Electrophysiological recordings and analysis

Day 2

Extracellular recordings

11.10.2024

Tobias Ackels

Purpose of the course

- The principles underlying neural signals
- Electrophysiological recording methods, in particular extracellular recordings
- Enable basic comprehension of neuroscience data and results

Goals of neuroscience

1. Understand the mechanisms by which the brain/nervous system carries out all functions (e.g. sensory processing, cognition, motor functions, etc.).

2. Understand what failures in those mechanisms lead to particular disorders of the brain.

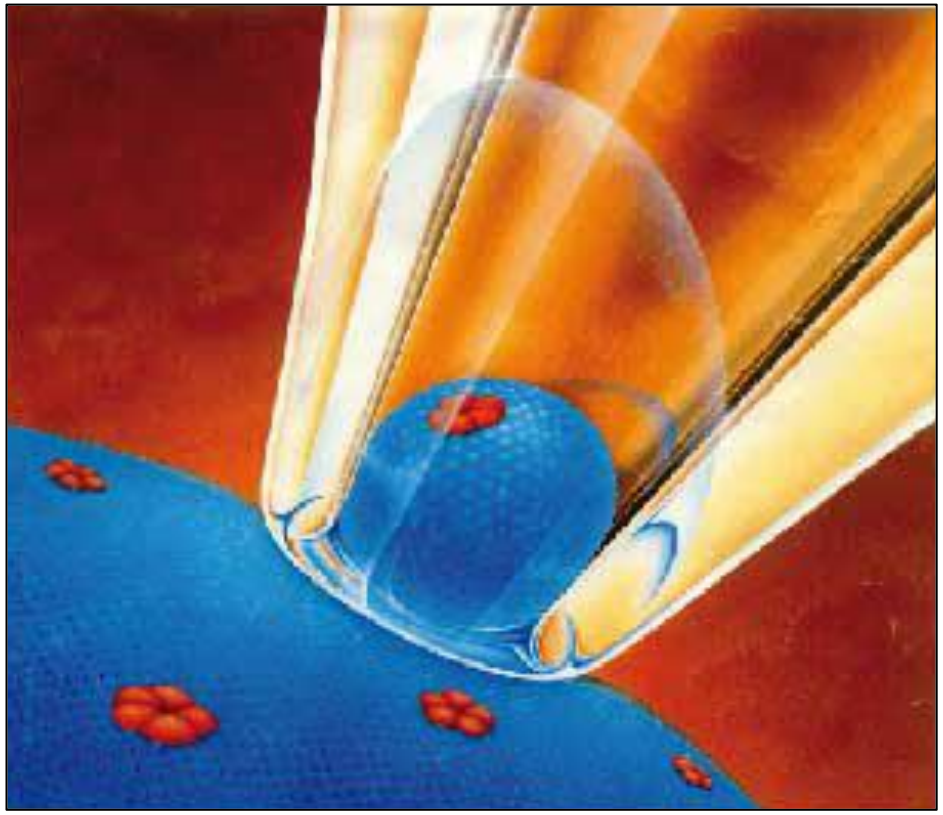
3. Develop treatments for those disorders in order to restore function.

Neural acquisition methods: Electrophysiology

- Acquisition of electrical signals of biological origin over time
- Various spatial scales:
    - Patch-clamp
    - Intracellular electrode recordings
    - Extracellular electrode recordings
    - Electrocorticography (ECoG)
    - Electroencephalography (EEG)

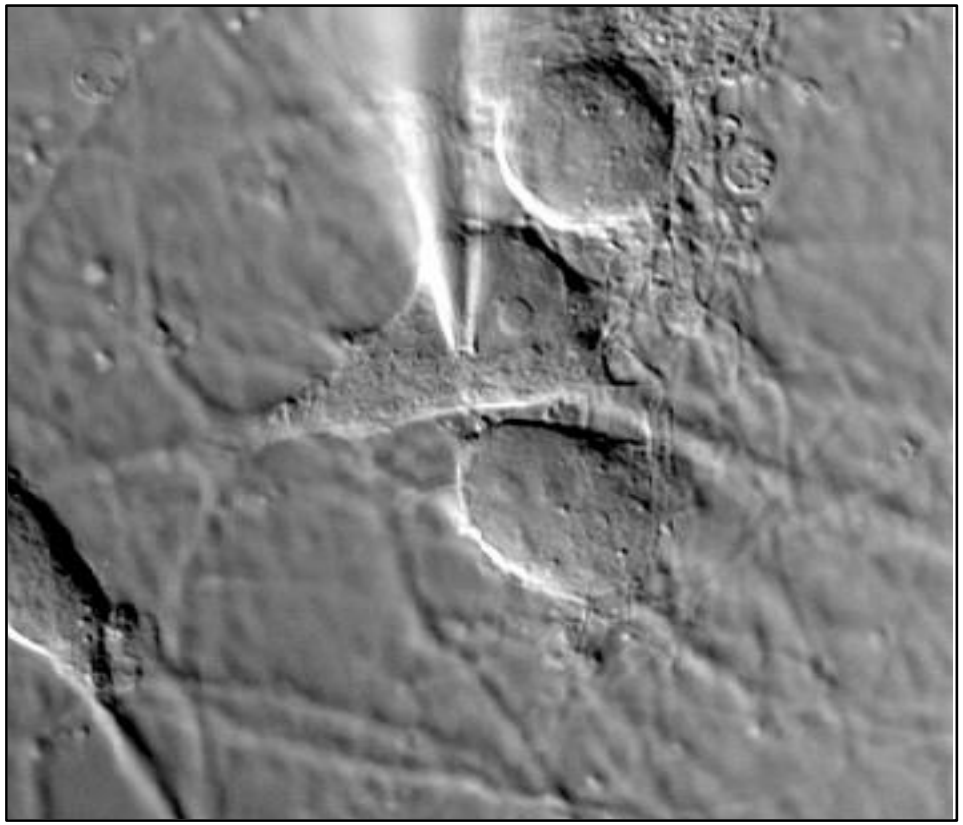
Electrophysiology: Patch-clamp

- Glass pipette seals membrane patch by suction.
- Measures voltage changes in solution inside pipette (electrolyte)
- Used to study properties of a small patch of membrane, even individual ion channels!



Electrophysiology: Intracellular recordings

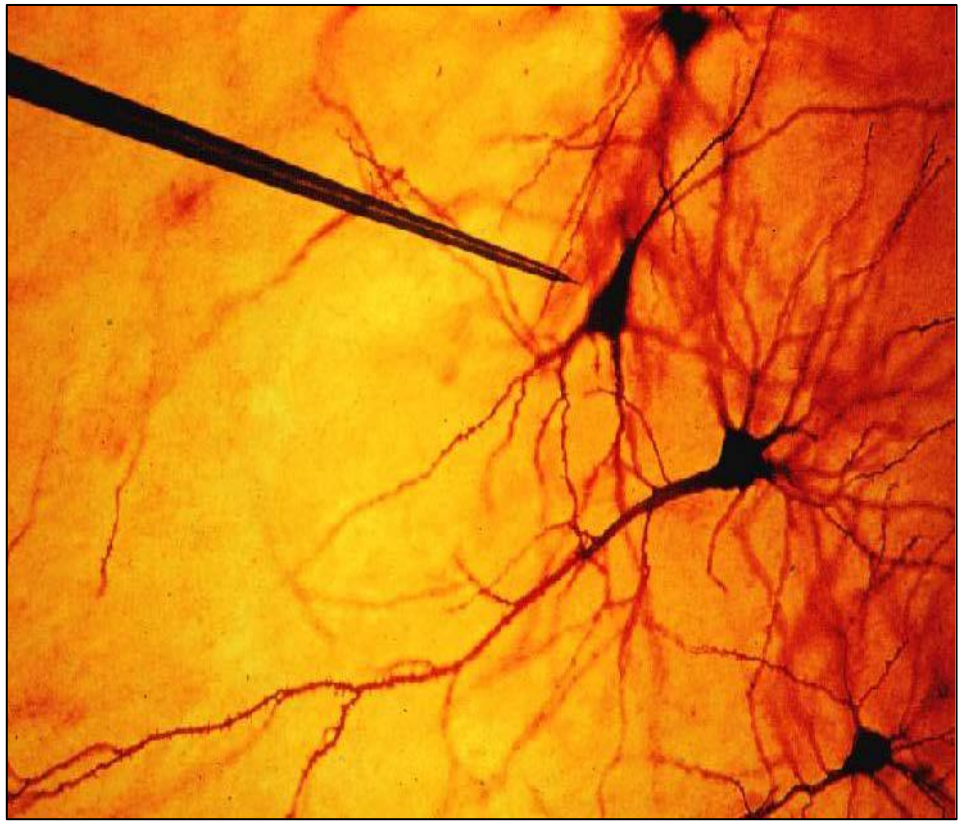
- Sharp glass pipette filled with electrolyte solution
- Pipette tip penetrates cell membrane of a single neuron
- Measure ionic currents across cell membrane of the cell body (for somatic patching)
- Current clamp & voltage clamp configurations



Electrophysiology: Extracellular recordings

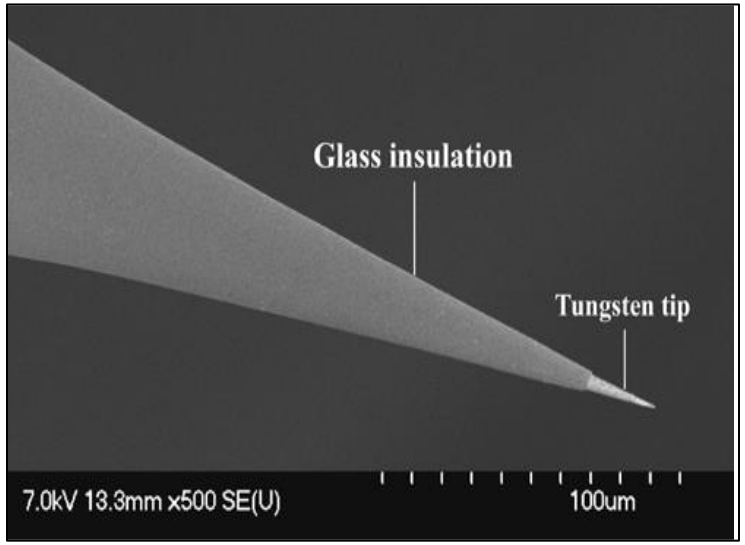
- Microelectrode made of metal (e.g. tungsten) coated with insulating material but with an exposed tip
- Acquires voltage readings in extracellular space

- Voltage signal has several components:
  - Noise
  - LFP
  - Single-unit spiking activity
  - Multi-unit spiking activity

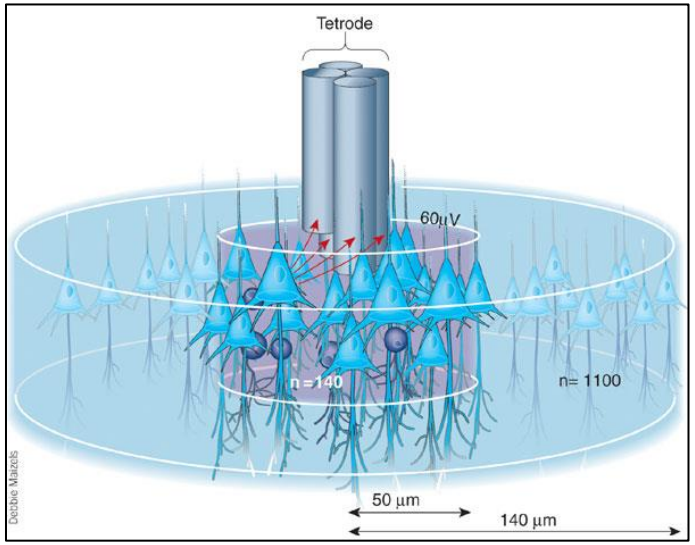


Types of microelectrodes

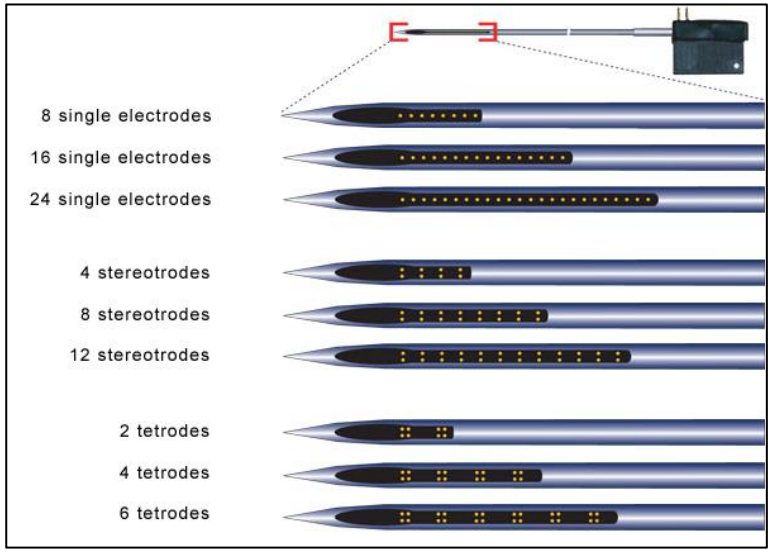
Single microelectrode



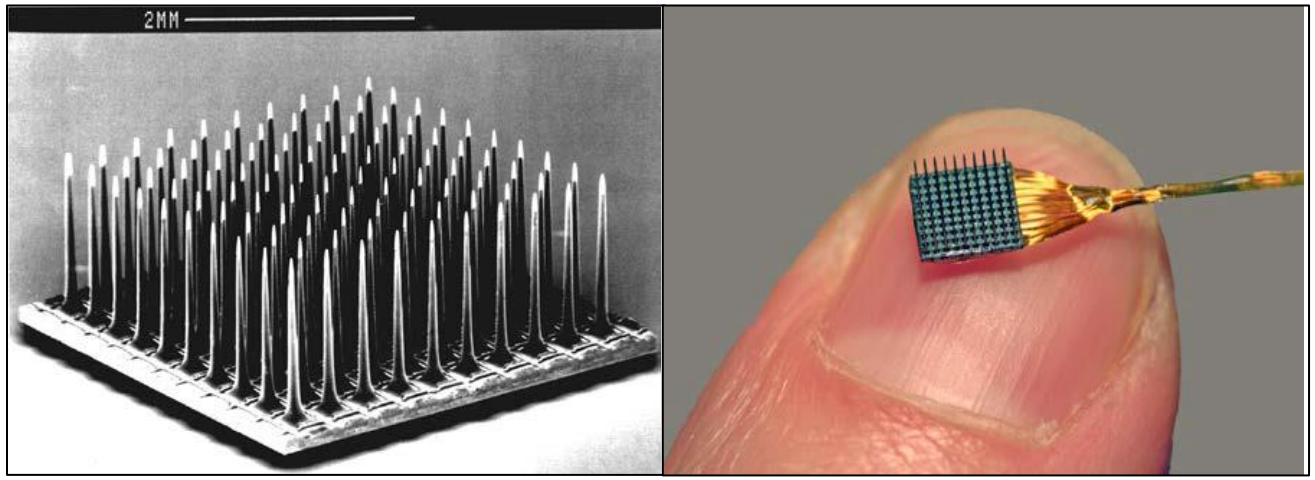
Tetrode



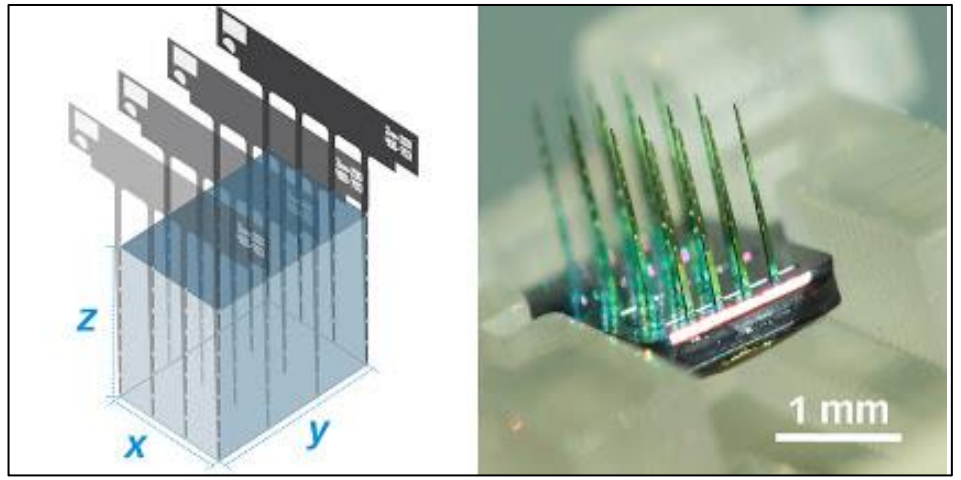
Linear electrode array



2D matrix electrode array



3D matrix electrode array



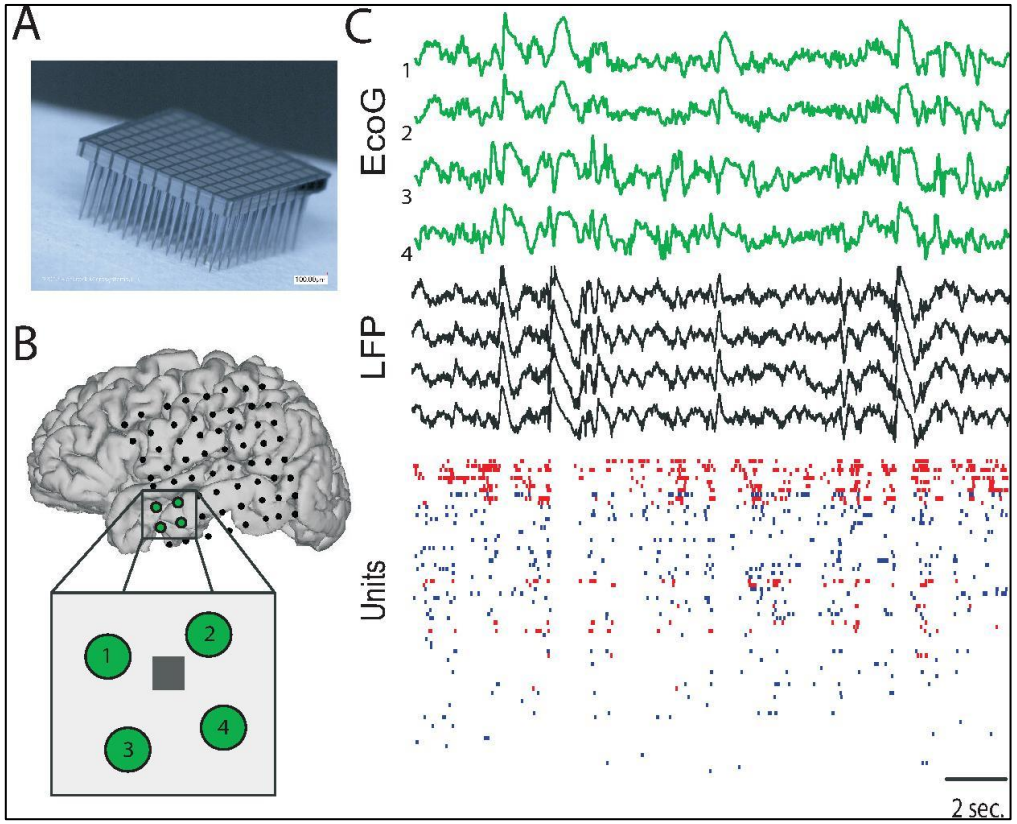
Electrophysiology: LFP

ECoG (electro-corticogram)

- Recorded from the surface of the brain using large subdural electrodes

LFP (local field potential)

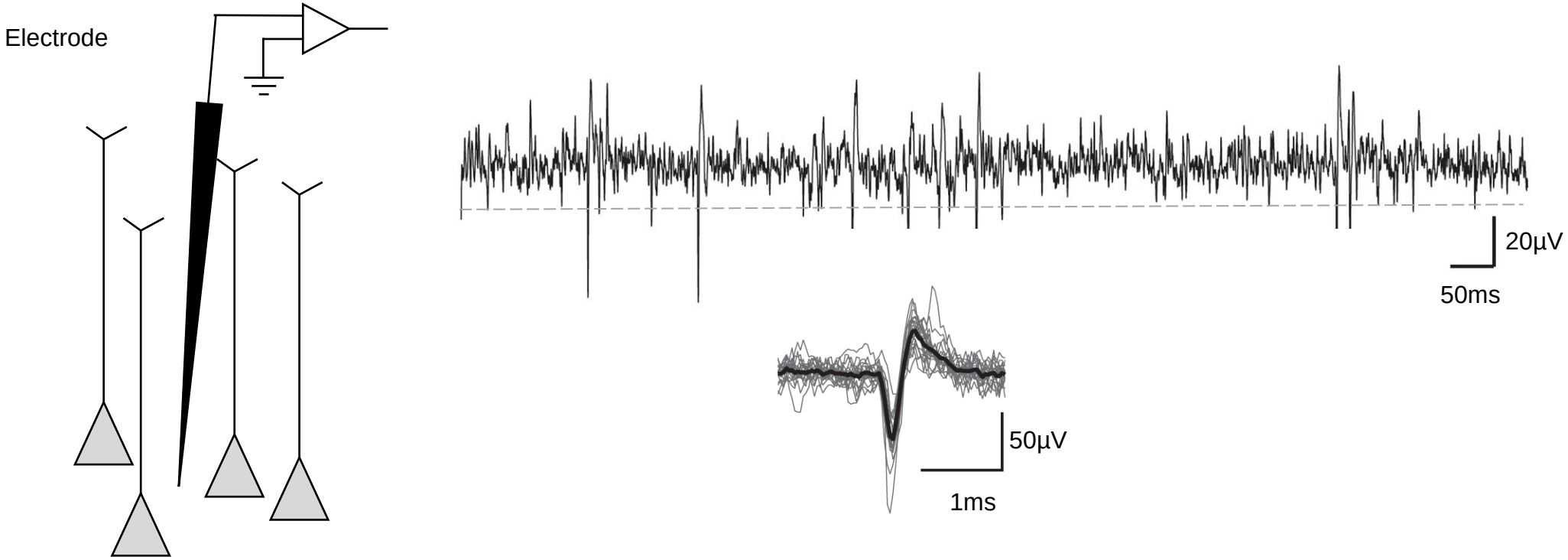
- Recorded in depth, from within the cortical tissue
- Electric potential in the extracellular space around neurons
- Current theory: LFPs are generated by synchronized synaptic currents arising on cortical neurons
- Filtered compound signal as electrode is separated from the sources by portions of cortical tissue
- Spatial resolution: 100s of microns



"Utah array" in human medial temporal cortex
Delta waves recorded during sleep

modified from Peyrache et al., 2012

Extracellular recording



Source separation (”spike sorting”)

Extracellular recording - Stereotrode

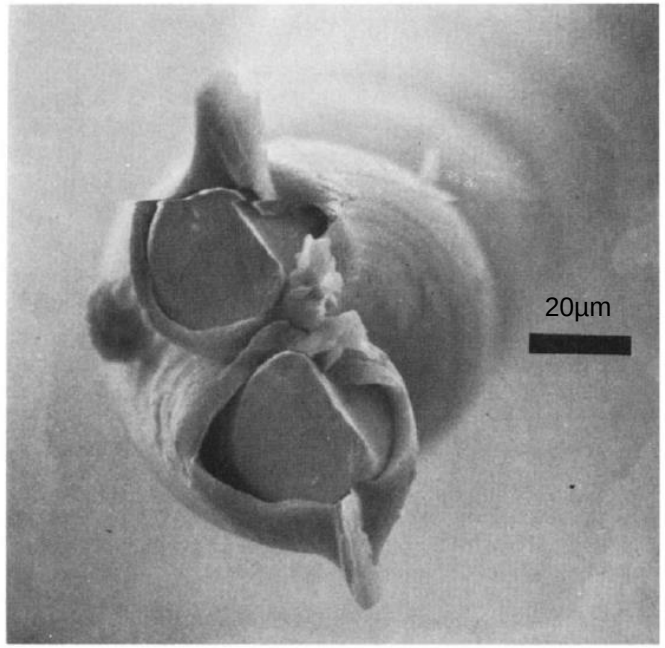
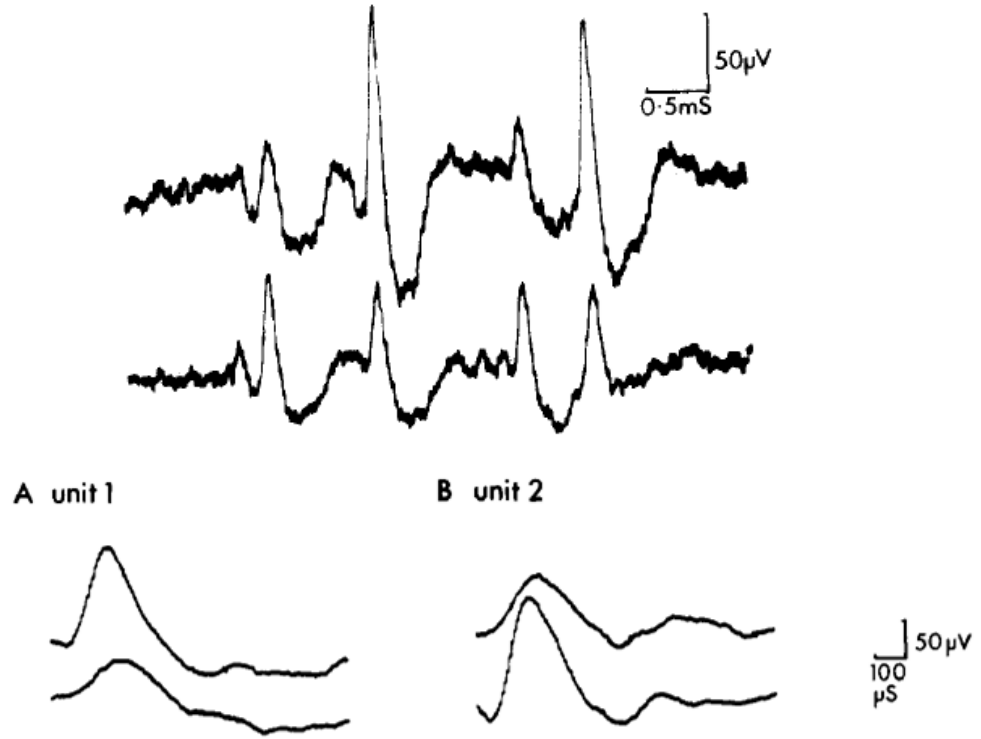
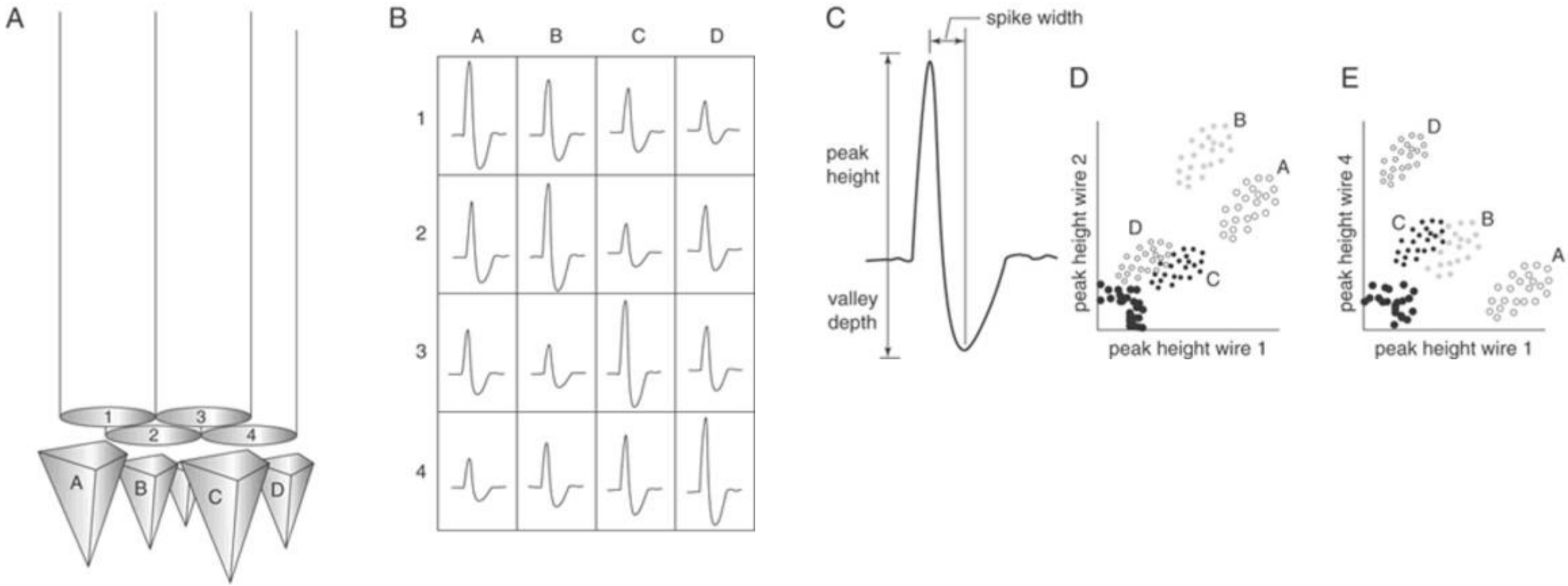


Fig. 1. Scanning electronmicrograph of a 'stereotrode' constructed by twisting together two lengths of 25 µm teflon-insulated wire, and cutting the ends with sharp scissors. Original magnification 240×. Calibration bar 20 µm.



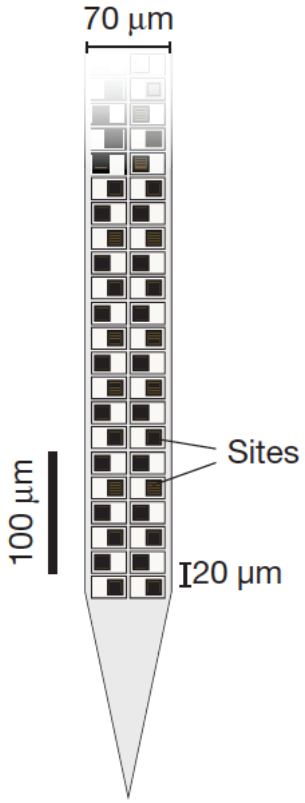
McNaughton, O'Keefe, Barnes (1983) *J Neuro Methods*

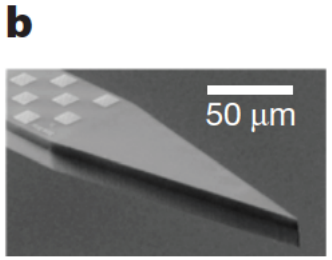
Extracellular recording - Tetrode

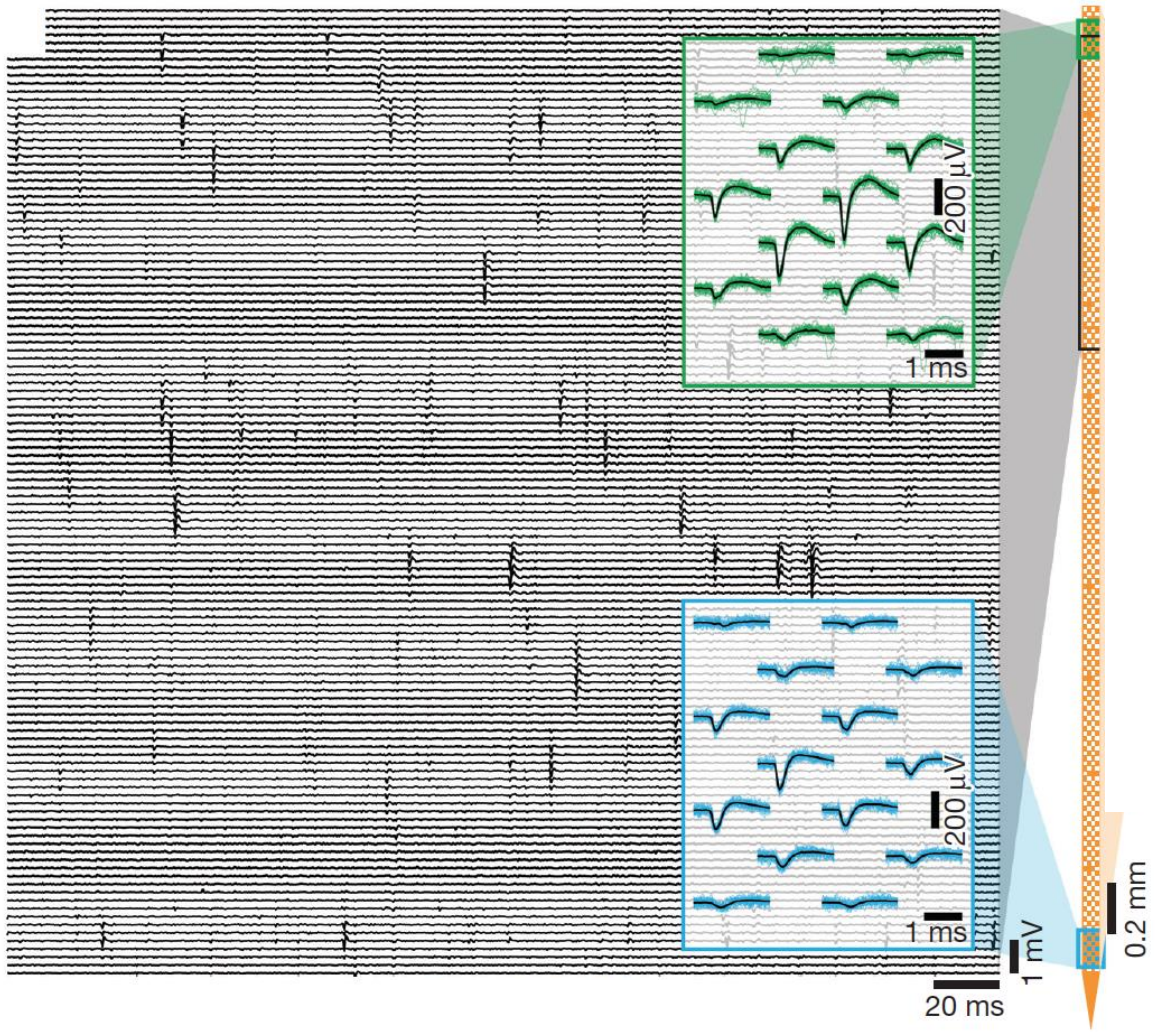


Nitz,D. (2003) *Current Protocols in Neuroscience*

Extracellular recording – Neuropixels probe

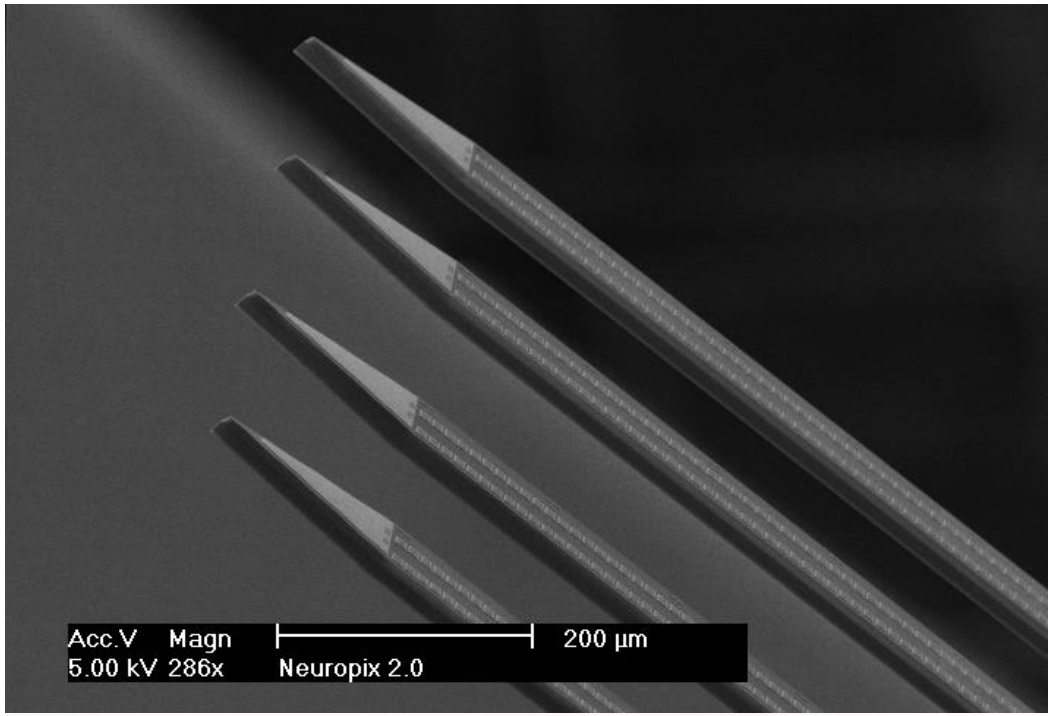


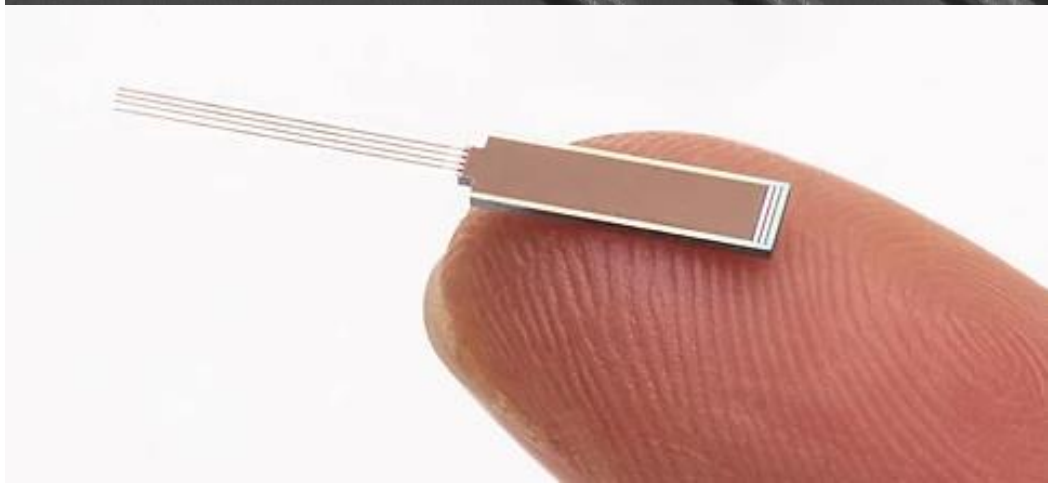




Jun, .., O'Keefe, T. Harris (2017) *Nature*

Extracellular recording – Neuropixels 2.0



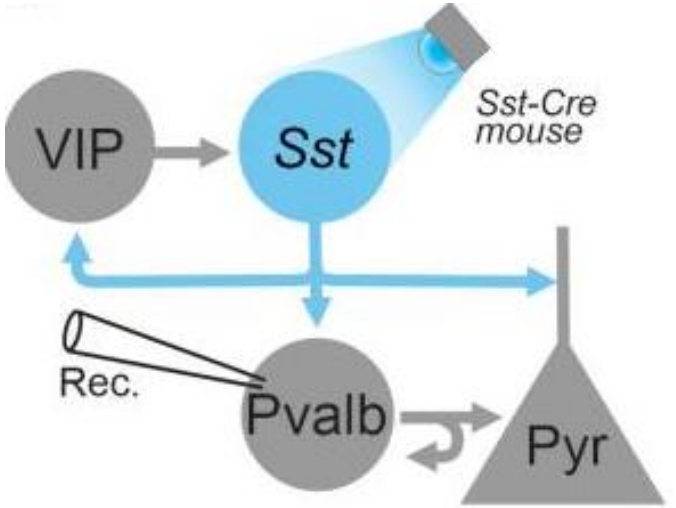


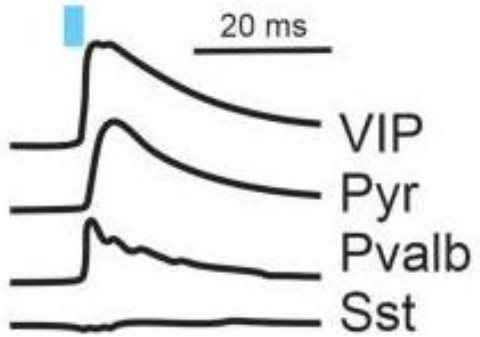
Extracellular recording – Neuropixels probe

Optogenetic tagging (“opto-tagging)

- Photostimulation-assisted identification of neuronal populations
- Combining electrophysiology with opsins targeted to defined subsets of neurons

Connectivity examination

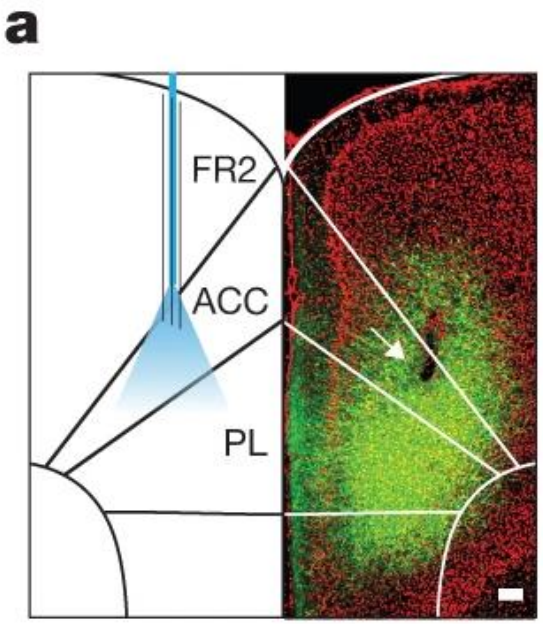




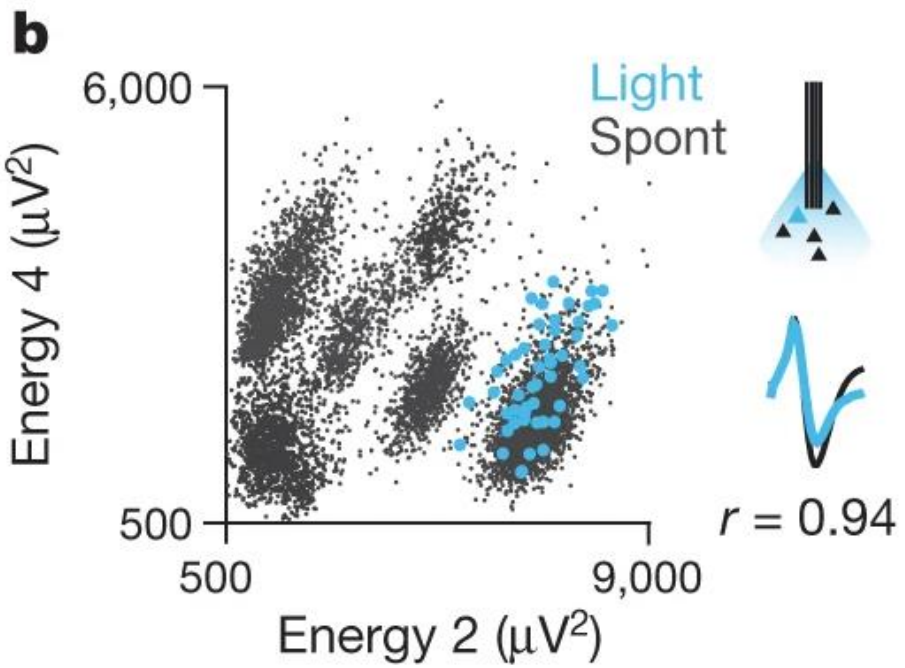
Lee et al (2020) *Front. Neur. Circ*

Optogenetic tagging ("opto-tagging)

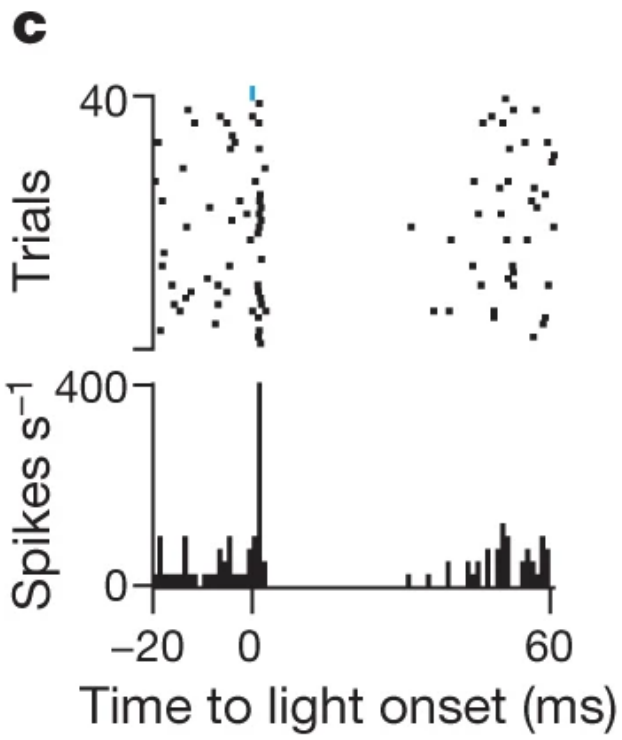
- Light-induced activation of genetically-defined interneurons in behaving mice



SOM-IRES-Cre mouse (green, ChR2; red, DAPI



Spikes plotted in waveform energy space from two tetrode channels

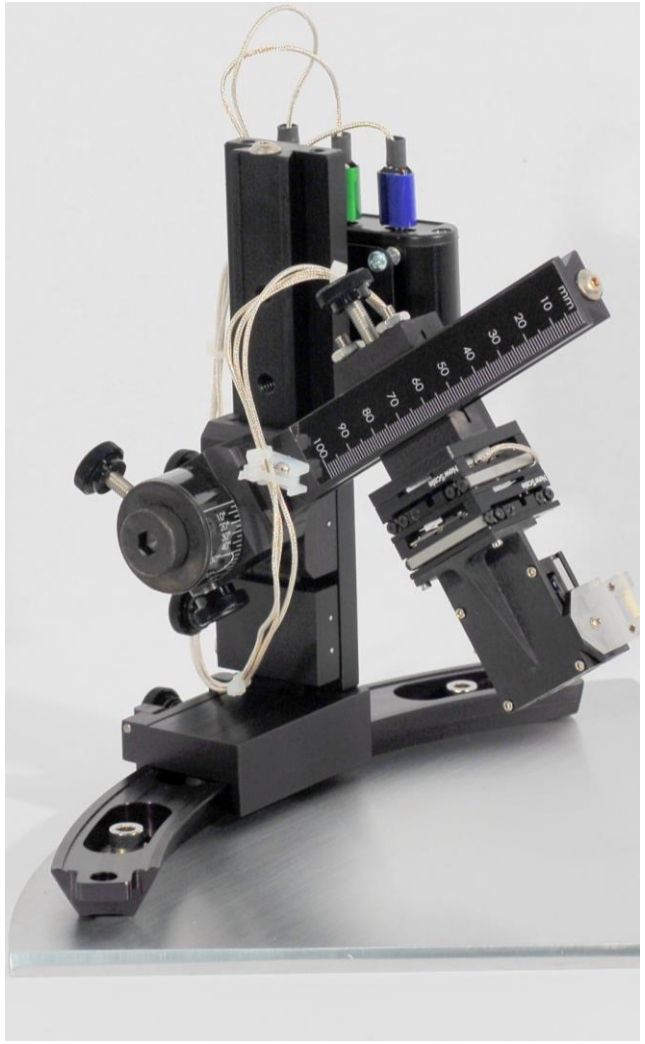


Spike raster (top) and peri-stimulus time histogram (PSTH) (bottom) for the light-activated cell in **b**

Kvitsiani et al (2012) *Nature*

Setup configuration

Micro manipulator

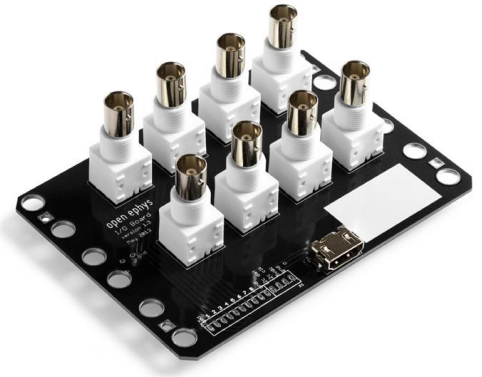


Probe

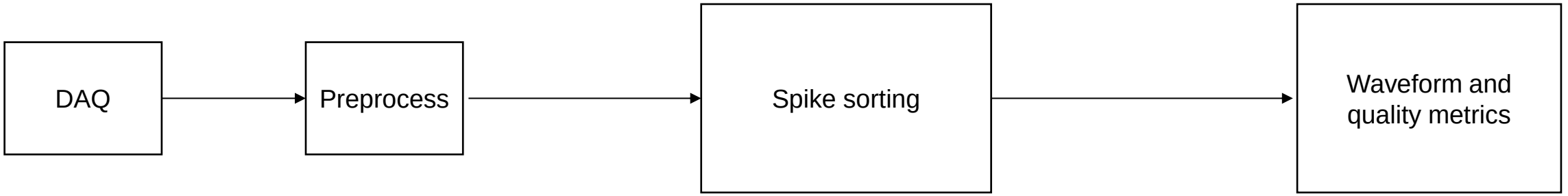
Head stage + adapter

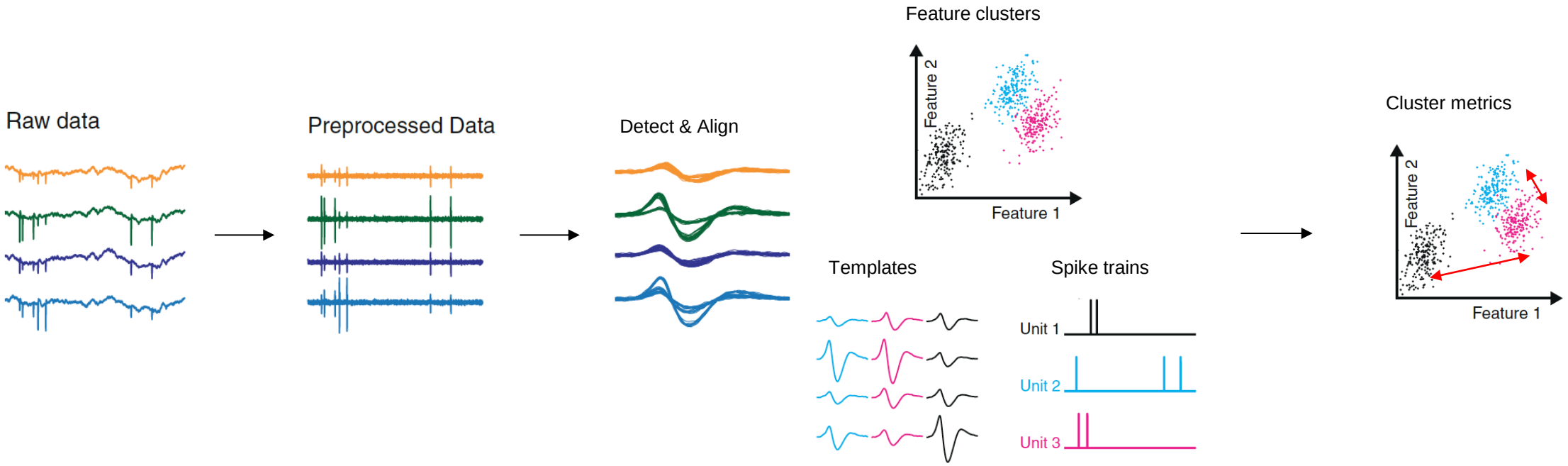
Acquisition board

External inputs



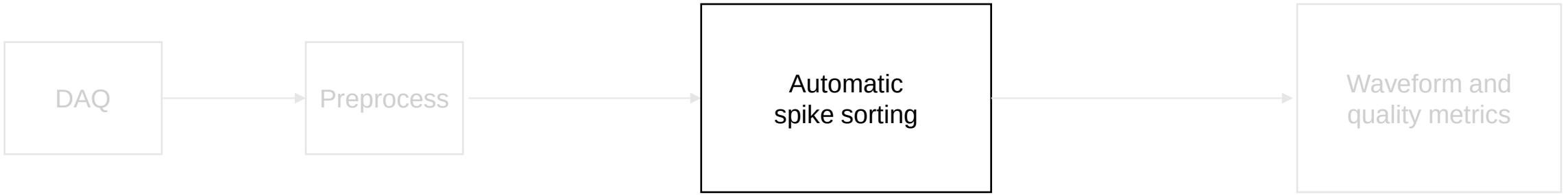
Spike sorting pipeline





Einevoll, .., K. Harris (2012), *Curr. Opin. Neurobiol.*

Automatic sorters

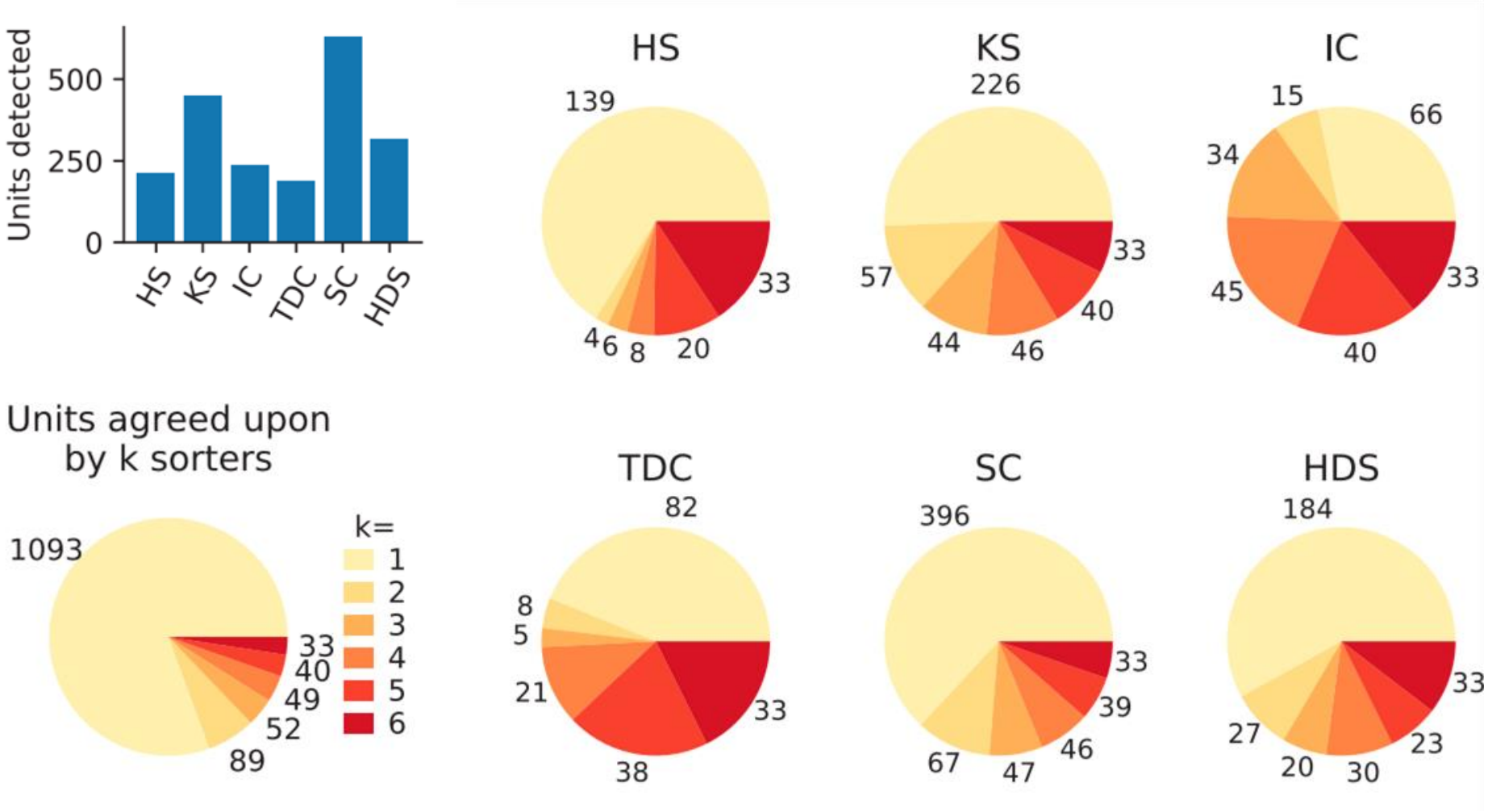


SpikeDetekt, KlustaKwik	(Rossant, .., K. Harris 2016 Nat Neuro)
Kilosort, (1,2,2.5,3,4)	(Pichatariu, .., K. Harris, 2016 NIPS)
MountainSort	(Chung, .., Greengard 2017 Neuron)
YASS	(Lee, .., Paninski 2017 NIPS)
SpyKING CIRCUS	(Yger, .., Marre 2018 eLife)
Herding Spikes, Herding Spikes 2	(Hilgen, .., Hennig 2018 Cell Reports)
JRCLUST, IronClust	(Jun, .., T. Harris, 2017 bioRxiv)
Tridesclous	(Garcia, Pouzat)
WaveClus	(Chaure, .., Quian Quiroga 2018 J Neurophys)

Different spike sorters – different results

- Spike sorting outputs are variable, i.e. not perfect

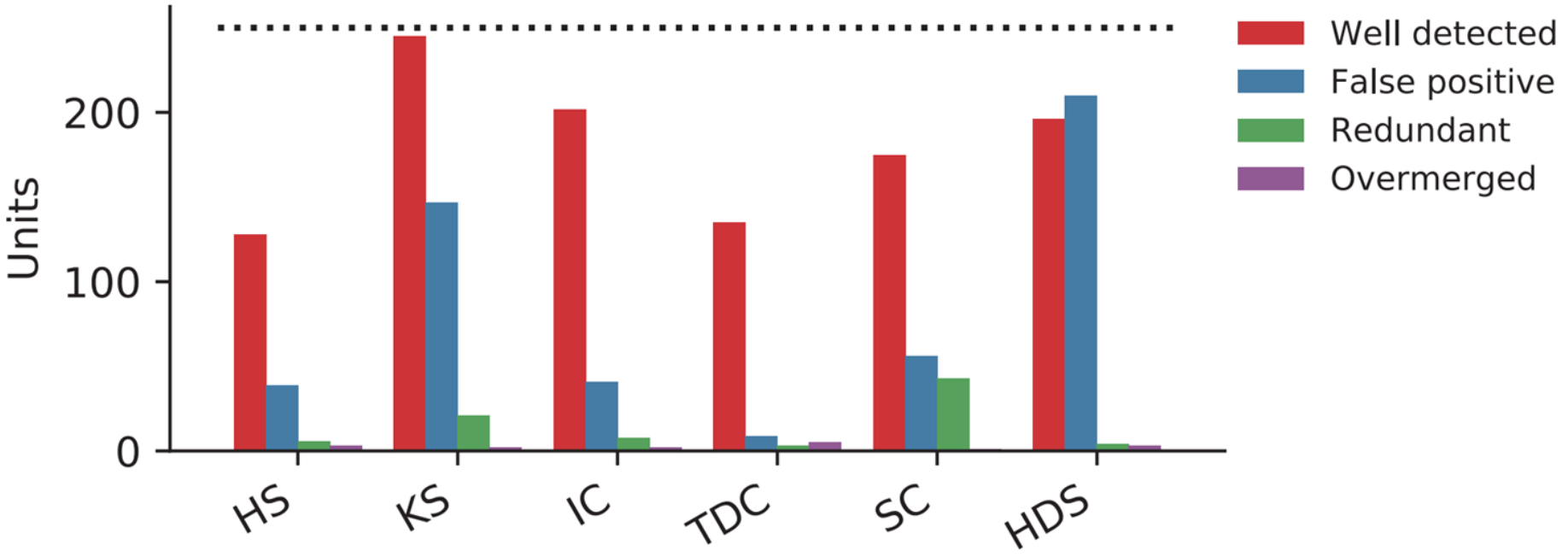
Different spike sorters – different results



HS = HerdingSpikes2
KS = Kilosort2
IC = IronClust
TDC = Tridesclous
SC = SpyKING Circus
HDS = HDSort

SpikeInterface (Buccino 2020, eLife)

Different spike sorters – different results



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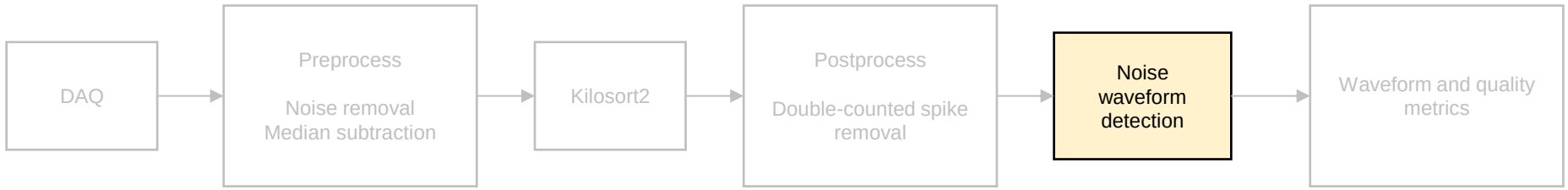
- Spike sorting outputs are variable, i.e. not perfect

→ Curation and experimenter judgement are important!

SpikeInterface (Buccino 2020, eLife)

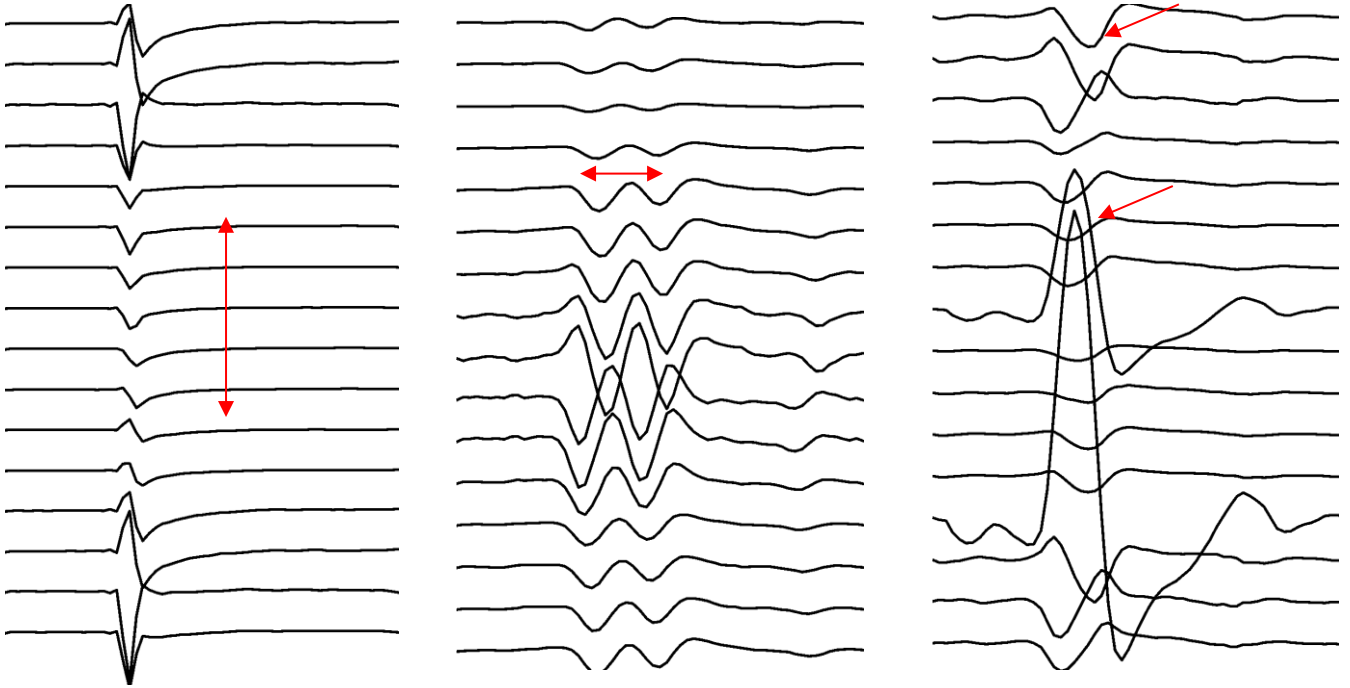
Allen Institute spike sorting pipeline

1. Preprocessing
2. Kilosort2
3. Postprocessing
4. Noise cluster detection
5. Waveform metrics
6. Quality metrics



Separation of clusters “noise” clusters:

1. Spatial spread of waveform peak
2. Waveform shape
3. Multiple spatial peaks



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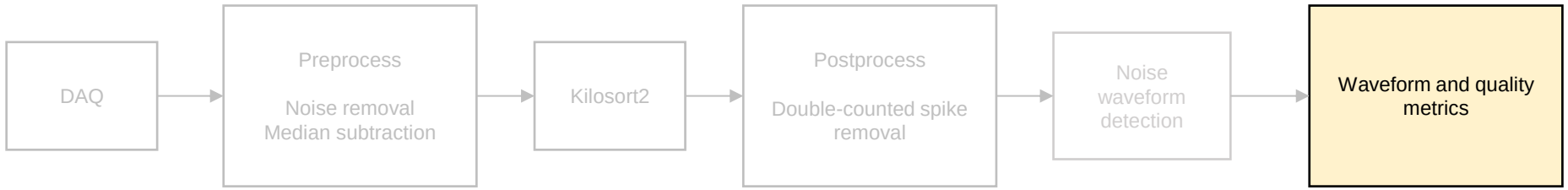
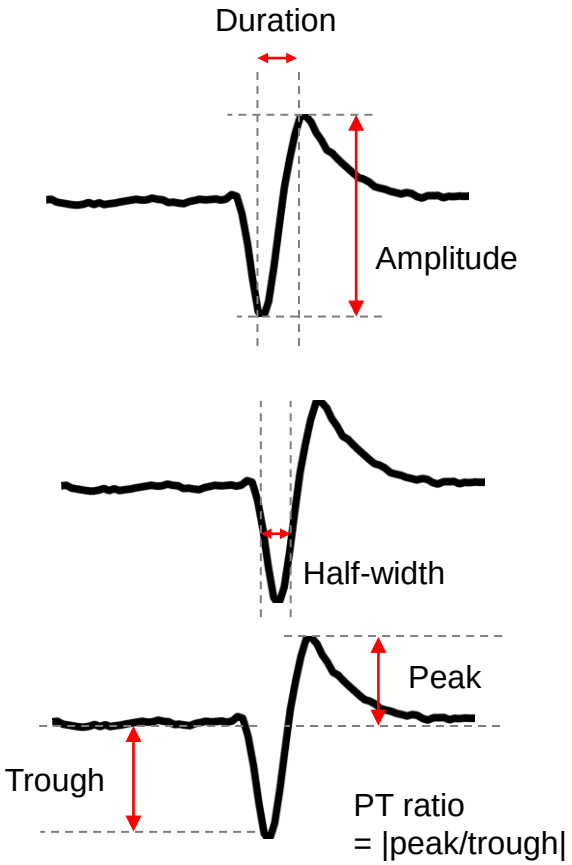
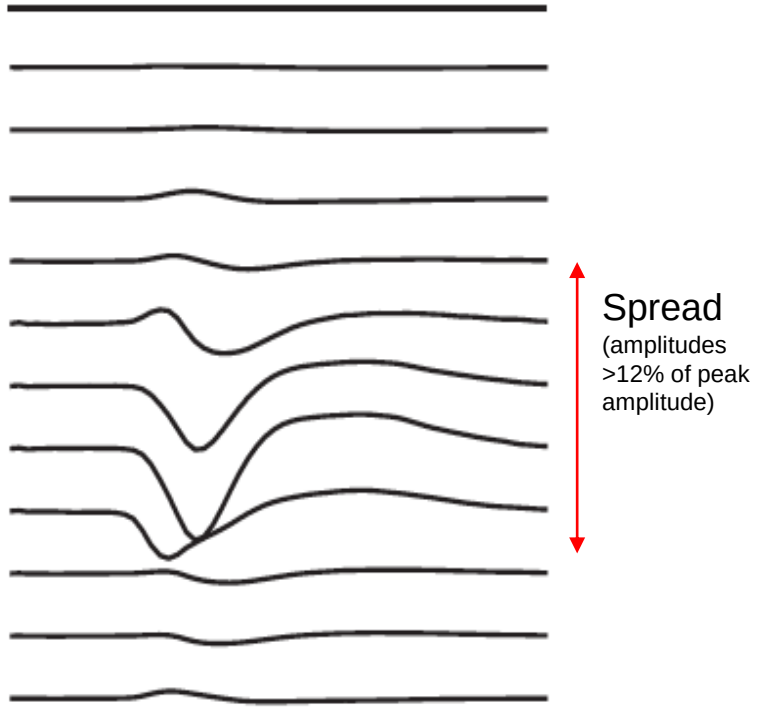


Waveform metrics:

Amplitude
Peak channel
Duration
Halfwidth
Spread (channels)
Peak-trough ratio





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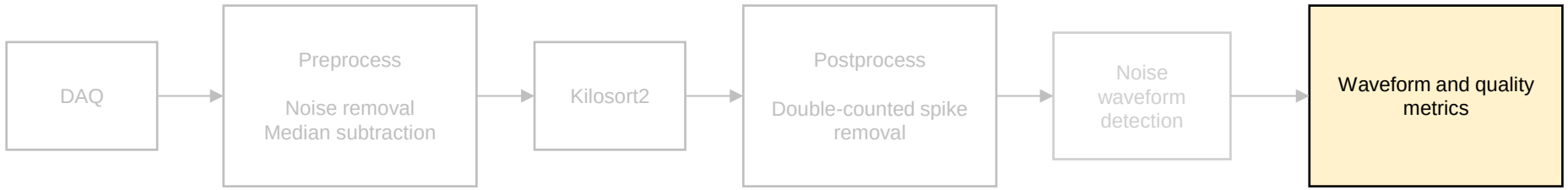
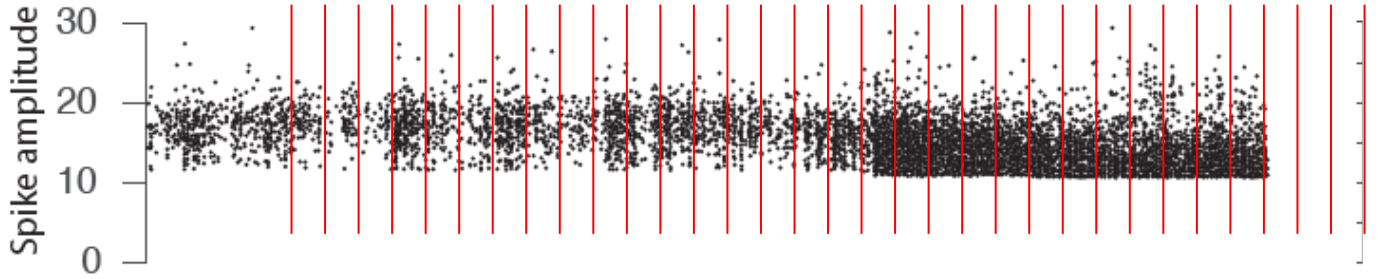


Quality metrics:

Presence ratio
Amplitude cutoff
ISI violations
Maximum drift
Signal-to-noise ratio
Isolation distance
L-ratio
d-prime
Nearest neighbours hit/miss rate
(Firing rate)

Presence ratio



Divide recording into 100 bins
Fraction of bins in which spike occurs

Allen Institute spike sorting pipeline

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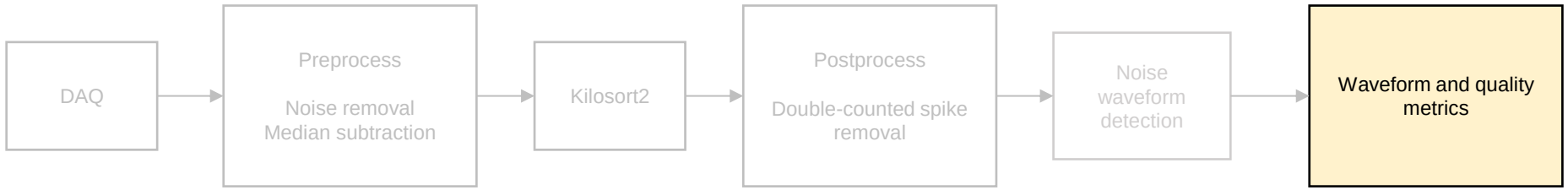

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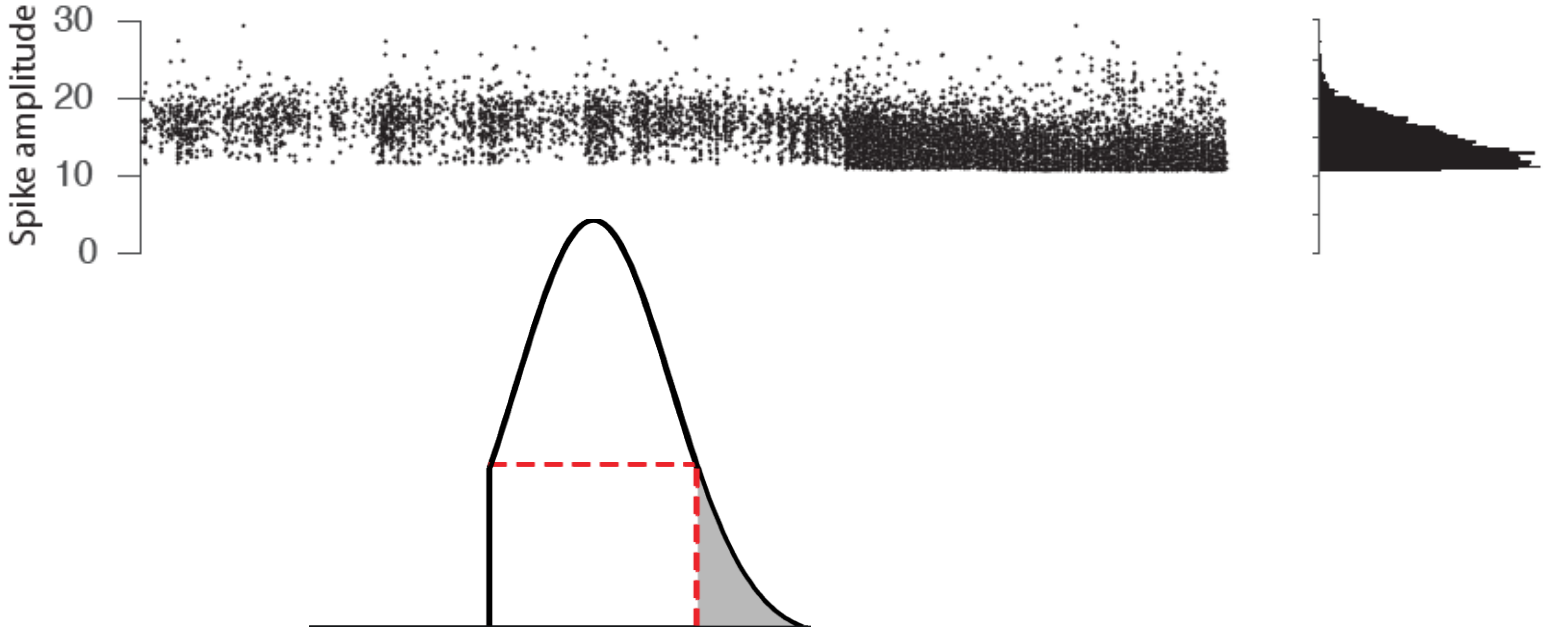
Amplitude cutoff



Allen Institute spike sorting pipeline

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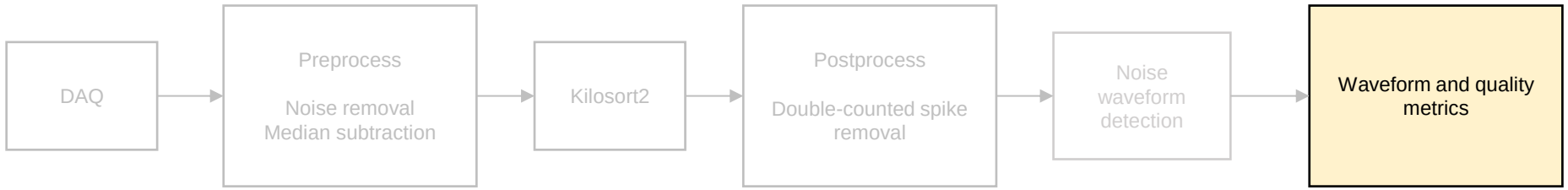

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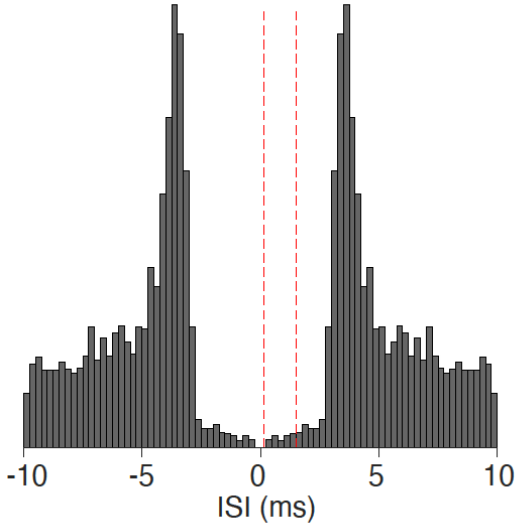
ISI violations

"Rate of contaminating spikes as a fraction of overall rate"



$$\frac{N_{contaminated}}{2(\tau_R - \tau_C) * N_{total} * F}$$

Hill, .., Kleinfeld (2011) *J Neurosci*

Allen Institute spike sorting pipeline

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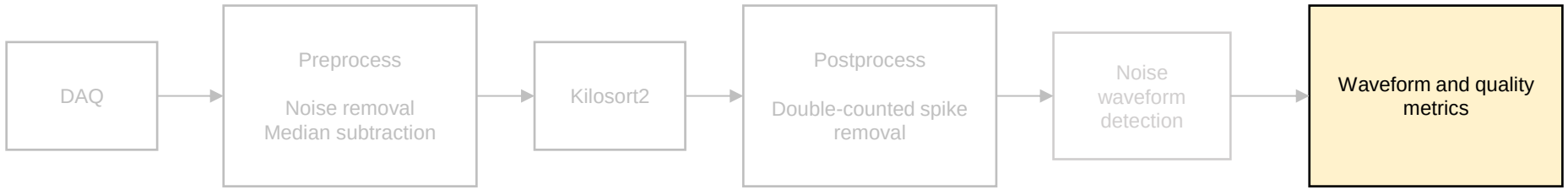

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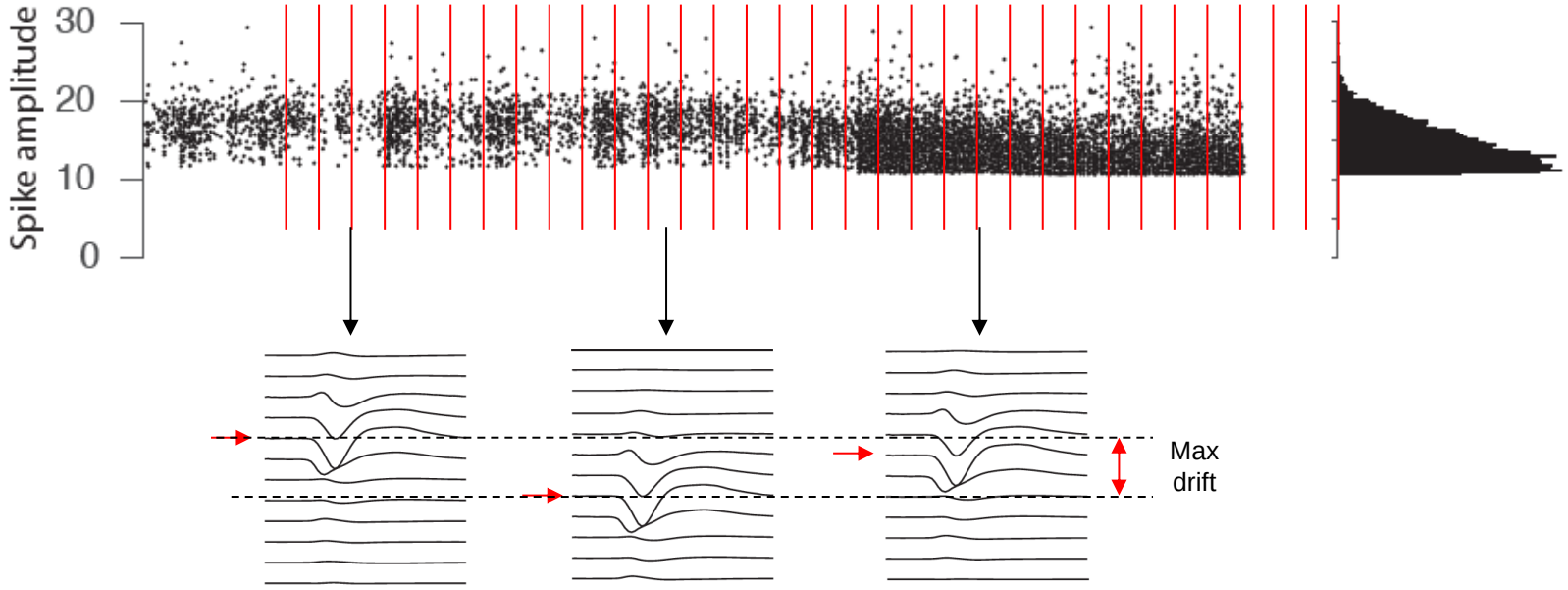
Maximum drift



Average max drift across all clusters
if > 80 um, discard whole recording

Allen Institute spike sorting pipeline

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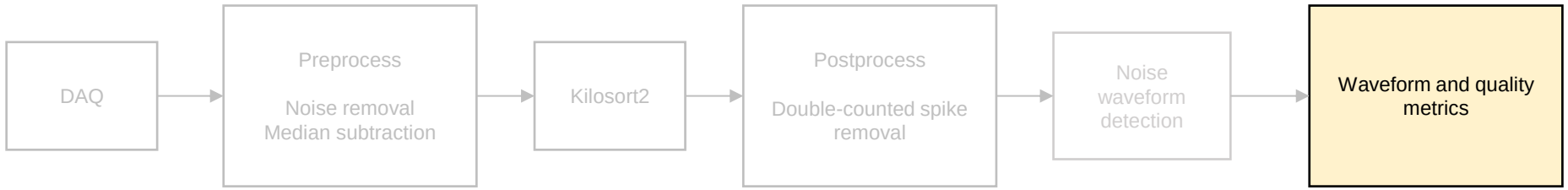

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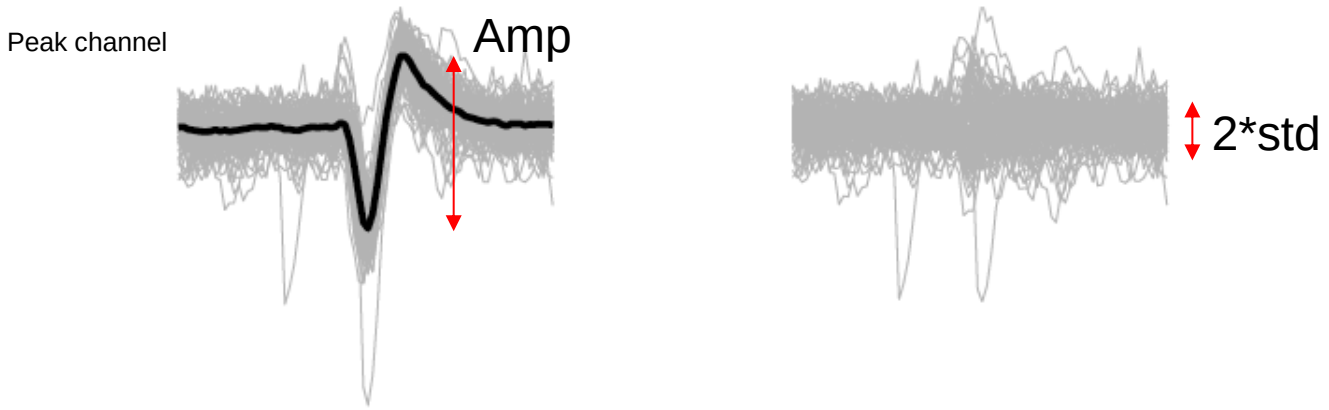
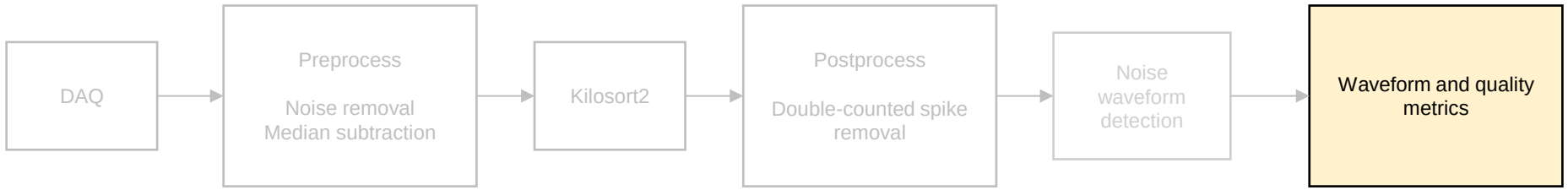
Signal-to-noise ratio



SNR = Amp / 2*std

Allen Institute spike sorting pipeline

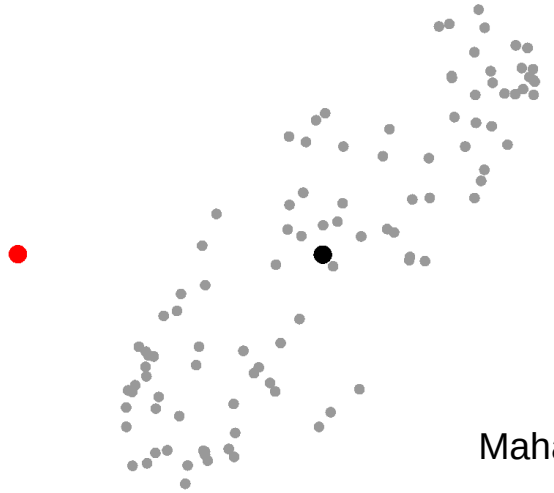
1. Preprocessing
2. Kilosort2
3. Postprocessing
4. Noise cluster detection
5. Waveform metrics
6. Quality metrics



Quality metrics:

- Presence ratio
- Amplitude cutoff
- ISI violations
- Maximum drift
- Signal-to-noise ratio
- Isolation distance
- L-ratio
- d-prime
- Nearest neighbours hit/miss rate
- (Firing rate)

Isolation distance



Mahalanobis distance

Distance of point from a distribution

Schmitzer-Torbert, .., Redish (2005) *Neurosci.*

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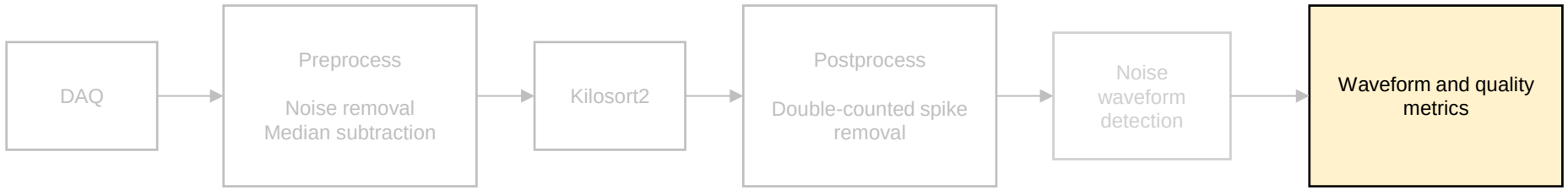

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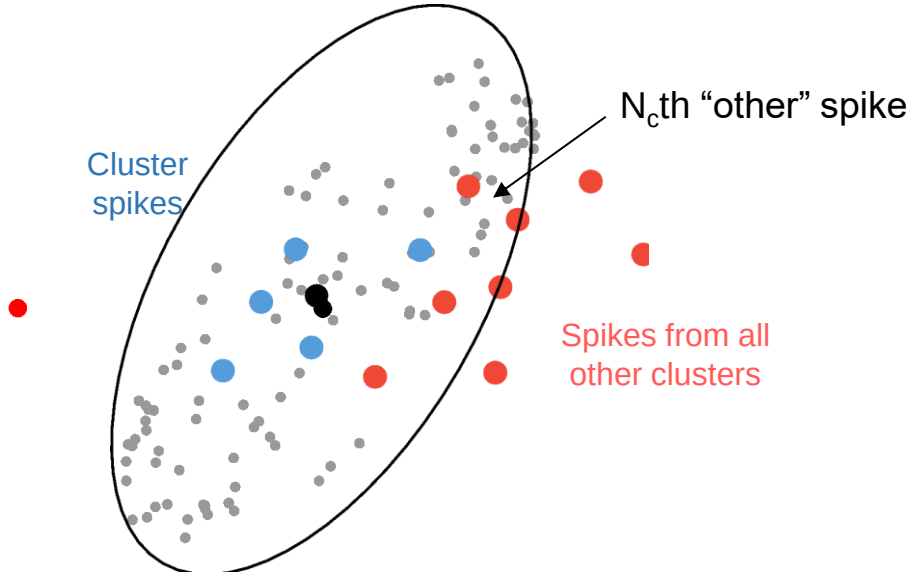

N_c = # spikes in cluster

Isolation distance = Mahalanobis distance of N_cth spike not in cluster

Schmitzer-Torbert, .., Redish (2005) *Neurosci.*

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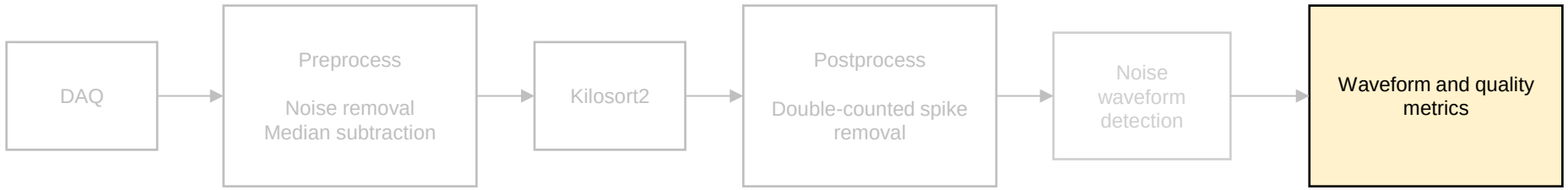

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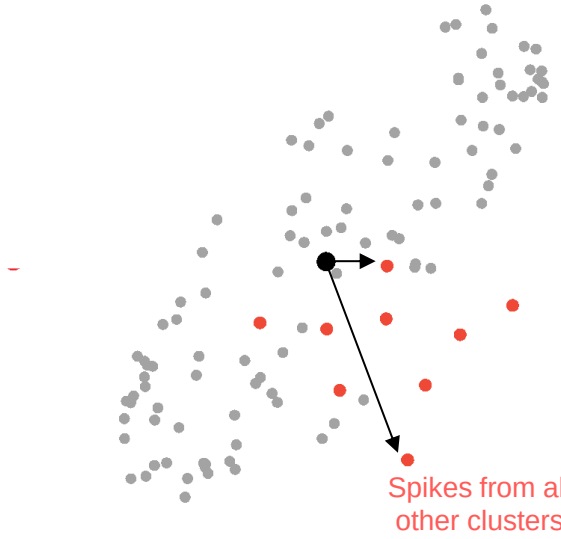
L-ratio



$$L_{ratio} = \frac{\sum_{i \notin C}(1 - CDF_{\chi^2_{df}}(D^2_{i,C}))}{N_C}$$

$1 - CDF_{\chi^2_{df}}(D^2_{i,C})$ high

"In any report including these cluster quality measures, it is important to clearly describe how features were calculated, in order that results from different labs can be more directly compared."

Schmitzer-Torbert, .., Redish (2005) *Neurosci.*

Allen Institute spike sorting pipeline

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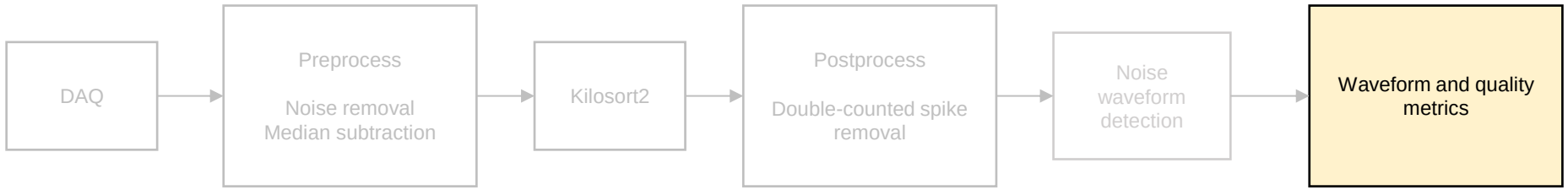

d-prime

Quality metrics:

Presence ratio
Amplitude cutoff
ISI violations
Maximum drift
Signal-to-noise ratio
Isolation distance
L-ratio
d-prime
Nearest neighbours hit/miss rate
(Firing rate)

Linear discriminant analysis to project cluster spikes and noise spikes onto one dimension.

Then:

$$d' = \frac{(\mu_C - \mu_O)}{\sqrt{\frac{1}{2}(\sigma_C^2 + \sigma_O^2)}}$$

Allen Institute spike sorting pipeline

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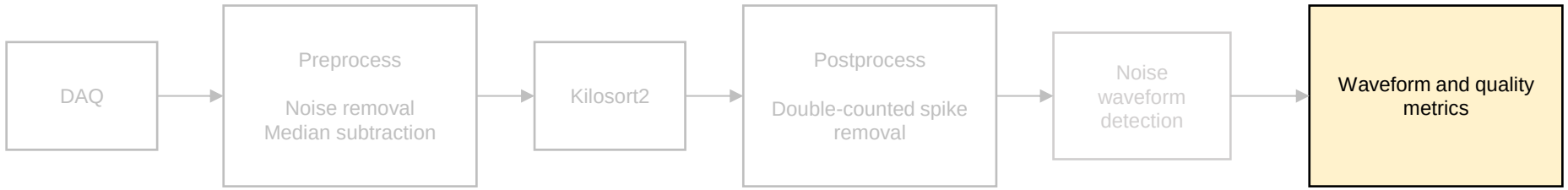
6. Quality metrics



Quality metrics:

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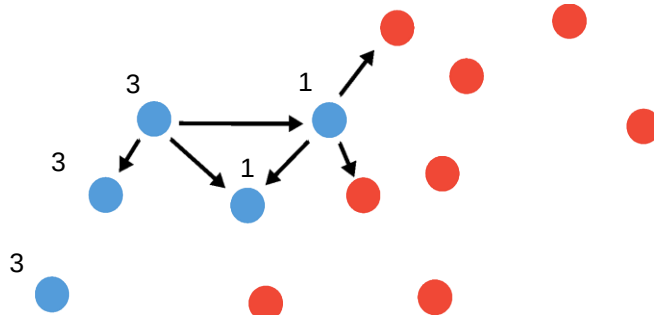
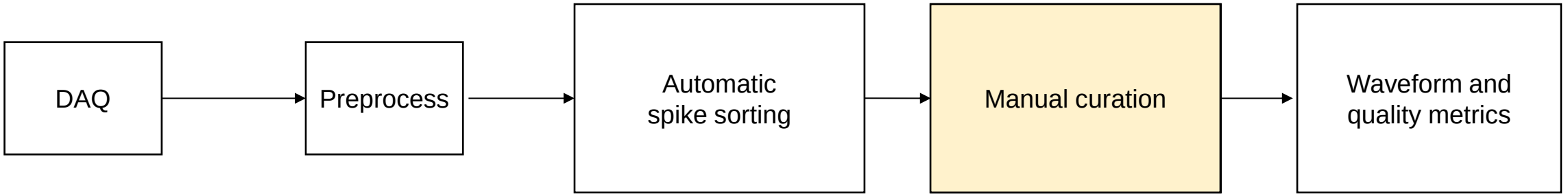
Nearest neighbours

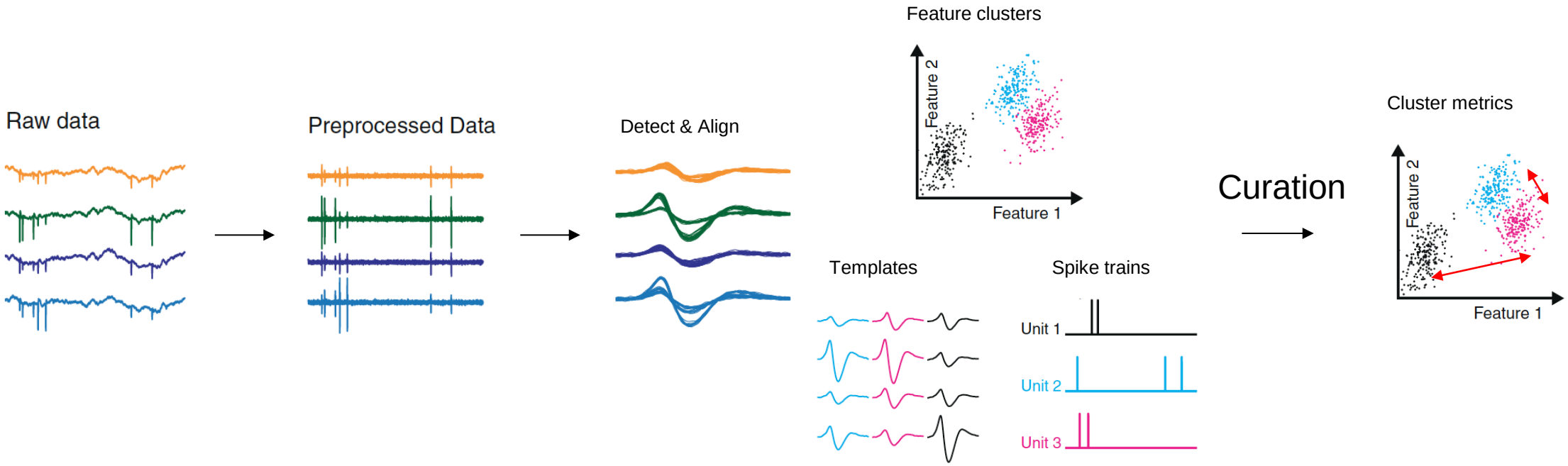


Hit rate = 11/15 = 0.73

Chung, .., Greengard (2017) *Neuron*

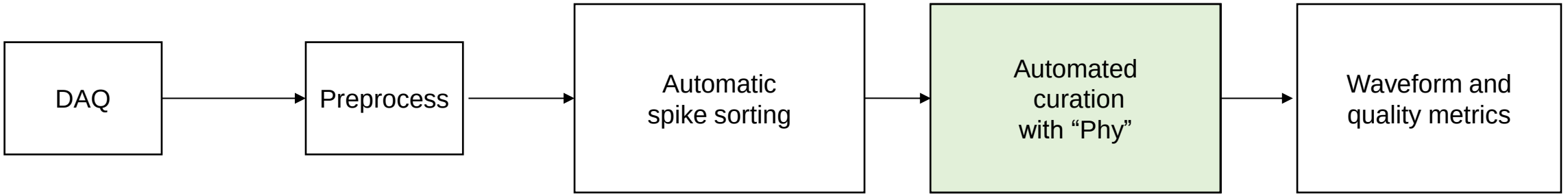
Curation

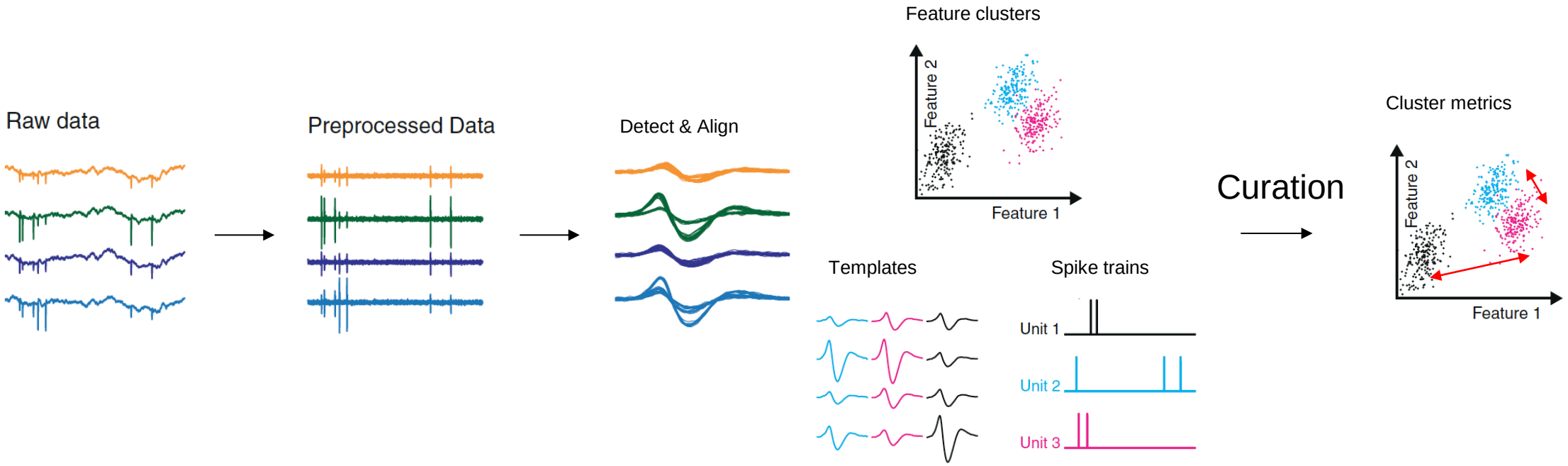




Einevoll, .., K. Harris (2012), *Curr. Opin. Neurobiol.*

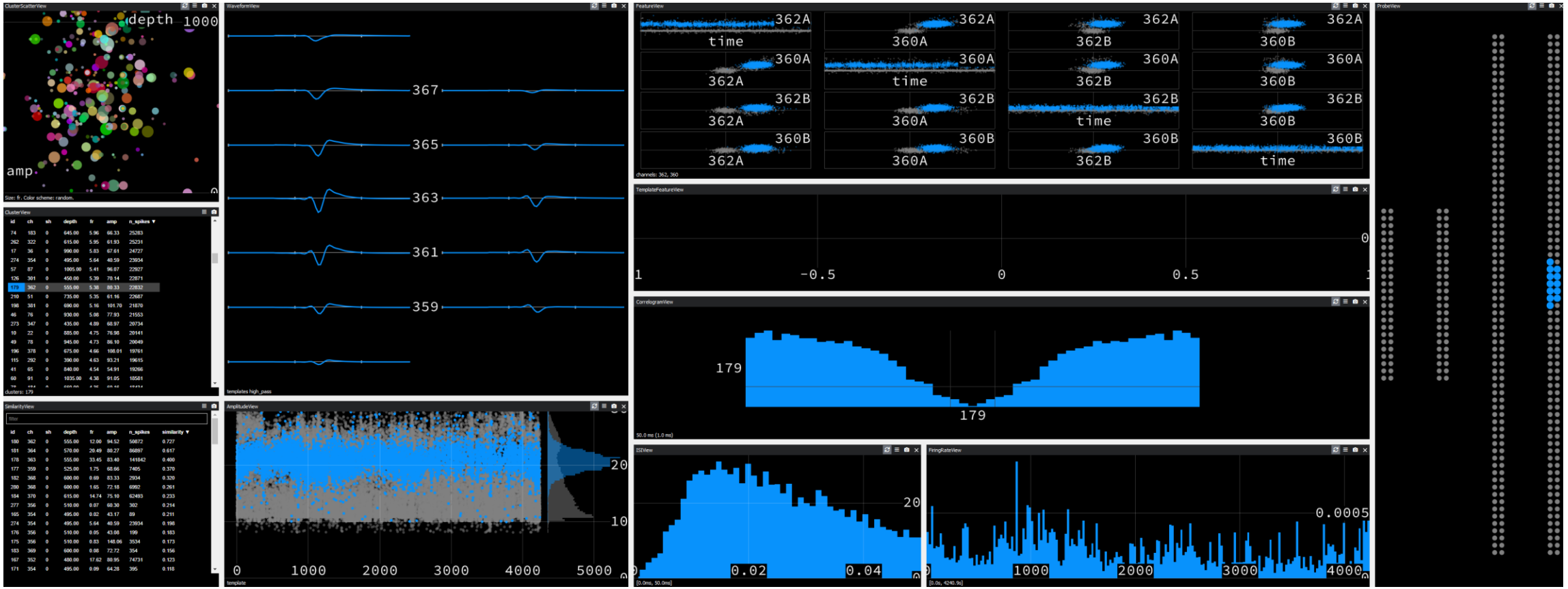
Curation





Einevoll, .., K. Harris (2012), *Curr. Opin. Neurobiol.*

Phy – brief demonstration



https://github.com/cortex-lab/phy/

Summary

- Different extracellular signals (ranging from EEG to single units)
- Rapid development of recording probes (high-density electrodes)
- Recordings can be combined with behaviour
- Target cells can be genetically defined
- Spike sorting outputs are **variable** and **need curation**

Resources

- Repository for practical part
  https://github.com/ackels-lab/BIGS-ephys2024
- Data resource by the Allen Institute
  https://allensdk.readthedocs.io/en/latest/visual_behavior_neuropixels.html
- Phy (Automated curation)
  https://phy.readthedocs.io/
- Lecture material
  https://software-skills.neuroinformatics.dev/courses/extracellular-analysis.html