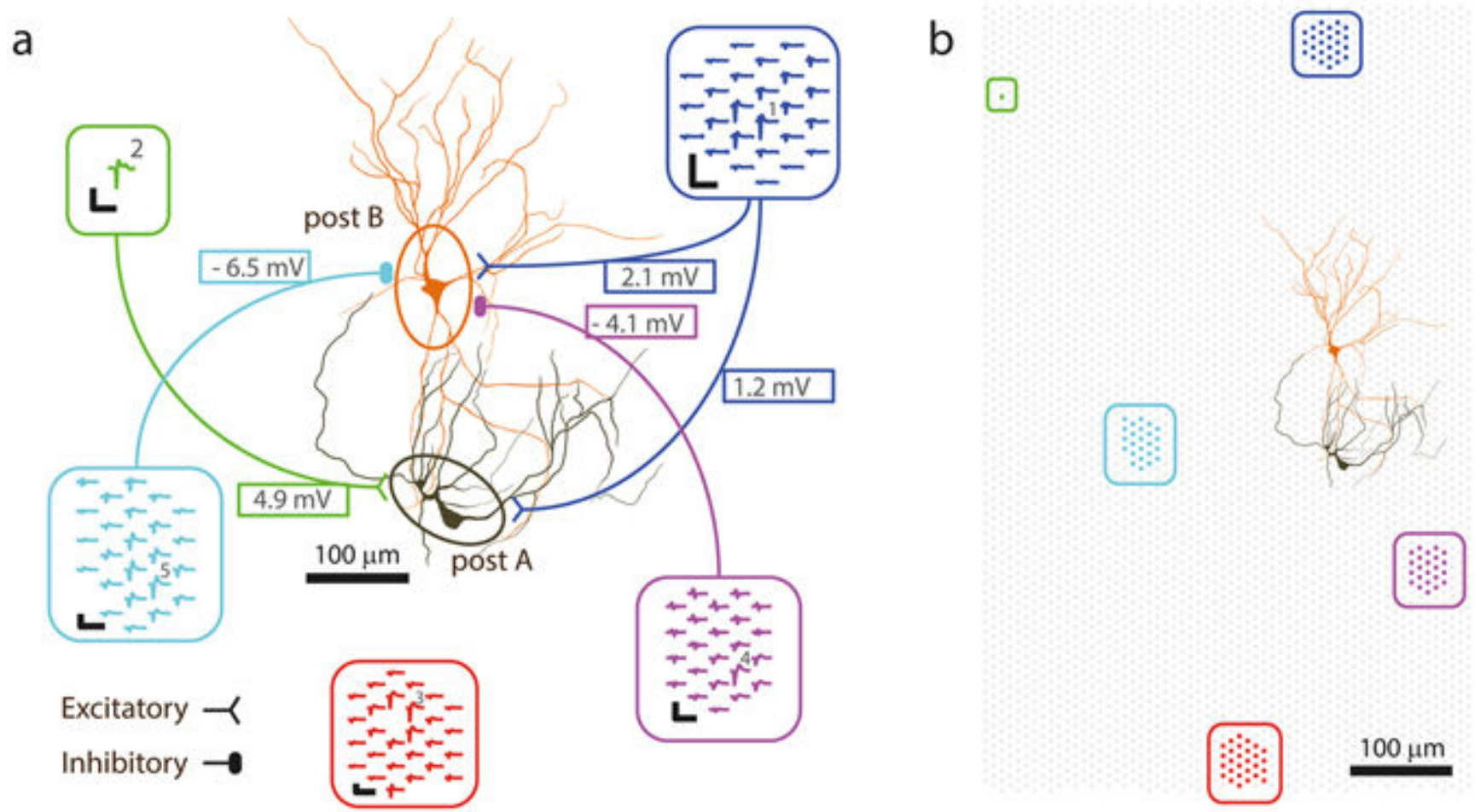
waveform

Experiment 4: Mapping PSPs based on spontaneous activity





Experiment 4: Connectivity diagram for the synaptic inputs



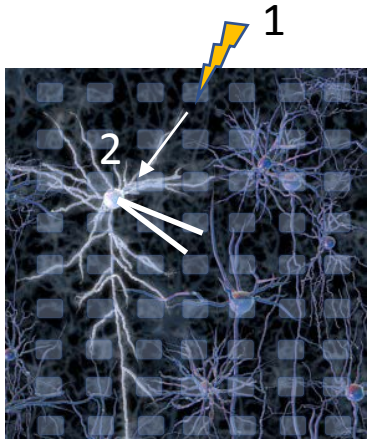
By just recording the spontaneous activity, assign excitatory/inihibitory postsynaptic input

Conclusions Method 1

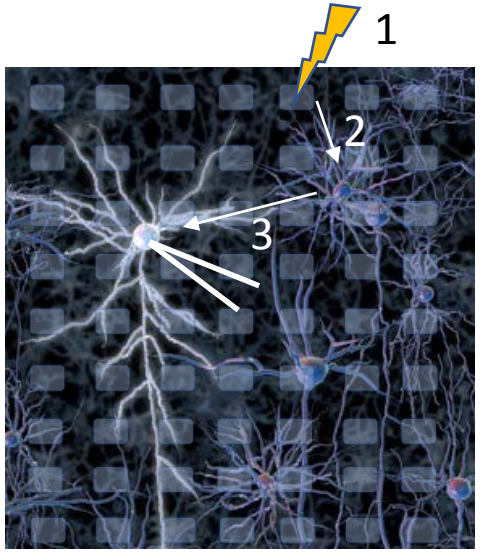
- Advanced alternative to calcium imaging for detecting population activity and single cell.
- However, no photobleaching, much better temporal and spatial (subcellular) resolution, and no need for genetic manipulation.
- Extracellular recordings coincide with intracellular recordings.
- Identification of subset of neurons within population responsible for generation of EPSP/IPSP (or not connected) → **spontaneous activtiy**.
- Electric footprint provides information on the morphology.
- **By just recording the spontaneous activity, assign excitatory/inihibitory postsynaptic input**.

Experimental procedure – Method 2

Intracellular recording from the patched neuron and extracellular recording from the MEA electrodes based on stimulation



- ‘Direct activation’
  - A neuron is patched (postsynaptic neuron)
  - Electrical stimulation of selected electrodes =>
  - causes a change in membrane potential =>
  - evokes APs in the patched neuron

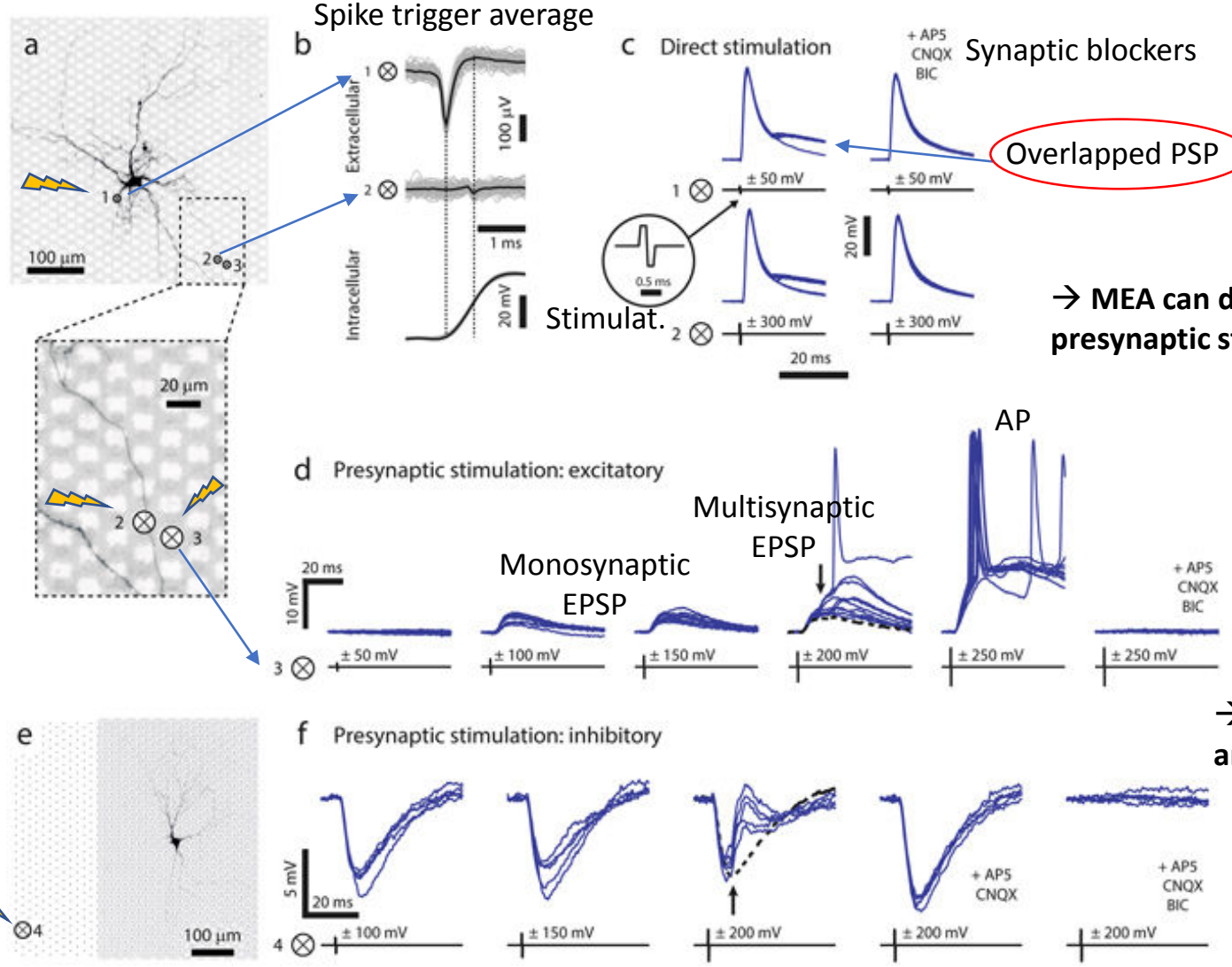


- ‘Presynaptic activation
  - A neuron is patched (postsynaptic neuron)
  - Electrical stimulation of selected electrodes =>
  - causes a change in membrane potential of preneuron =>
  - evokes AP in a presynaptic neuron =>
  - leads to postsynaptic potential/AP in the patched neuron

Experiment 5: Evoking postsynaptic signals through HD-MEA electrical stimulation

Fluorescence image

Spike trigger average



→ **MEA can distinguish between direct and presynaptic stimulation.**

→ **MEA can distinguish between mono and multisynaptic stimulation.**

With increase in amplitude → IPSP diminishes → multisynaptic EPSP.

Experiment 6: Stimulation-triggered PSPs from multiple presynaptic inputs

A neuron was patched

↓

Voltage stimulation were applied at ±100 mV, ±150 mV, ±200 mV, and ±250 mV amplitudes

↓

Subset of electrodes evoked EPSPs in the patched neuron (b)

↓

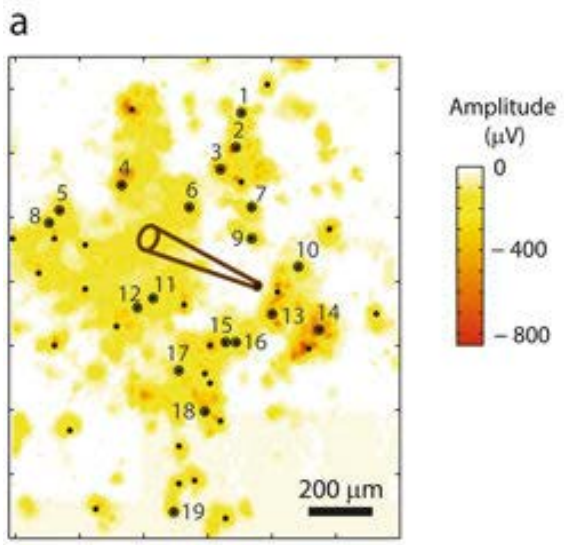
Lowest stimulation voltage that evoked EPSPs were identified

↓

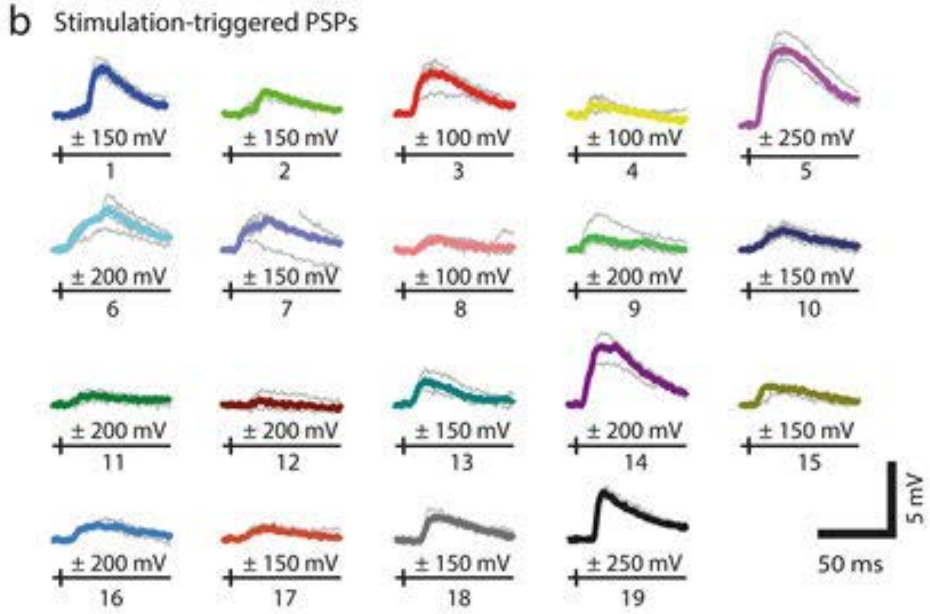
Multi-neuronal monosynaptic input to the patched cell was recorded

↓

How many preneurons can be found?



Response from patched postneuron



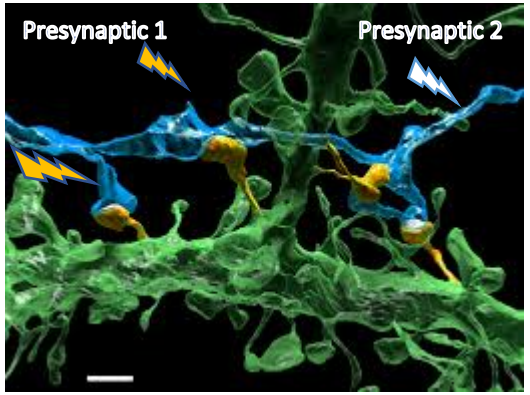
→ **Stimulating 19 different electrodes leads to PSPs in postneuron. But from how many preneurons do signals come from?**

Experiment 6: Multiple stimulation of presynaptic neurons

Neurons could be stimulated at different sites close to the soma and along the axon. Axonal outgrowth can extend over large distances across the array. Therefore, it is reasonable to assume that specific presynaptic neurons were stimulated multiple times through different stimulation electrodes

'Are all PSPs originated from different presynaptic cells?'

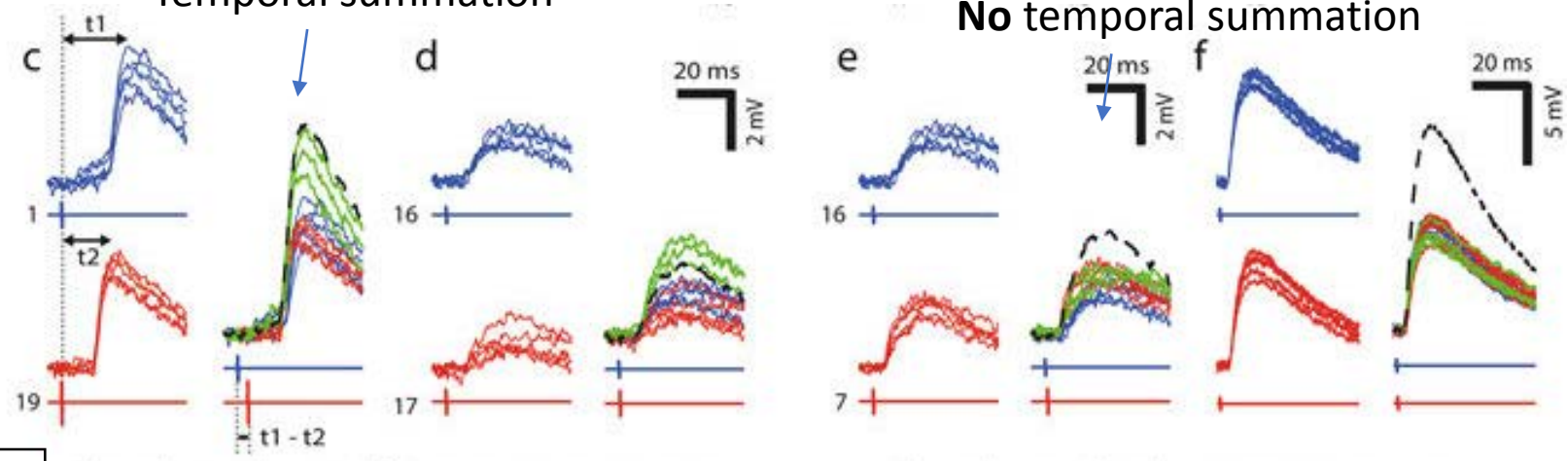
=> paired presynaptic stimulation was used to test whether PSPs with similar amplitudes were caused by stimulating the same presynaptic neuron through different electrodes.



Same presynaptic	⫩	Similar amplitude
Diff presynaptic	⫩	Summed up amplitude

Temporal summation

No temporal summation



Electrodes stimulate two different presynaptic neurons

Electrodes stimulate the same presynaptic neuron

→ **MEA can distinguish whether electrode records from one (at different sites) or multiple preneurons.**

Experiment 7: Short-term plasticity measurements for multiple synaptic inputs

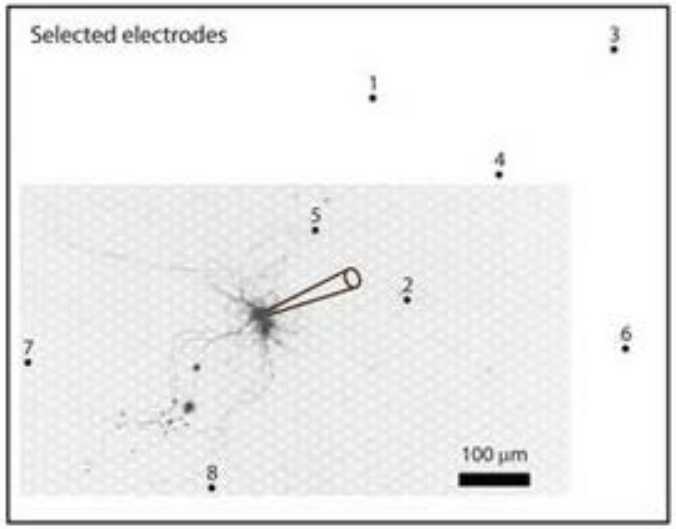
A neuron was patched

↓

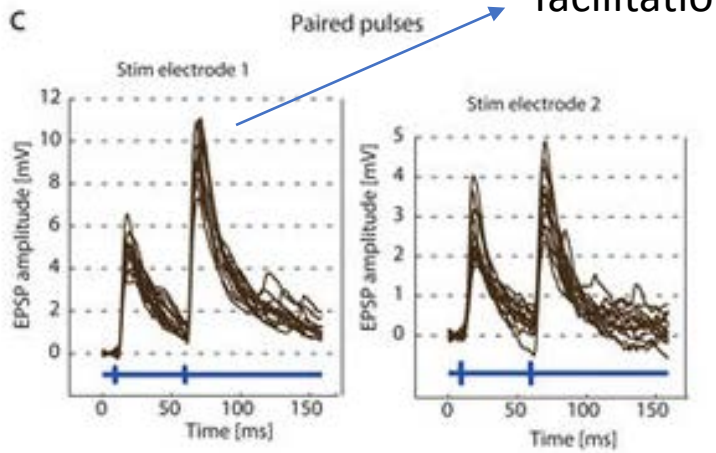
Eight electrodes that reliably evoked PSPs were selected (b)

↓

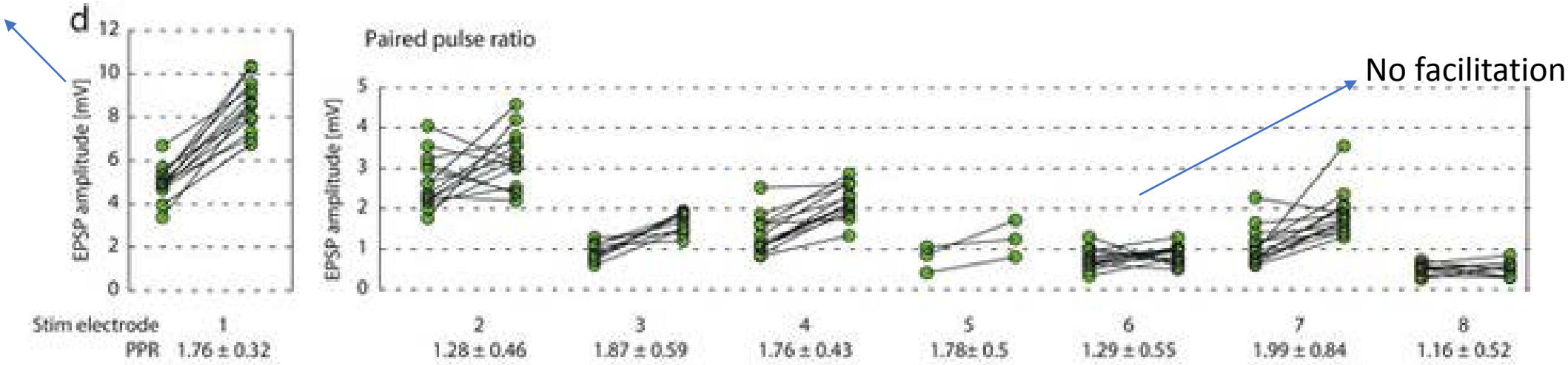
Paired-pulse protocol was executed; Pairs of stimulation at 50 ms interval



facilitation



Strong facilitation



No facilitation

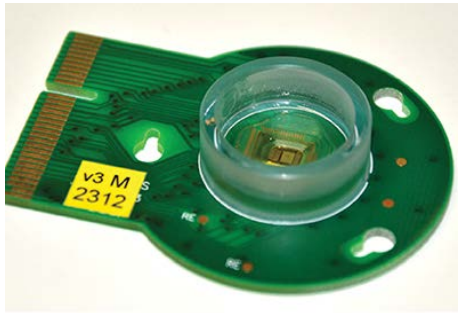
Conclusions Method 2

- MEA can distinguish between direct and presynaptic stimulation.
- MEA can distinguish between mono and multisynaptic stimulation.
- MEA can distinguish whether electrode records from one (at different sites) or multiple preneurons.
- MEA can be used to study neuronal adaptation and plasticity (short-term and long-term).

Overall thoughts

- Information processing in complex networks is contingent on single cells and their interplay in populations.
- CMOS MEA’s ultra-dense electrode arrays allow measurement of electrical signals at very high spatial and temporal resolutions.
- Combination of **MEA**, **patch clamp**, and **microscopy** enable insights into the population dynamics of complex neuronal networks on various levels.

Outlook



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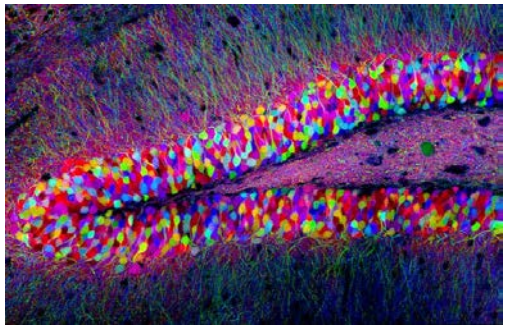


→ Network dynamics.

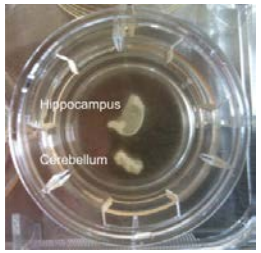
→ Single-cell properties of selected cells.

→ Identification of cellular subsets.

Applications in biology

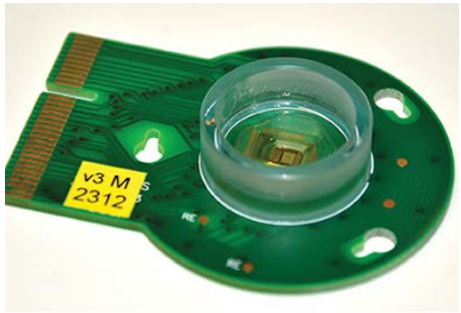
- Compound screenings
- Genetic manipulations
- Disease modelling
  - →Effect on network dynamics
  - →Effect on specific cellular subpopulations
- Relationship neurons – glia.

Applications in biology



+ treatment

- treatment



Record neuronal network dynamics over long time → identify changes in e.g. synchronisation.

Identify vulnerable populations using fluorescent markers (e.g. only GABAergic neurons show decreased synchronisation).

Patch cells from different populations and assess intrinisic ephys properties and compare to global network dynamics.

Thank you for your attention!