Gentle introduction to neural optogenetics data

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CONICET

OUTLINE

1) How is this data obtained?

   -technical aspects of virus-laser-opto-neural stuff

2) Where can I find it?

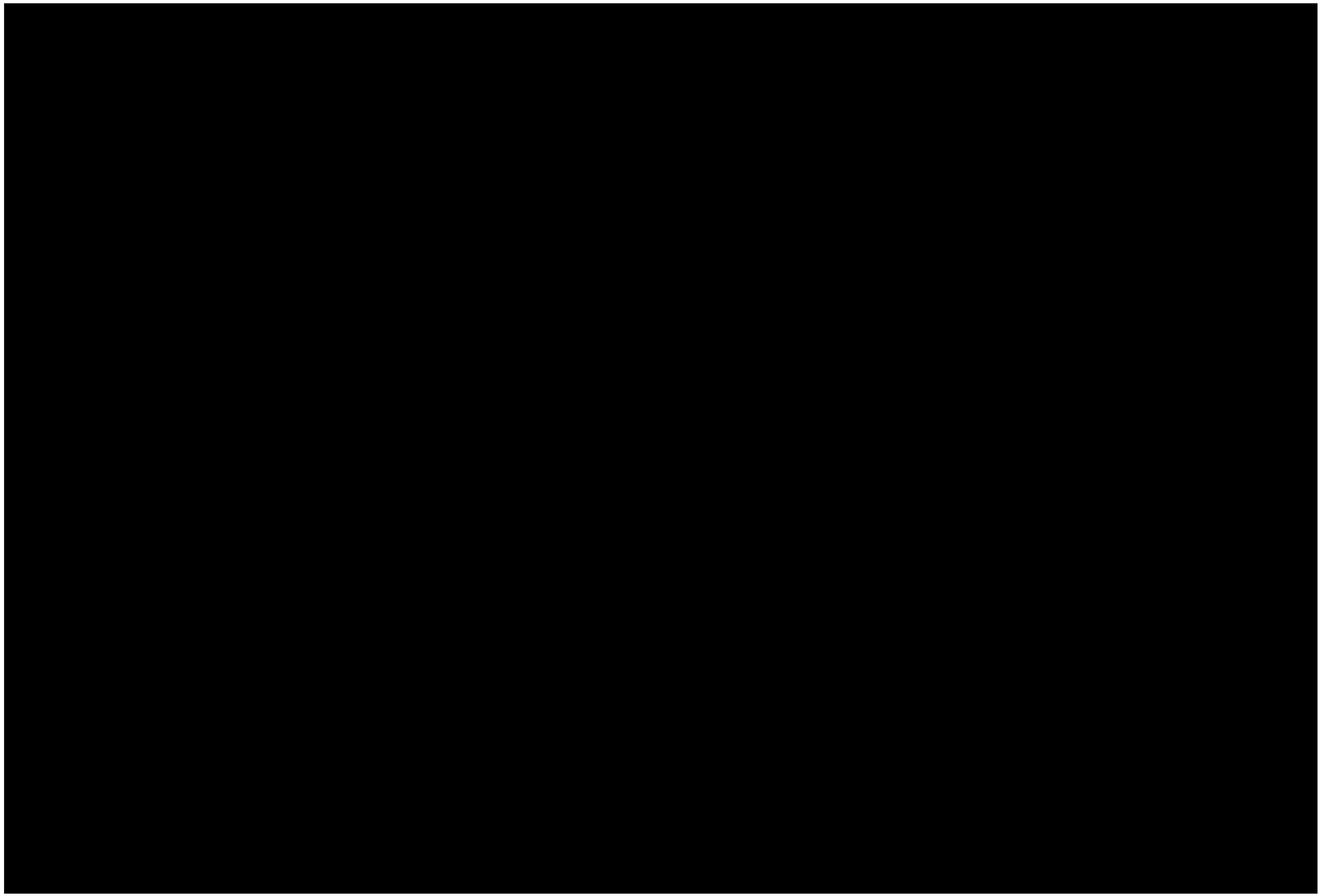
   -almost no need for new experiments!

3) Example of results from others

   -what is being published as hot results nowadays?

4) Preliminary results from our lab

Whole-brain functional imaging at single-neuron resolution on a zebrafish



Ahrens MB, Orger MB, Robson DN, Li JM, Keller PJ. Whole-brain functional imaging at cellular resolution using light-sheet microscopy. Nat Methods. 2013 May;10(5):413-20. doi: 10.1038/nmeth.2434. Epub 2013 Mar 18. PMID: 23524393.

So… what is Optogenetics?

Light is used to record activity and stimulate selectively genetically modified neurons

1) Neuron modification

Engineered viral vectors are injected into the brain.
These viruses carry the optogenetic actuator (what causes the neuron to be reactive to light stimulus) attached to a promoter (what causes the actuator to bind to certain cells).

Another way to do this is by creating transgenic mice, so the actuator can be introduced into mice zygotes.

2) Light stimulus

Using a LED or a DPSS (laser), by illuminating the body of the neurons, an action potential is caused.

Problem: Not all the cells may react at the same level, even if the illumination is even throughout the region.

3) optical recording

It is mostly done by calcium imaging (GCaMPs). When a neuron fires an action potential, its calcium levels spike. These neurons are genetically modified (binding them to a calcium indicator) to emit fluorescence when calciums levels spike and that can be measured via 2-photon microscopy

—> How is this data obtained

1) How is this data obtained?

Recording

Probes

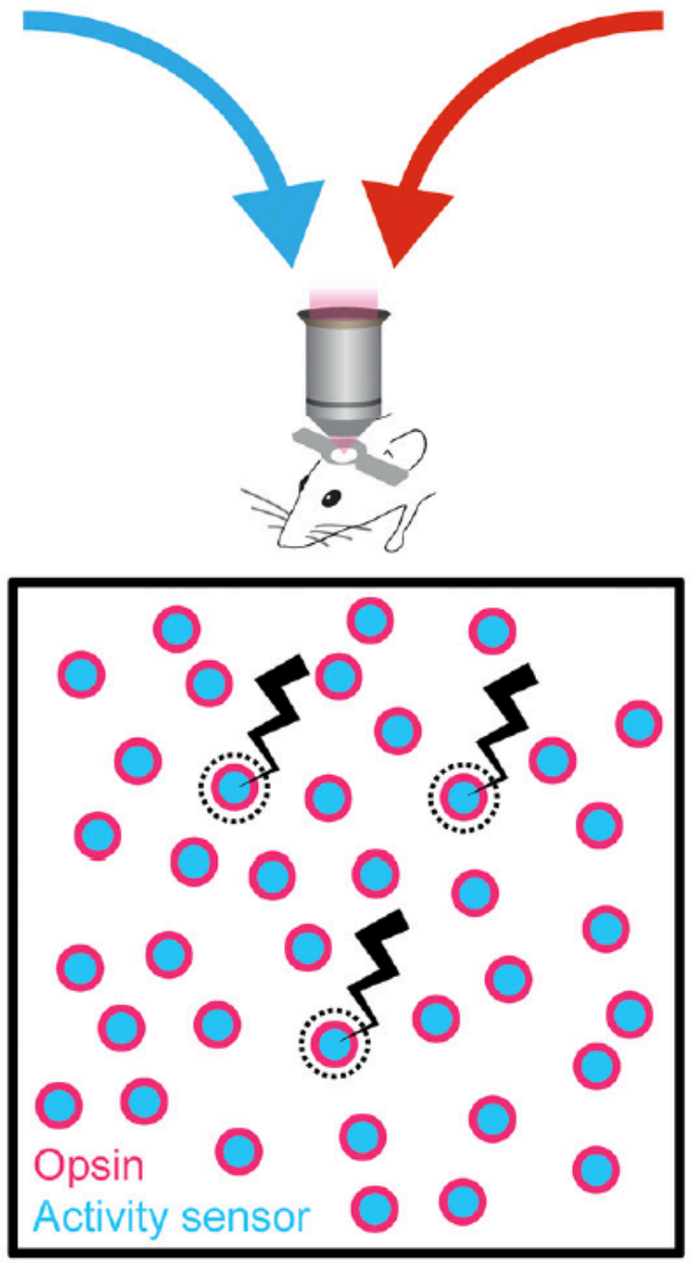
[Ca^{2+}]	GCaMP, Twitch, RCaMP2, YC
Voltage	Quasars, ASAP1, ArcLight

Expression

Electroporation
Virus
Transgenics

Optics

1P	fiberscope
2P	galvo resonant galvo SLM AOD



Manipulation

Probes

Activation	ChR2, ReaChR C1V1, Chronos, Chrimson
Inactivation	eNpHR, Arch, Jaws, ACRs

Expression

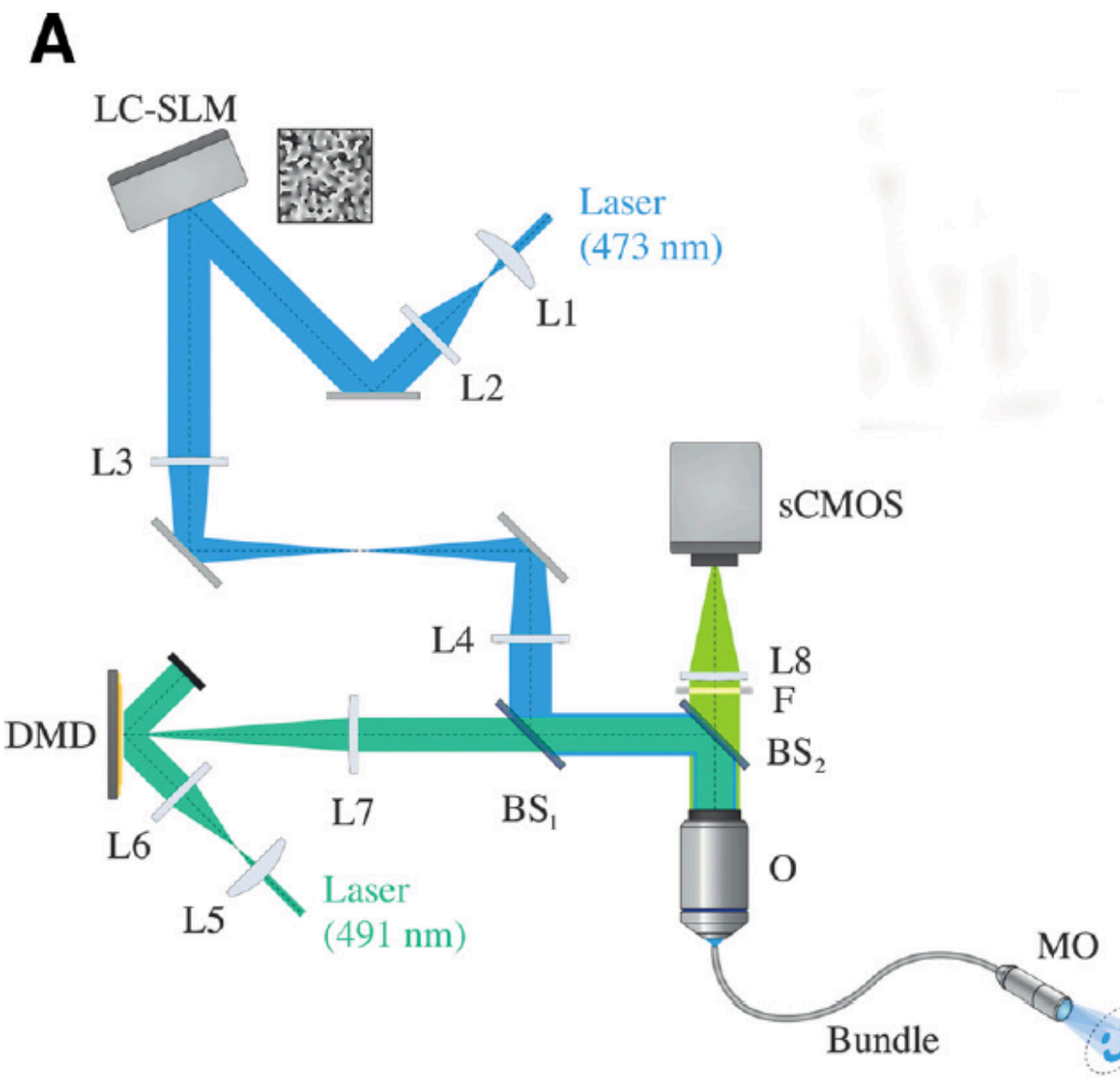
Electroporation
Virus
Transgenics

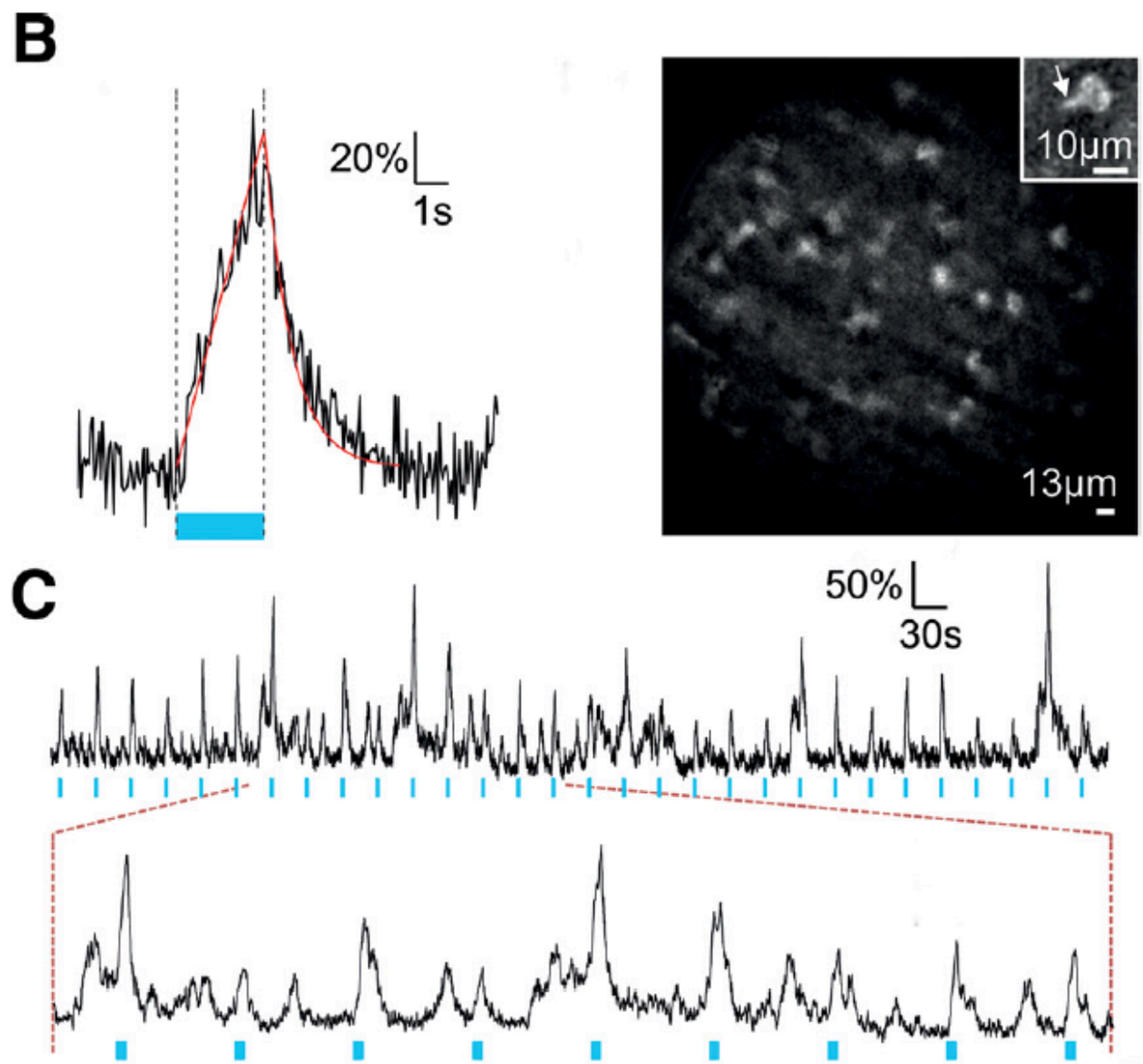
Optics

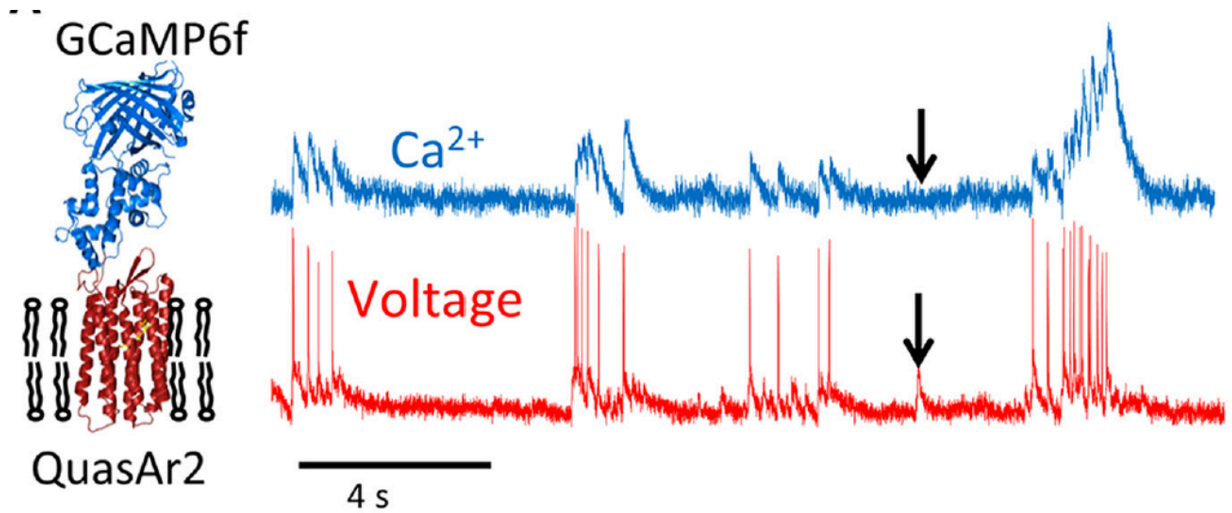
1P	fiberscope, DMD, SLM
2P	SLM + galvo TF AOD

Emiliani V, Cohen AE, Deisseroth K, Häusser M. All-Optical Interrogation of Neural Circuits. J Neurosci. 2015 Oct 14;35(41):13917-26. doi: 10.1523/JNEUROSCI.2916-15.2015. PMID: 26468193; PMCID: PMC4604230.

—> Experimental set up







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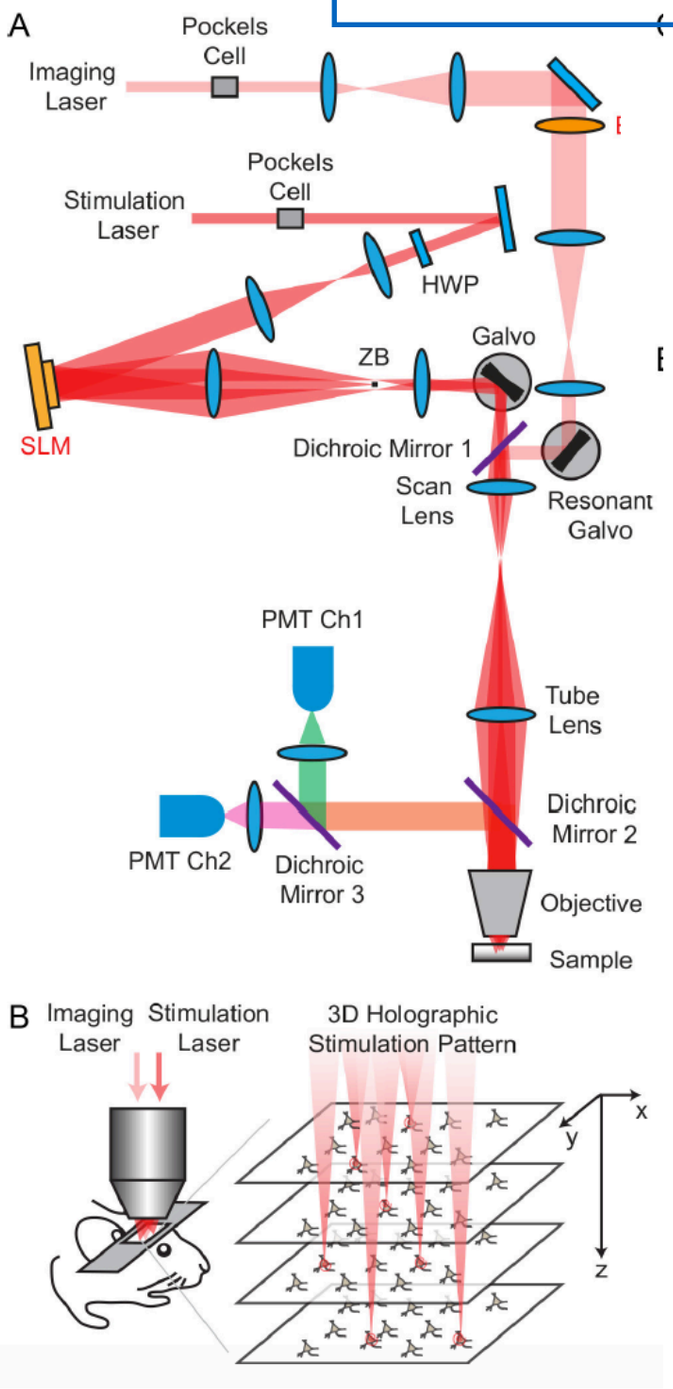
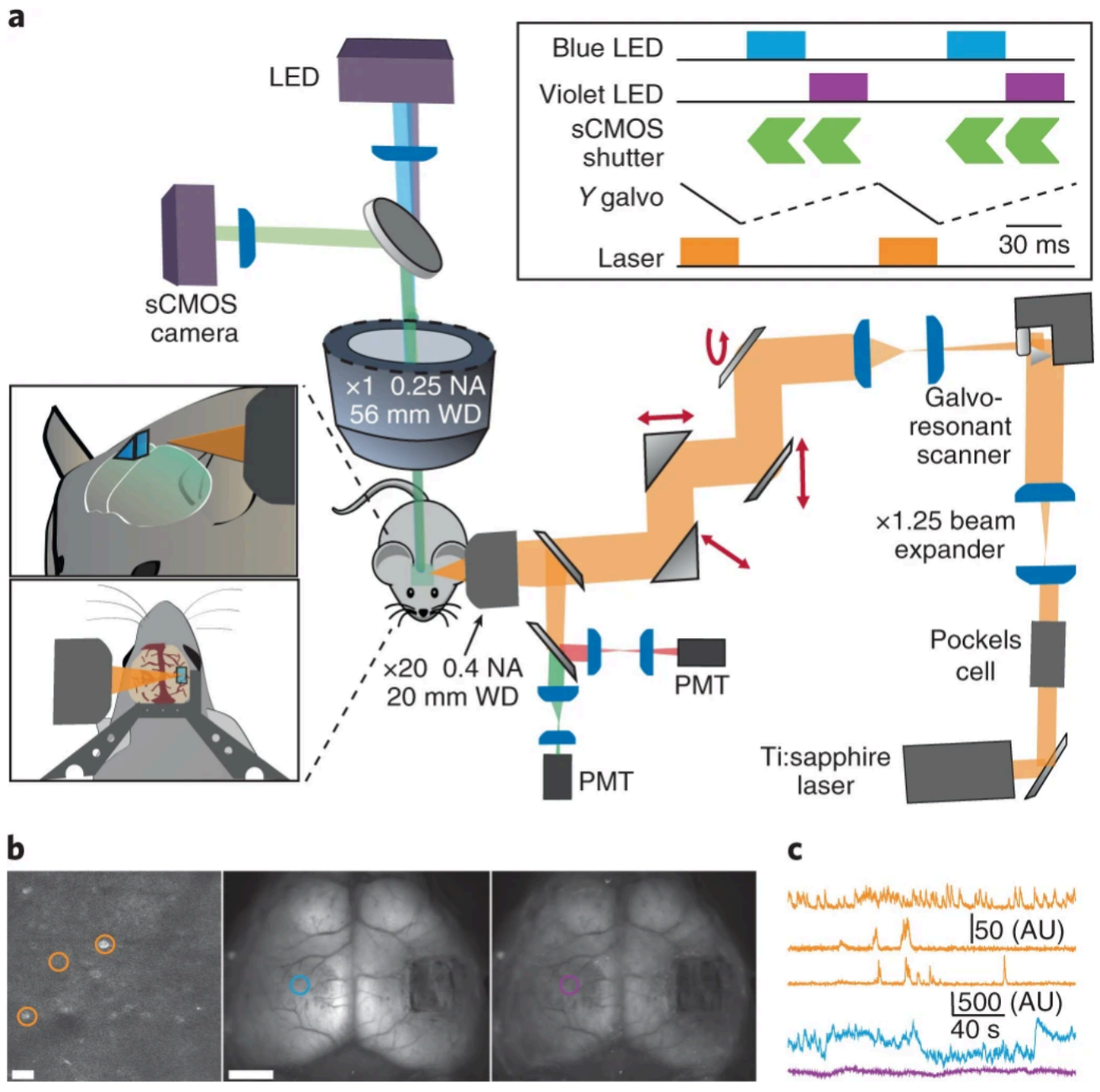
2-photon calcium imaging set up





Yang, W., Carrillo-Reid, L., Bando, Y., Peterka, D. S., & Yuste, R. (2018). *Simultaneous two-photon imaging and two-photon optogenetics of cortical circuits in three dimensions. eLife, 7.* doi:10.7554/elife.32671

Barson, D., Hamodi, A.S., Shen, X. *et al.* Simultaneous mesoscopic and two-photon imaging of neuronal activity in cortical circuits. *Nat Methods* **17,** 107–113 (2020). https://doi.org/10.1038/s41592-019-0625-2

—> Datasets

2) Where can I find it?

-Google dataset search
-Allen Brain Observatory: calcium imaging of visual areas
-Stringer's Dataset: Calcium imaging of visual cortex and behavioural aspects (pupil diameter and whisking)

https://datasetsearch.research.google.com/

"Calcium imaging mice"
"Optogenetics mice"

Allen Brain Observatory

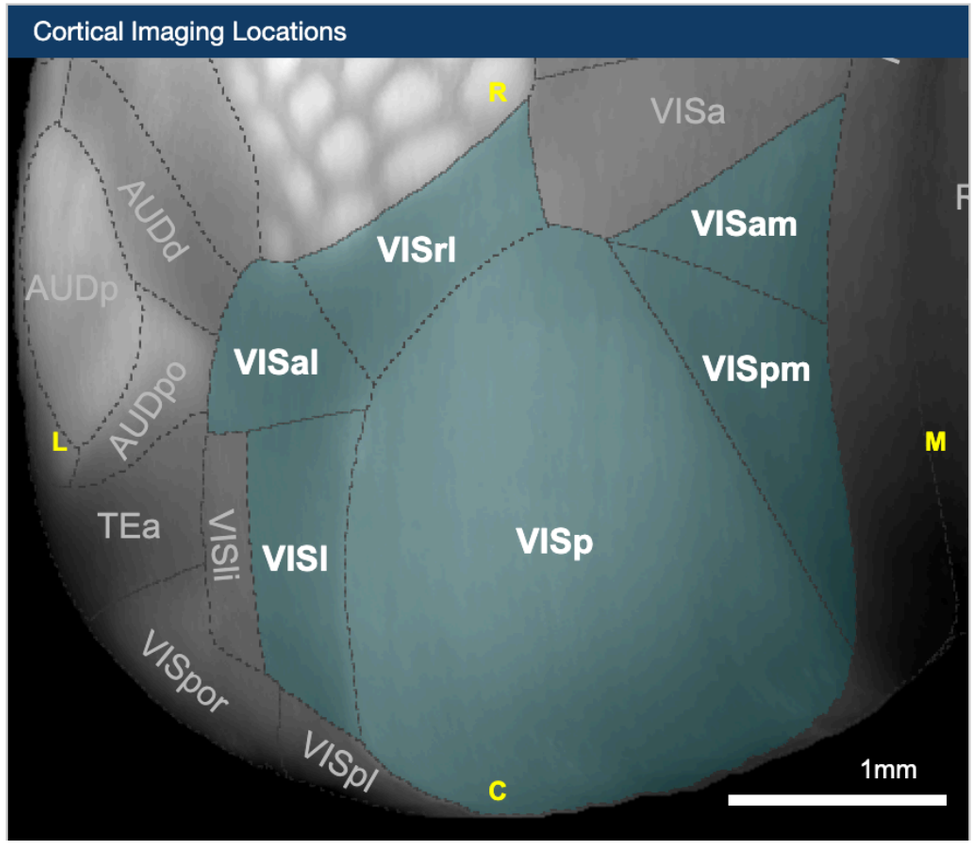
ALLEN INSTITUTE for BRAIN SCIENCE

About the Allen Brain Observatory

The Allen Brain Observatory presents the first standardized *in vivo* survey of physiological activity in the mouse visual cortex, featuring representations of visually evoked calcium responses from GCaMP6-expressing neurons in selected cortical layers, visual areas and Cre lines.

Key features of the dataset:

- Searchable data from hundreds of two-photon calcium imaging sessions across multiple visual areas and depths in the visual cortex
- A variety of data visualization summaries capturing visual coding properties of single cell and cell population responses to sensory stimuli
- Standardized spatial mapping of cellular responses to five types of rich visual stimuli, surveyed from transgenic mouse lines
- Raw data and analysis modules available for download via the Allen Brain Atlas application program interface (API) and Allen Software Development Kit (SDK)



—> Visual Stimulus

Visual Stimulus

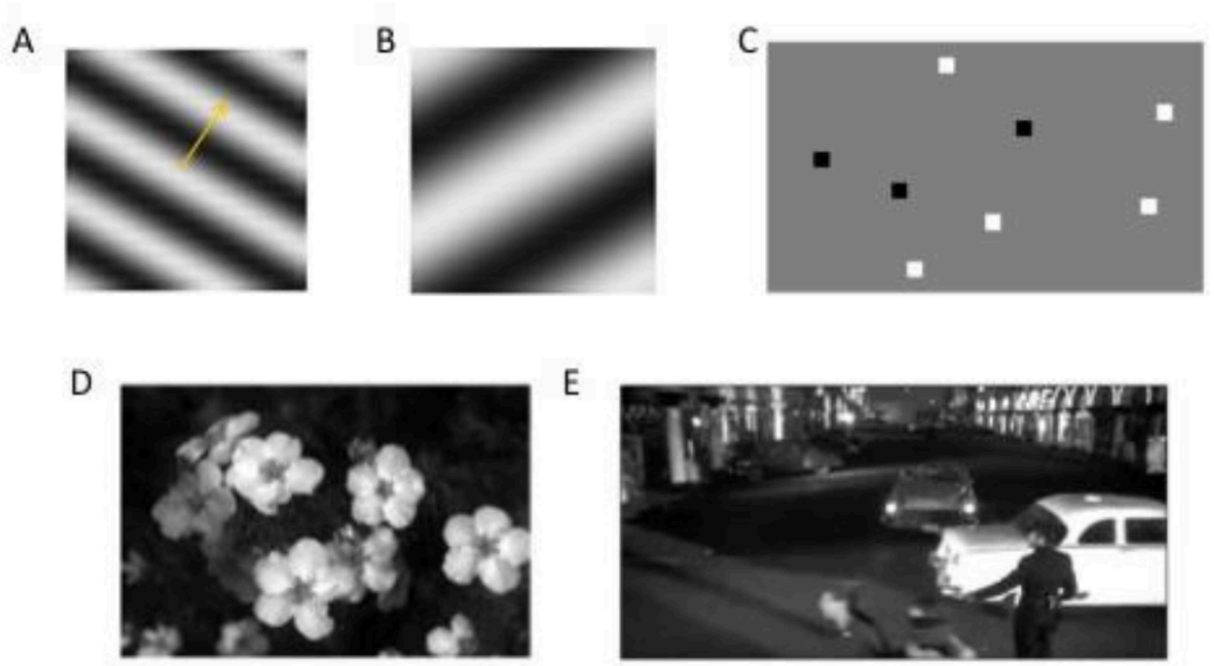


Figure 1. Stimulus set for the Allen Brain Observatory – Visual Coding. A) Drifting gratings B) Static gratings C) Locally sparse noise D) Natural scenes E) Natural movies.

Allen Brain Observatory

Session A

Stimulus	min
Drifting Gratings	30
Natural Movie 1	5
Natural Movie 3	20
Spontaneous Activity	5
Inter-stim gray	2
Total	**62**

Session B

Stimulus	min
Static Gratings	25
Natural Images	25
Natural Movie 1	5
Spontaneous Activity	5
Inter-stim gray	2
Total	**62**

Session C

Stimulus	min
Locally Sparse Noise 4 deg	37
Natural Movie 1	5
Natural Movie 2	5
Spontaneous Activity	10
Inter-stim gray	1
Total	**58**

Session C2

Stimulus	min
Locally Sparse Noise 4 deg	24
Locally Sparse Noise 8 deg	23
Natural Movie 1	5
Natural Movie 2	5
Spontaneous Activity	10
Inter-stim gray	1
Total	**60**

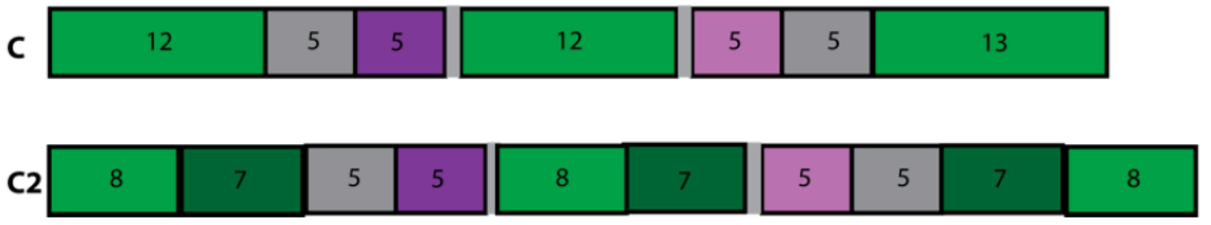
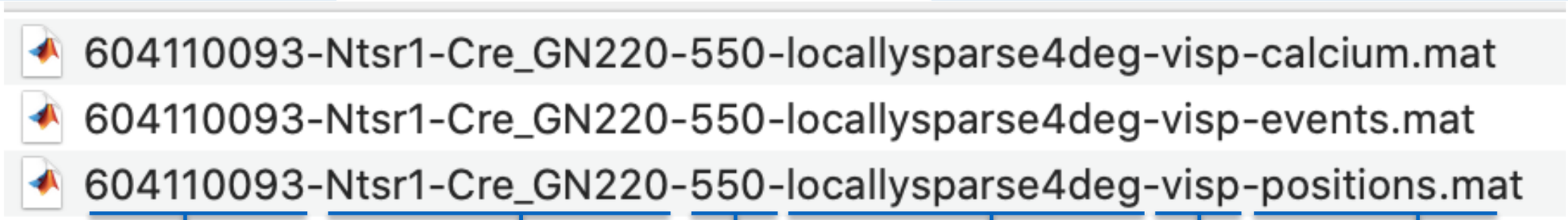


Figure 4. Sequence of visual stimuli across three sessions.

—> Data files

Data files

Allen Brain Observatory



(mouse experiment ID)-(transgenic line)-(depth um)-(stimulus type)-(brain area)-(file type).mat

Explanation of terms:

mouse experiment id: The Allen Brain Observatory identification number for the experiment this file corresponds to. This ID corresponds to a particular mouse imaged at a particular depth over 10 different stimulus types.

transgenic line: The transgenic line of the animal used for this experiment.

depth: The depth in um that this experiment occurred at. Typically 175-275um is layer 2/3 and >550um is layer 6.

stimulus type: The shorthand name for the stimulus shown to the animal for this subset of the recording.

brain area: The area of recording.

file type: Either "events": lambda matrix of deconvolved events, or "calcium": raw calcium traces, or "positions": cell positions.

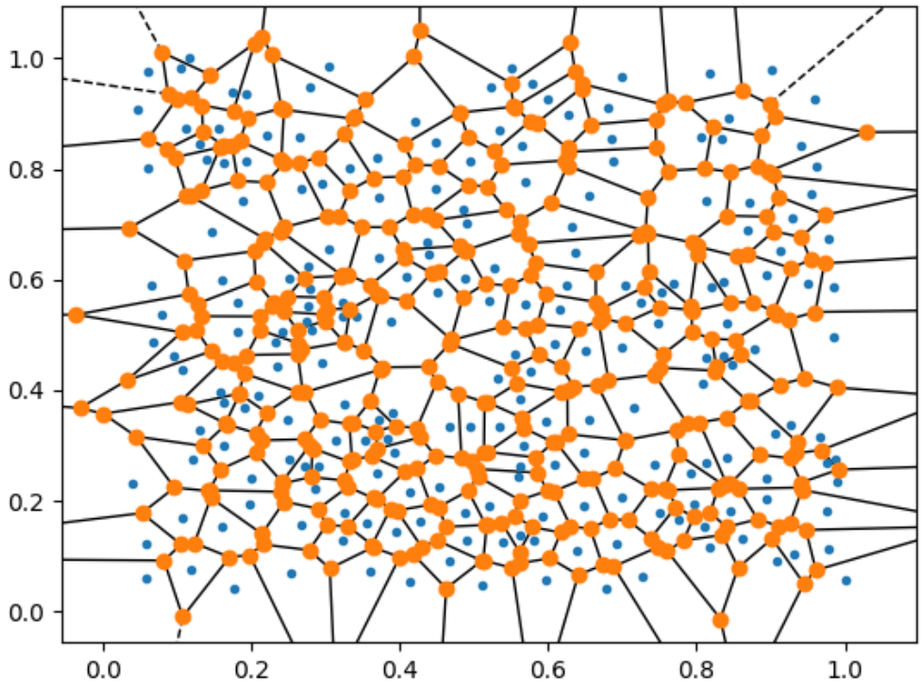
Data type
All datasets are matrices of size (row-major) NxM. N is the number of cells for the respective experiment; M is 2 for "positions", or the number of time bins for "events" or "calcium" file types.

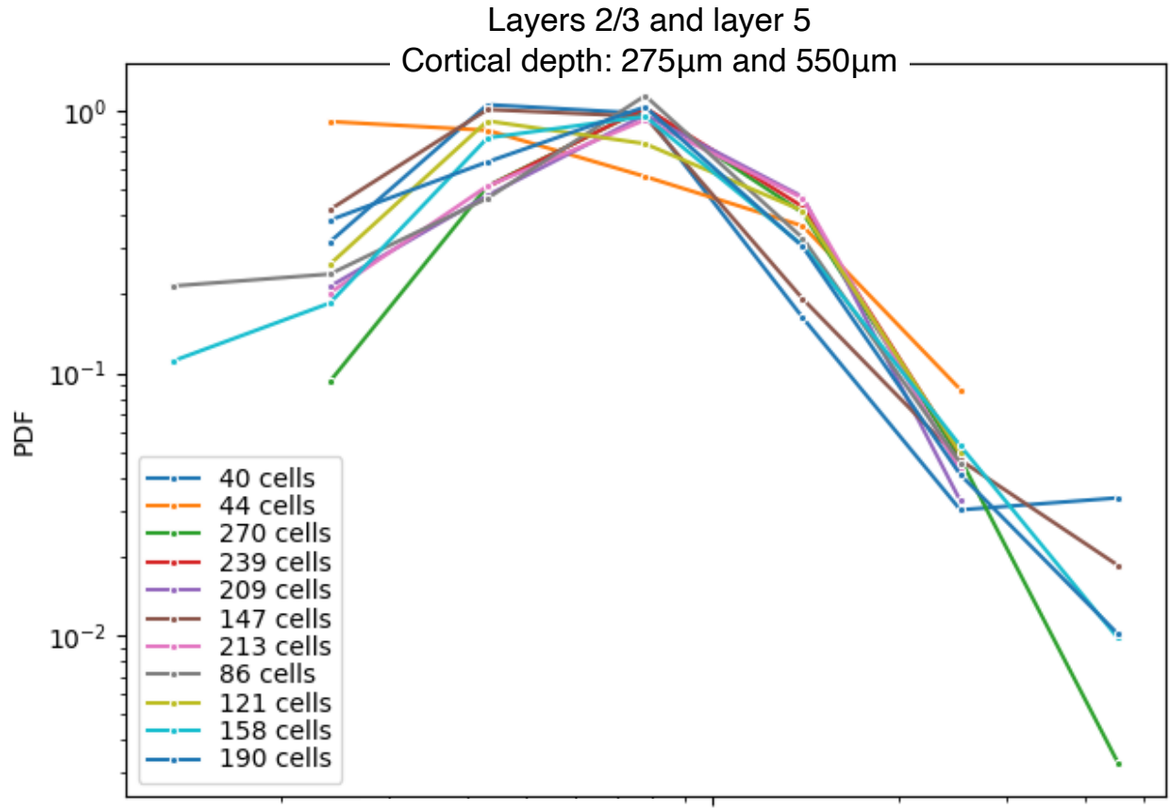
—> Structural analysis

Structural analysis of the ROI's measured in the Allen Dataset

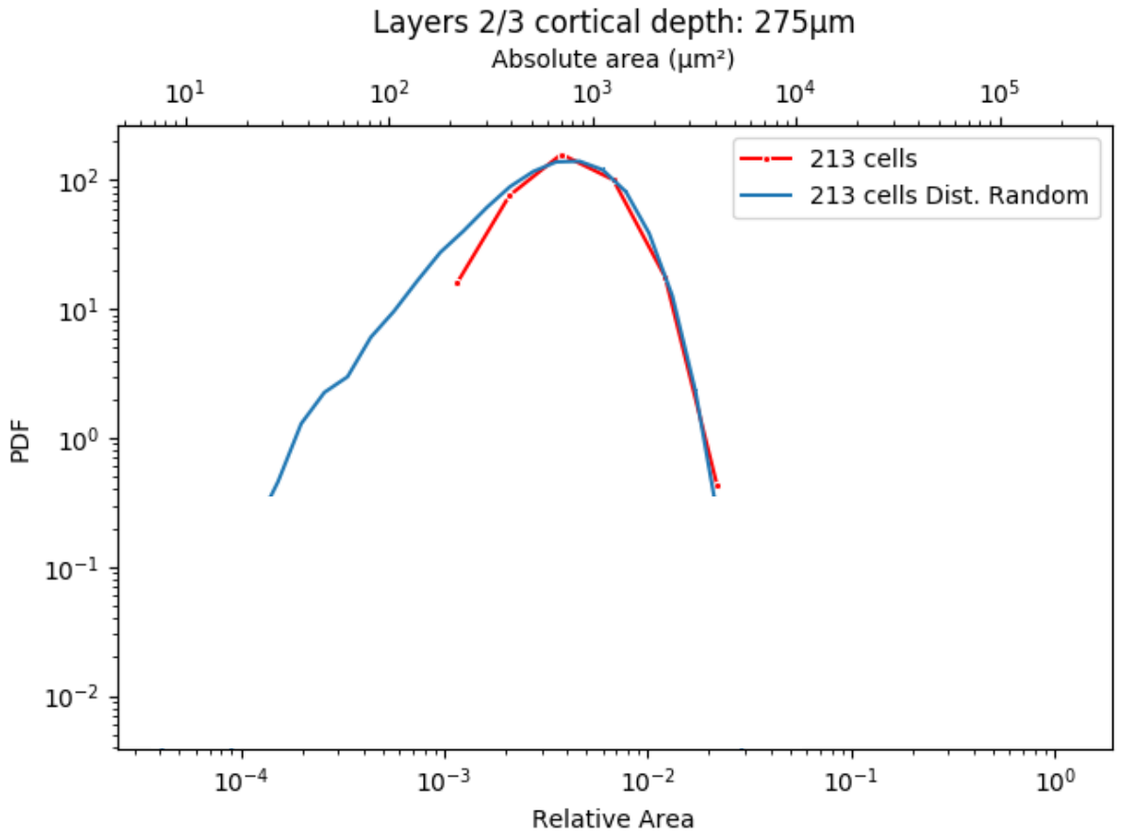
Jeremi Ochab

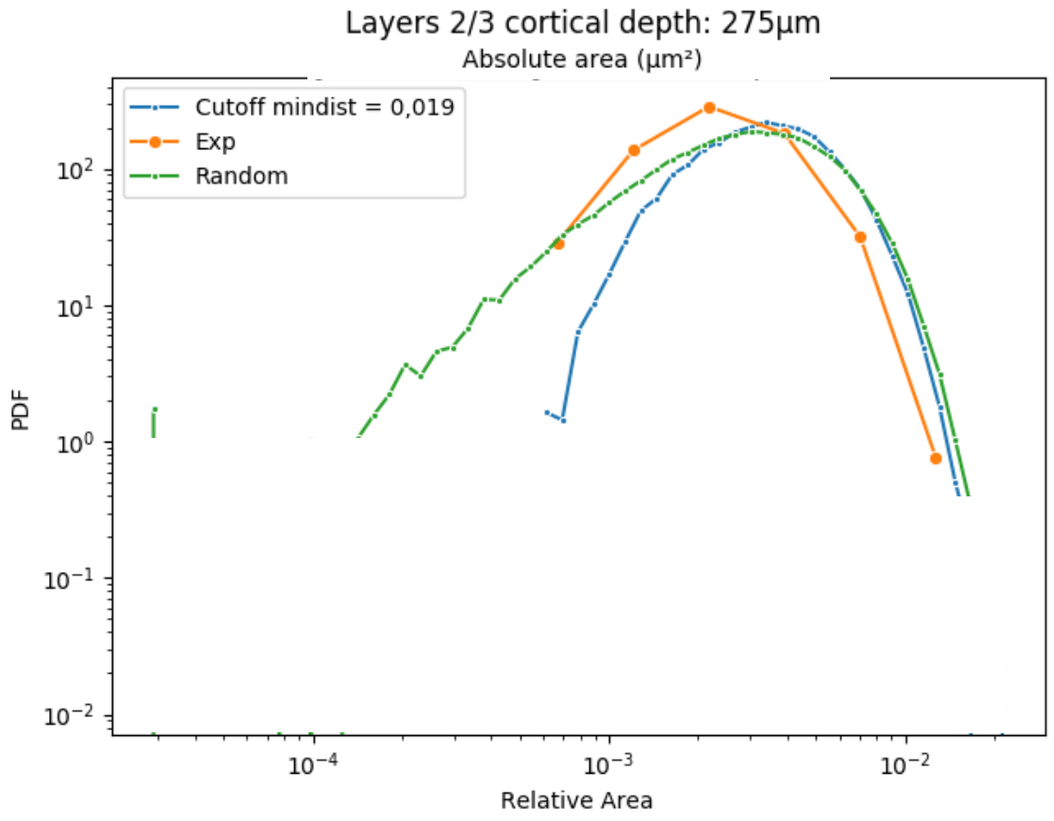
209 Voronoi cells



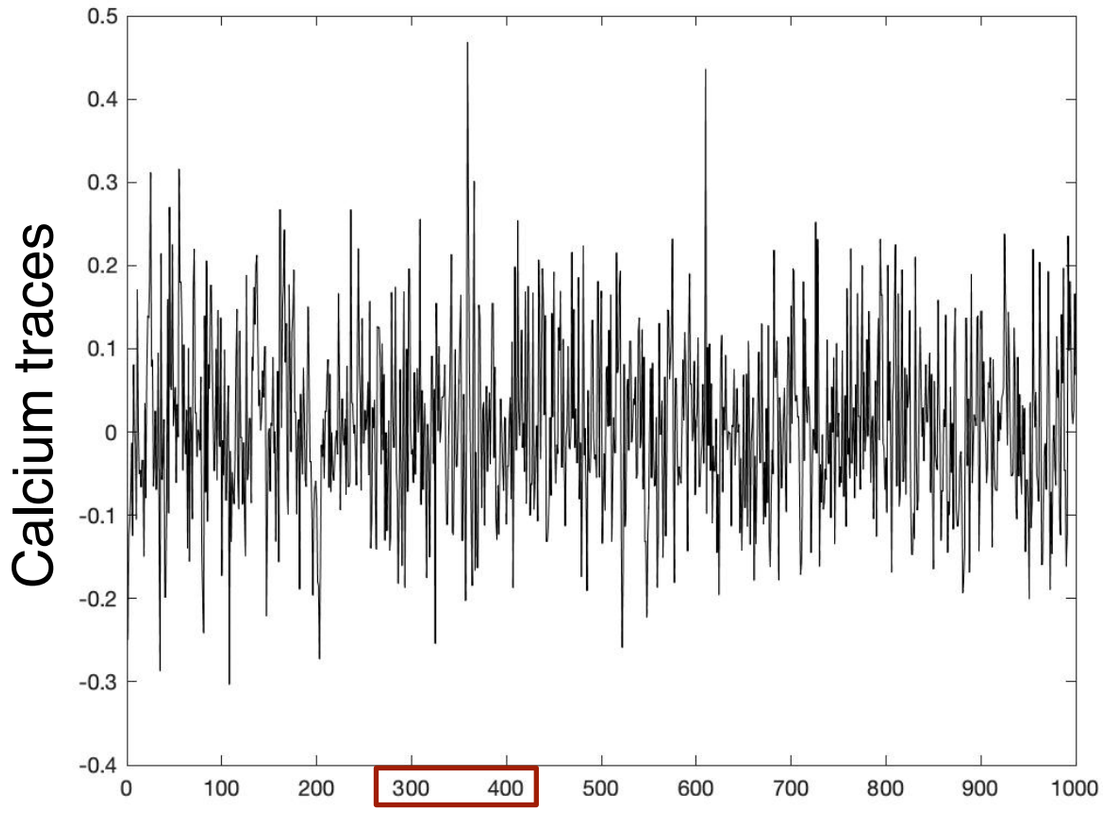


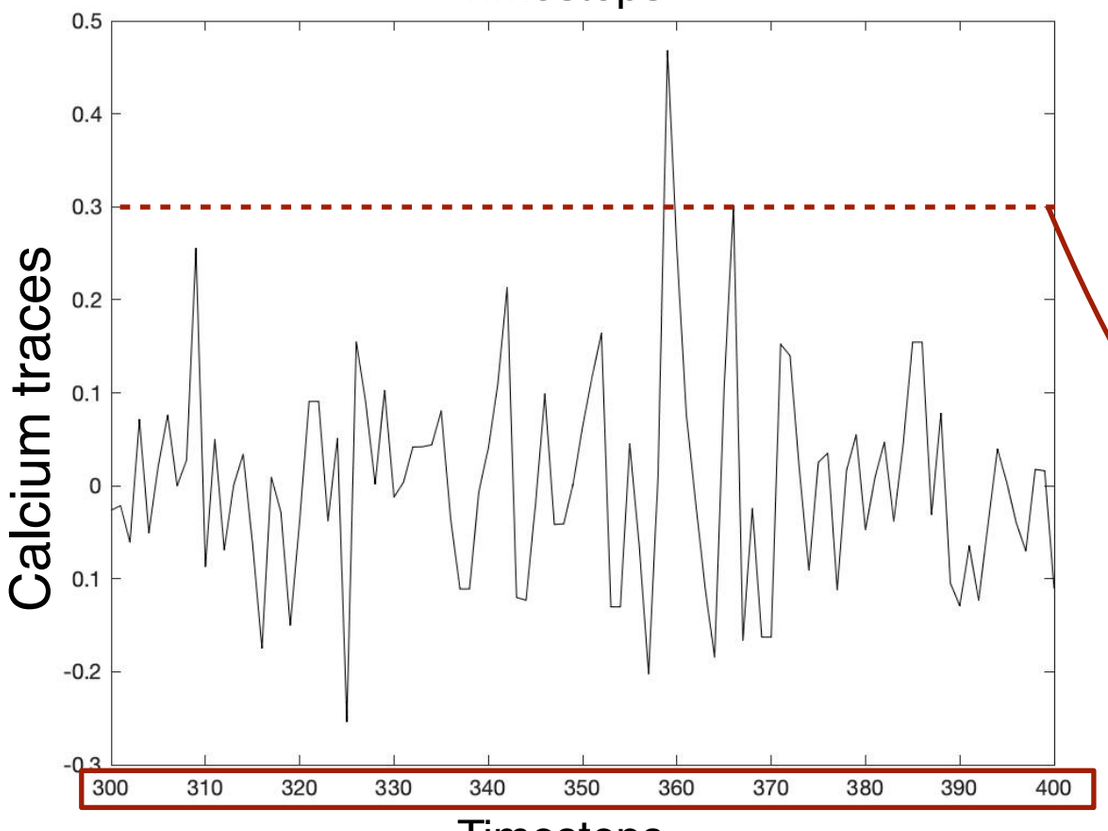
Normaliced areas 10^0





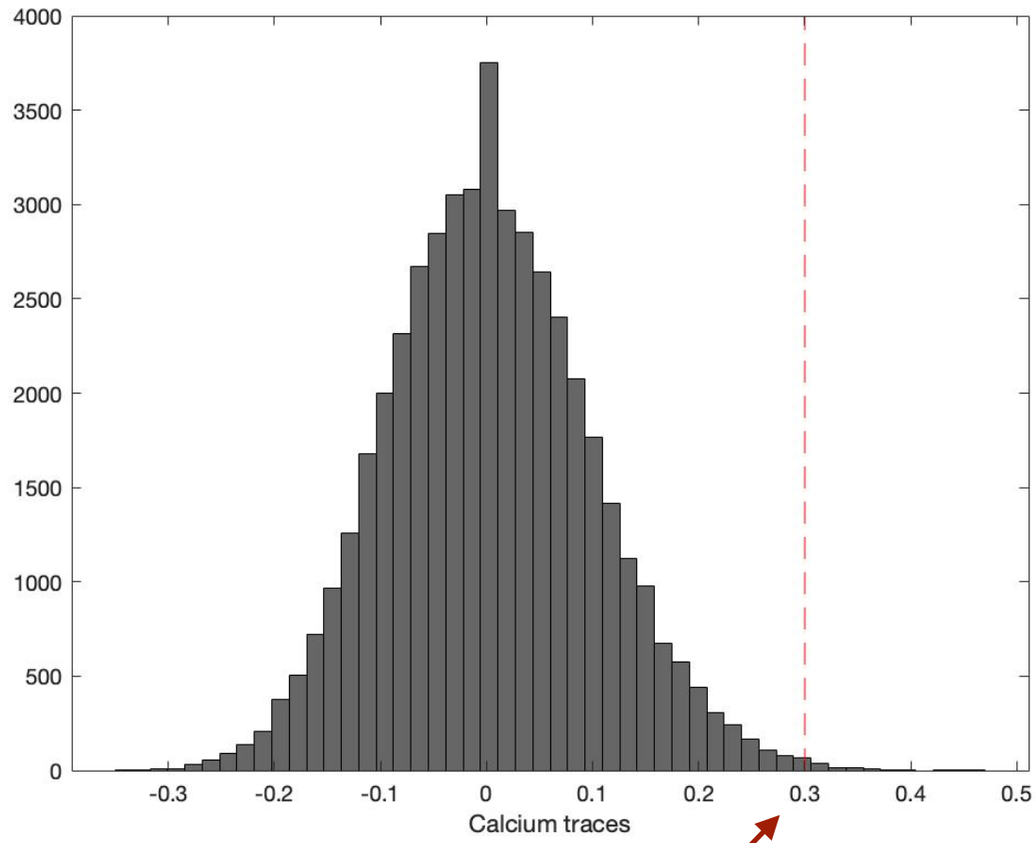
Raw calcium traces





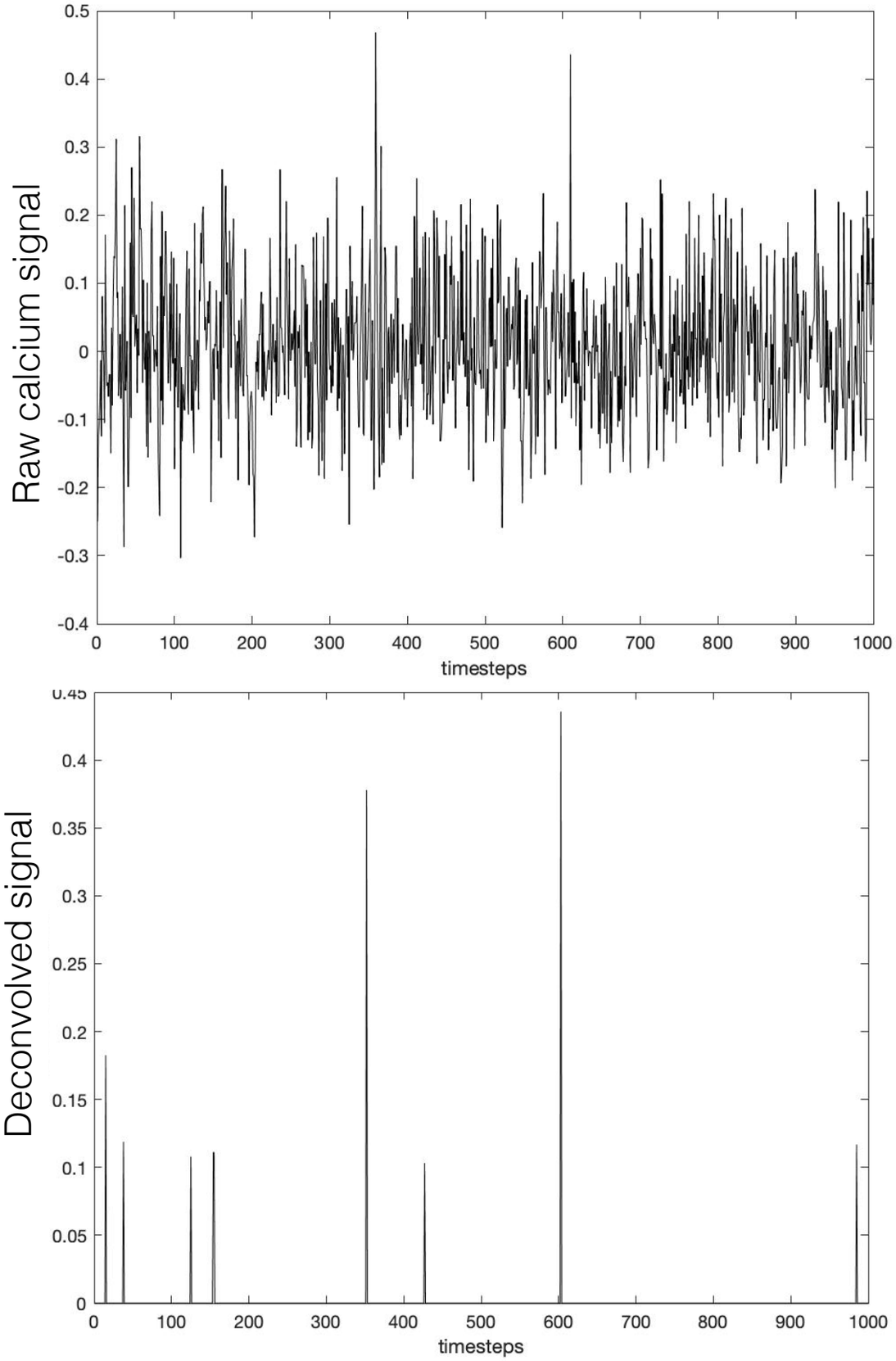
Allen Brain Observatory

In this experiment, the calcium fluorescence signal was recorded for around 47000 time-steps, over the course of several minutes



—> Deconvolved signal

Events



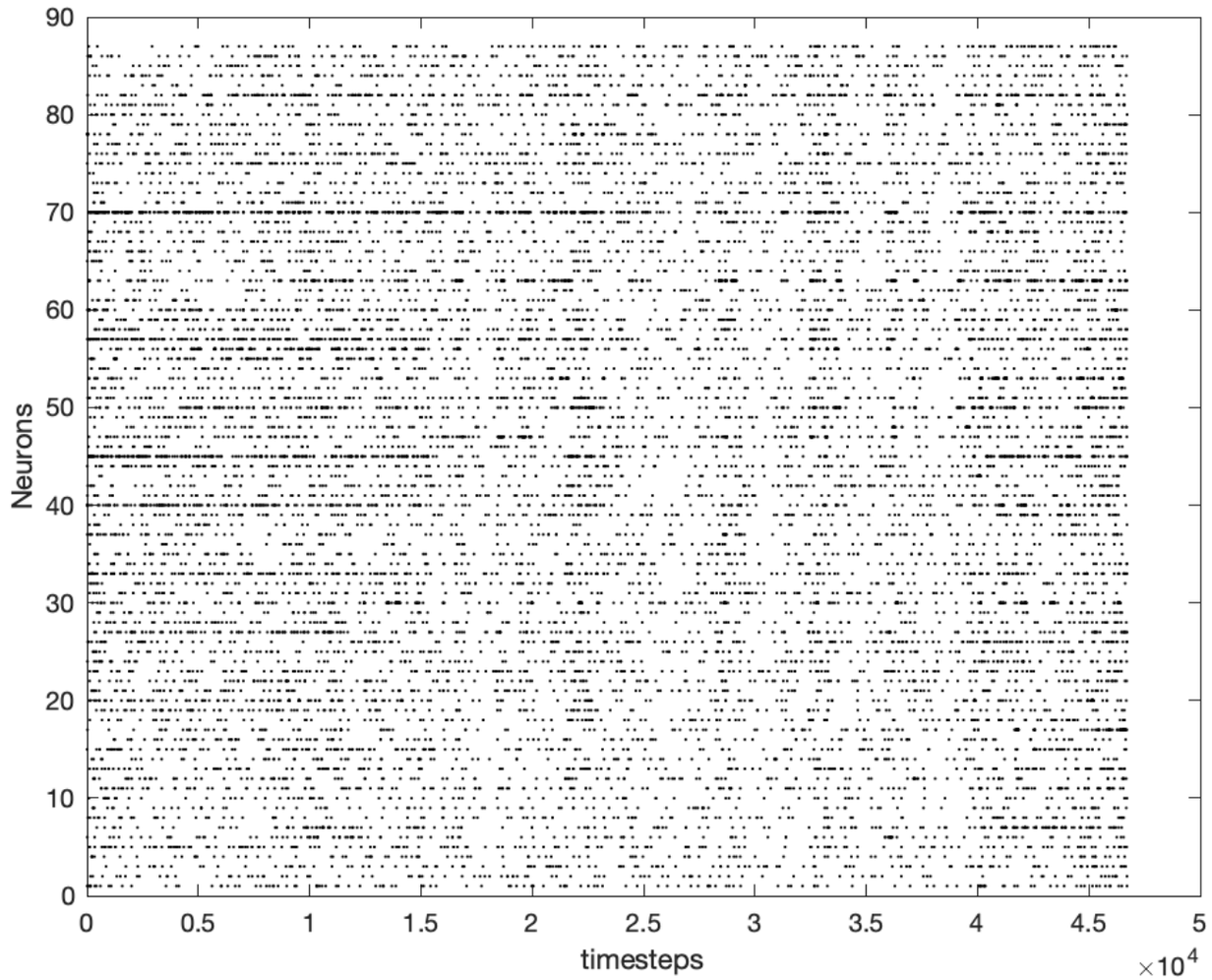
Allen Brain Observatory

The signal is deconvolved to estimate the timing of spikes

Suite2p

http://www.suite2p.org/

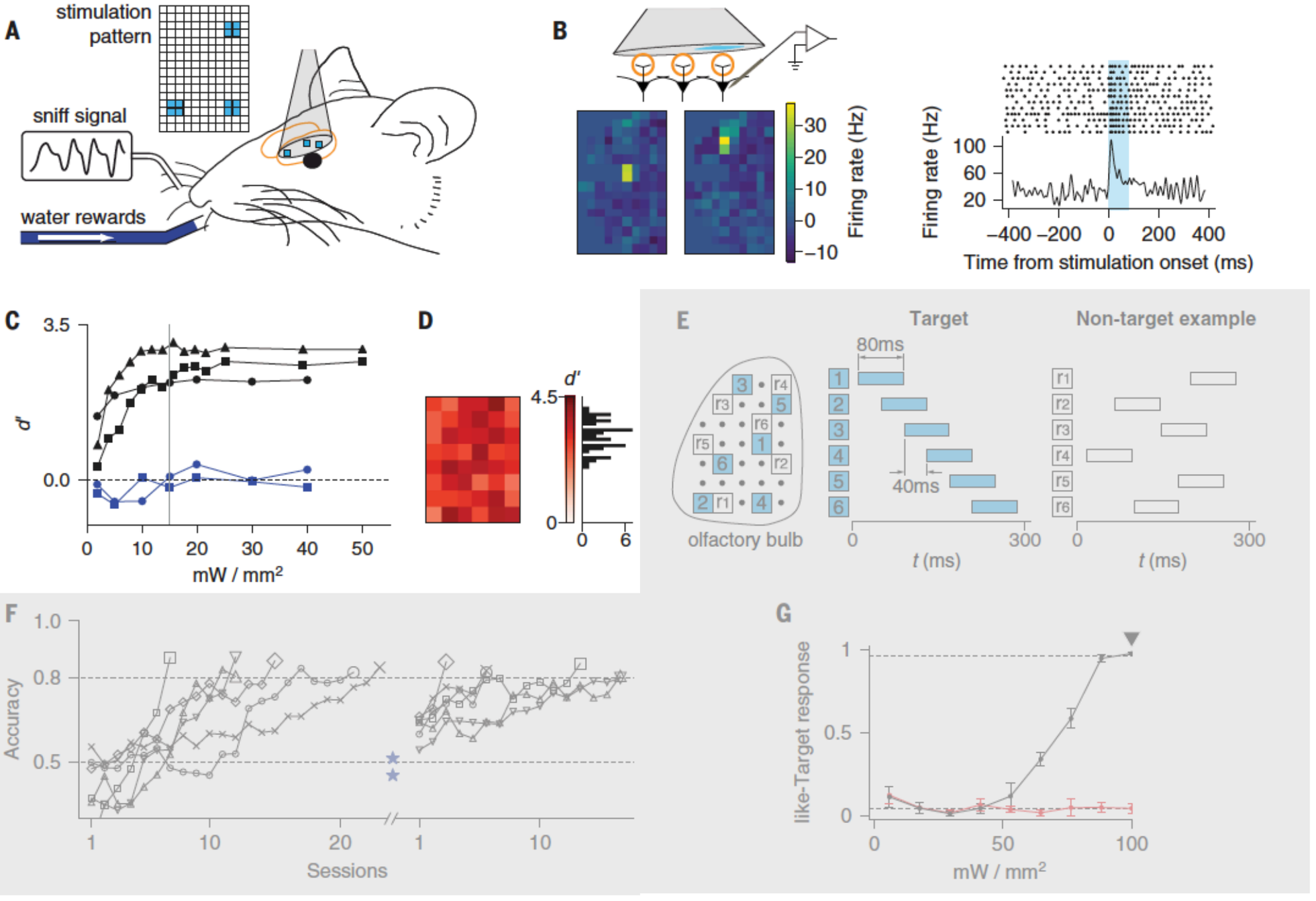
Raster plot showing events activity of all neurons



—> Optogenetic stimulation of olfactory bulb - Rinberg et al.

3) Hot results from other labs

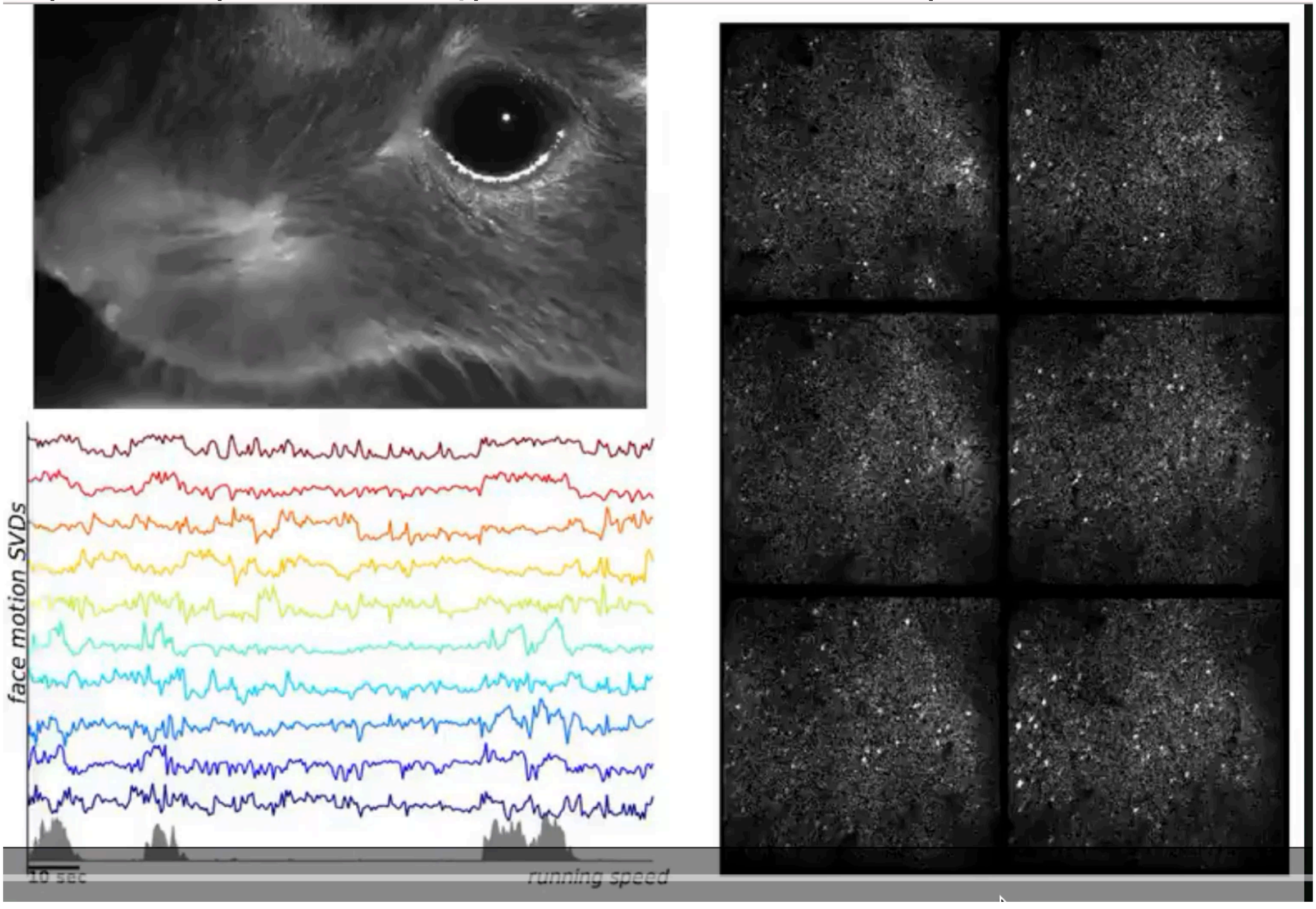
Optogenetic stimulation of olfactory bulb in mice



Chong E, Moroni M, Wilson C, Shoham S, Panzeri S, Rinberg D. Manipulating synthetic optogenetic odors reveals the coding logic of olfactory perception. Science. 2020 Jun 19;368(6497):eaba2357. doi: 10.1126/science.aba2357. PMID: 32554567.

—> Stringer et al.

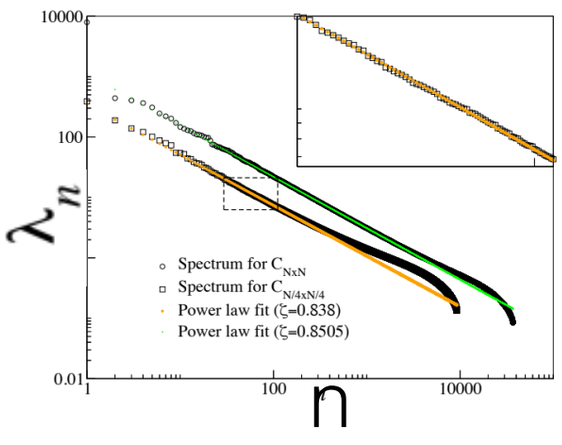
Hollywood style: Recording of calcium traces and spontaneous behaviour

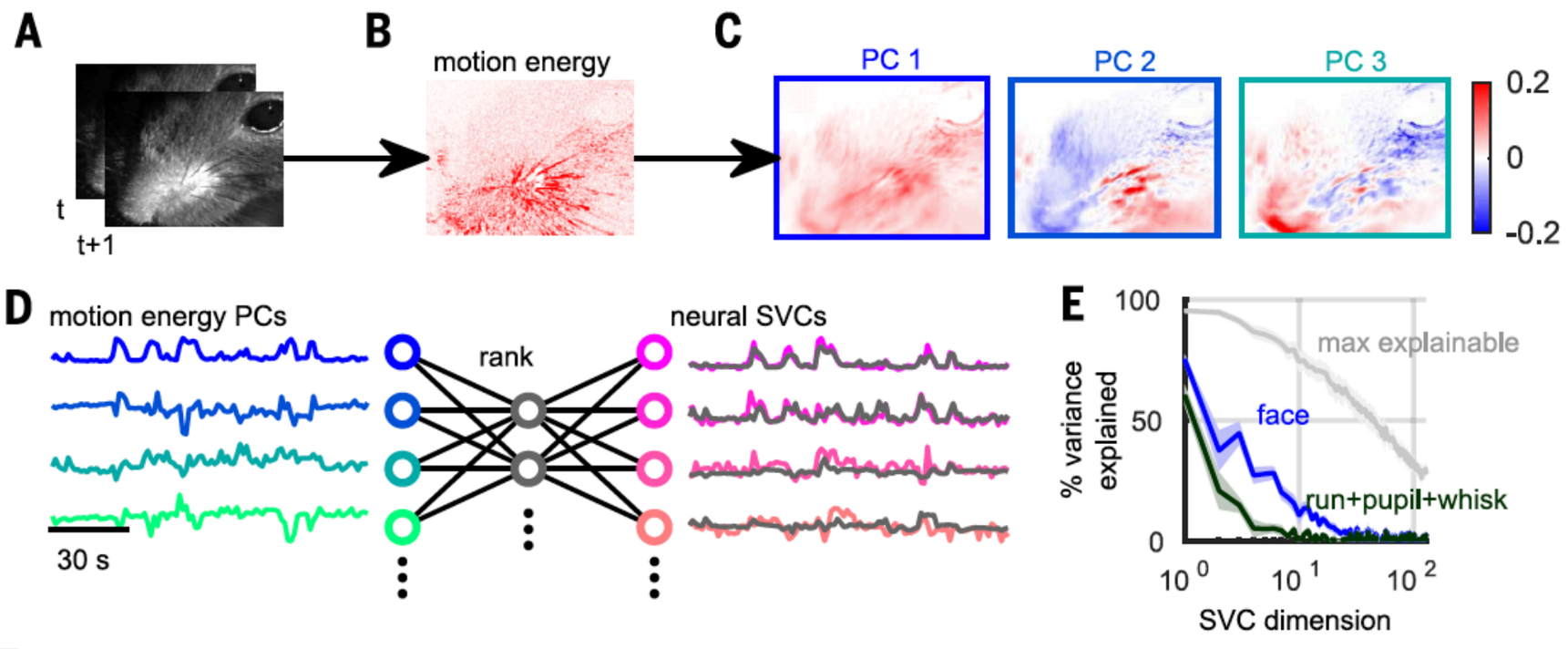


Stringer C, Pachitariu M, Steinmetz N, Reddy CB, Carandini M, Harris KD. Spontaneous behaviors drive multidimensional, brainwide activity. Science. 2019 Apr 19;364(6437):255. doi: 10.1126/science.aav7893. Epub 2019 Apr 18. PMID: 31000656; PMCID: PMC6525101.

news flash!! brain exhibits collective dynamics

1000 neurons!!





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Vinayak, Prosen, T., Buča, B., & Seligman, T. H. (2014). *Spectral analysis of finite-time correlation matrices near equilibrium phase transitions. EPL (Europhysics Letters), 108(2), 20006.* doi:10.1209/0295-5075/108/20006

—> Machine learning + network states

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Simple approaches to identify network states

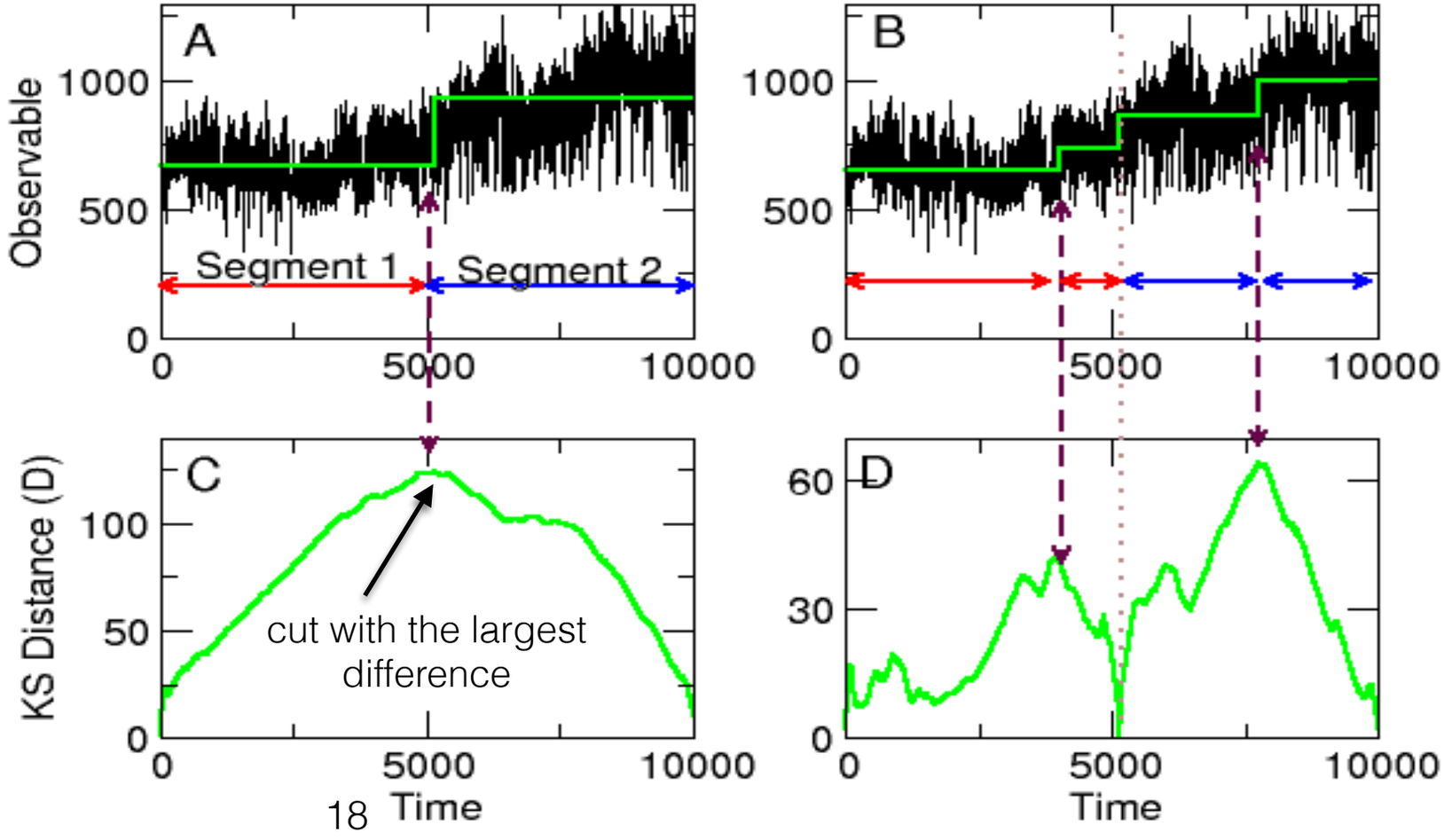
Sabrina Camargo & Aylen de Florian

Motivation:

- On one side, animal behaviour or arousal increases the repertoire of network states.
- On the other, averaging neural responses over trials might “throw the baby out with the bathwater”.
- As an alternative to averaging, we parcel quasi-stationary “network states” using non-parametric segmentation* and machine learning.

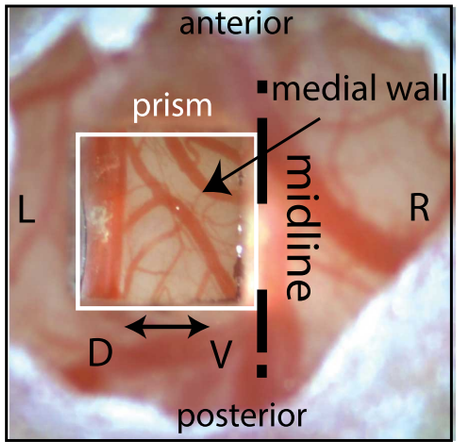
The first 2 steps of the K-S segmentation algorithm which finds (at each step) the two most dissimilar segments.

Further steps repeat the same, until no more cuts are possible, because of length or statistical significance



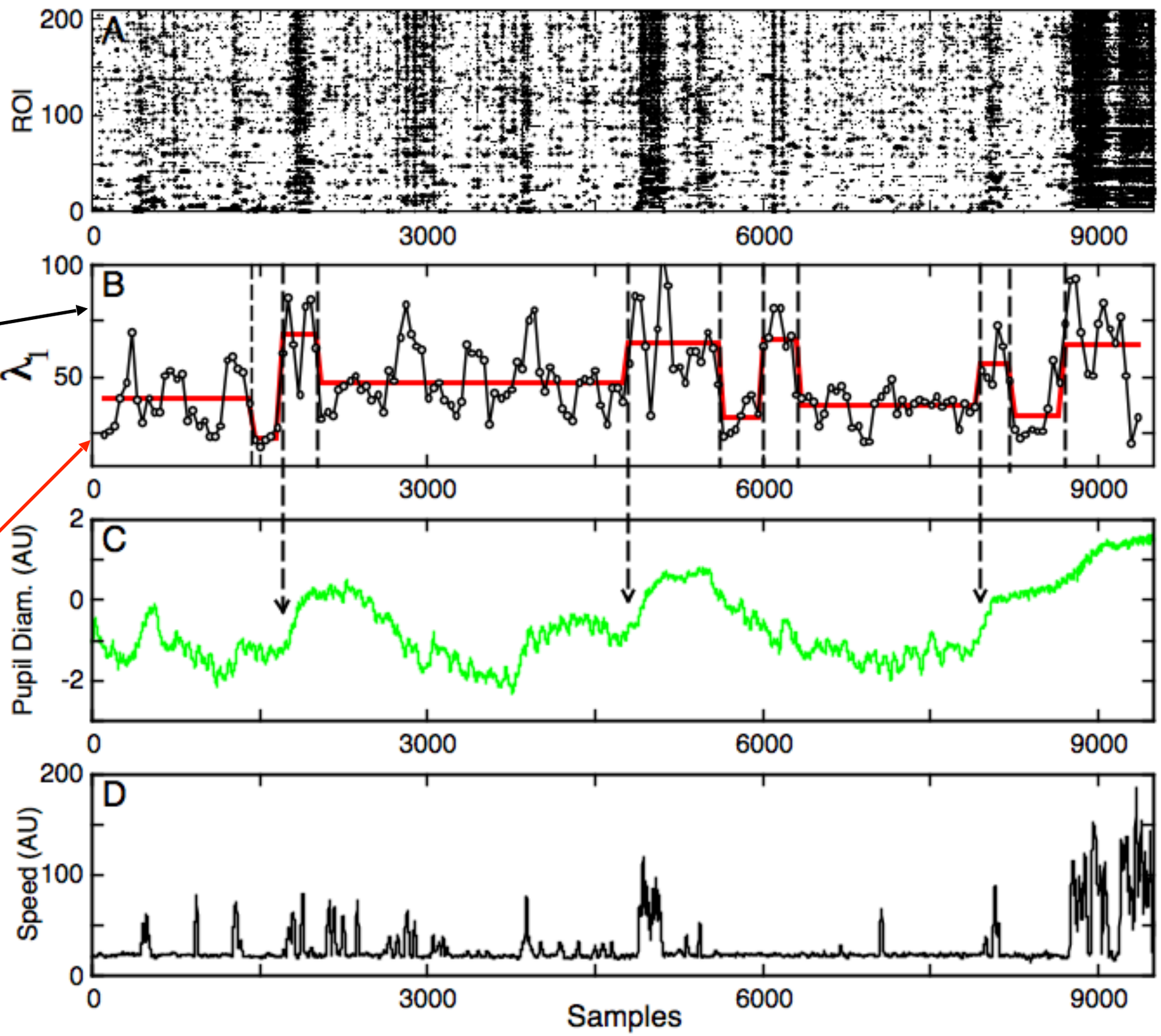
* S Camargo, SMD Queirós, C Anteneodo, Nonparametric segmentation of nonstationary time series, Physical Review E 84, 046702 (2011).

Example of network states identification from ~200 anterior cingulate cortex neurons (resting /running)



(**circles**)Time series of the largest Eigenvalues of the (sliding) correlation matrix of Panel A.

(**red line**) Segments of quasi-stationary Eigenvalues statistics identified by the K-S segmentation.



* MPFC/Cingulate unpublished Calcium data from Plenz et al.

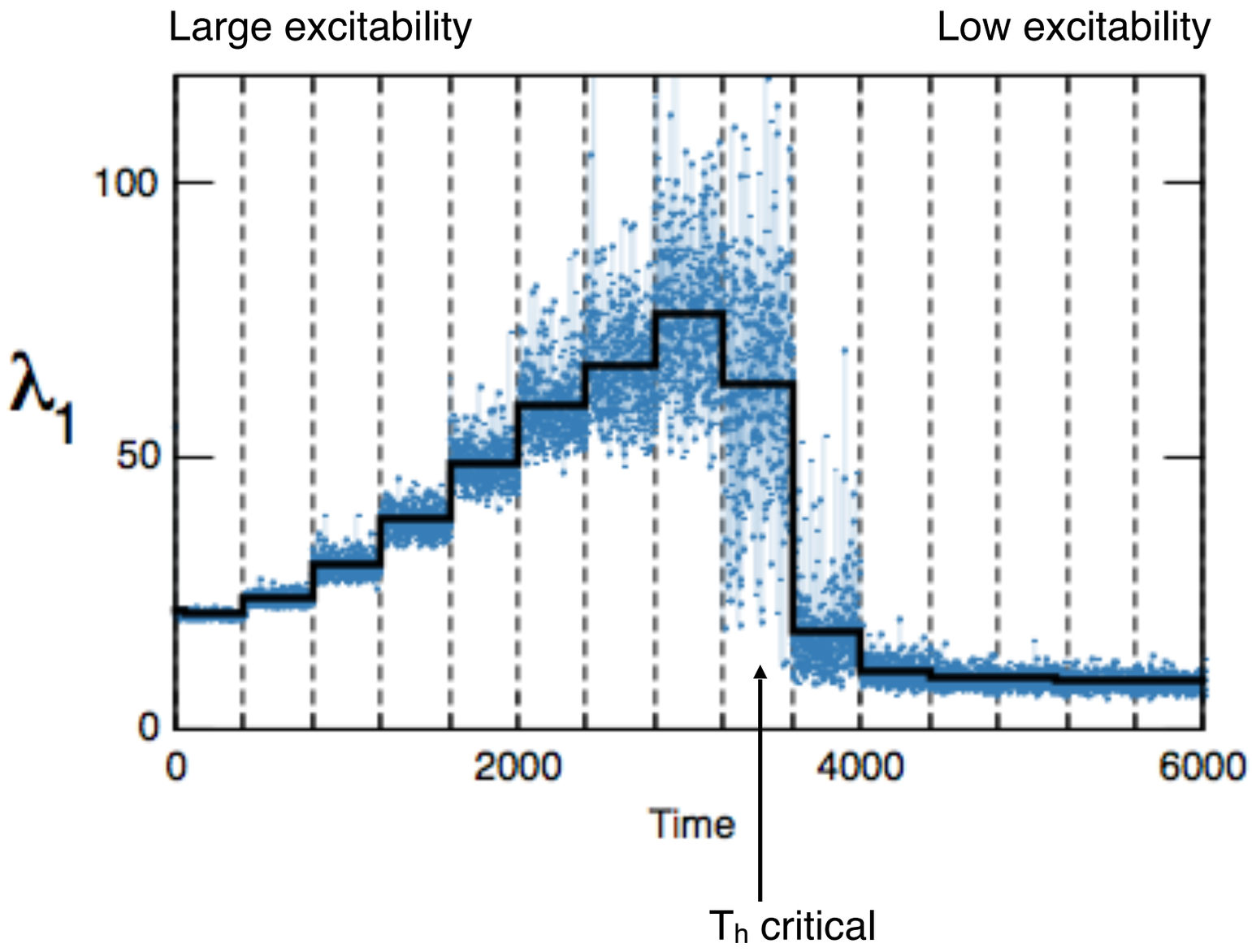
next -> K-S segments, are states?

A numerical simulation* shows that the K-S segments correspond to network states of stationary correlations

Simulation of a spiking network model* in which the excitability is decreased every 400 time **steps**.

The **blue traces** are the largest Eigenvalues of the correlation matrix.

The **dark line** denotes the segments of *stationary correlations* identified by the KS-method.



* Haimovici. et al. Brain organization into resting state networks emerges at criticality on a model of the human connectome, Physical Review Letters 110(17), 178101 (2013)

next -> what kind of states?

Machine learning confirms that quasi-stationary network states exhibit unique fine structure correlation

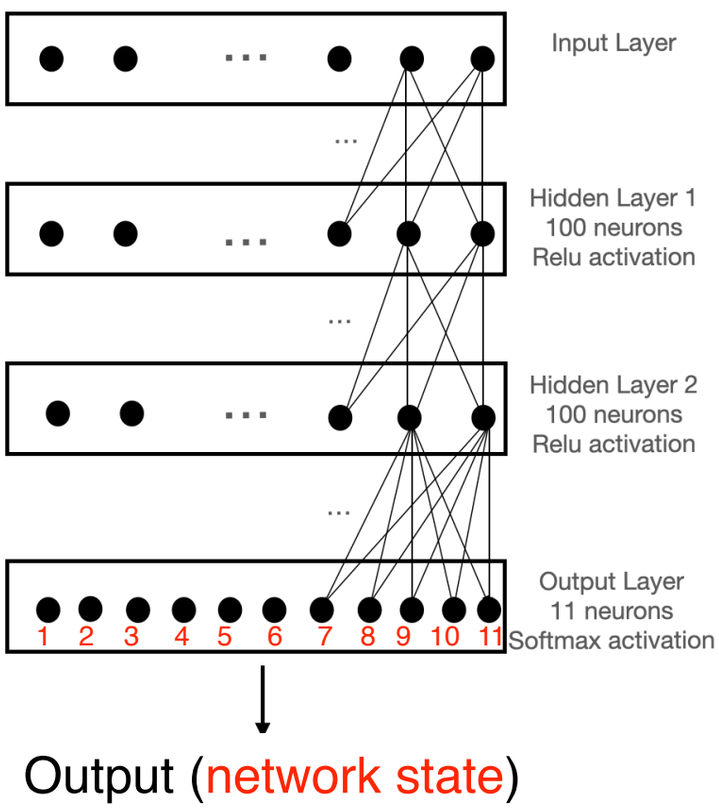
Neural Network

Input (209 roi's)

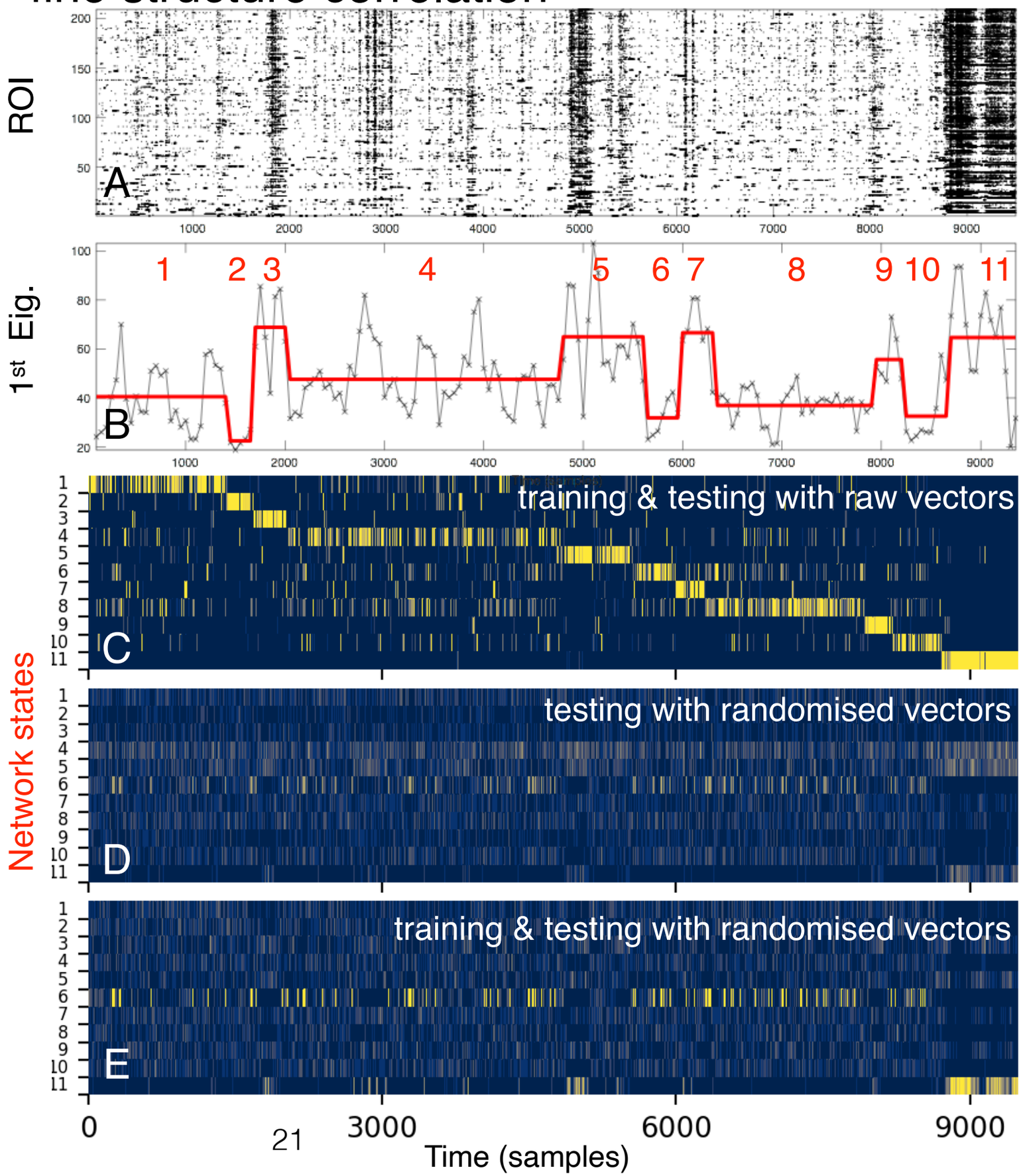
01000 ··· 1100001000000

Shuffling (optional)

11100 ··· 0000000001000



Output (network state)



Summing up

- In freely behaving animals, the richness of experimental results might not be fully exploited by averaging neural responses.
- We describe a simple approach based on Kolmogorov-Smirnov segmentation to identify segments with quasi-stationary statistics (aka “network states”).
- A second step using ML on a very simple feedforward neural network is able to determine the nature of the segmented neural states, in terms of *firing rate* or *fine structure of the correlations*.

Summary

We reviewed the bases of Optogenetics, focusing especially on optical recording techniques.

We discussed the databases available and the type of analysis being done in 2 recent papers, along with our preliminary analysis combining ML and KS-segmentation.

Overall, the optogenetics data offers ample opportunities for the application of statistical physics and ML tools.

References

Ahrens MB, Orger MB, Robson DN, Li JM, Keller PJ. Whole-brain functional imaging at cellular resolution using light-sheet microscopy. Nat Methods. 2013 May;10(5):413-20. doi: 10.1038/nmeth.2434. Epub 2013 Mar 18. PMID: 23524393.

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Thanks!

Questions?

Experimental setup

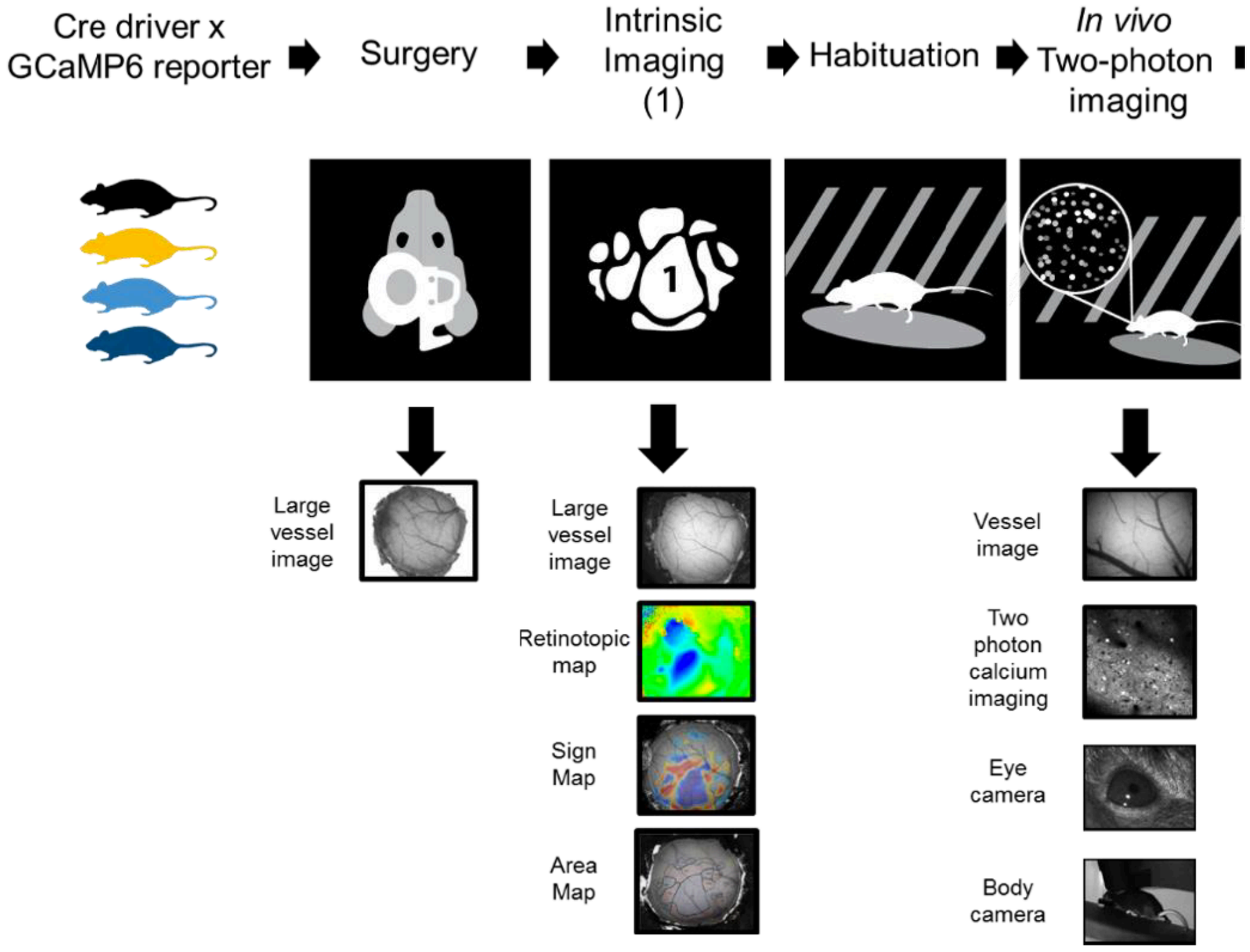
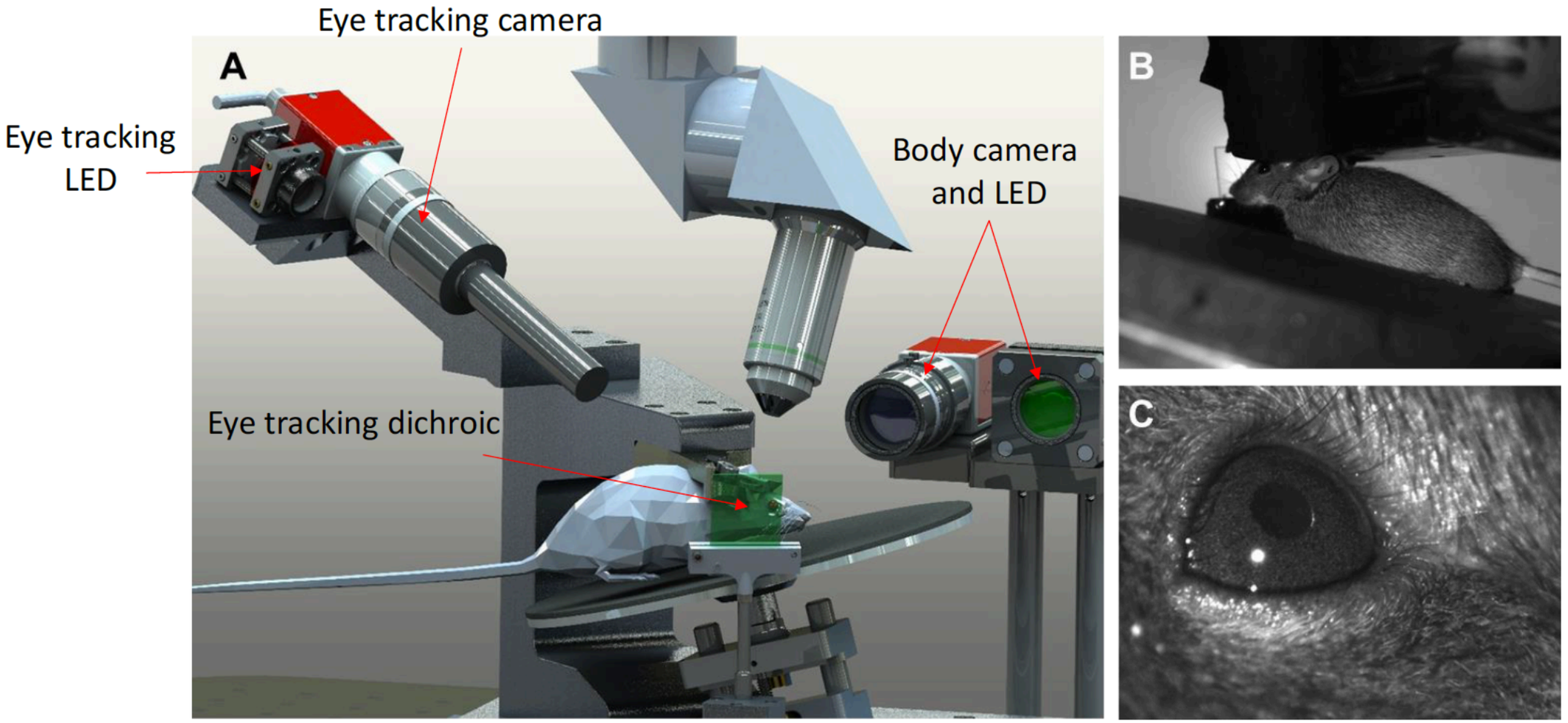
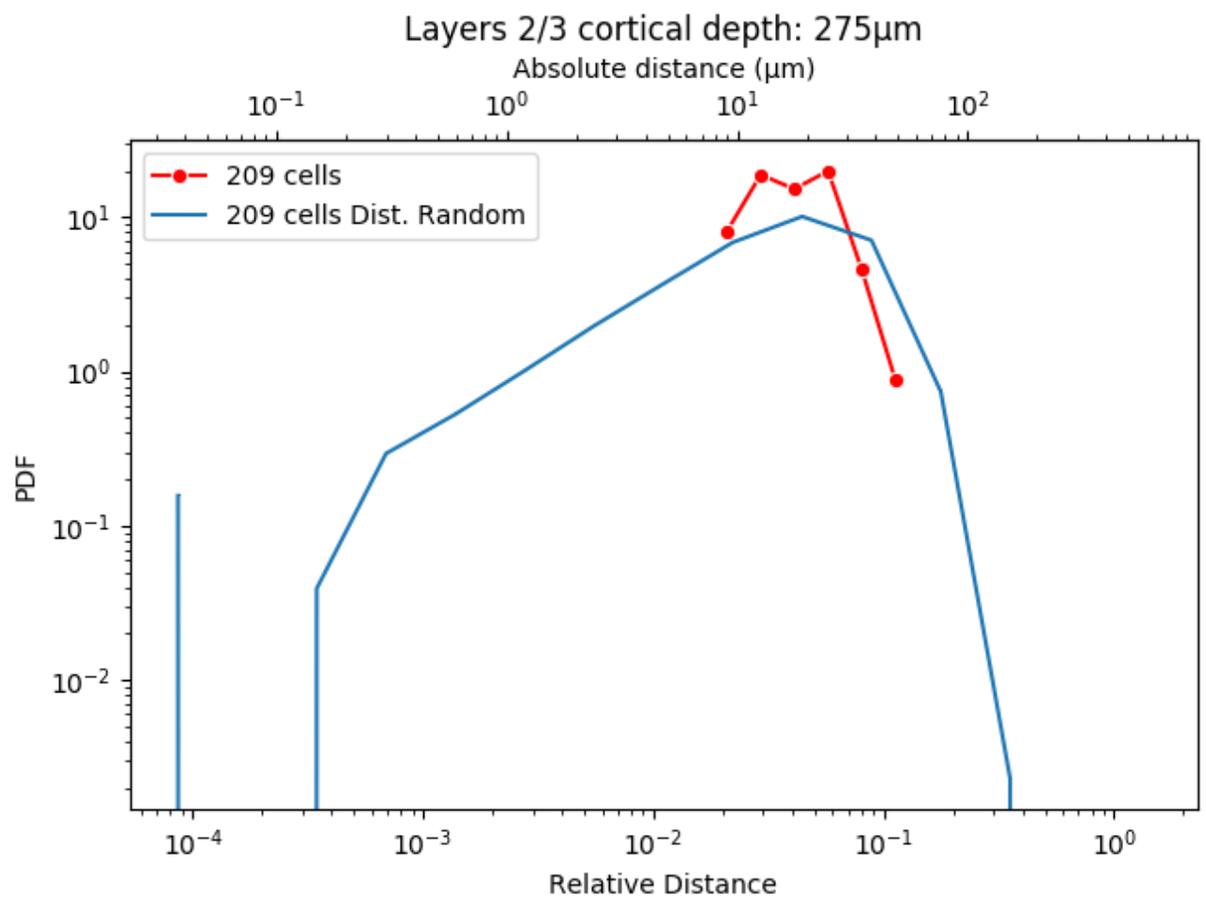


Figure 1. Schematic of the Allen Brain Observatory data acquisition workflow. Mice expressing genetically encoded calcium sensors in subsets of cortical neurons received implantation of a custom headframe. Intrinsic signal imaging was used to map functional visual areas and retinotopy. Mice were habituated to the passive sensing task. *In vivo* two-photon calcium imaging was collected across multiple sessions during a diverse set of stimuli. Eye tracking, body position and running data was collected.


A
Eye tracking camera
Eye tracking
LED
Body camera
and LED
Eye tracking dichroic
B
C



We created a ‘random’ distribution of points that conserved the same distribution of distances to nearest neighbour.