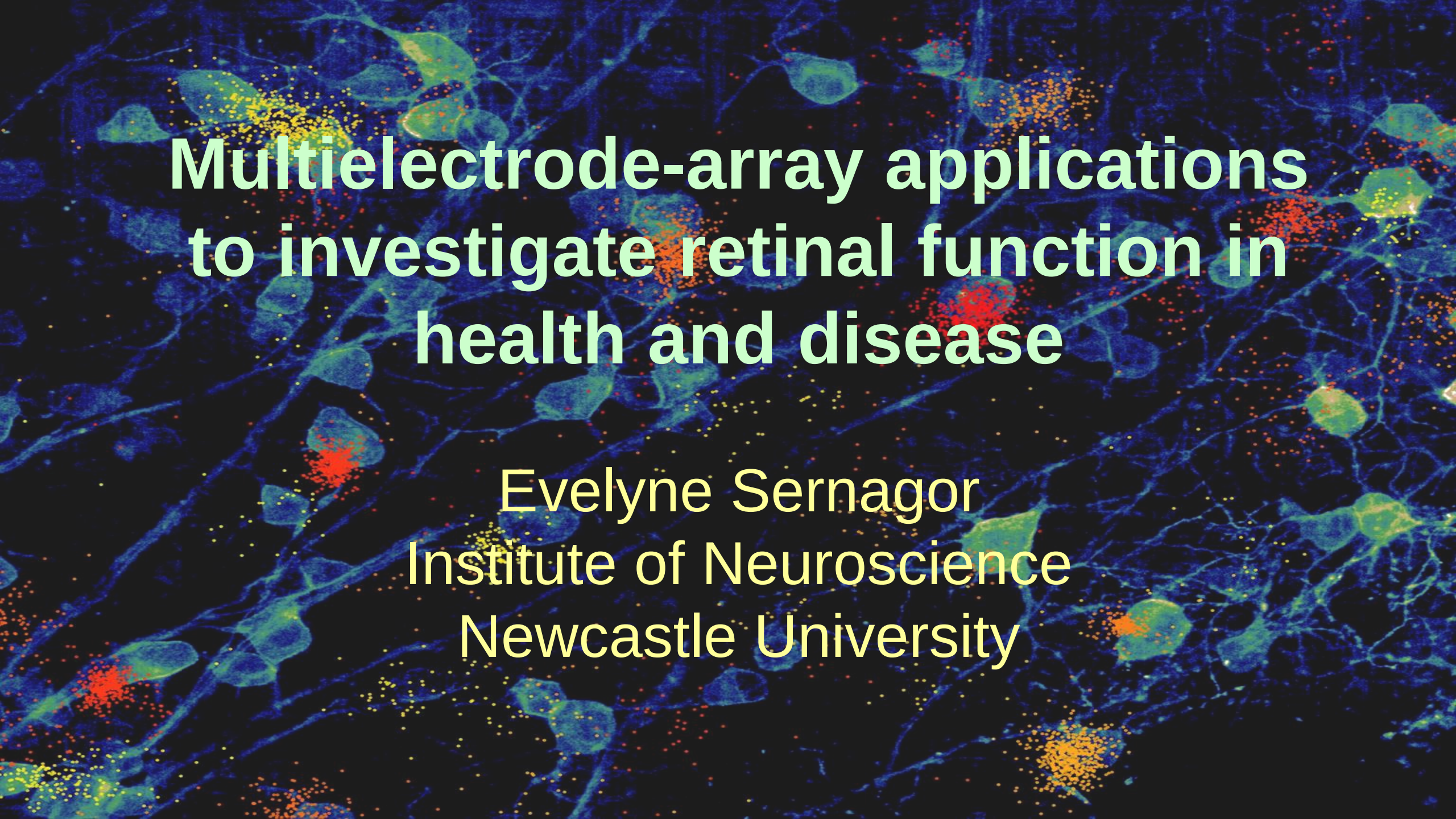


Multielectrode-array applications to investigate retinal function in health and disease

Evelyne Sernagor
Institute of Neuroscience
Newcastle University

Newcastle
Gerrit Hilgen, John Barrett, Cyril Eleftheriou

Edinburgh
Matthias Hennig, Martino Sorbaro, Oliver Muthmann, Sahar Pirmoradian

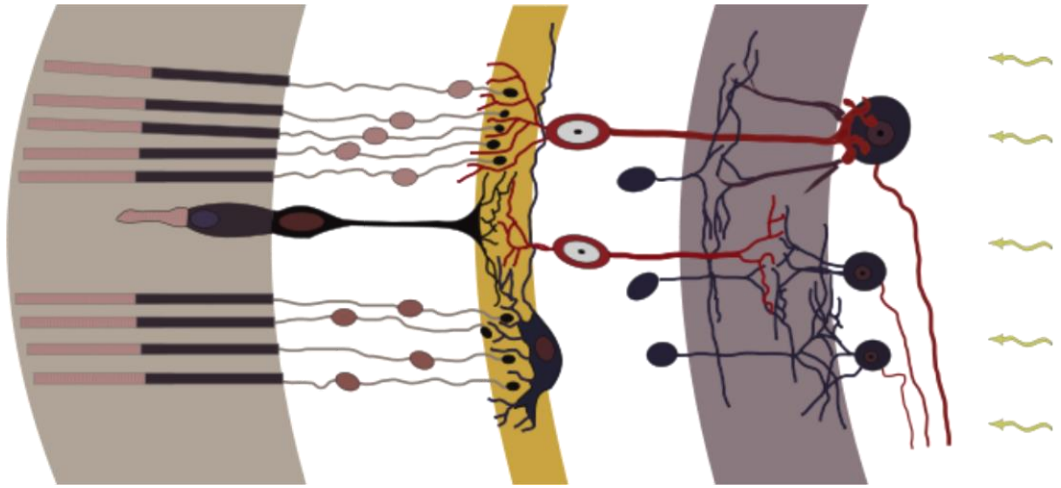
INRIA (Sofia Antipolis)
Bruno Cessac, Pierre Kornprobst, Geoffrey Portelli, Daniela Pamplona

IIT (Genova)
Luca Berdondini, Alessandro Maccione, Mauro Gandolfo, Ibolya Kepiro

Cambridge
Stephen Eglen

Retinal information processing

Visual scenes → Information processing → Spike trains
RETINAL CODE



Brain visual areas

Plasticity in RGC signalling

In health (during development and adulthood)

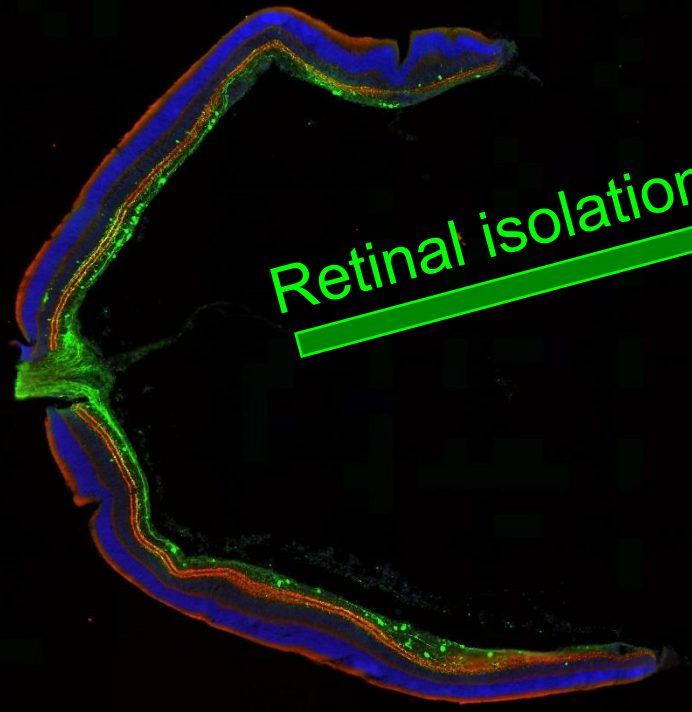
- Spatiotemporal properties of spontaneous waves of activity sweeping across the neonatal RGC layer (important for guiding the wiring of visual connections in the retina and in retinal projections).
- RGC responses to light in various conditions
- RGC classification

In disease (degeneration and repair)

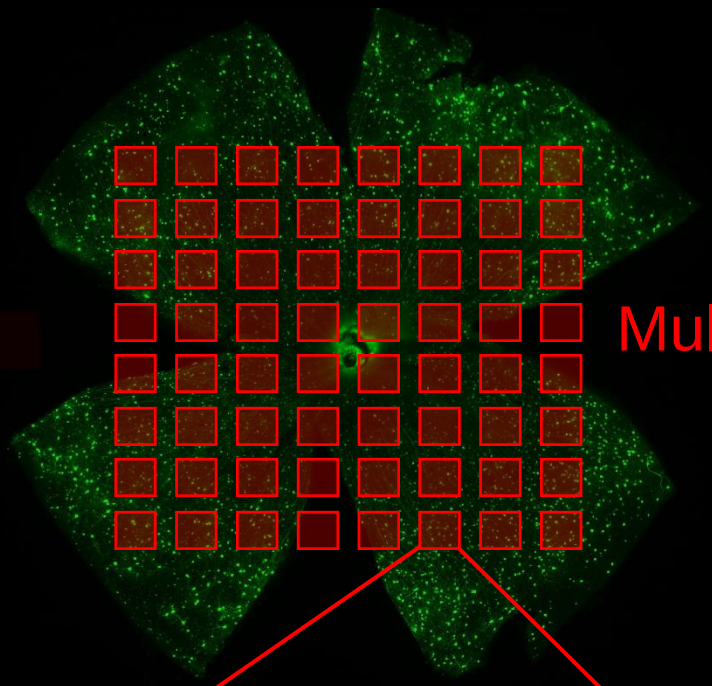
- Targeting RGCs for direct electrical or optogenetic stimulation in outer retinal dystrophies (rod/cone degeneration)
- Stem cell repair

All these projects require very close collaboration with computer scientists, physicists, mathematicians, engineers

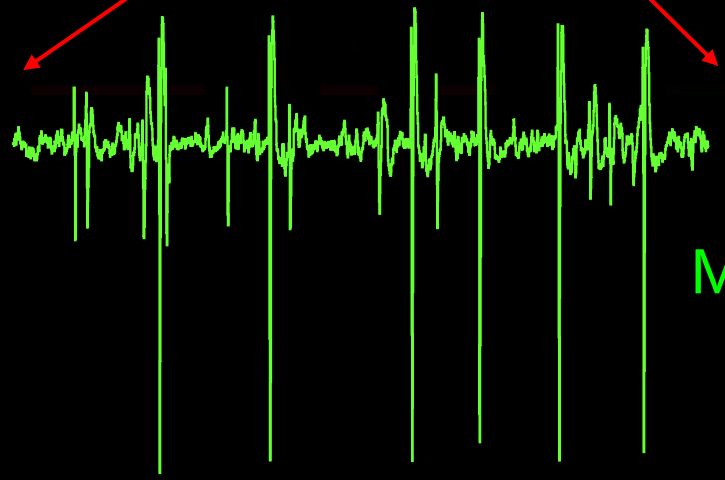
How can we record from many retinal ganglion cells simultaneously?



Retinal isolation and flattening

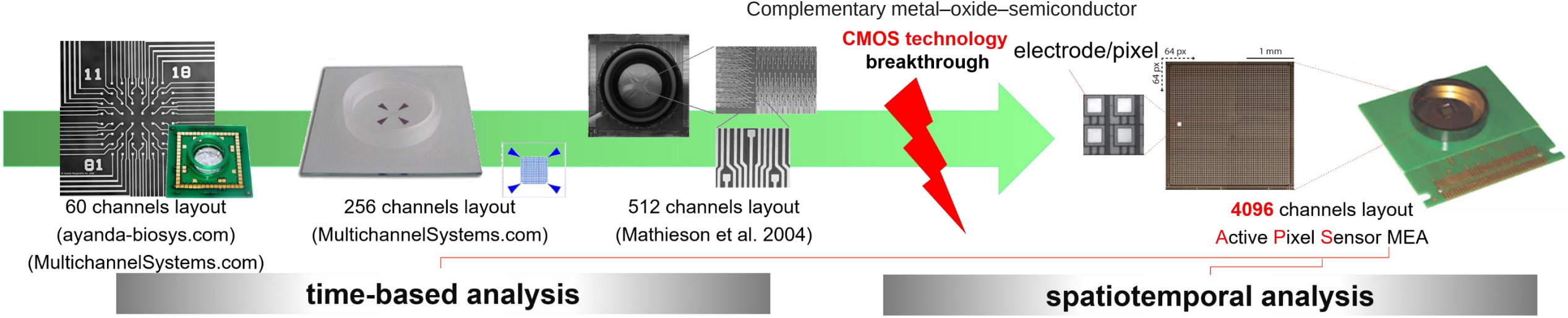


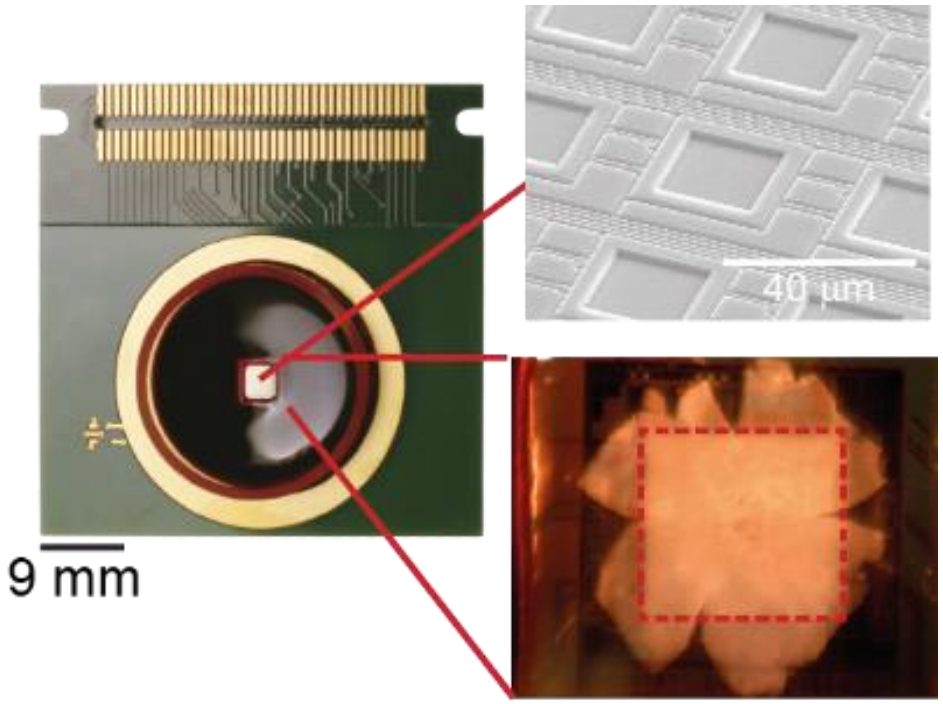
Multielectrode array (MEA)



Eye cross-section

Multi unit signals





From Maccione et al., J Physiol. 2014

Active Pixel Sensor (APS) MEA (3Brain)

Camera chip

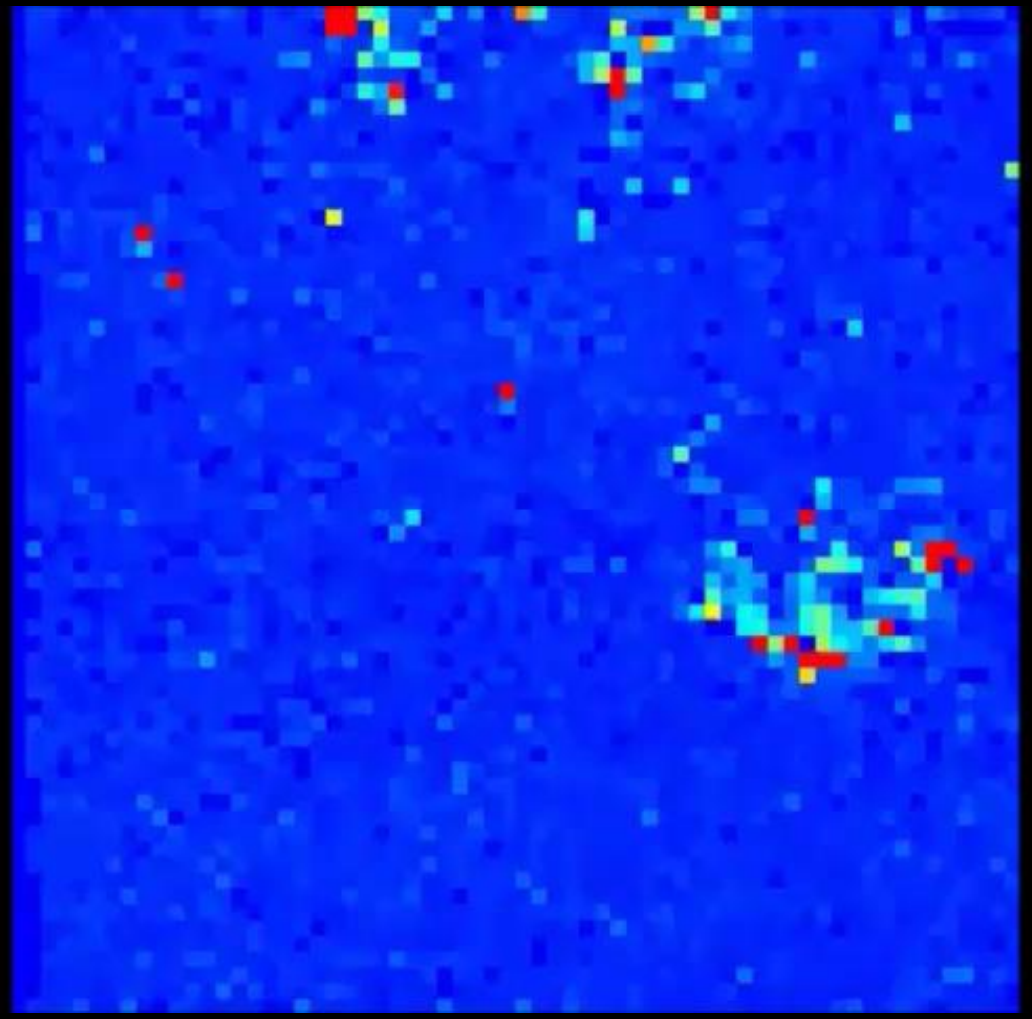
Pixels are metallic electrodes instead of light sensors

- 4,096 electrodes (64x64 array) – 7.12 mm^2
- Spatial resolution: 21 µm (el. pitch 42 µm)

Resolution comparable to neuronal somata in intact networks

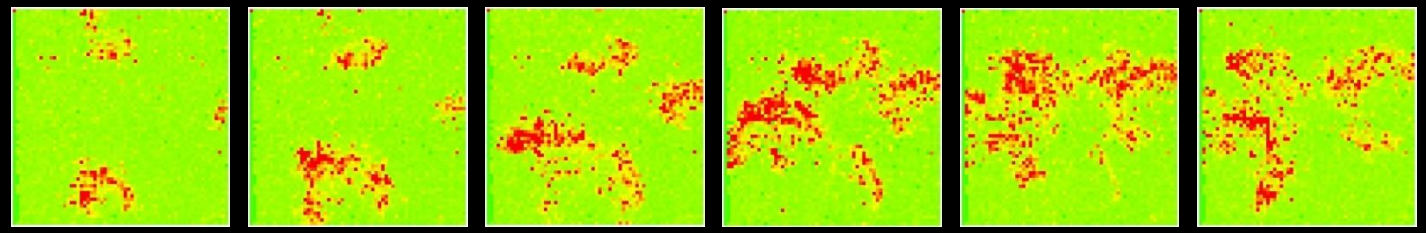
- Acquires at full frame rate of 7.7kHz (new model @18kHz)

The APS MEA gave us a completely new, pan-retinal perspective of network activity, with unprecedented spatial and temporal resolution



Retinal waves

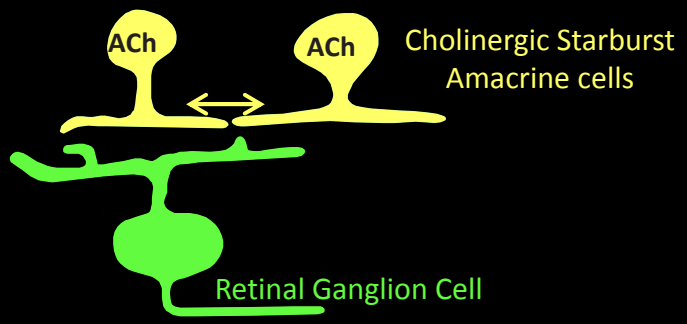
Sweep across the RGC layer during a limited period in perinatal life



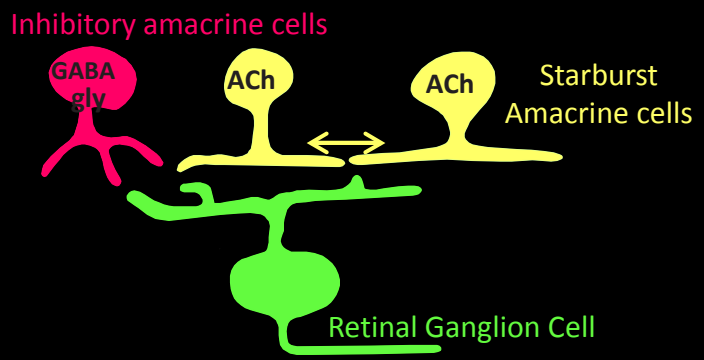
Strong evidence that they guide wiring throughout the visual system

Disrupting this early activity may lead to irreversible disorders (amblyopia)

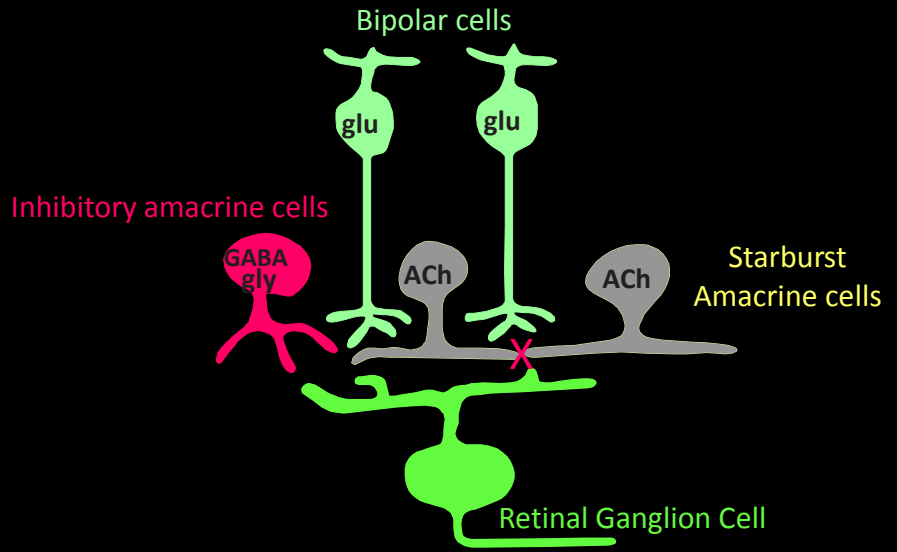
Stage II (late gestation to P9)



Stage II + GABA (P4-P9)



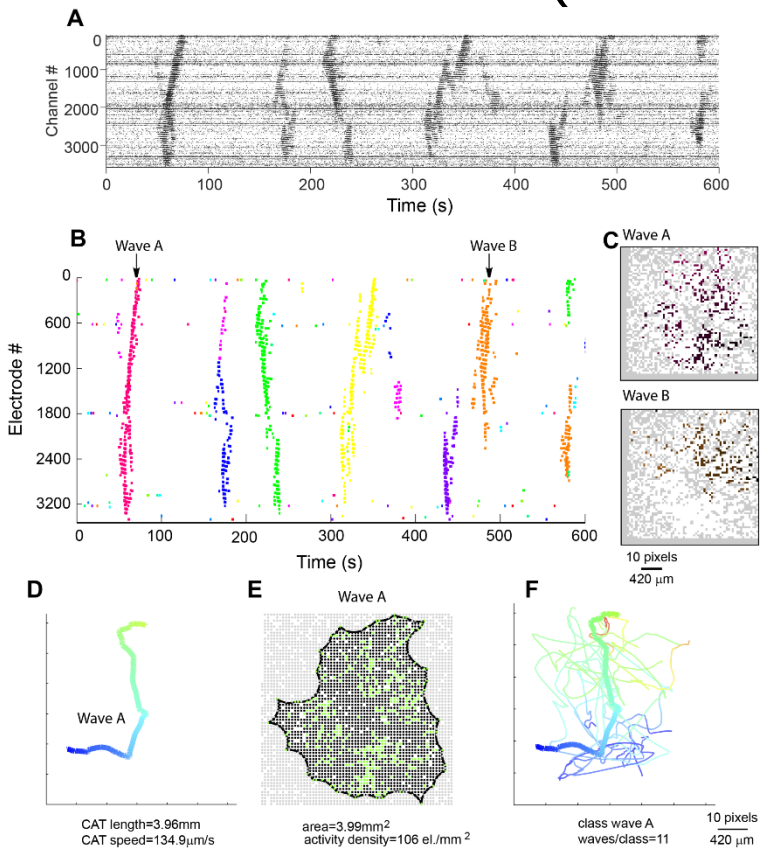
Stage III (P10-P13)

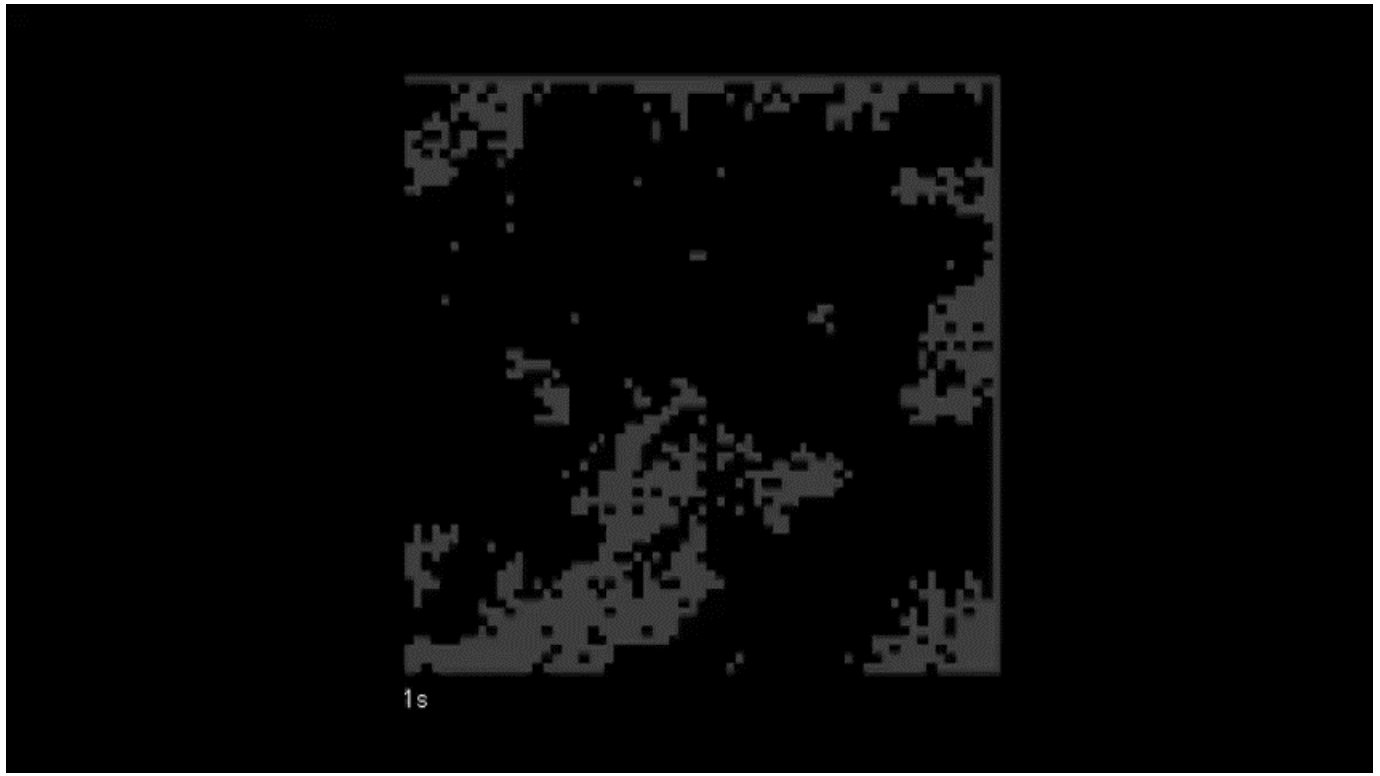


Eye opening occurs at P12

Various tools were developed (Matlab and R), allowing us to perform detailed longitudinal analysis of wave spatiotemporal properties

Matthias Hennig and Oliver Muthmann (Edinburgh), Stephen Eglen (Cambridge), Mauro Gandolfo (IIT Genova)



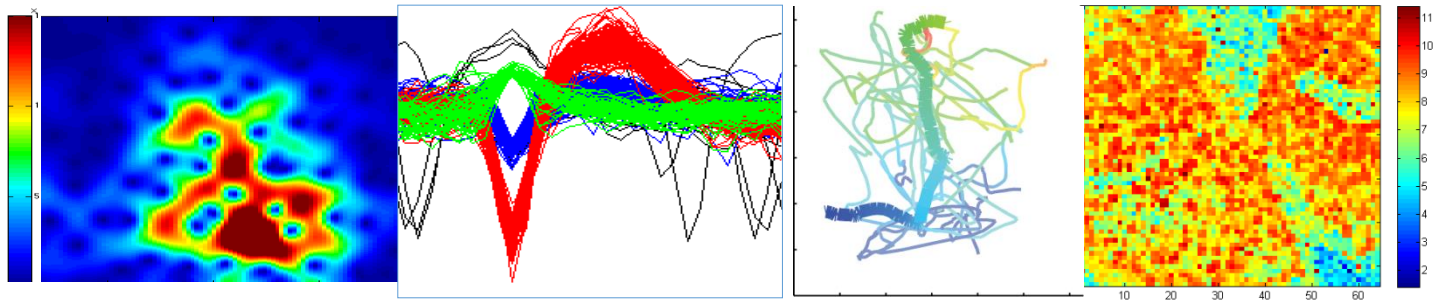


Maccione et al, J Physiol 2014

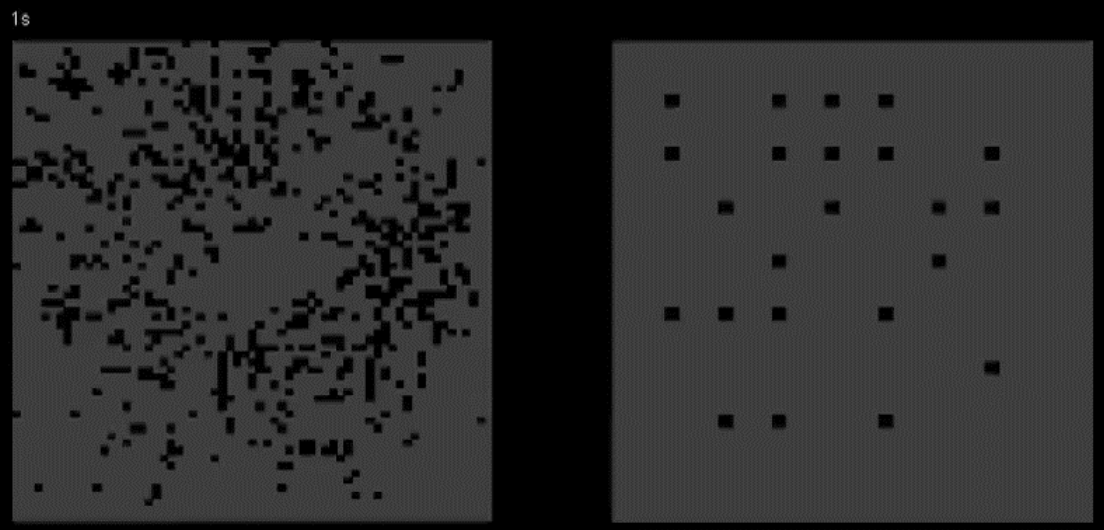
Retinal waves analysis tools were deployed on CARMEN

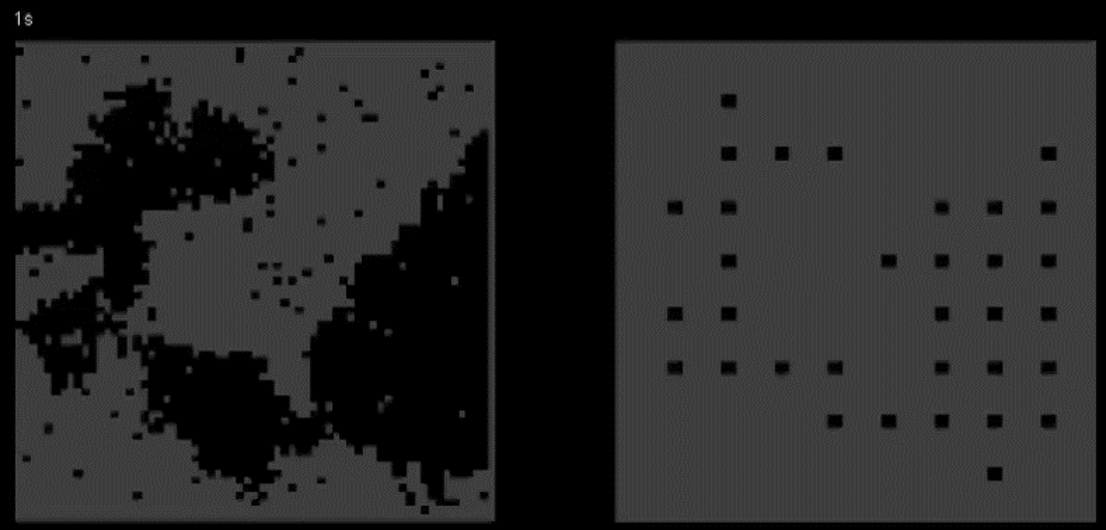
Code **A**nalysis, **R**epository & **M**odelling for **e-N**euroscience

Portal based collaborative facility allowing neuroscientists to share neurophysiological data and analytical tools. Accessed via standard web browsers.



Wave patterns change with development





Stage II waves:
Slow
Widespread
Relatively sparse cellular recruitment
Random propagation patterns

Stage III waves:
Faster
Spatially restricted
Denser (more cells recruited)
Few, repetitive propagation patterns

From Maccione et al. J Physiol 2014

To investigate receptive field properties, signals must be separated and assigned to single neurons
SPIKE SORTING/CLUSTERING

In extracellular recordings (*in vitro* and *in vivo*):

-Each electrode records from several adjacent cells

-Spikes belonging to different cells traditionally separated according to waveform features (Principal Components Analysis)

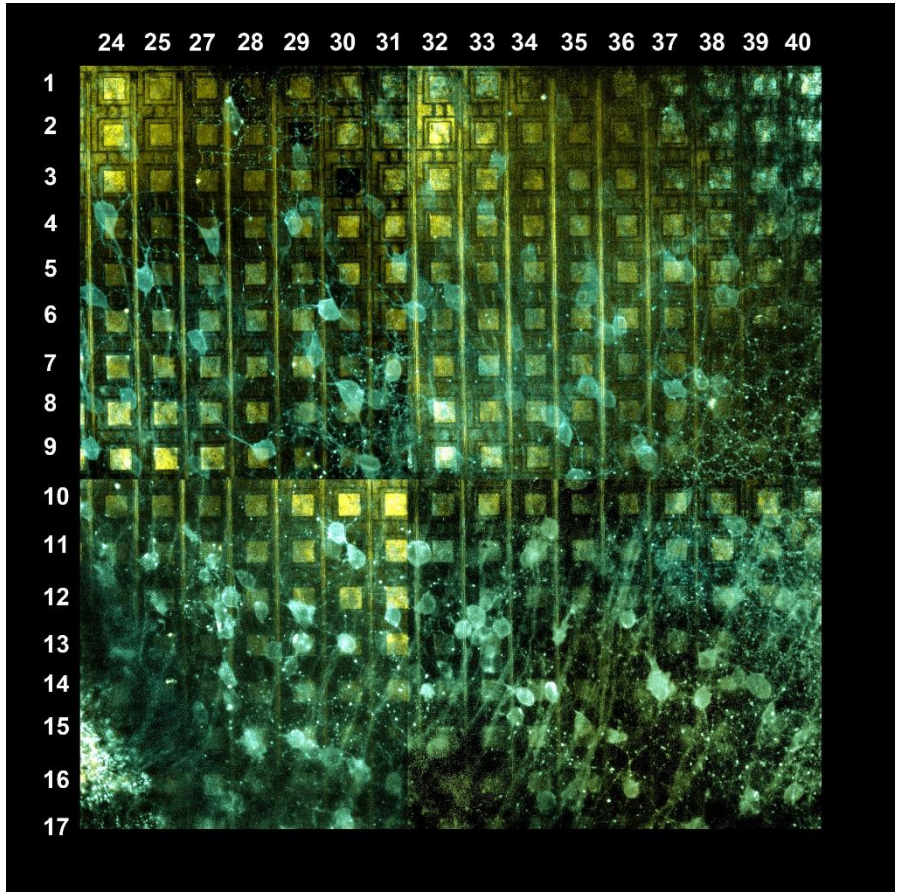
Spikes in high density arrays

-Each electrode records from several adjacent cells

-Spikes generated by a single cell are often recorded by several adjacent electrodes

-Different cells may generate similar looking signals on a single electrode

-It becomes extremely challenging to accurately separate signals originating from distinct cells (e.g. signals from On RGC and Off RGC can be pooled into a false On-Off cell)



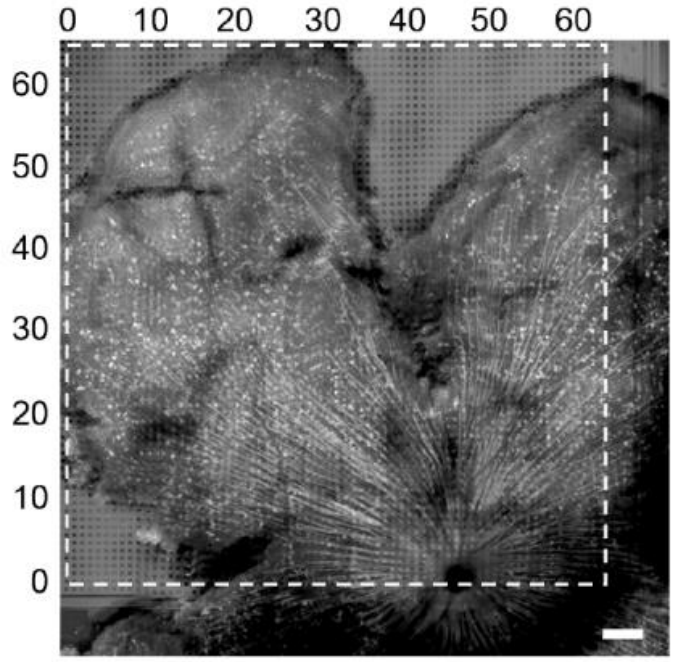
Thy1-EYFP, expressed in ~40% of RGCs

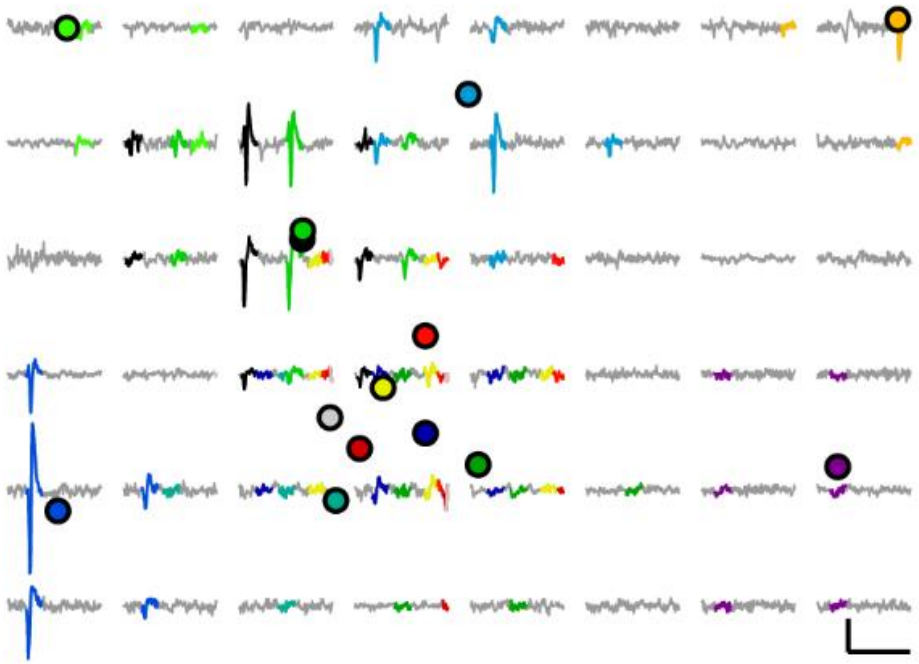
New fast and automated method exploiting the dense sampling of single neurons by multiple electrodes

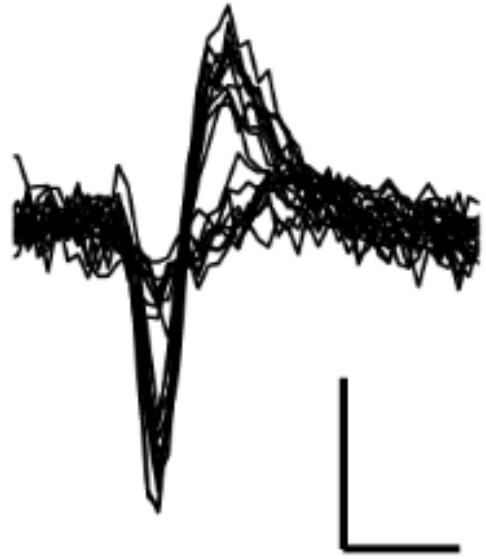
Matthias Hennig, Oliver Muthmann, Martino Sorbaro (Edinburgh)

Based on spike clustering according to spatial current source locations (X/Y) and dominant spike shape features (principal components)

First step: finding the centre of mass for coincident spikes on neighbouring electrodes





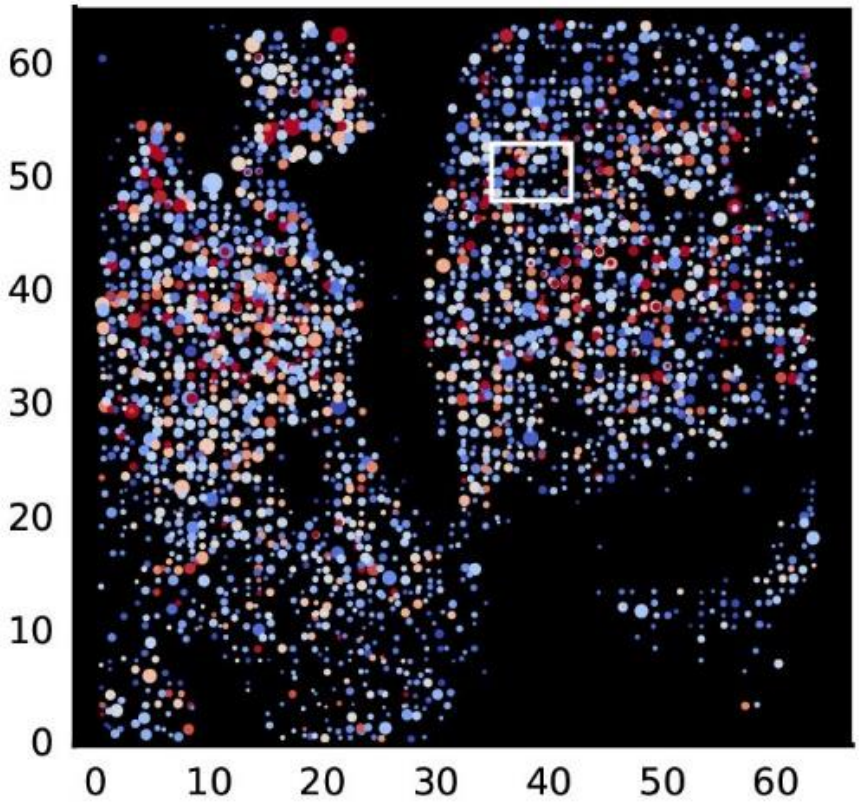


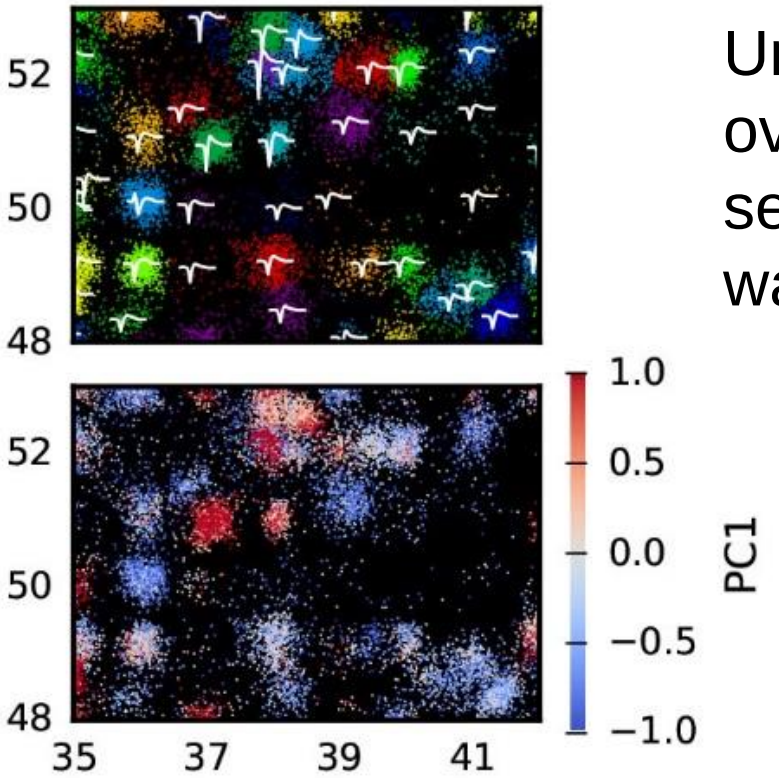
2 cells, clustering necessary

Second step: spike clustering

Shape features extracted from average waveforms are combined with spatial locations.

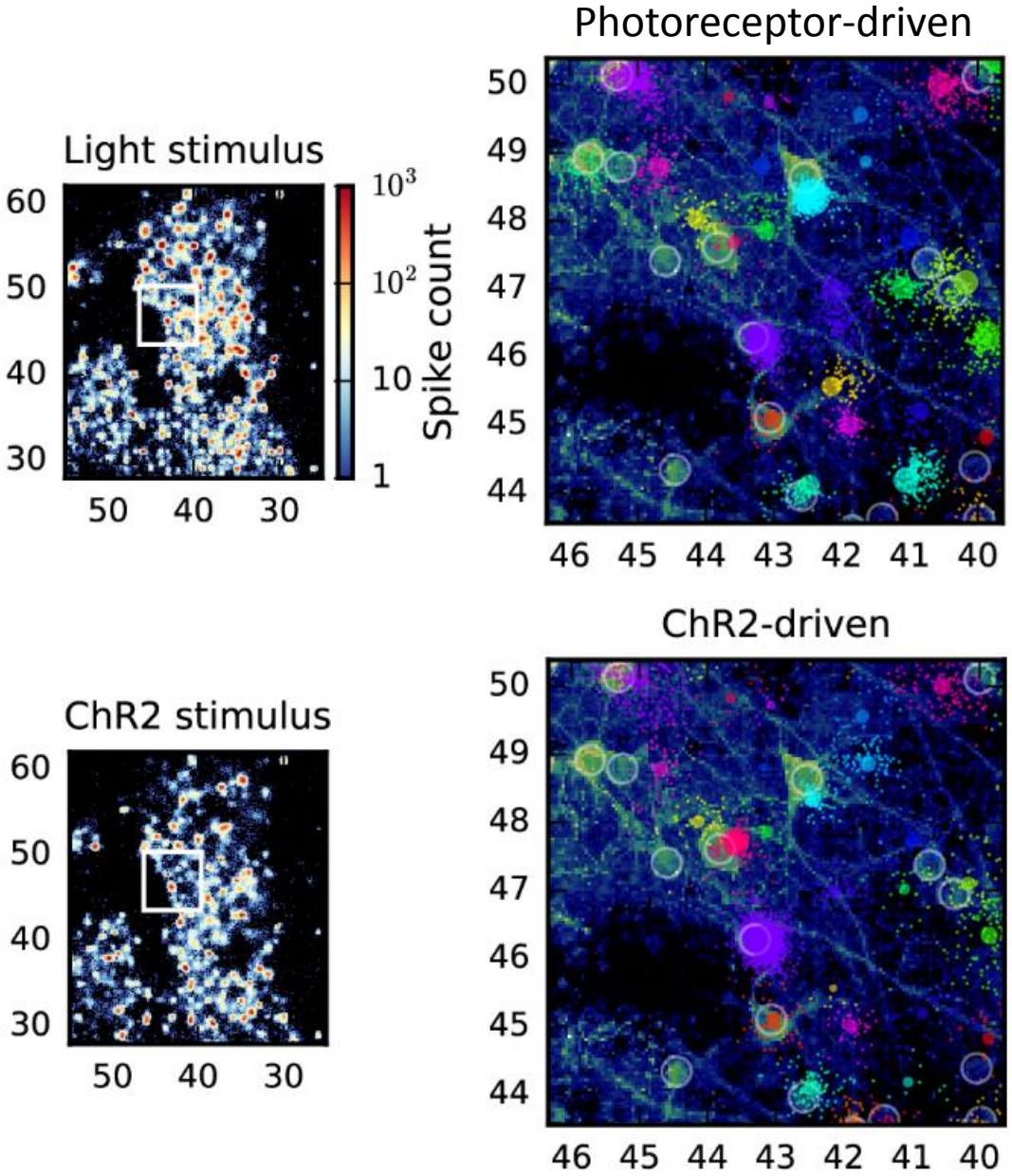
Clustering requires only 4 dimensions: X, Y, PC1, PC2
Can be performed in minutes for millions of events





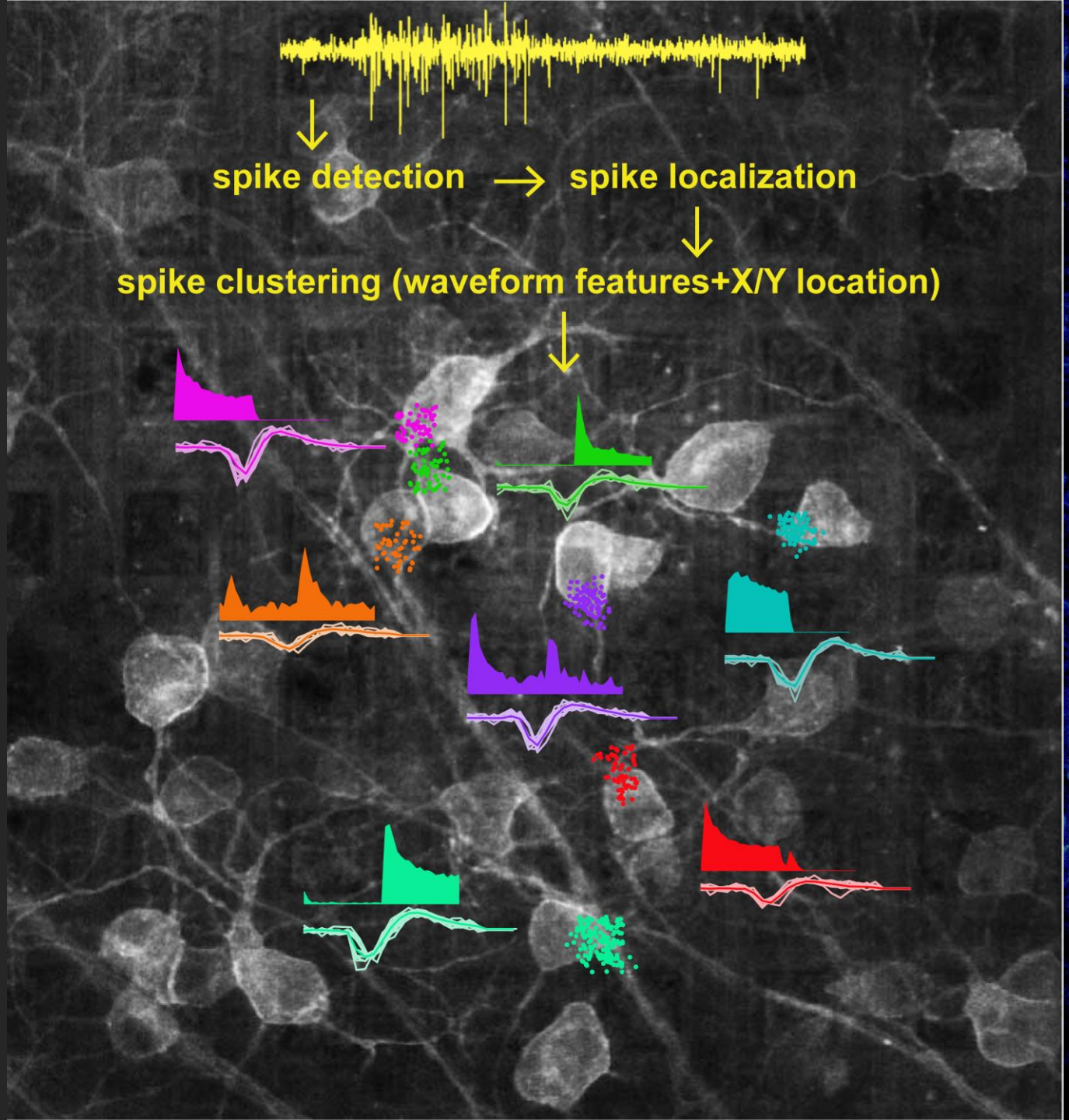
Units may spatially overlap, but are well separated by their waveform features

Method validated with optogenetics



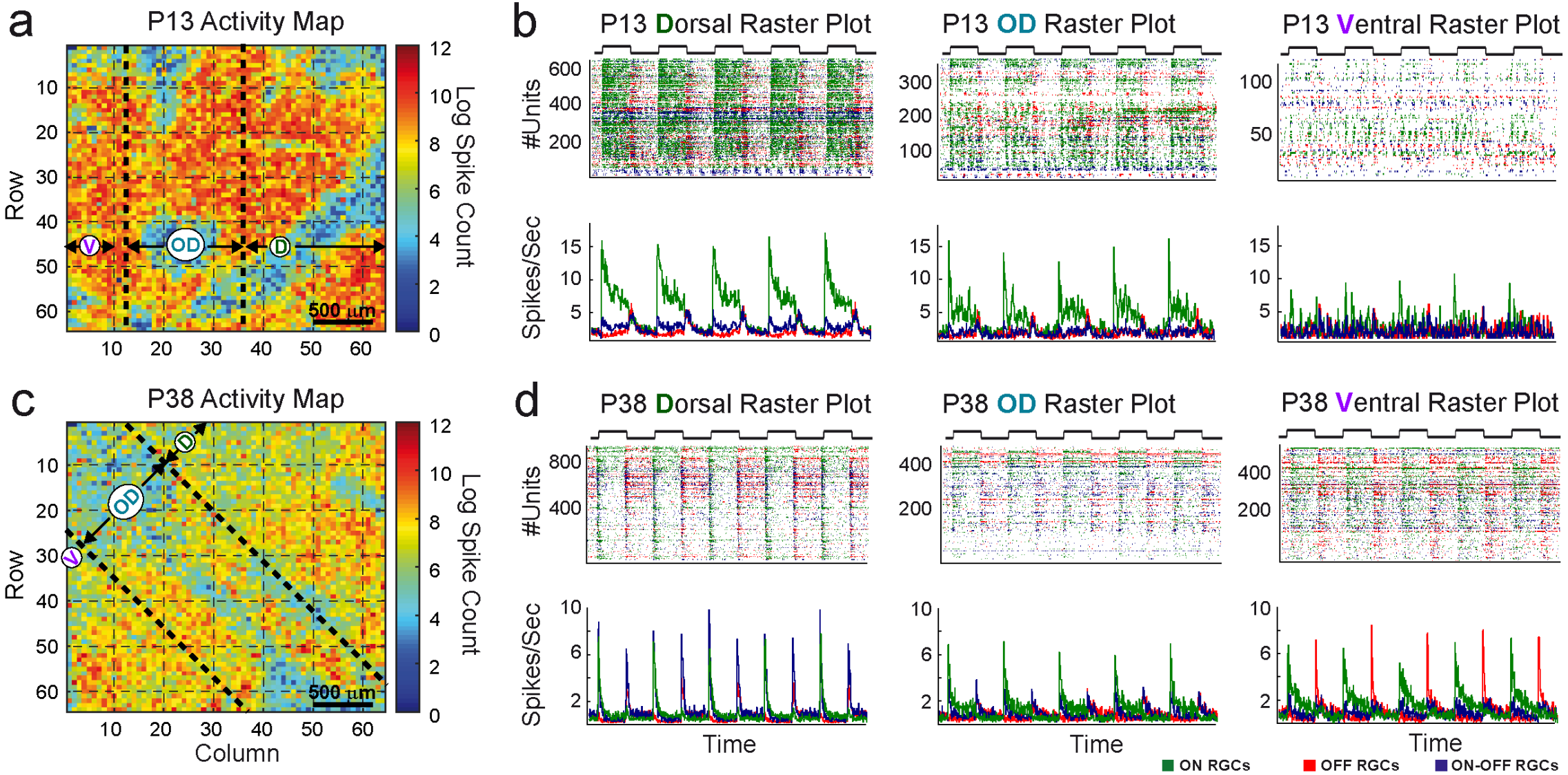
We use Thy1 as a promoter for ChR2 expression → 40% RGCs express ChR2 and YFP

Responses from photoreceptors pharmacologically blocked



Paper in press in Cell Reports

Developmental study of RGC light responses
At eye opening, dorsal RGCs have the strongest responses to light



Hilgen et al. 2017, Sci Rep. 2017 Feb 10;7:42330

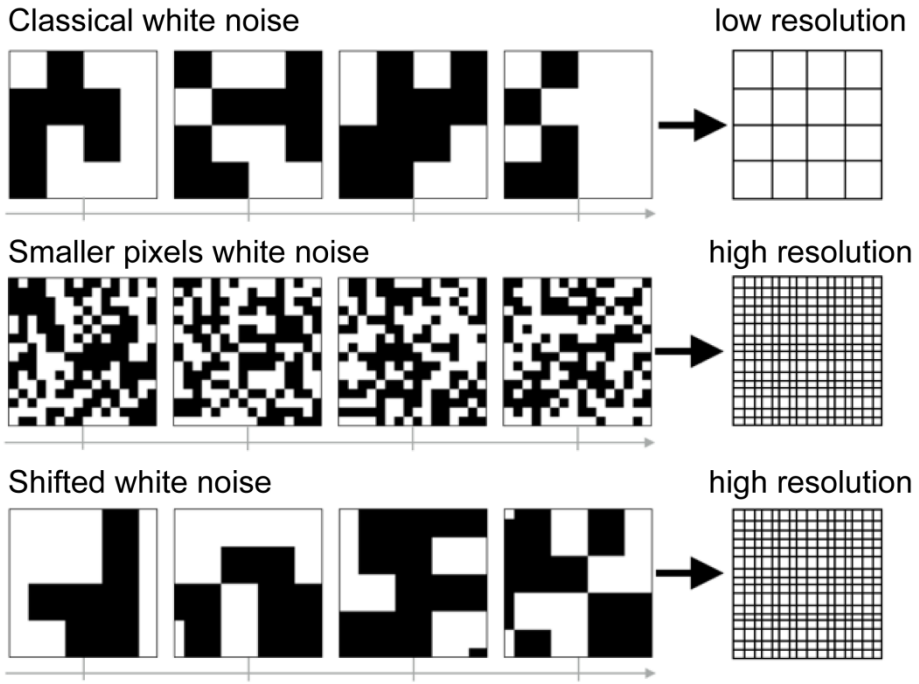
High resolution receptive field measurements using spike-triggered average responses to white noise

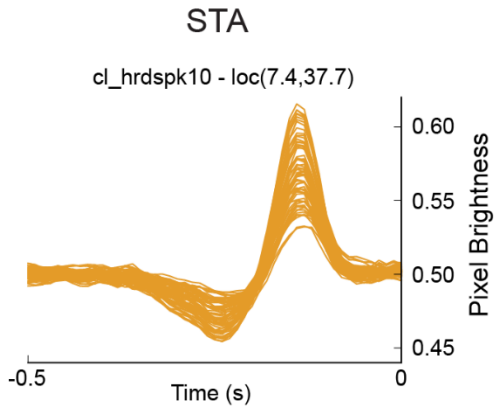
(Pierre Kornprobst, Bruno Cessac, Daniel Pamplona, INRIA, Matthias Hennig, Sahar Pirmoradian, Edinburgh)

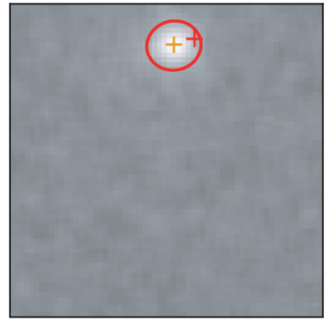
Reducing the size of the pixels too much decreases the likelihood to detect light responses

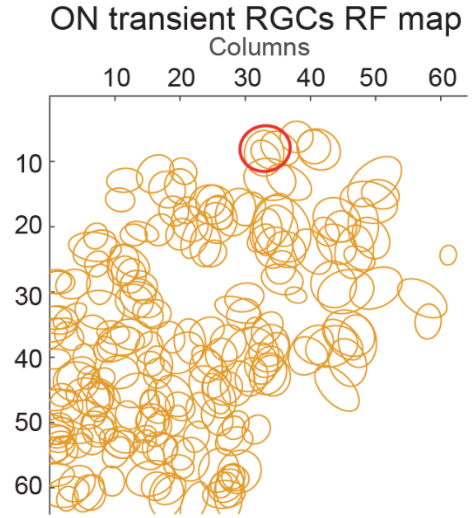
Shifted white noise has large pixels (160 μm), hence stronger responses

The resolution is given by the shift (40 μm)









This high resolution approach allowed us to measure RFs in very young cells with unstable light responses

Hilgen et al. 2017, Sci Rep. 2017 Feb 10;7:42330

RGC population encoding of visual information

Rate coding:
Most traditional view
Information is encoded by changes in firing rate of individual neurones

Latency coding:
Was demonstrated in the salamander retina (Gollisch and Meister, 2008)
Concerted spiking of RGC pairs encodes spatial information

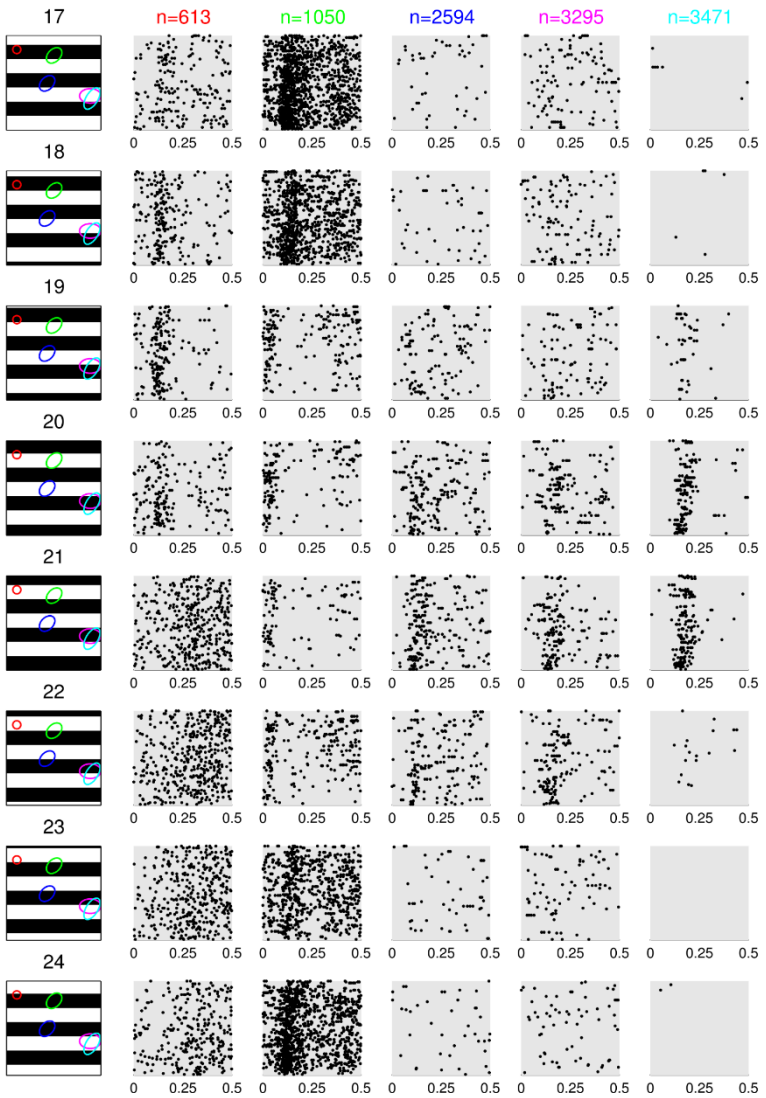
We did not find any spatiotemporal information in the relative latencies of RGC pairs responding to light in the mouse retina

RGC population response described with relative activities, or ranks, provides more relevant information than classical independent spike count- or latency- based codes

The wave of first stimulus-evoked spikes (WFS) is an accurate indicator of stimulus content

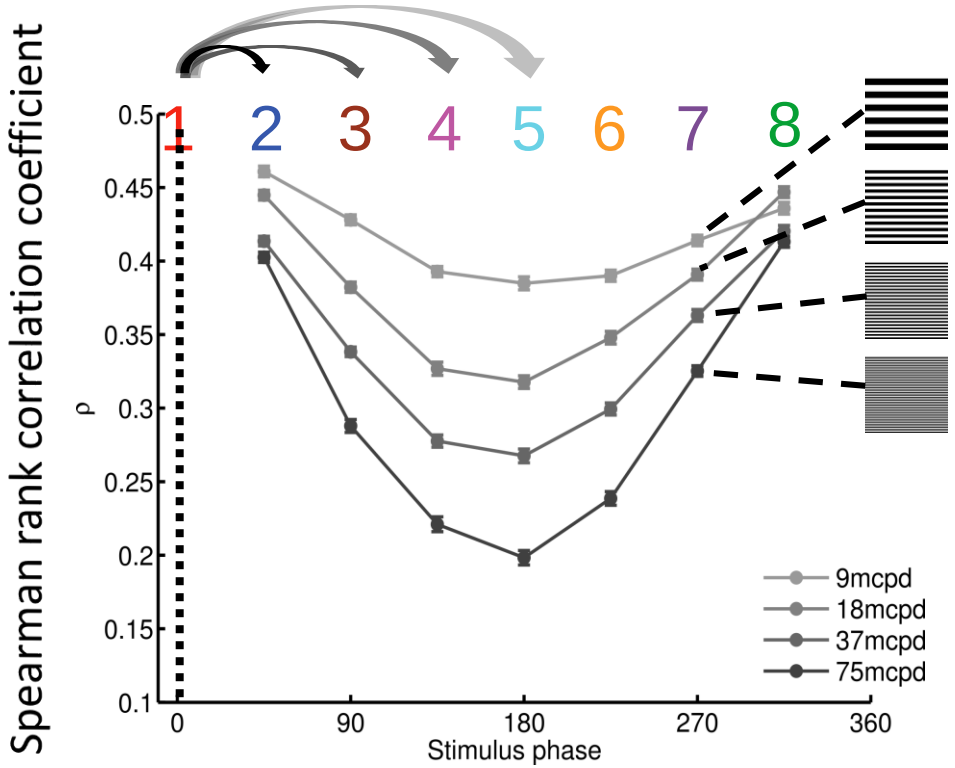
Portelli et al., eNeuro May 2016, 3 (3) ENEURO.0134-15.2016

Stimuli: stationary gratings of varying spatial frequencies presented at 8 different phases



There is no latency tuning to the grating phase
Stimulus modulates spike count for some cells

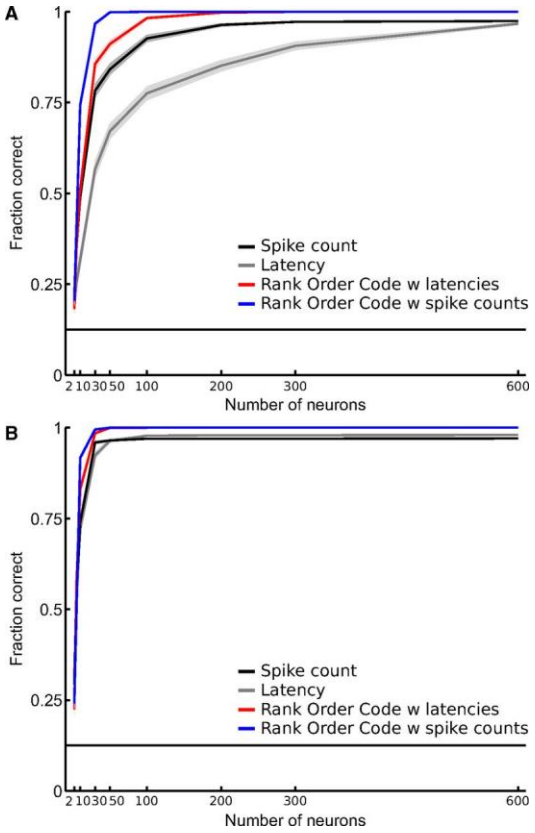
Spearman's Rank Correlation Coefficient ρ: nonparametric measure of statistical dependence used to quantify the differences between the WFS obtained with gratings of different phases



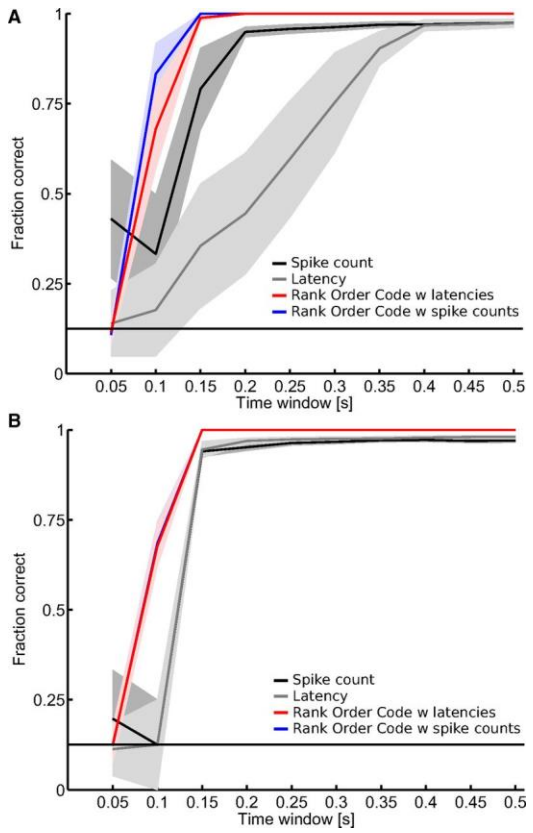
The ρ varies cyclically with the phase of the gratings

A classical supervised Bayesian classifier was used to test the independent spike count code, the independent latency code, the WFS, and a correlated spike count code. Discrimination task: identifying the phase φ ∈ {0, 45, 90, 135, 180, 225, 270, 315}° among the 8 gratings for a given spatial frequency.

Discrimination performance as a function of the number of RGCs

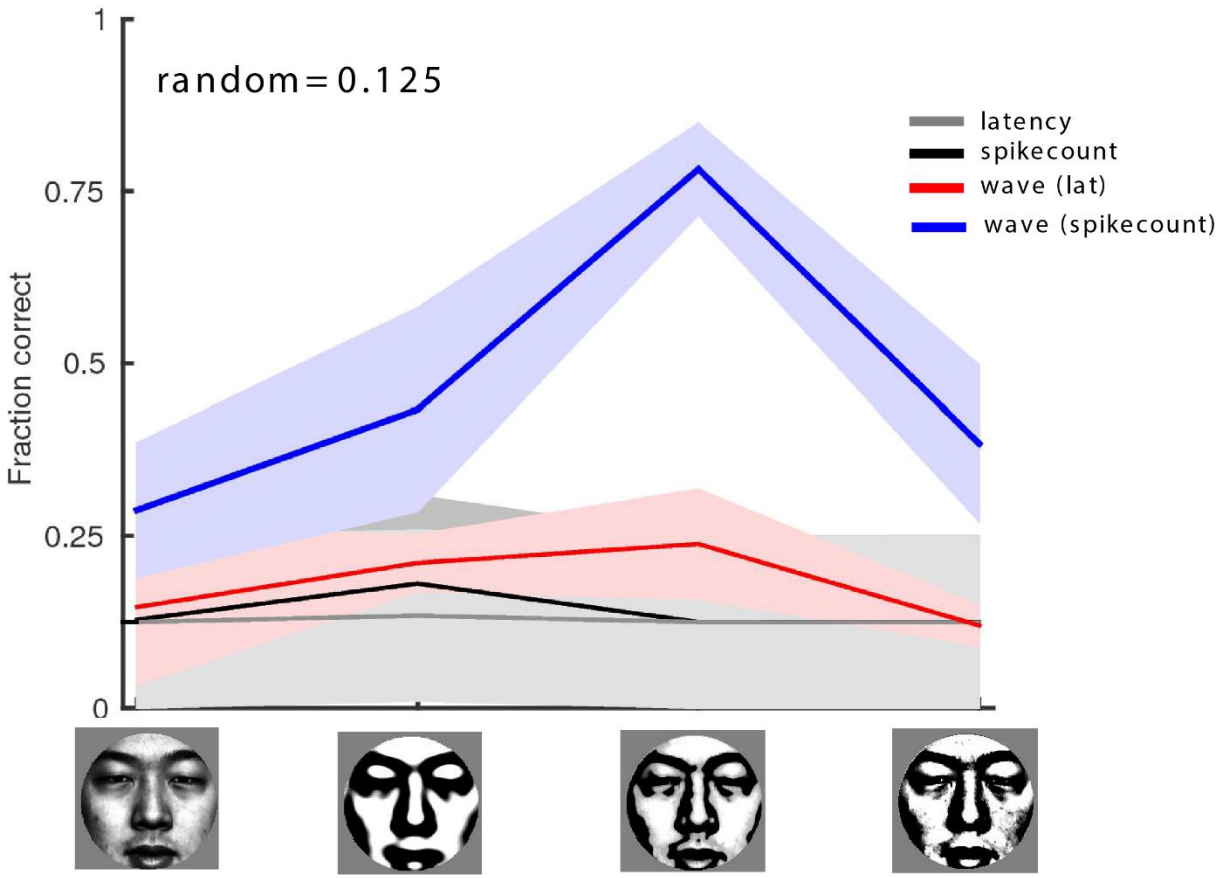


Discrimination performance as a function of the time window after the stimulus onset



Same approach to study discrimination between transformations of natural images

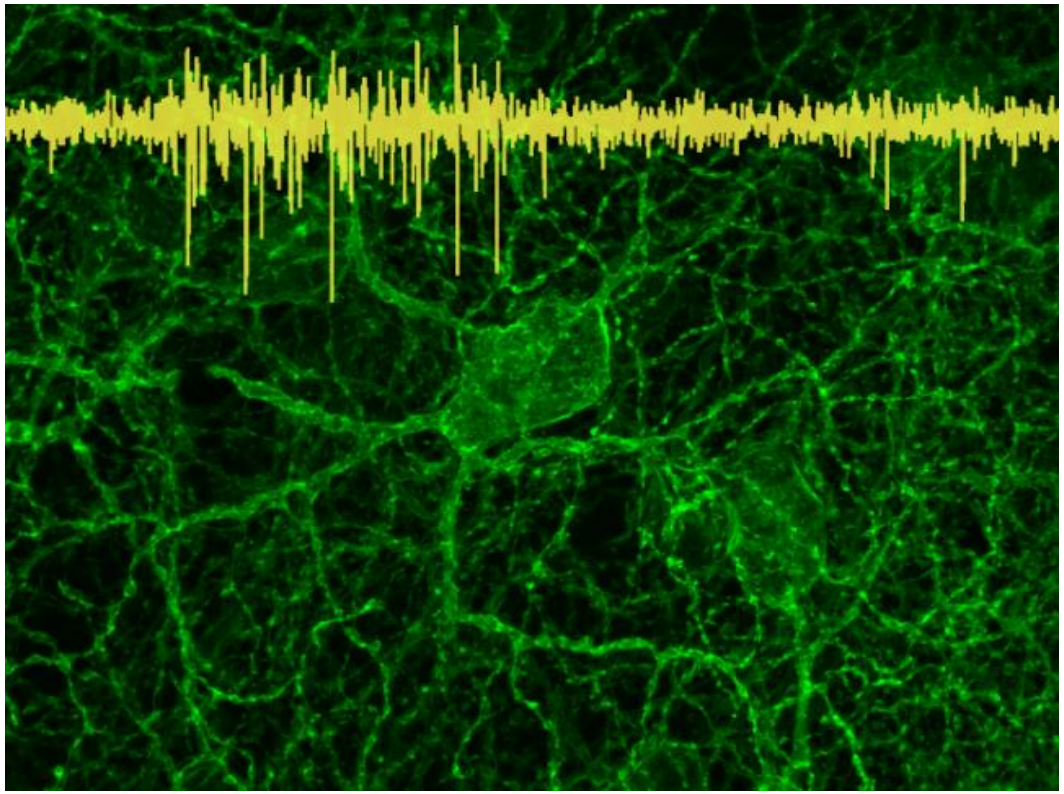
Work in progress…

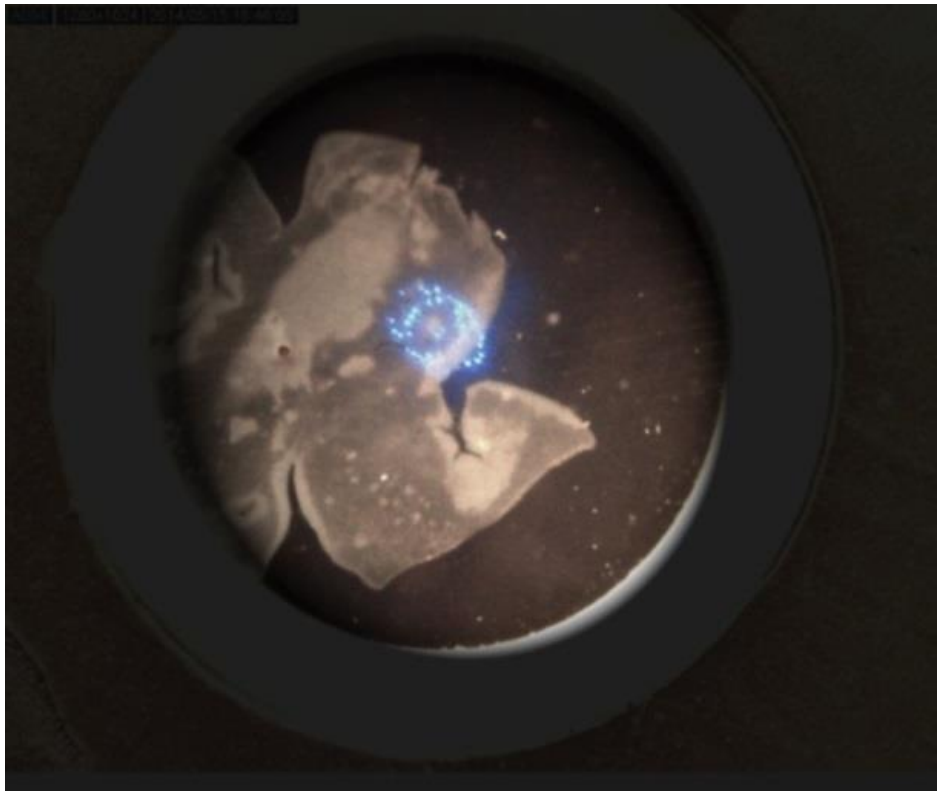


Exciting preliminary results

May be extremely important for the design of retinal prosthetics

Optogenetic stimulation of retinal ganglion cells in a mouse model of retinitis pigmentosa (John Barrett)



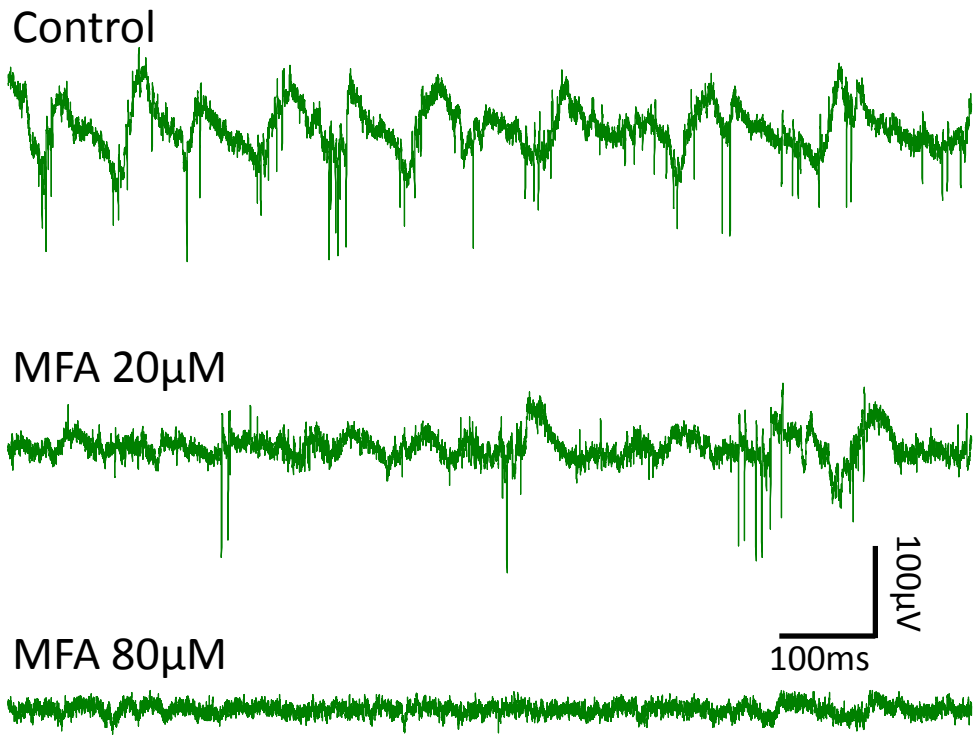


Thy1-ChR2 rd1 mouse

Spontaneous oscillations in dystrophic retinas

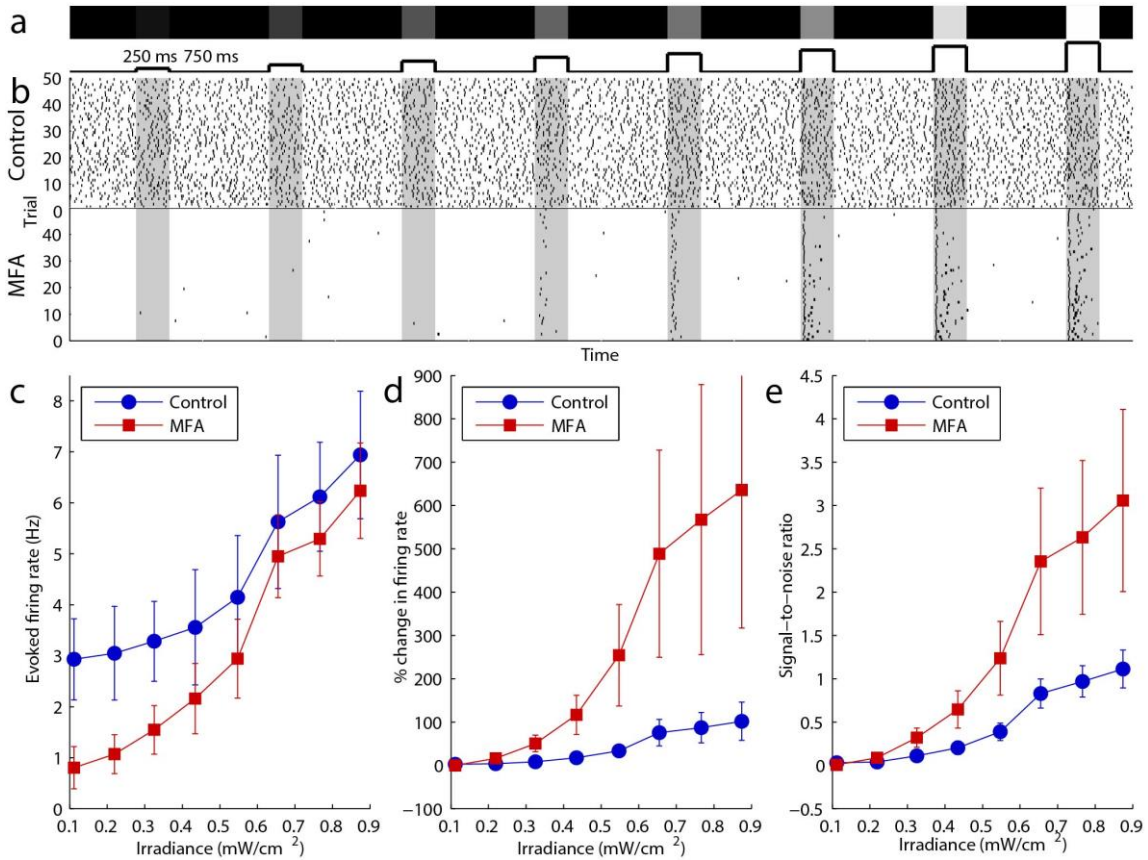
These oscillations reduce the signal-to-noise ratio of evoked responses

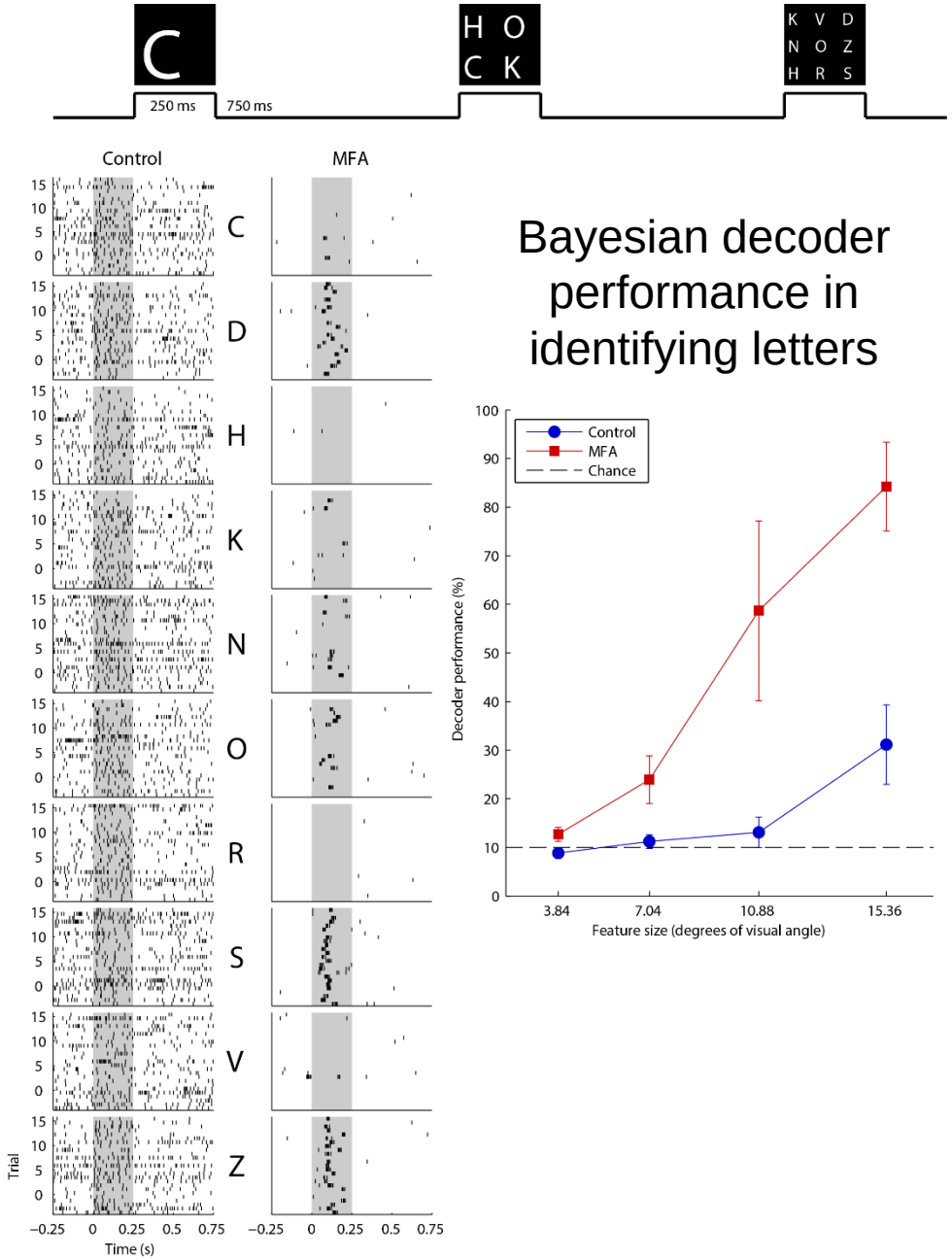
They can be silenced with gap junction blockers



MFA: meclofenamic acid

Barrett et al., Front Neurosci 2015





Bayesian decoder performance in identifying letters

Barrett et al., Sci Rep 2016

Newcastle
Gerrit Hilgen, John Barrett, Cyril Eleftheriou
Edinburgh
Matthias Hennig, Martino Sorbaro, Oliver Muthmann, Sahar Pirmoradian

INRIA (Sofia Antipolis)
Bruno Cessac, Pierre Kornprobst, Geoffrey Portelli, Daniela Pamplona

IIT (Genova)
Luca Berdondini, Alessandro Maccione, Mauro Gandolfo, Ibolya Kepiro

Cambridge
Stephen Eglen

RENVISION

SEVENTH FRAMEWORK PROGRAMME

BBSRC
bioscience for the future

wellcome trust

CARMEN
ENHANCING COLLABORATION IN NEUROSCIENCE

EPSRC

Newcastle University

3·Brain
high resolution technology