# A Biological Position Sensitive Detector: the Retina

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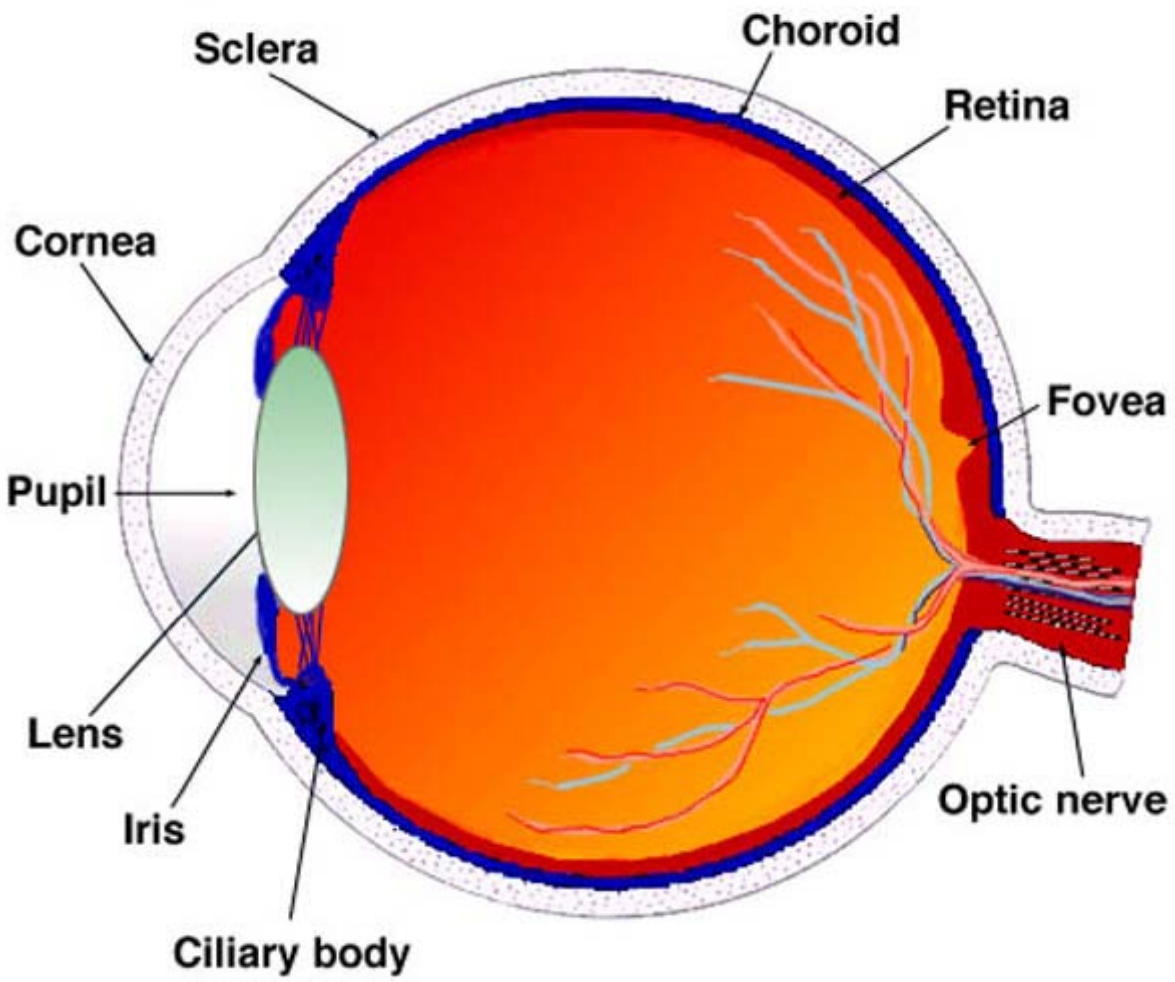
SCIPP/UCSC
2 September 2008

1. Architecture
2. Measurement of functional properties
3. Functional Organization
4. What can be done when the retina malfunctions?: retinal prosthesis studies
5. Fabrication: retinal development studies (first steps)
6. Conclusions and Outlook

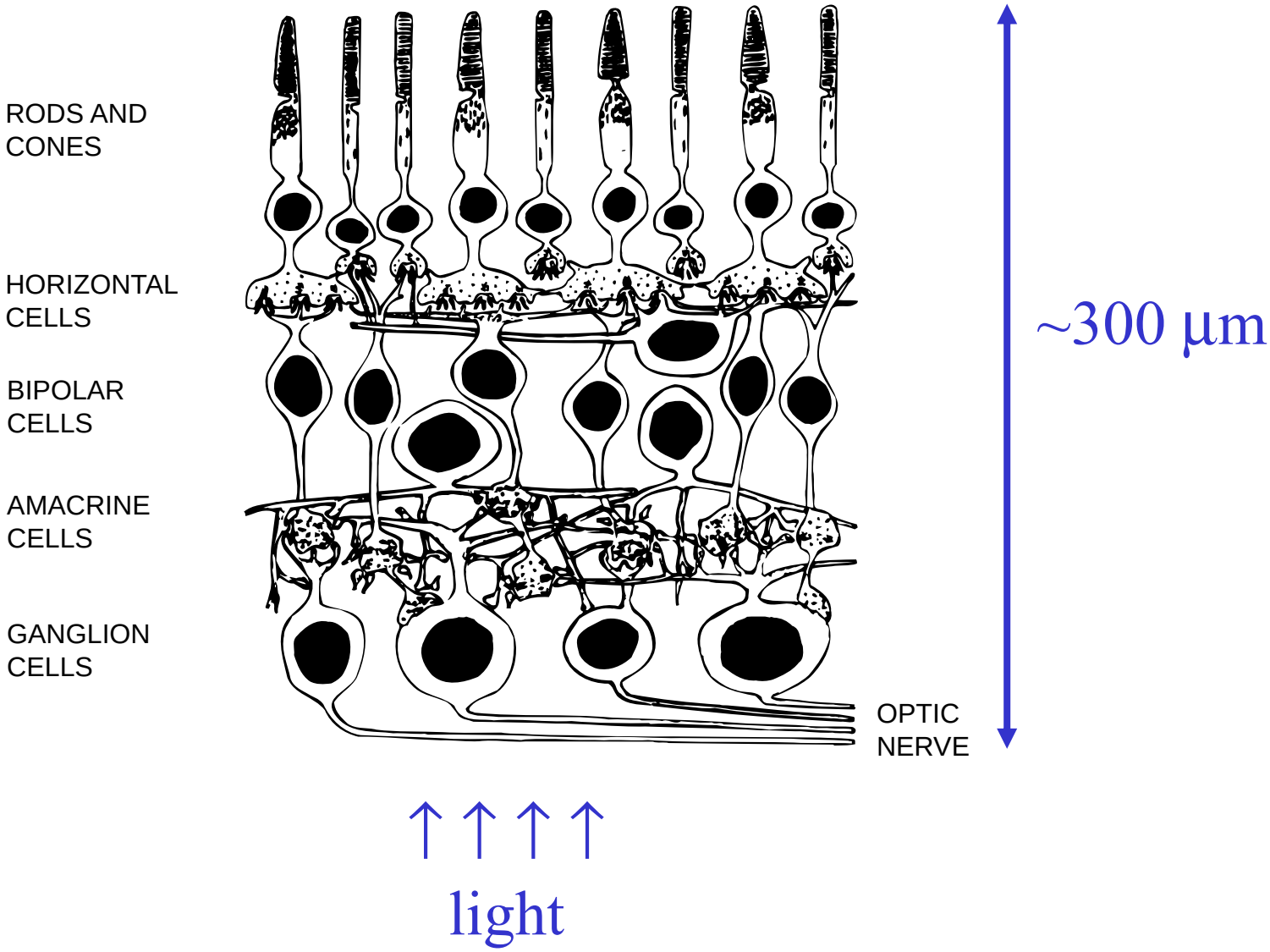
# Collaborators

- SCIPP/UC Santa Cruz:
  A. Grillo, M. Grivich, S. Kachiguine, D. Petrusca, A. Sher
- AGH U. of Science and Technology, Krakow (I C design):
  W. Dabrowski, P. Hottowy (now at UC Santa Cruz)
- U. Glasgow (electrode array fabrication):
  D. Gunning, K. Mathieson
- The Salk Institute (neurobiology):
  E. J. Chichilnisky, G. Field, J. Gauthier, M. Greschner, C. Sekirnjak, J. Shlens

# The Eye


Sclera
Choroid
Retina
Cornea
Fovea
Pupil
Lens
Iris
Optic nerve
Ciliary body

# The Retina

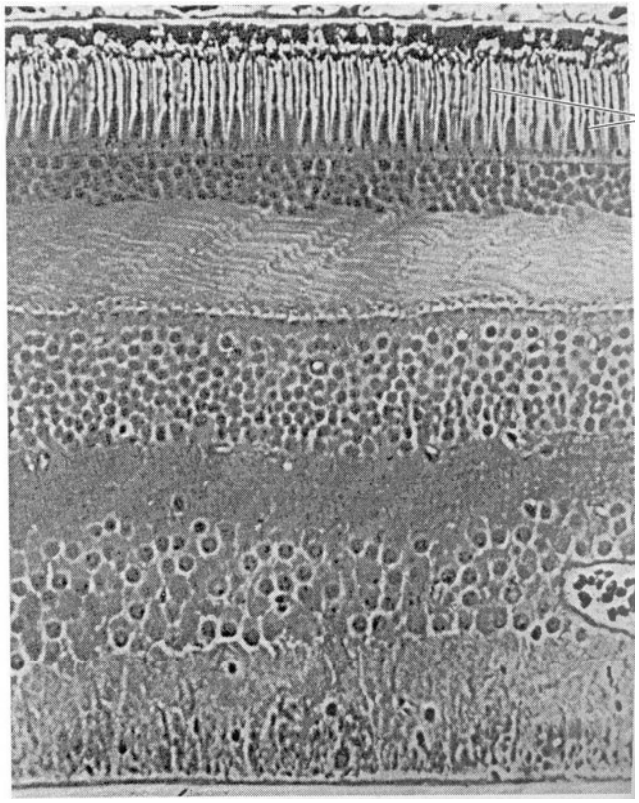

RODS AND
CONES
HORIZONTAL
CELLS
BIPOLAR
CELLS
AMACRINE
CELLS
GANGLION
CELLS
OPTIC
NERVE
~300 µm
light

# The Retina: A Biological Pixel Detector

- thickness: ~300 μm
- active area: 10 $cm^2$
- number of pixels: $10^8$
- number of output channels: $10^6$
- compression factor (# input channels/# output channels): 100:1
- output signal width: ~1ms
- output format: visual information encoded by pattern of digital signals ("spikes") on multiple parallel channels
- spatial resolution: down to 2 μm
- 3D (depth perception): stereoscopic vision
- radiation hardness: non-rad-hard
- technology: mature, reliable, and in wide-spread use

# The Retina

photoreceptors



outer plexiform layer

inner nuclear layer
(horizontal, bipolar,
amacrine cell bodies)

inner plexiform layer

ganglion cell layer

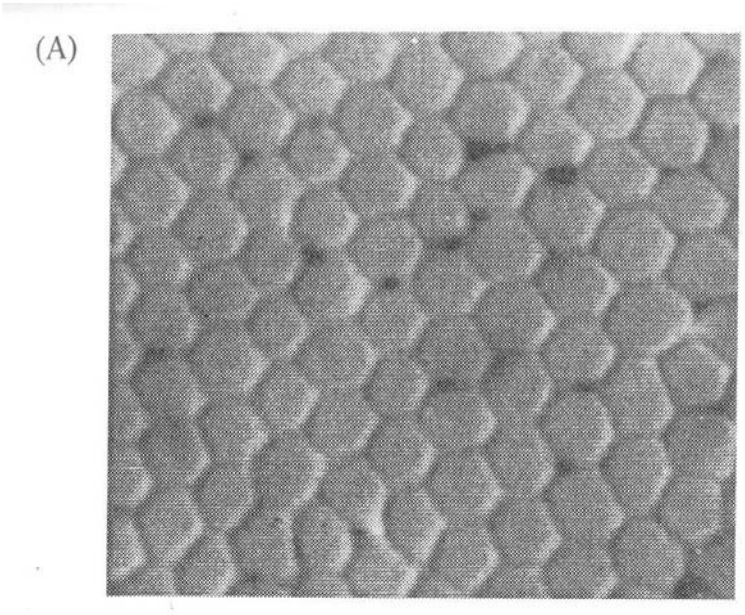
nerve fiber layer

↑ ↑ ↑ ↑

light

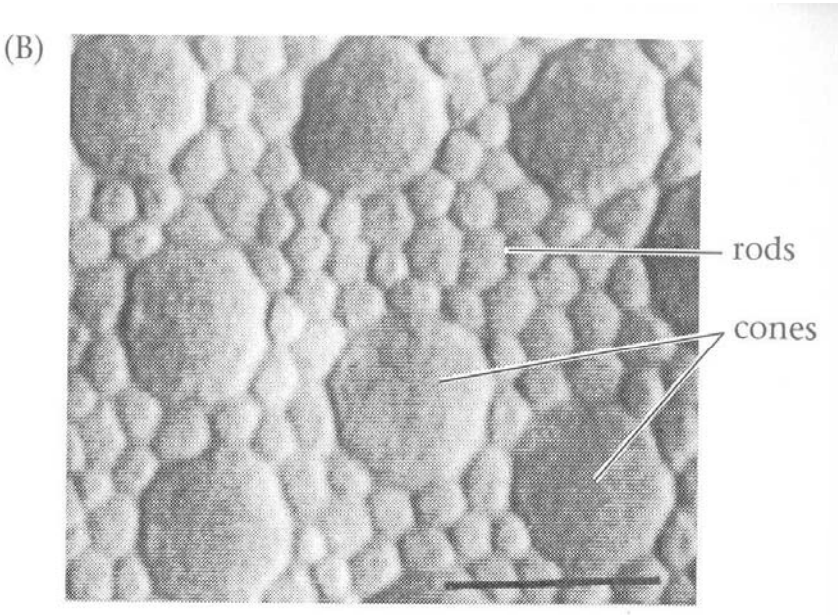
# The Retina:
# pixel detector layout

cones in the fovea

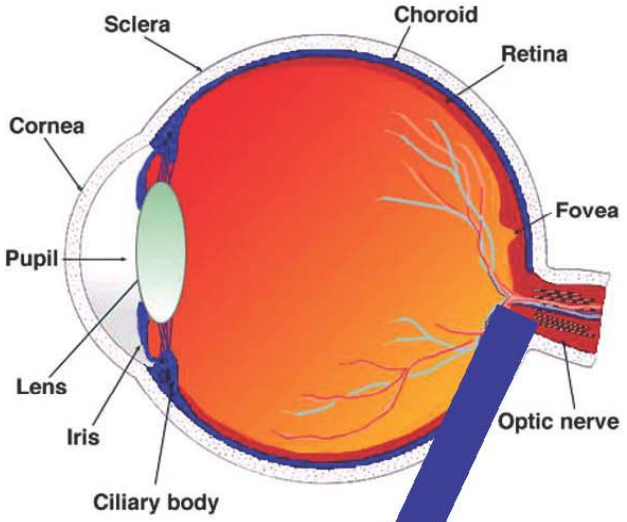



center-to-center spacing = 2.5 μm

rods and cones in the periphery




10 μm


Sclera
Choroid
Retina
Cornea
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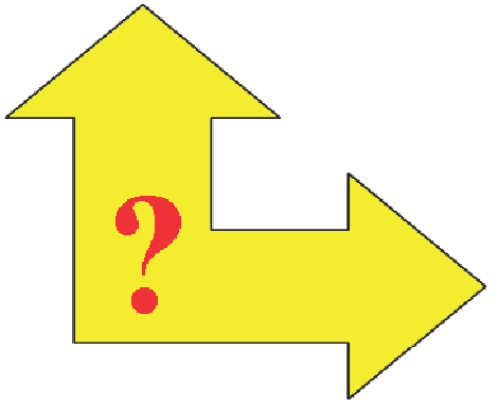
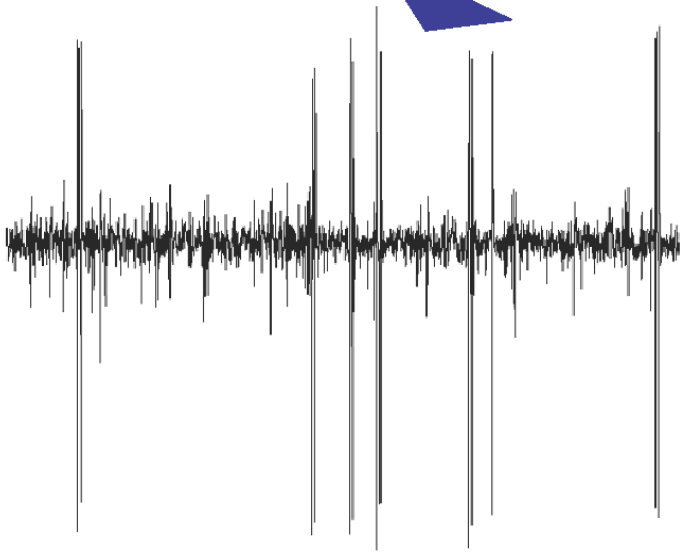
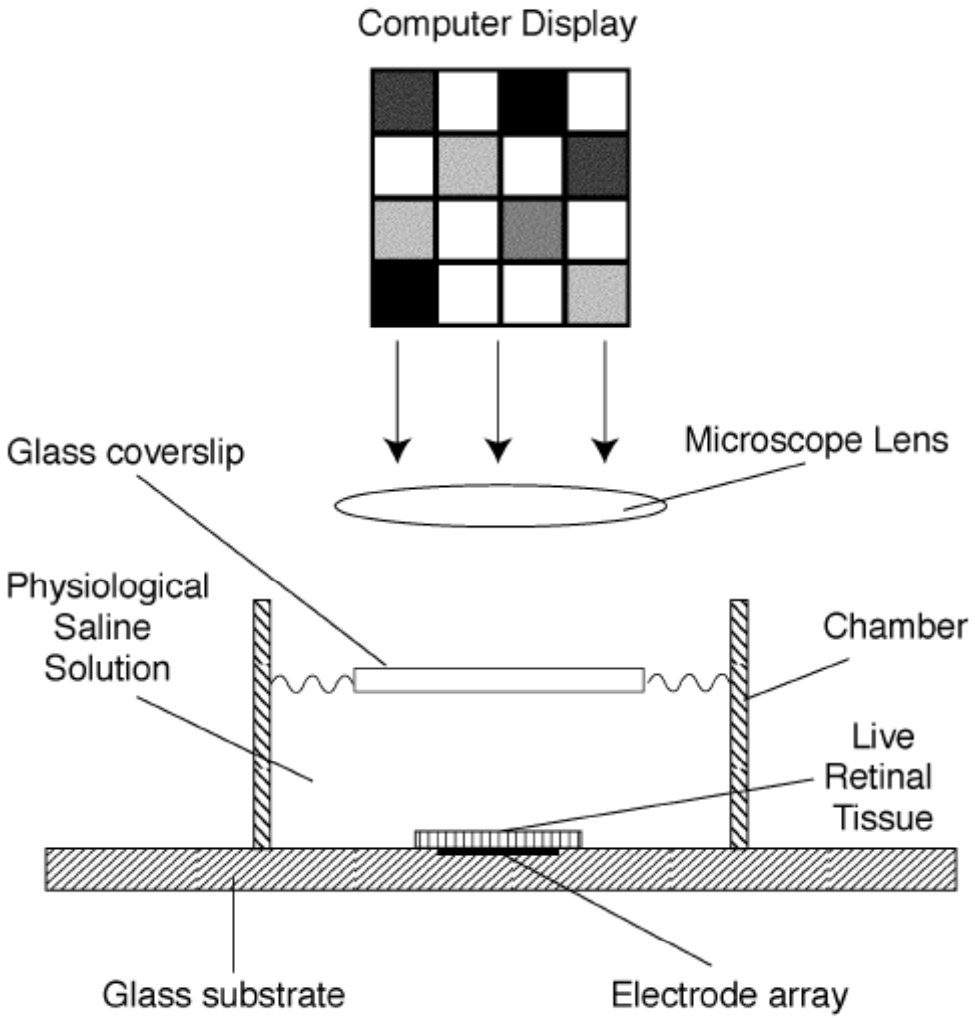
Ciliary body

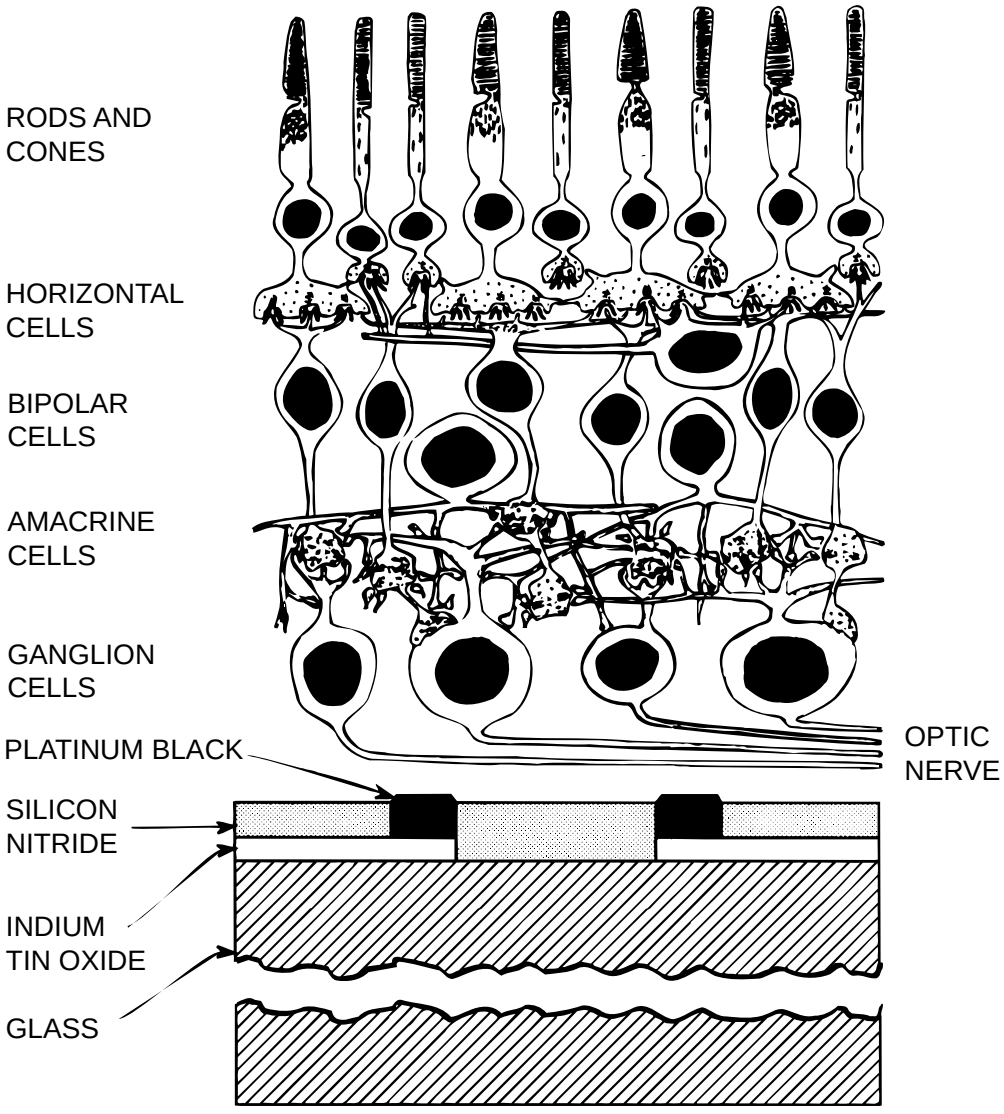

?



# Measurement of Functional Properties

## (based on work by Meister, Pine and Baylor)




RODS AND
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HORIZONTAL
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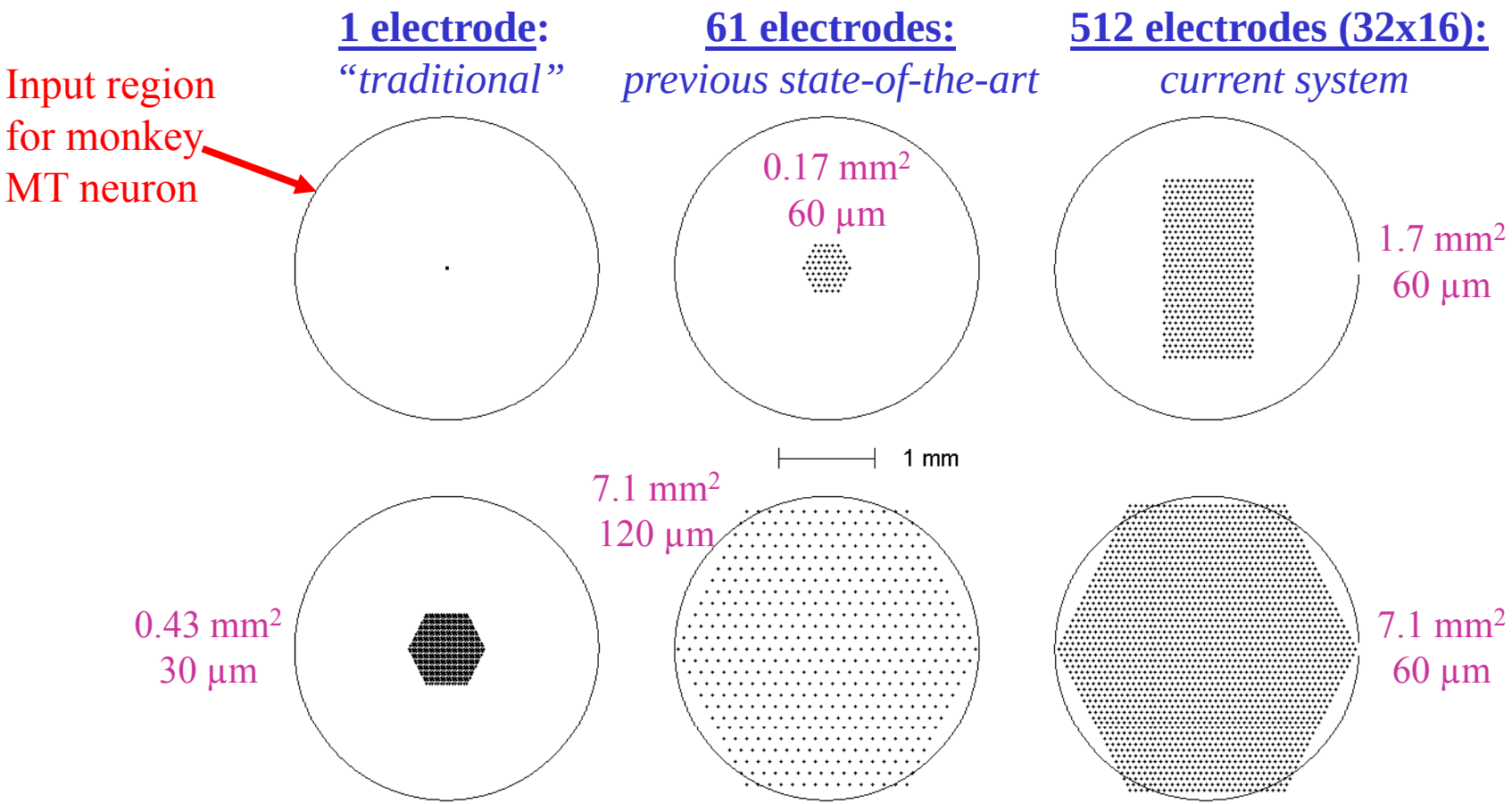
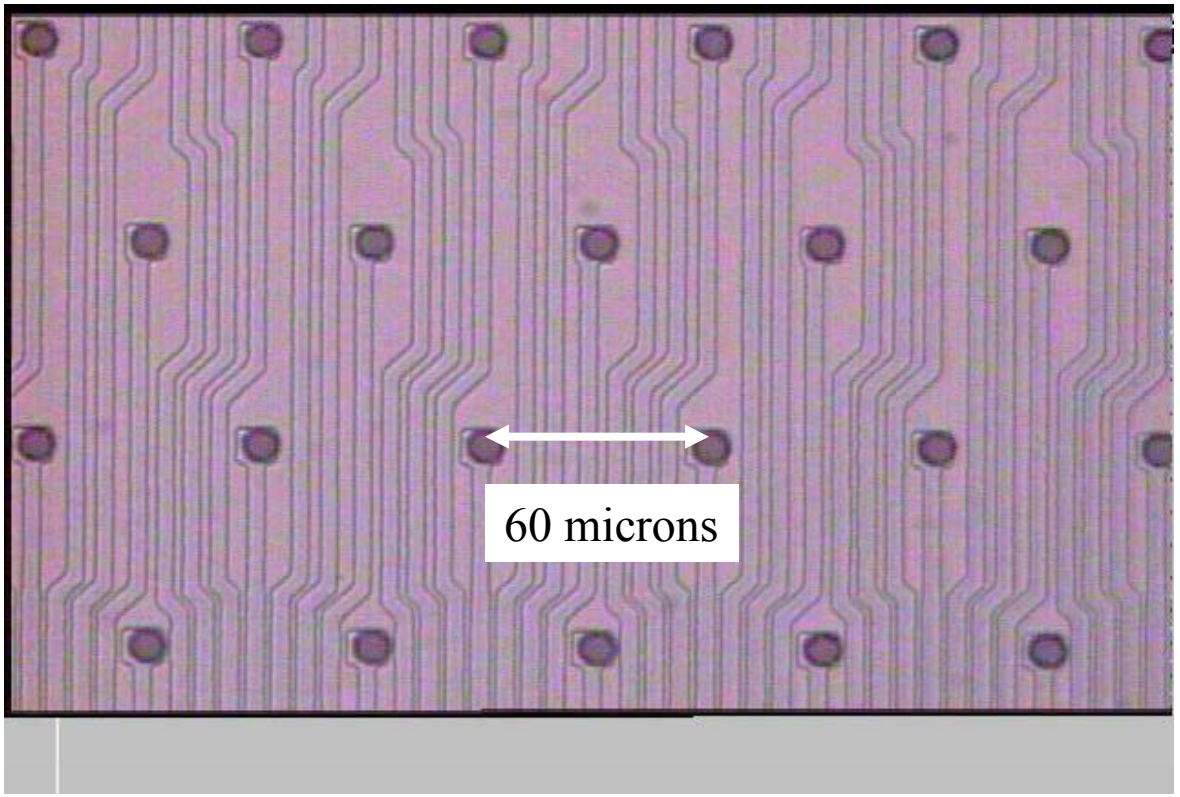
AMACRINE
CELLS
GANGLION
CELLS
PLATINUM BLACK
OPTIC
NERVE
SILICON
NITRIDE
INDIUM
TIN OXIDE
GLASS

# Electrode Array Geometries

(Electrode diameters = 5 μm; area and electrode spacing given below.)



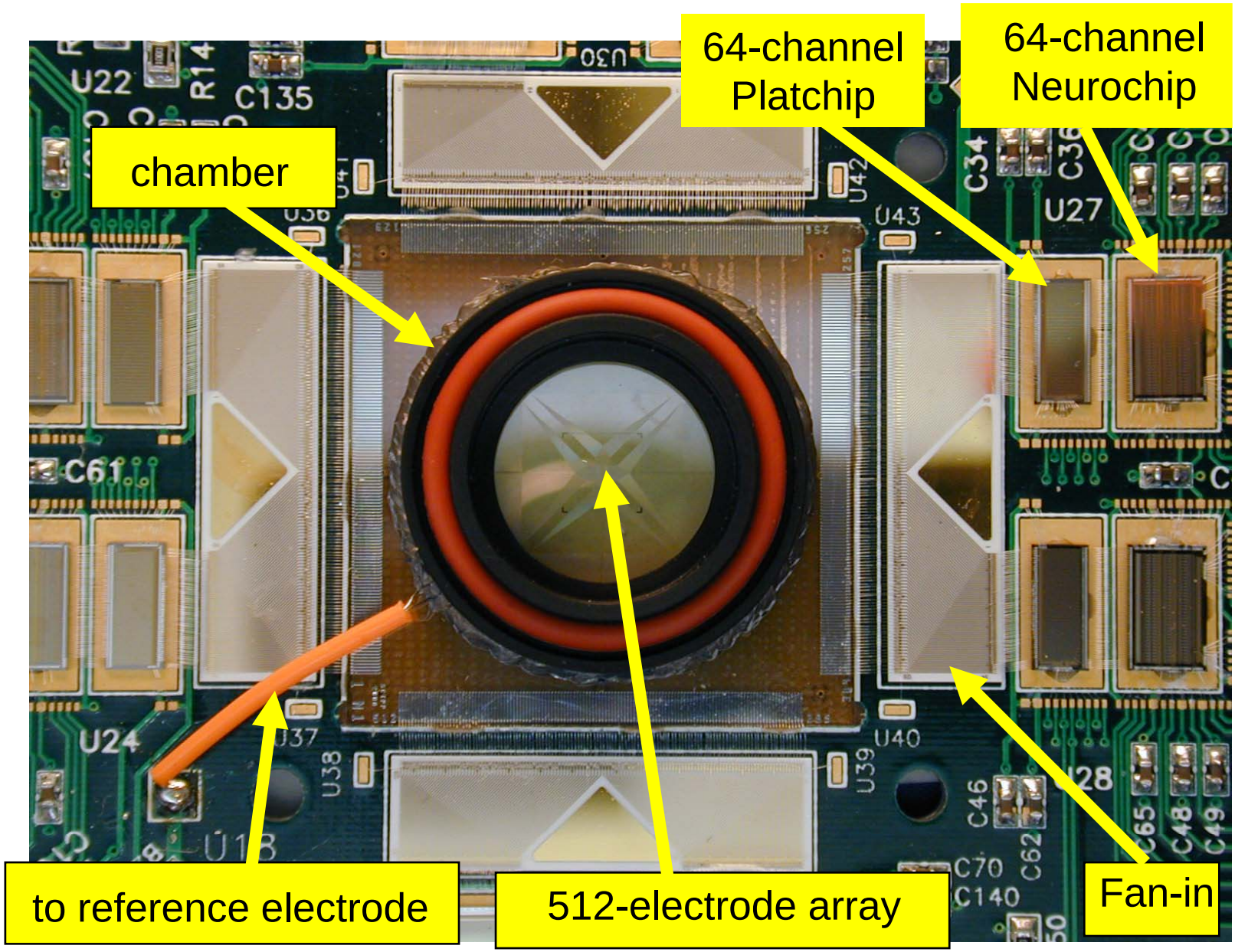
# Section of
# 512-electrode Array (32x16)

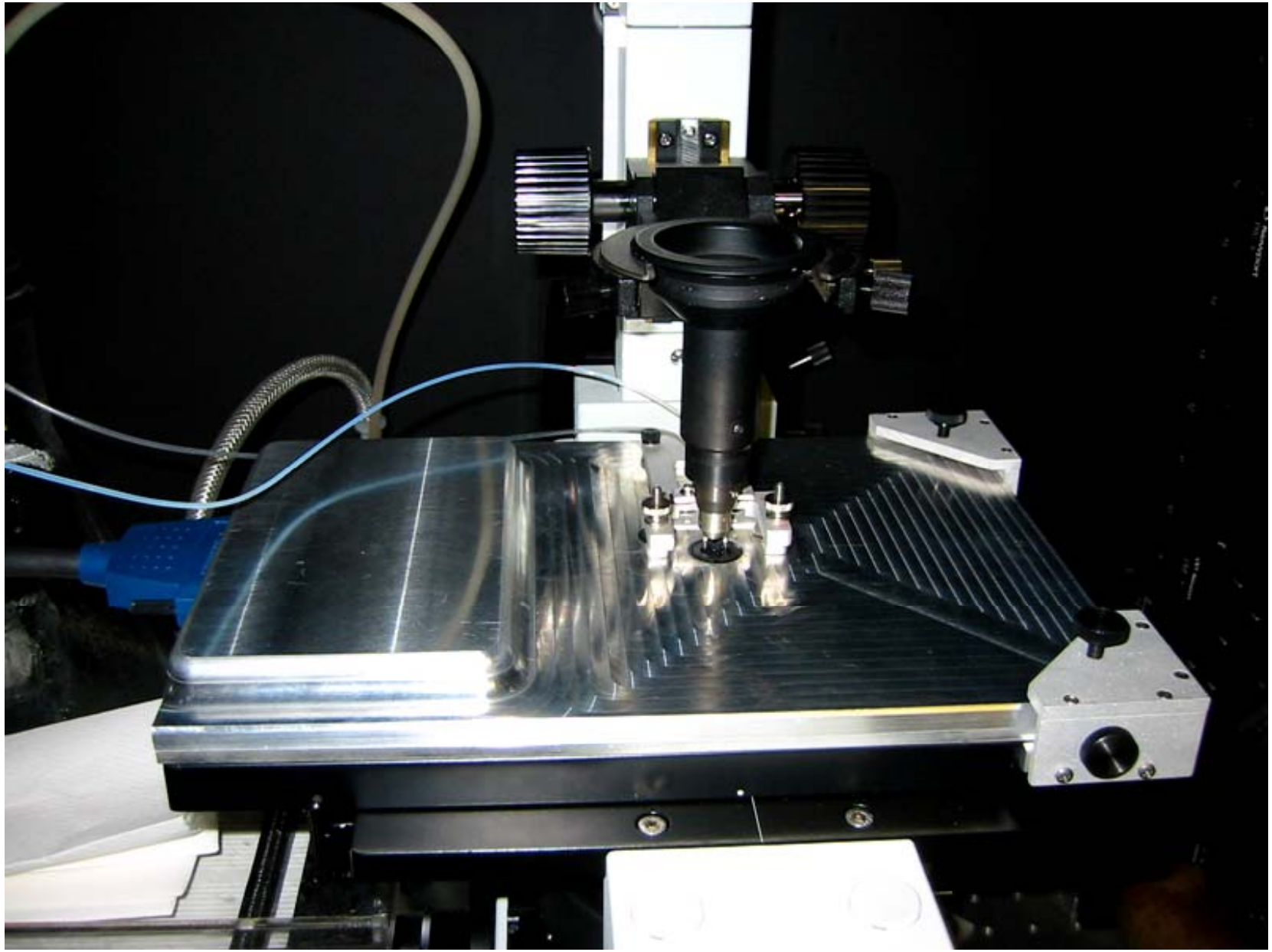



Electrode diameter = 5 μm

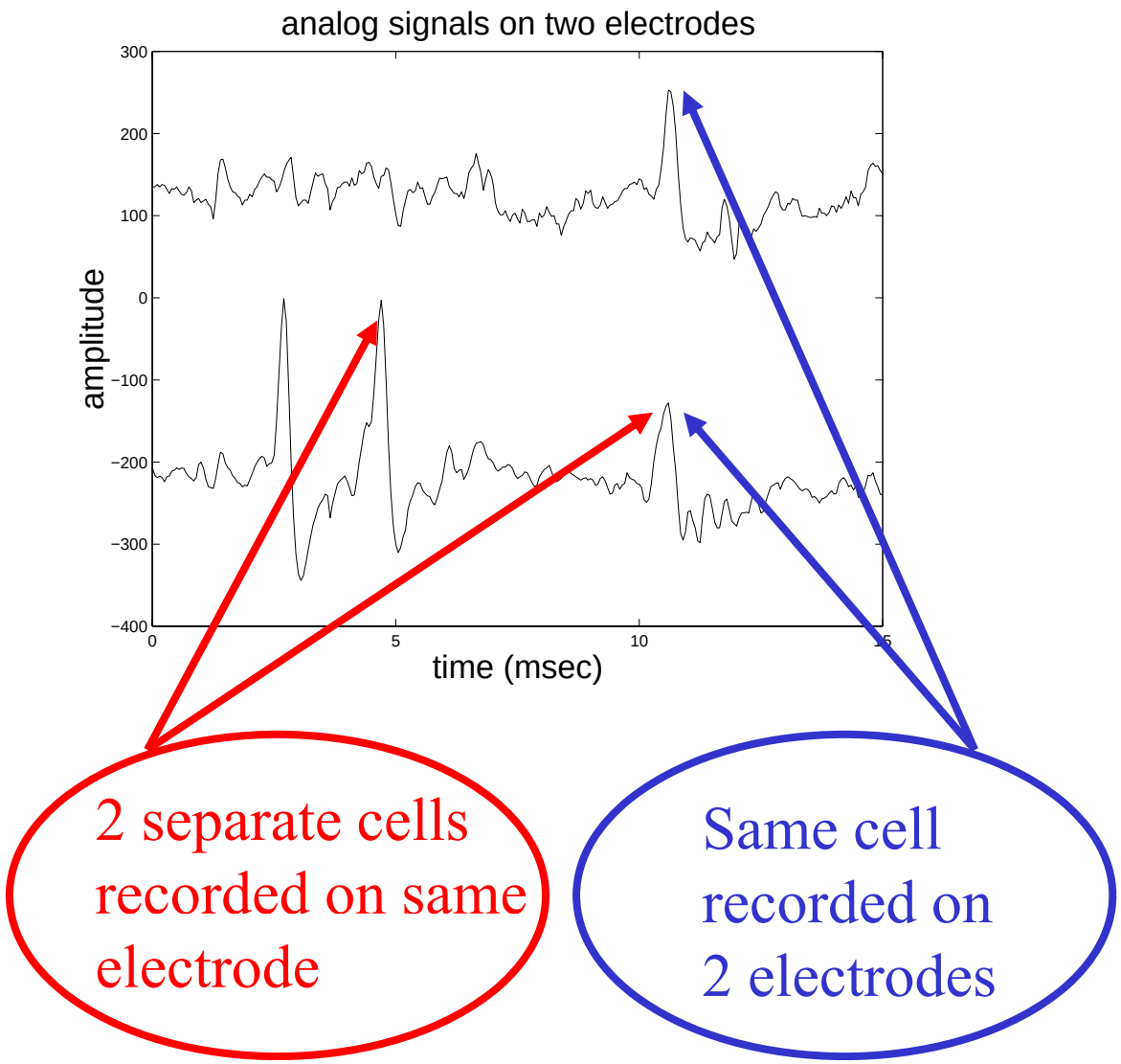
Litke et al., IEEE Trans. Nucl. Sci. (2004) 1434

# Section of 512-electrode “Neuroboard”





# Spikes on electrodes ⇒ spikes from identified neurons

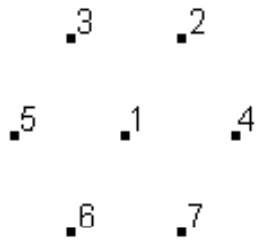


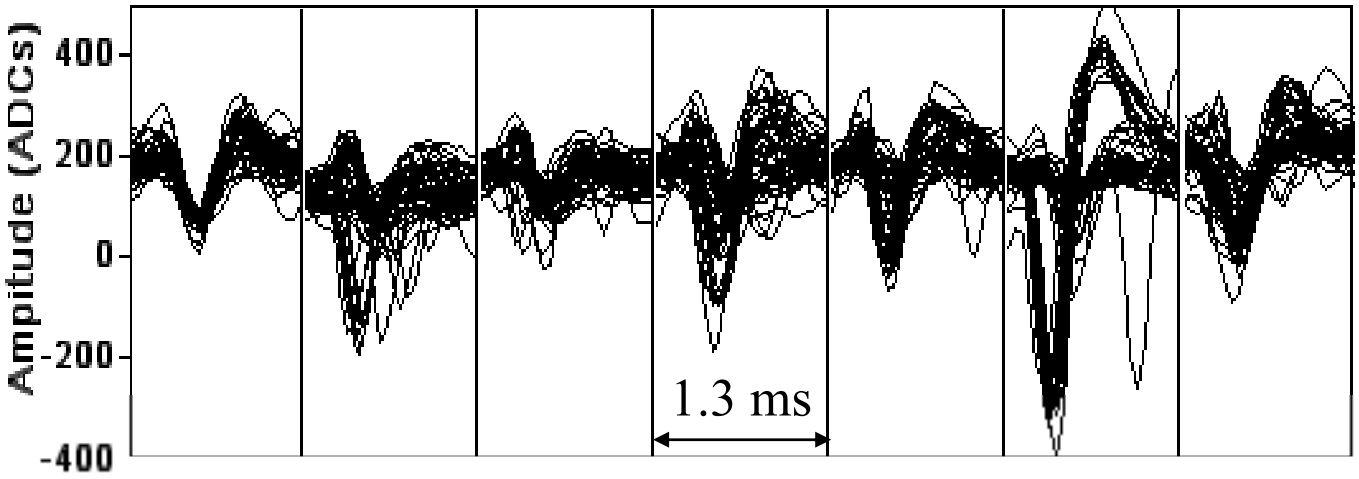
# Neuron Identification

## (signals on electrodes ⇒ spikes from identified neurons)

7x26=182 measurements

Multiple electrodes



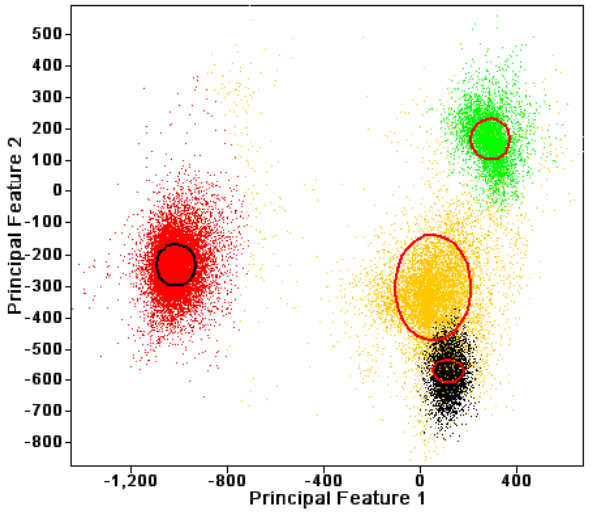

Electrode # 1 2 3 4 5 6 7

- Principal Components Analysis

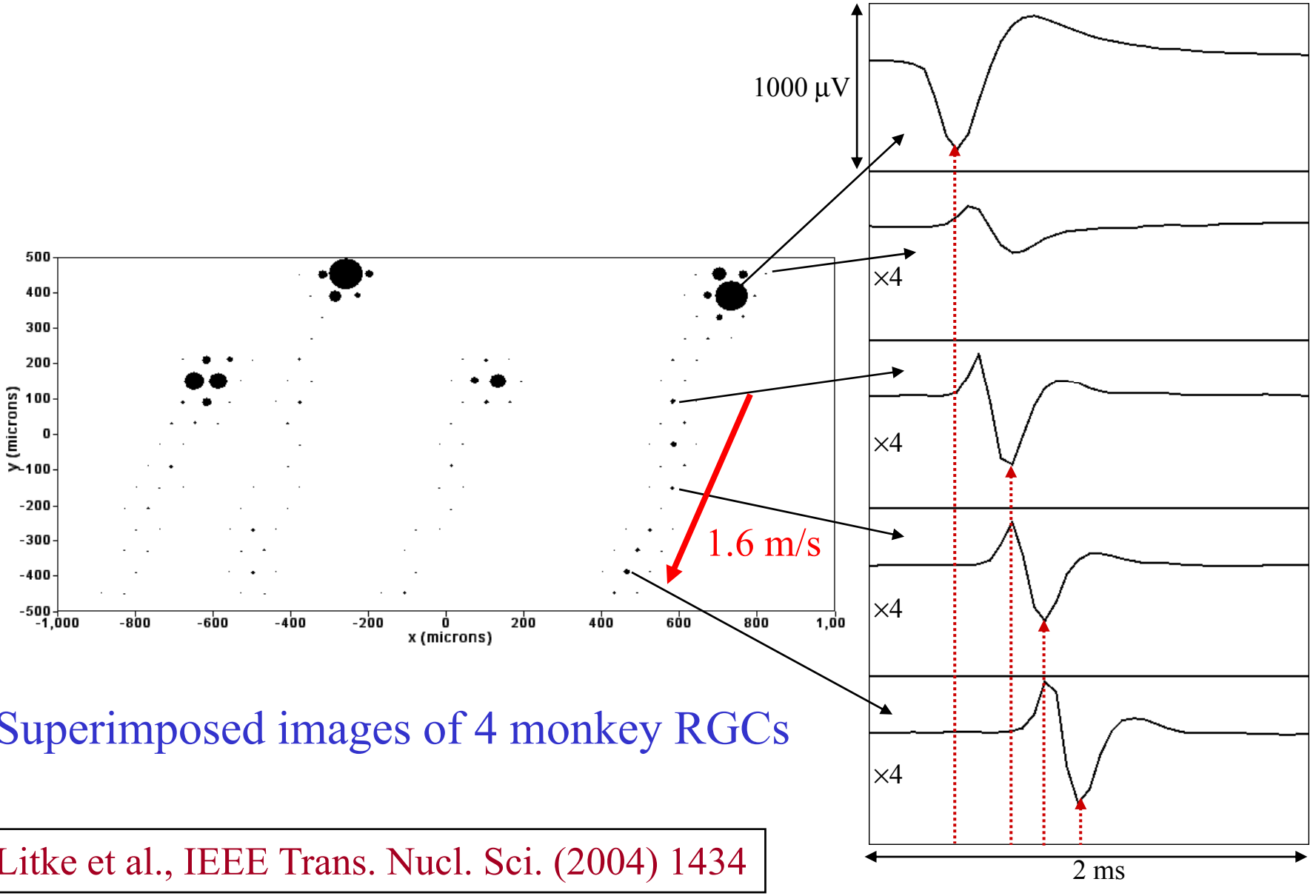
Find ~5 most significant variables that are linear combinations of the 182 measurements

- Multidimensional Clustering

⇒ Identified Neurons



# Electrophysiological Imaging

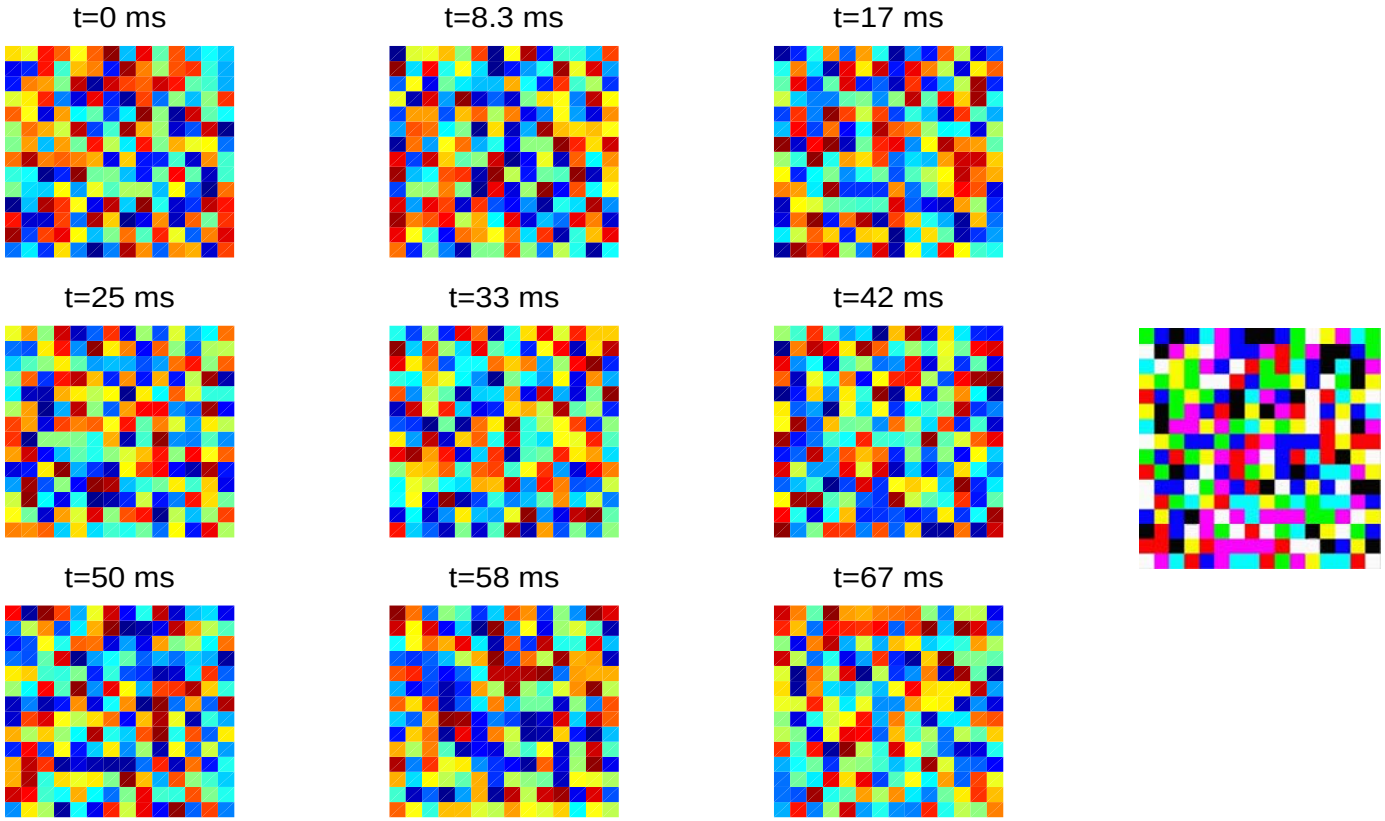



Superimposed images of 4 monkey RGCs

Litke et al., IEEE Trans. Nucl. Sci. (2004) 1434

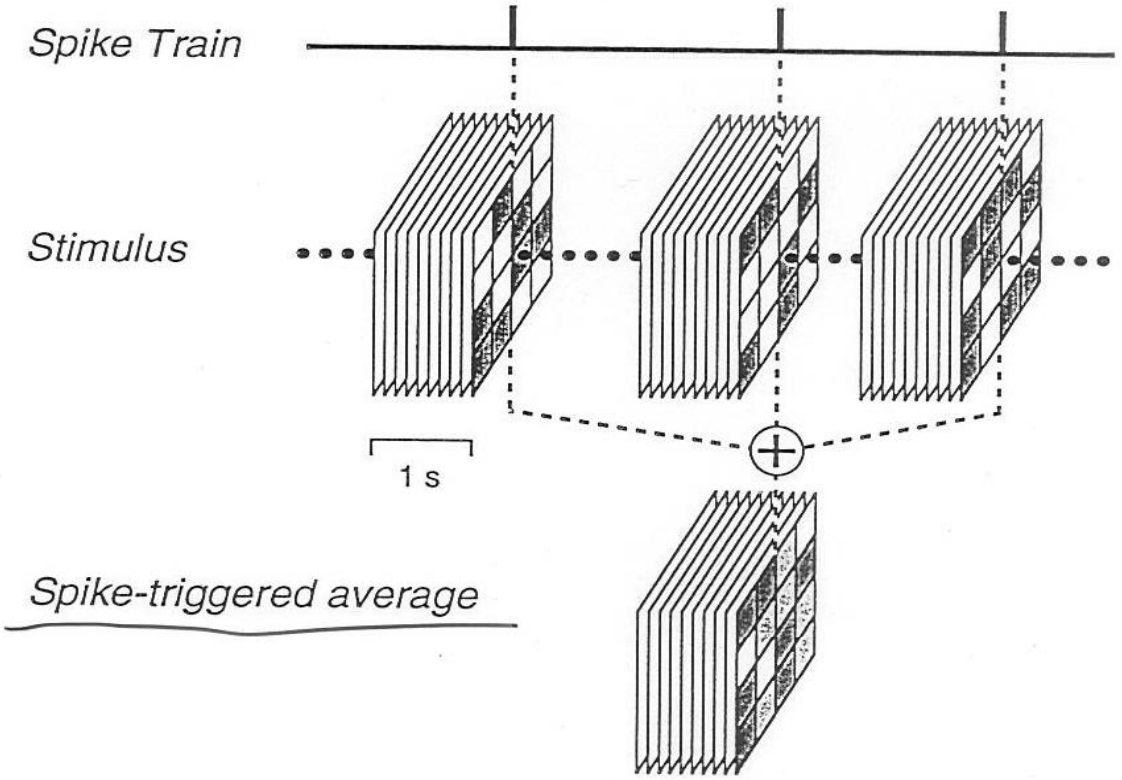
## measure the response properties of identified neurons

⇒ white noise analysis: use time sequence of random checkerboard images



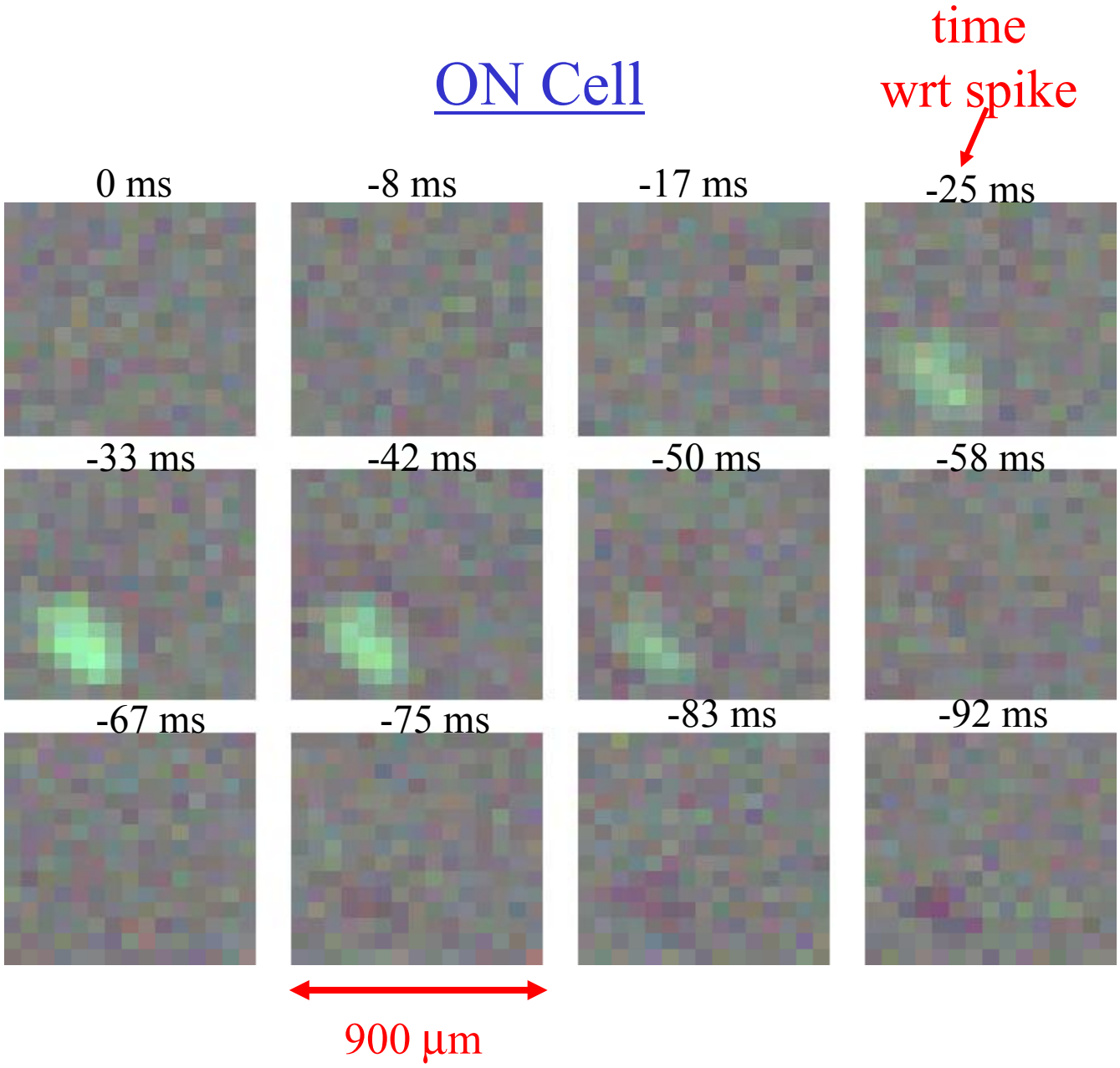

⇒ measure the "spike-triggered average" (sta) response for each neuron

# Spike-triggered Average

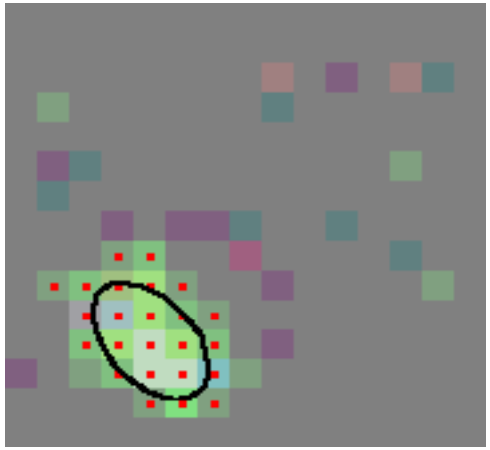


# Monkey Retinal Ganglion Cell

## ON Cell

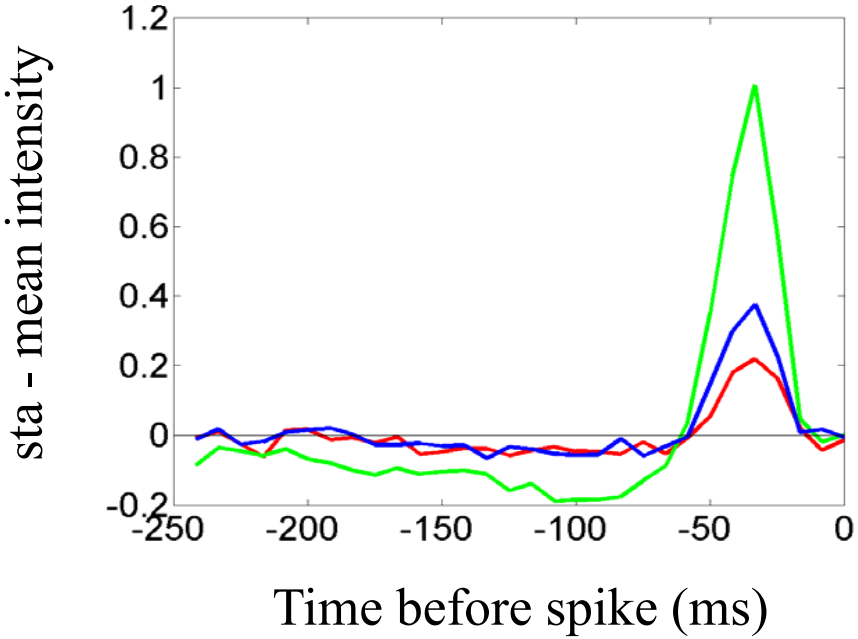


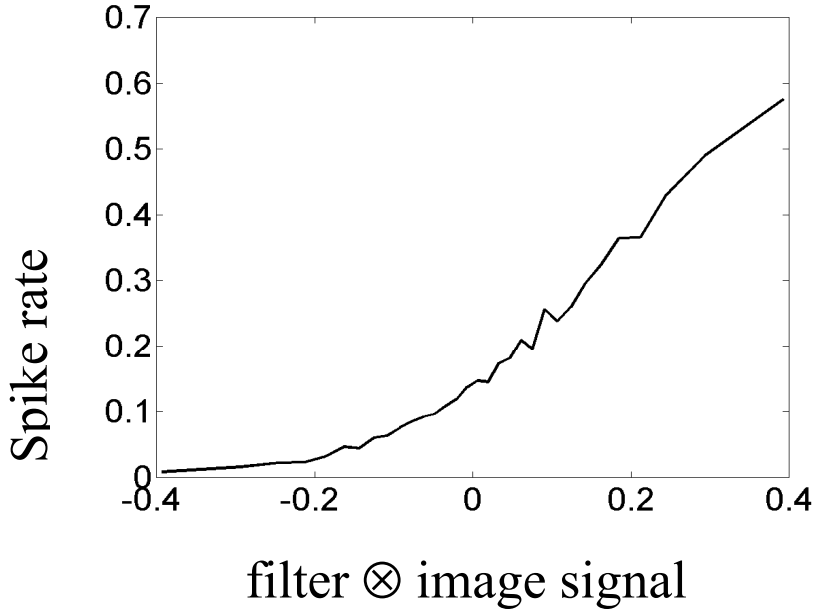
Spike-triggered
average image
at time of maximum
absolute intensity



900
μm

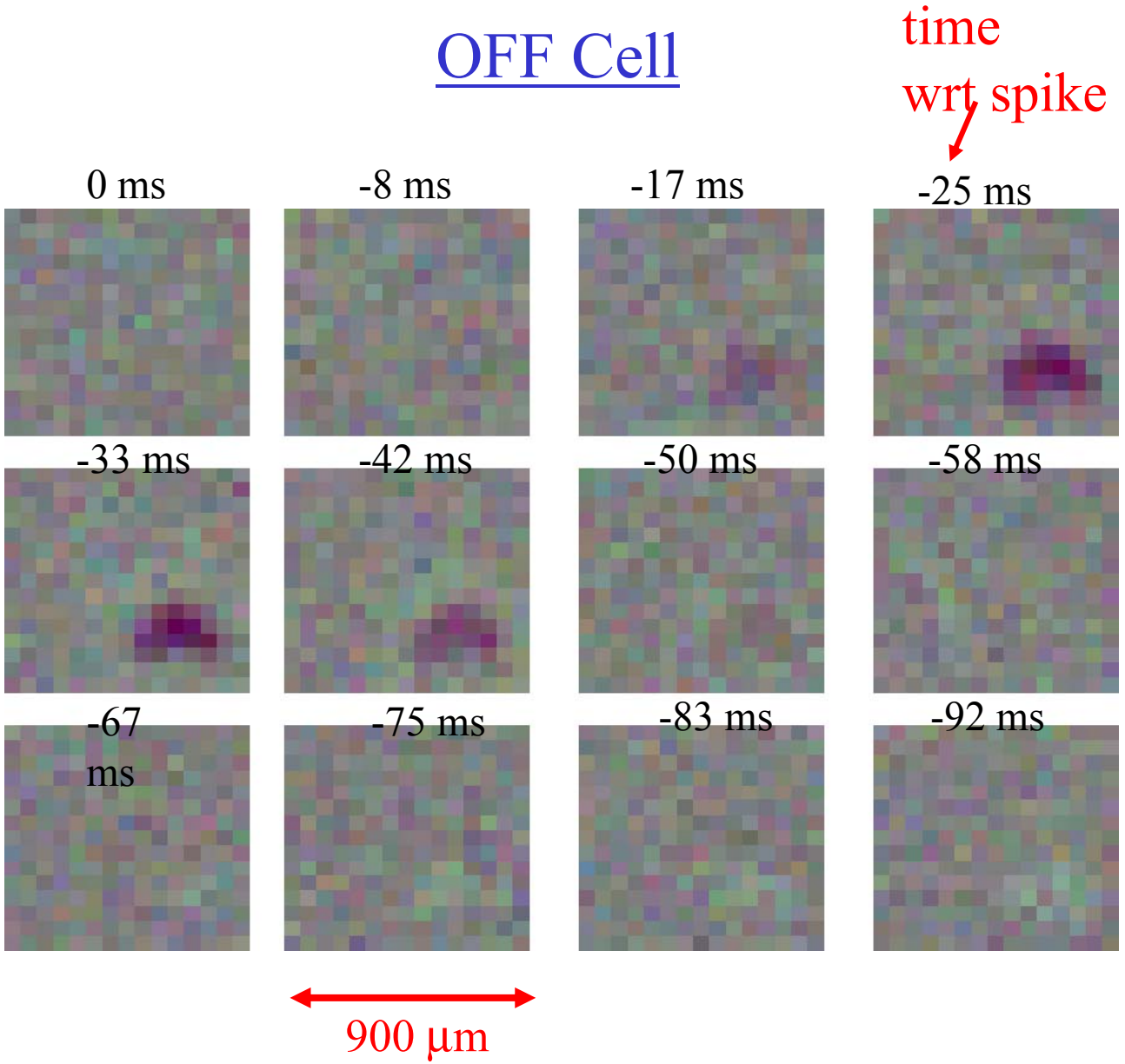
## ON cell: sta time filter



# Monkey Retinal Ganglion Cell

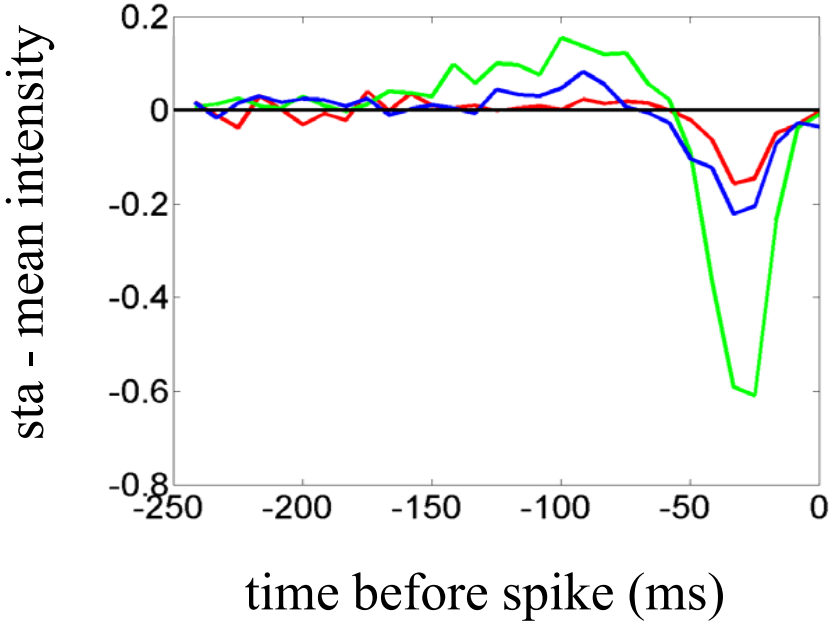
## OFF Cell

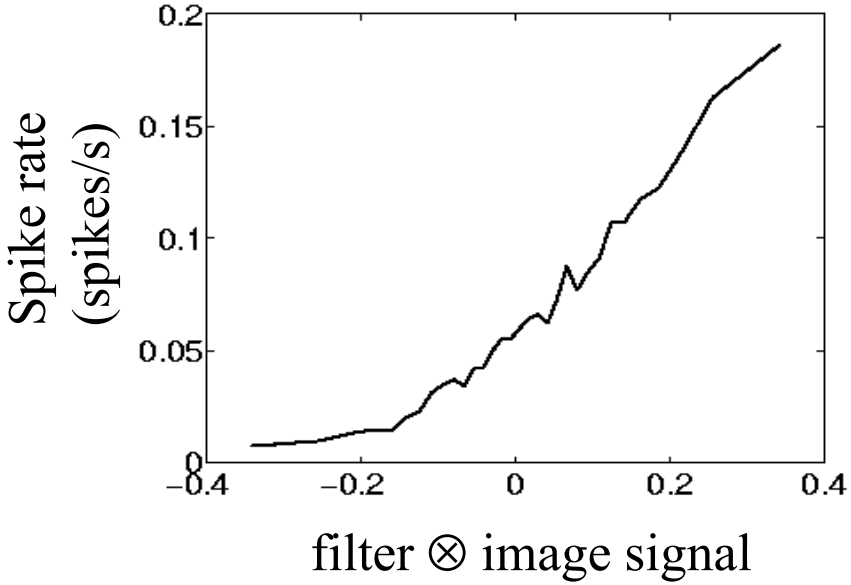


Spike-triggered
average image
at time of maximum
absolute intensity

900
μm

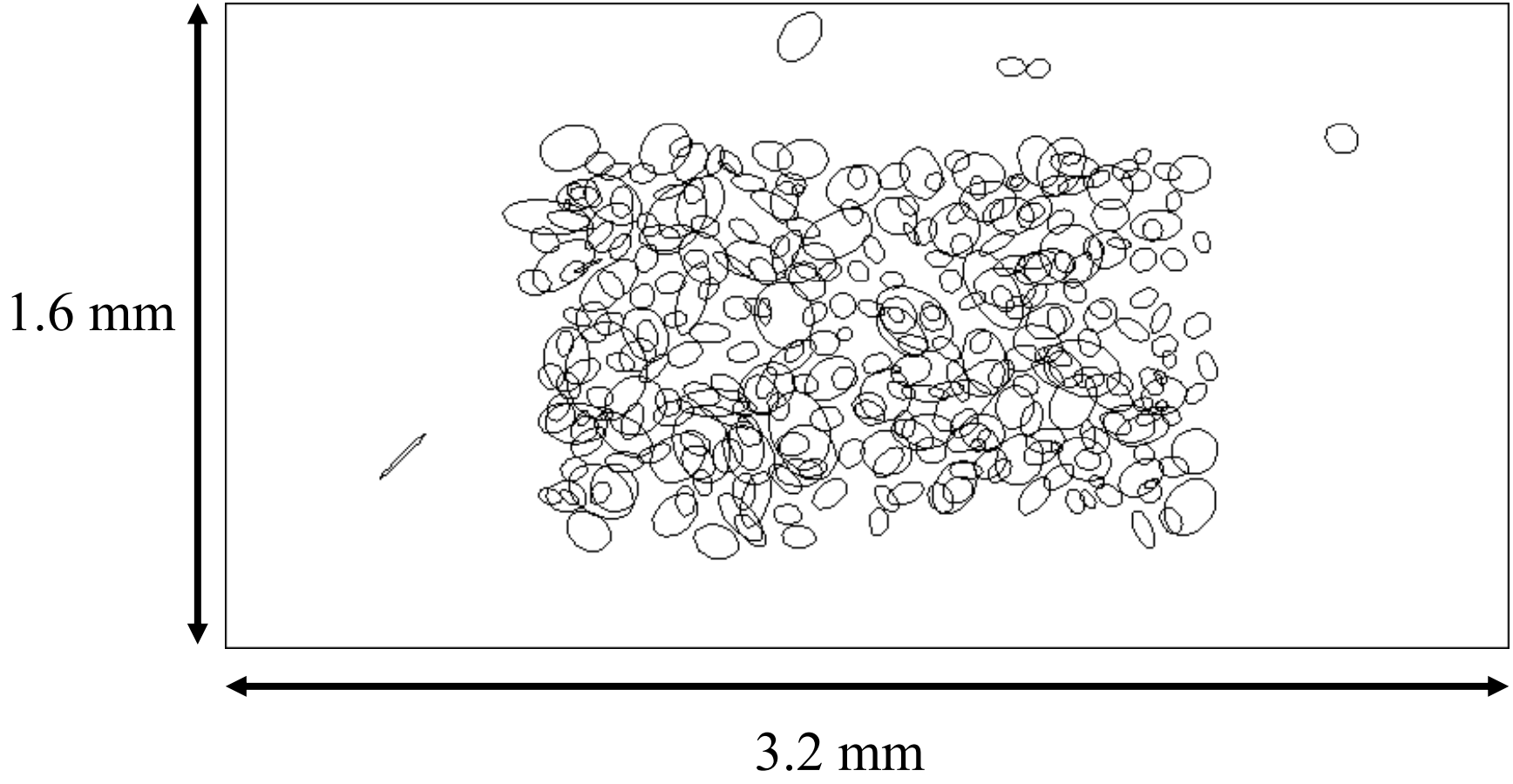
OFF cell: sta time filter



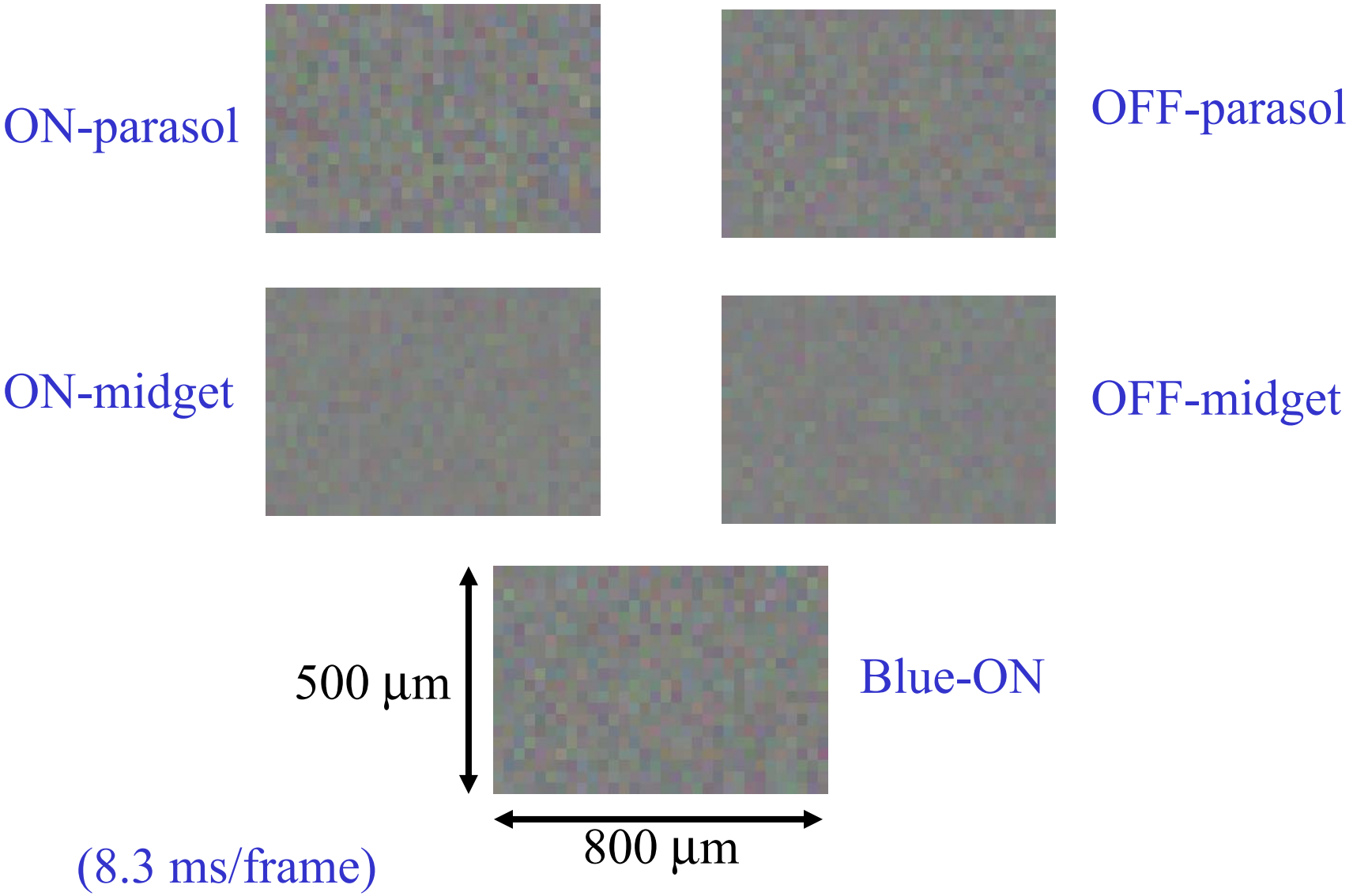



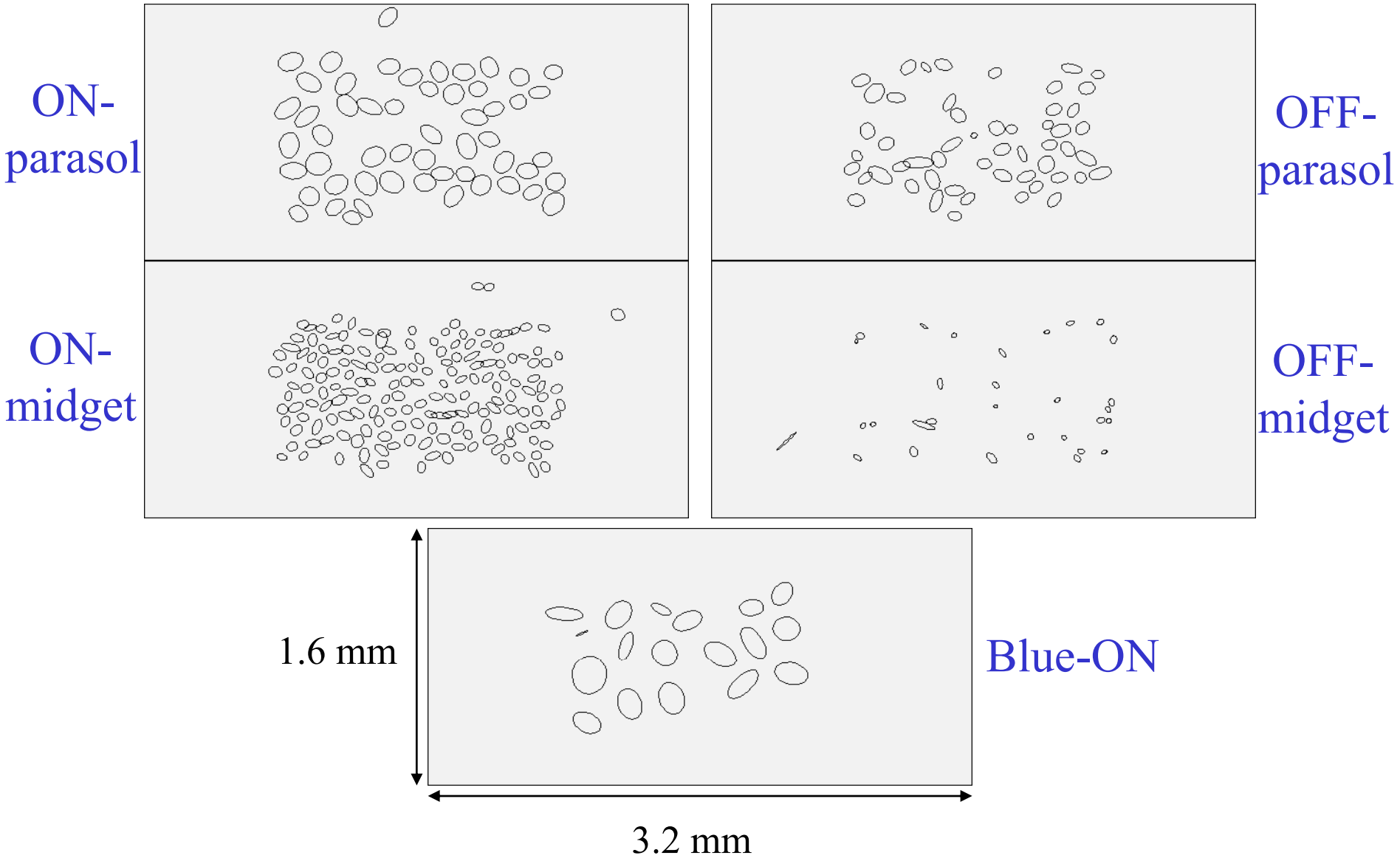
# Some first results with monkey retina

Light-sensitive regions ("receptive fields") for 338 identified neurons



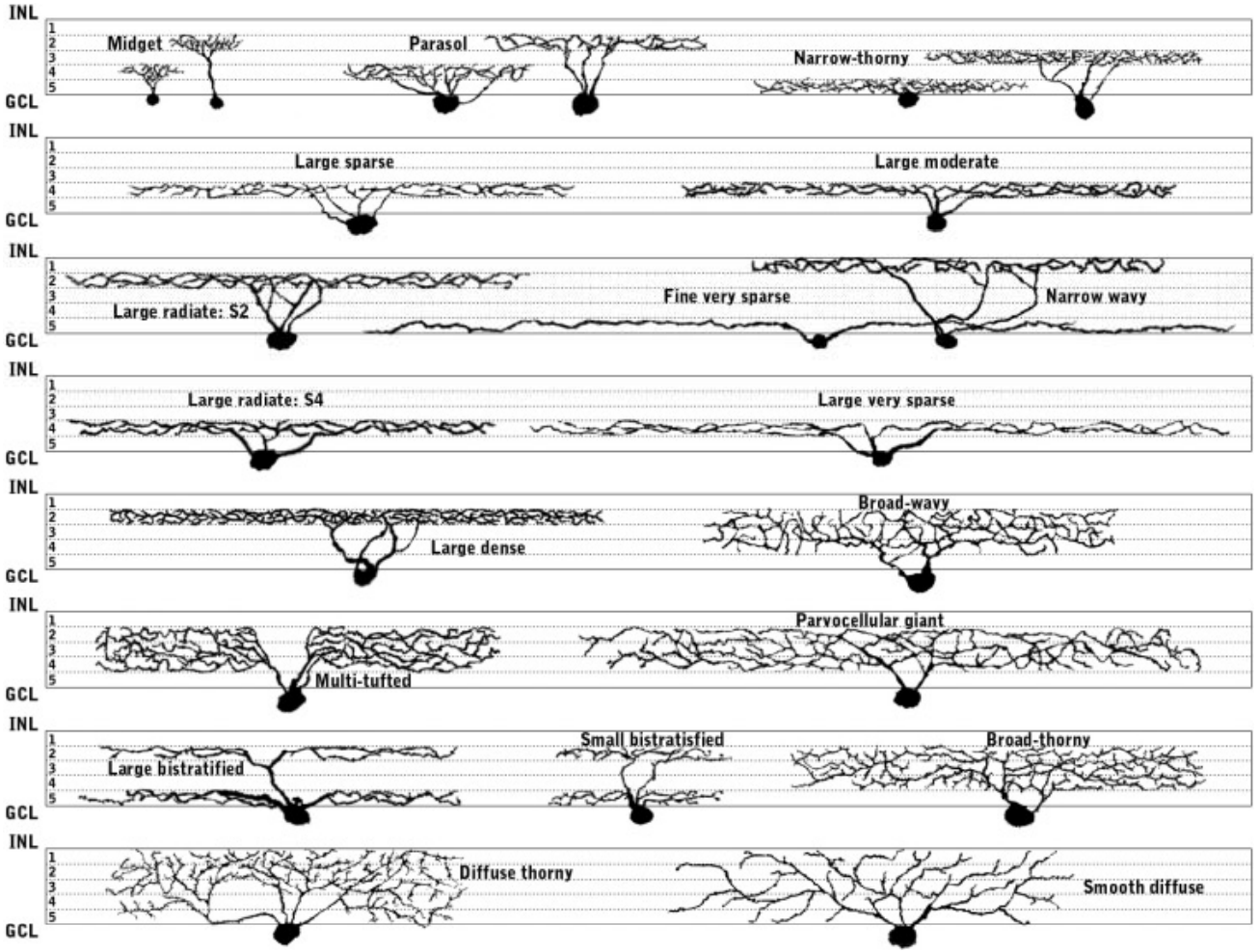
# Spatial/temporal response properties of individual neurons ("spike-triggered average")




ON-
parasol
OFF-
parasol
ON-
midget
OFF-
midget
1.6 mm
Blue-ON
3.2 mm

# Five identified monkey RGC classes (already well-known), but this is just the tip of the iceberg.

From anatomical studies, it is estimated that there are at least 22 distinct types of monkey RGCs.




Yamada, Bordt, and Marshak, Visual Neuroscience 22 (2005) 383.

## Search for "missing" primate functional cell types

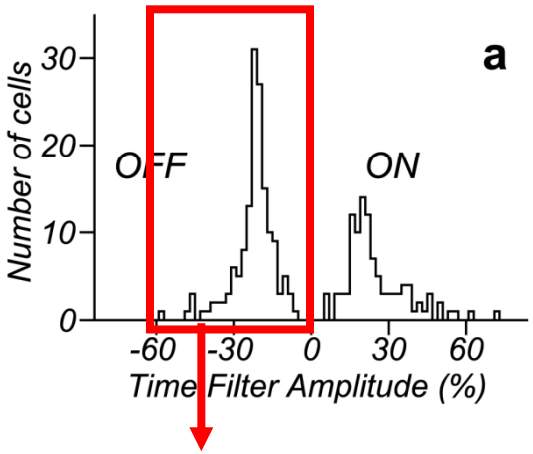
- Anatomical studies indicate cells have large area ("wide-field")
- combined with the "mosaic principle" (cells of a given type tile the visual field)

  $\Rightarrow$ missing cell types have low spatial density – they make up only a small fraction (few %) of the primate RGCs

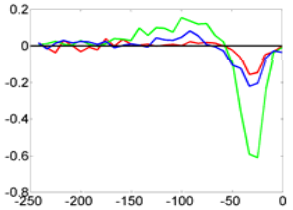
## Search Strategy

- use large area/high density arrays to look for a significant number of cells with similar functional properties in a single preparation (statistics!)
- confirm these are ganglion cells with EIs
- confirm cells form a mosaic
- verify in several preparations

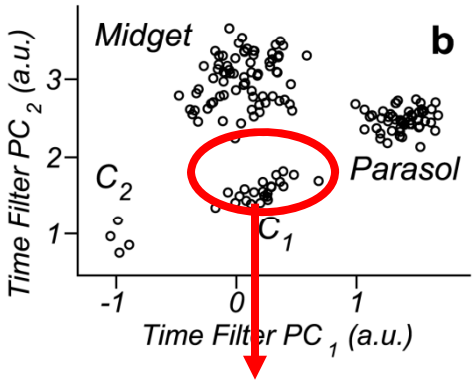
All identified cells




sta time filter amplitudes

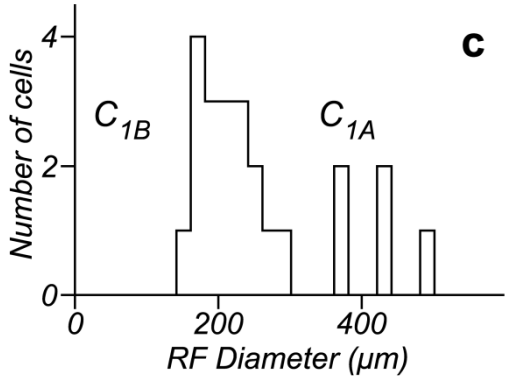



OFF cells




PCA: sta time filter waveforms

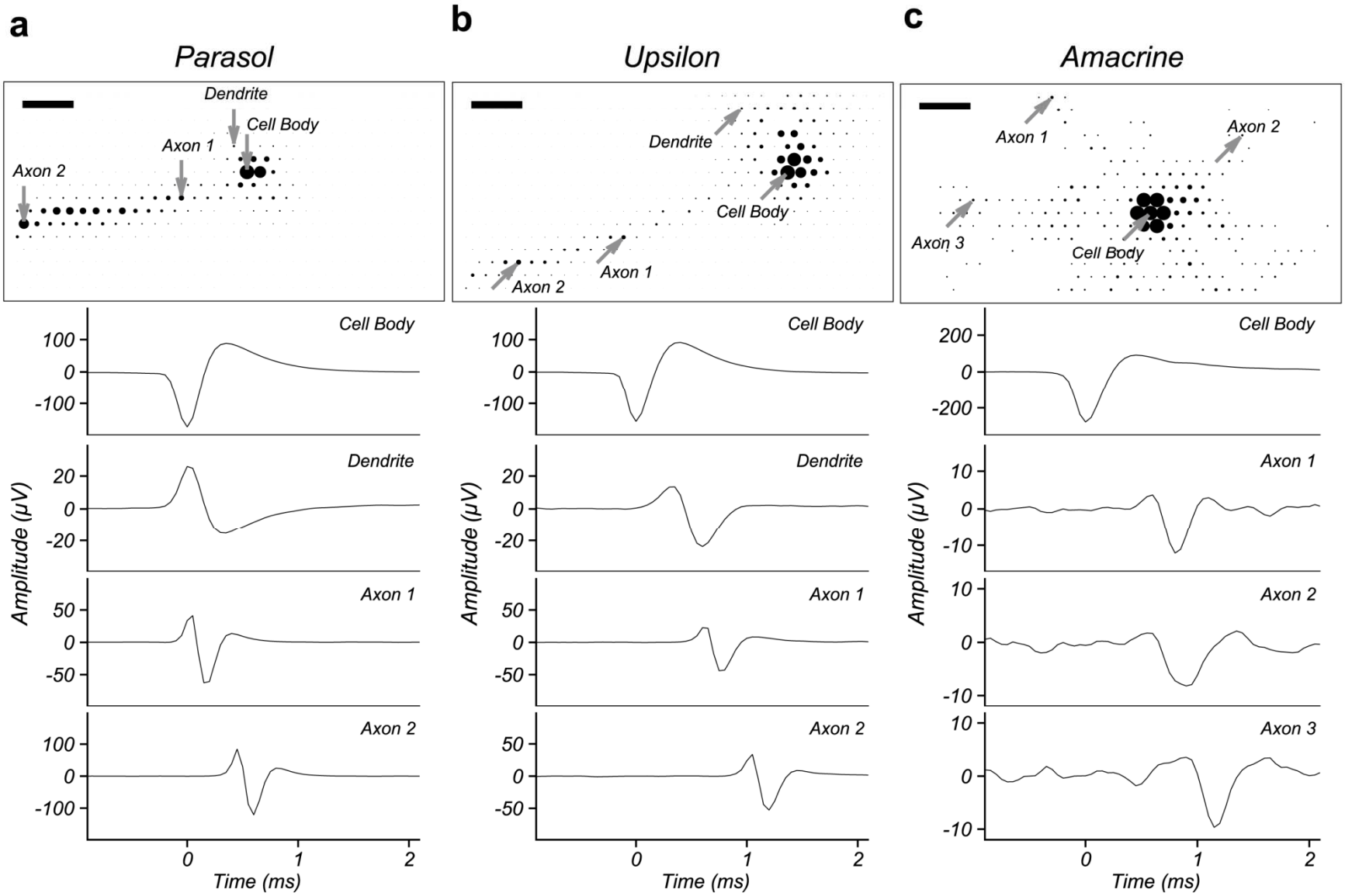
C1 cells



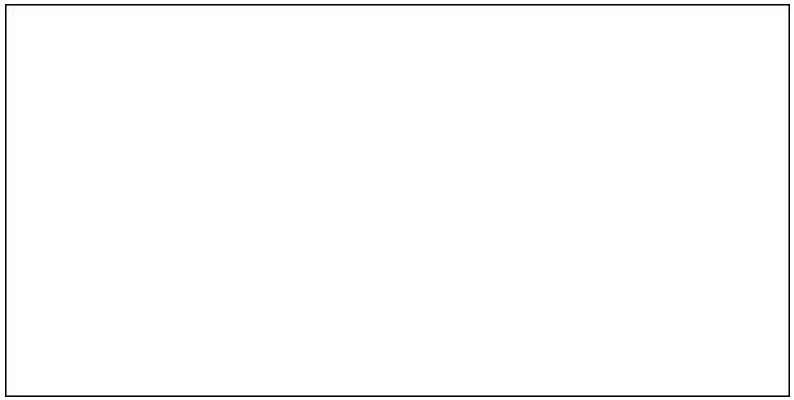

receptive field diameter

D. Petrusca et al., J. Neuroscience (2007)

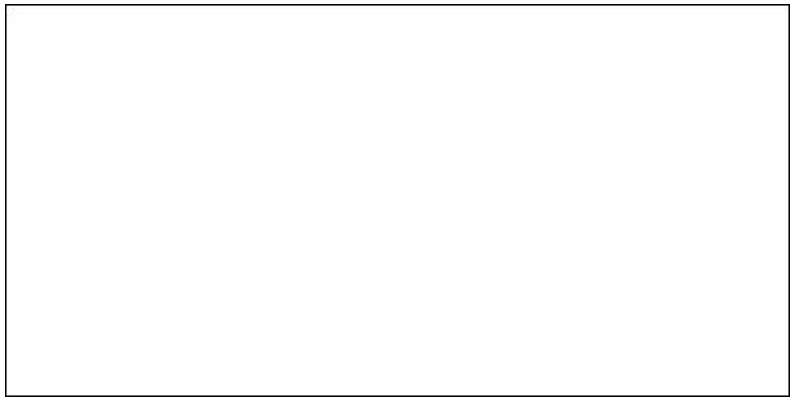
Parasol C1A C1B



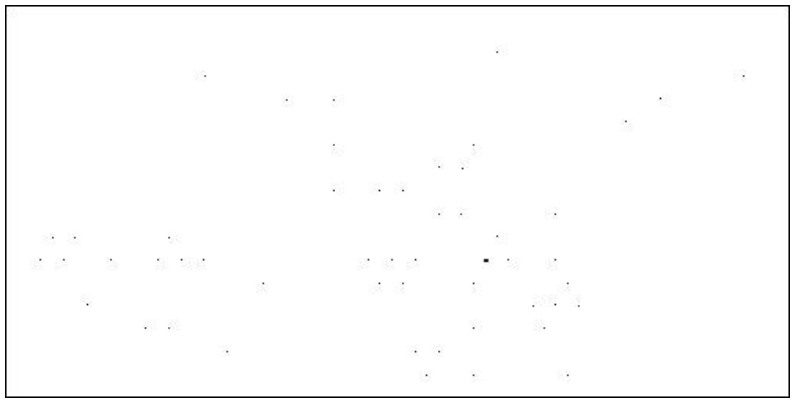
OFF Parasol



C1A
("Upsilon")



C1B
"polyaxonal spiking amacrine"

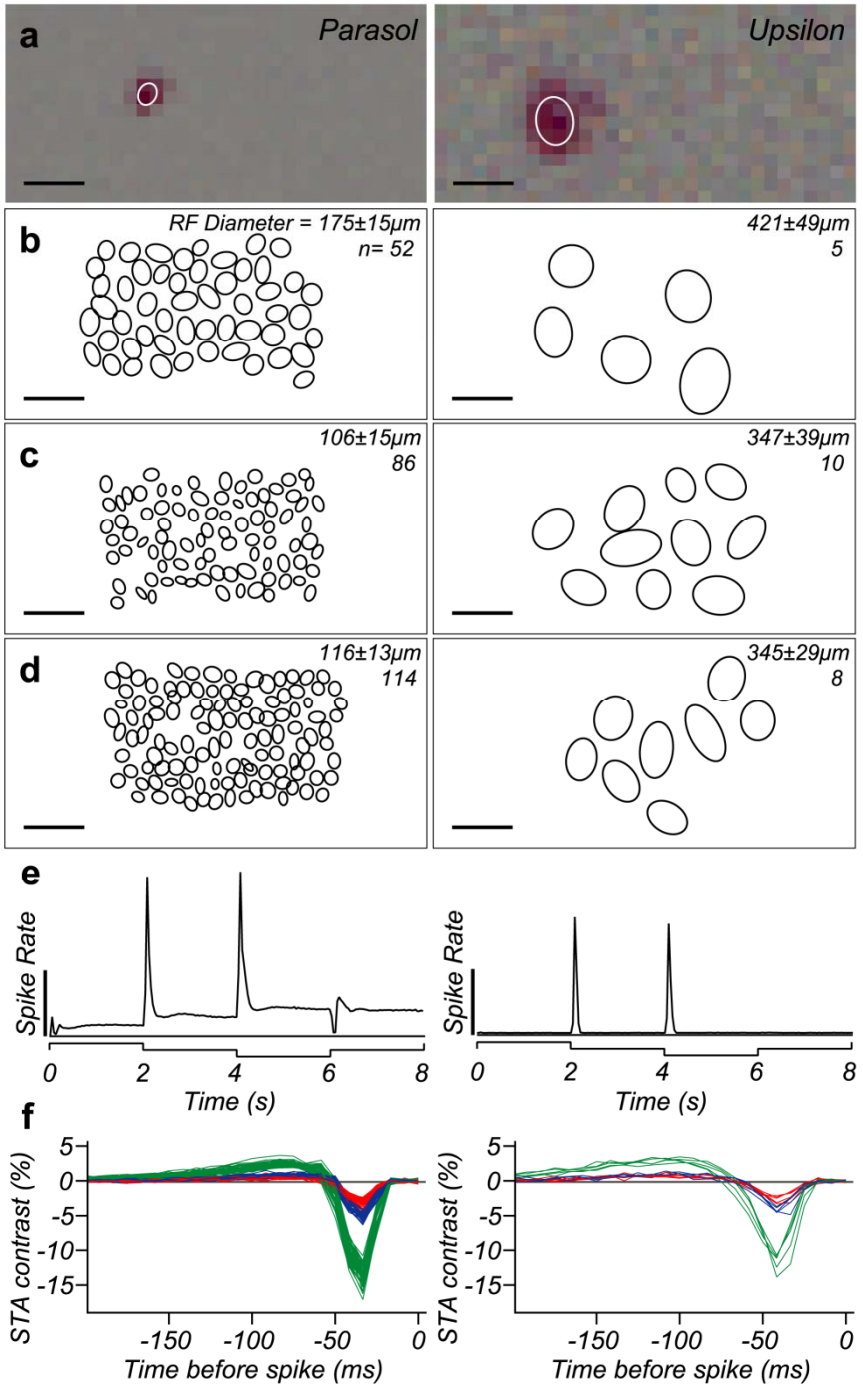


Receptive field

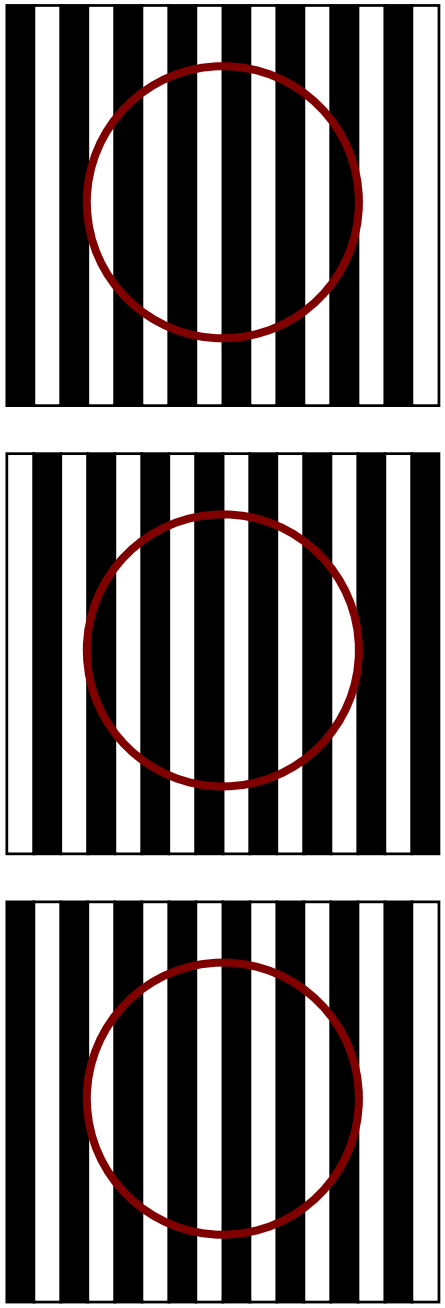
Mosaics in 3 preparations

Response to diffuse light steps

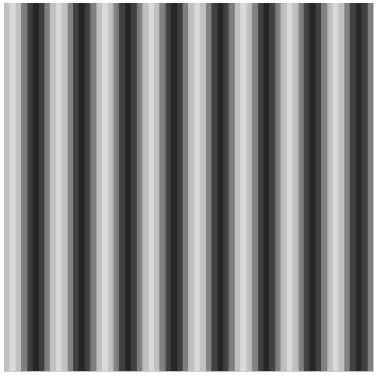
Sta time filter



# Linear/nonlinear summation of the visual image over the RF of the RGC



Contrast reversing gratings at temporal freq. F1

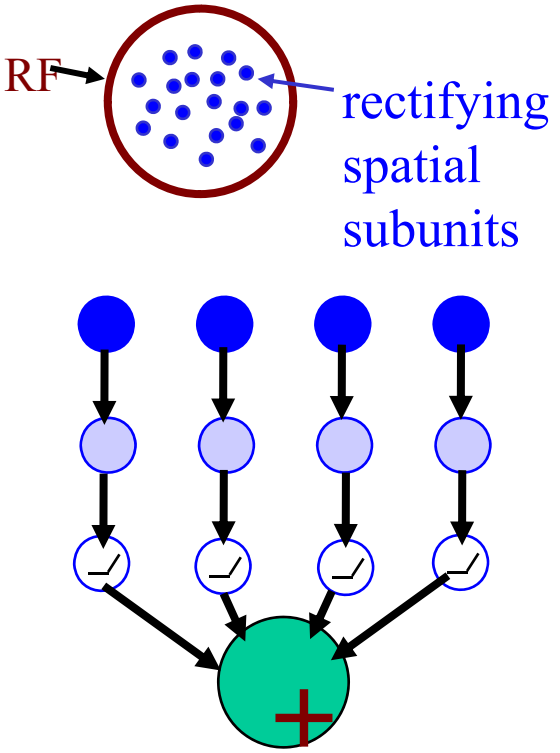


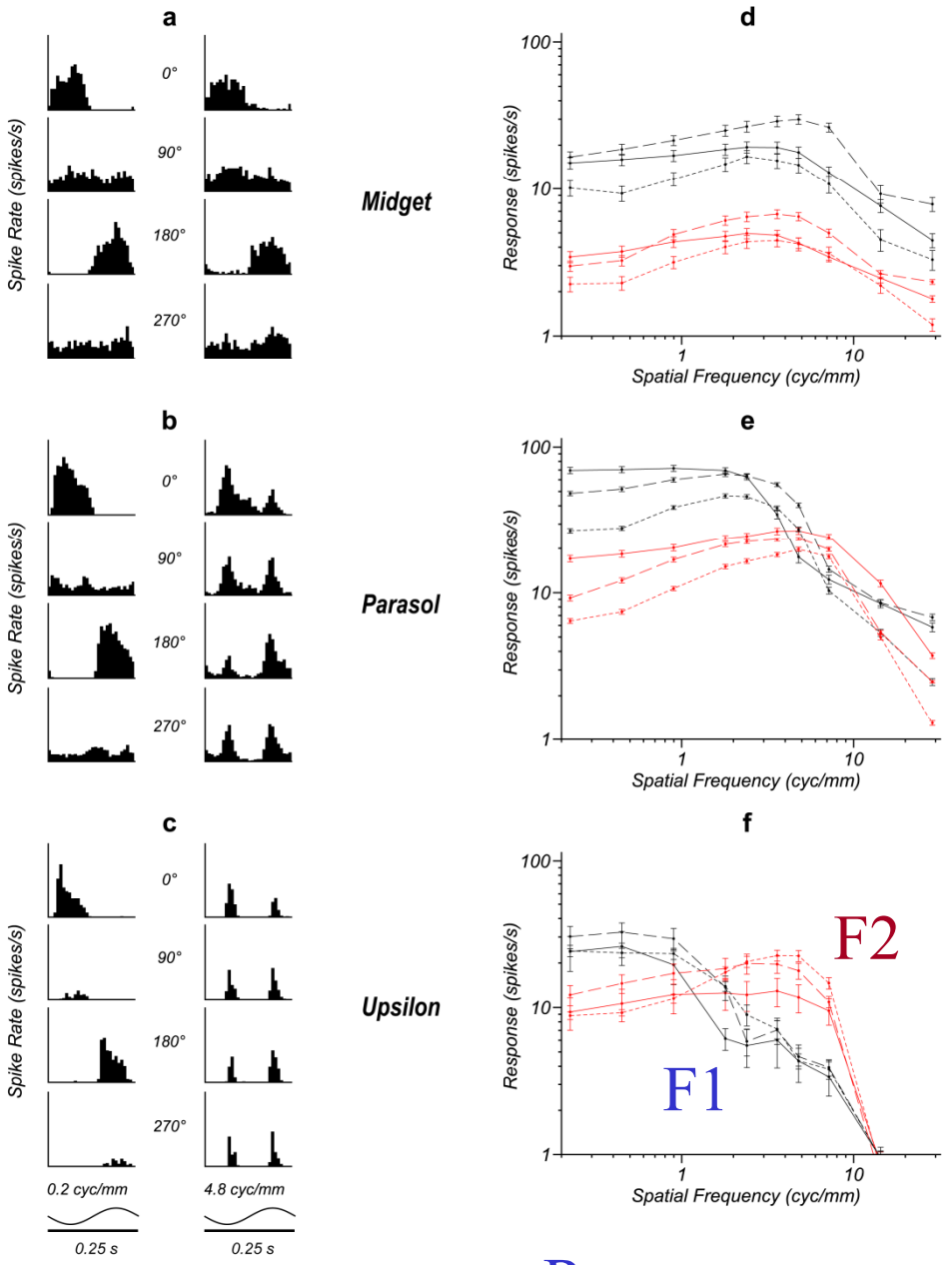
Linear summation over RF ("X-like cell")

**Response mainly at fundamental freq. F1;**
(dependent on spatial phase; "null position")

Nonlinear summation over RF ("Y-like cell")

**Response mainly at second harmonic F2 (freq. doubling);**






Spatial freq.: low med.

Response versus spatial frequency

# Properties of OFF upsilon primate RGCs

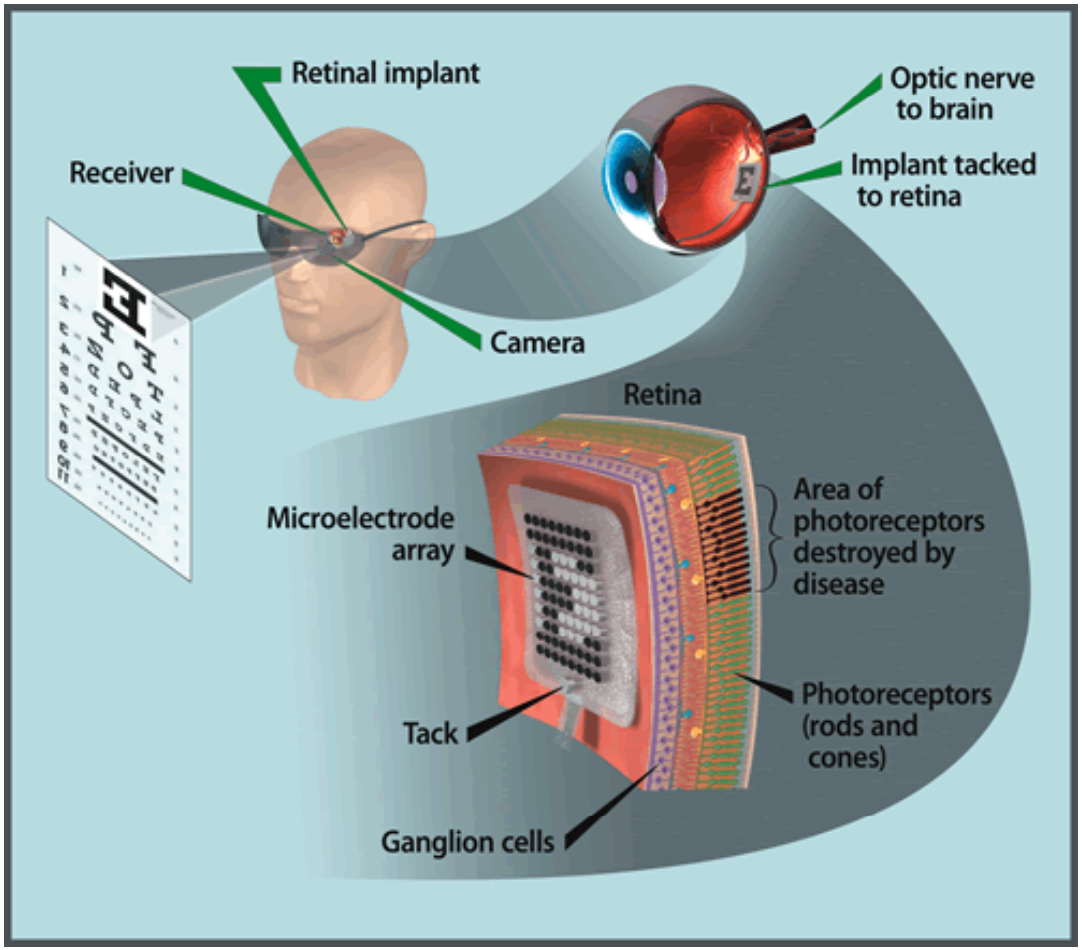
- large receptive field – RF diameter ~3 times OFF parasol RF
- rapid and highly transient response to light
- highly nonlinear spatial summation (Y-like RGC)

Speculation: upsilon cells play a role in motion perception
- detection of moving objects or moving textured patterns

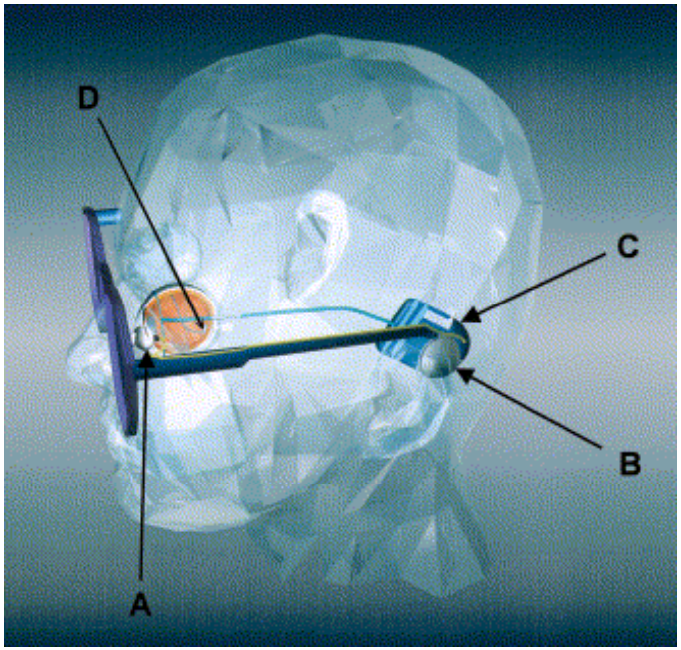
# Retinal Prosthesis

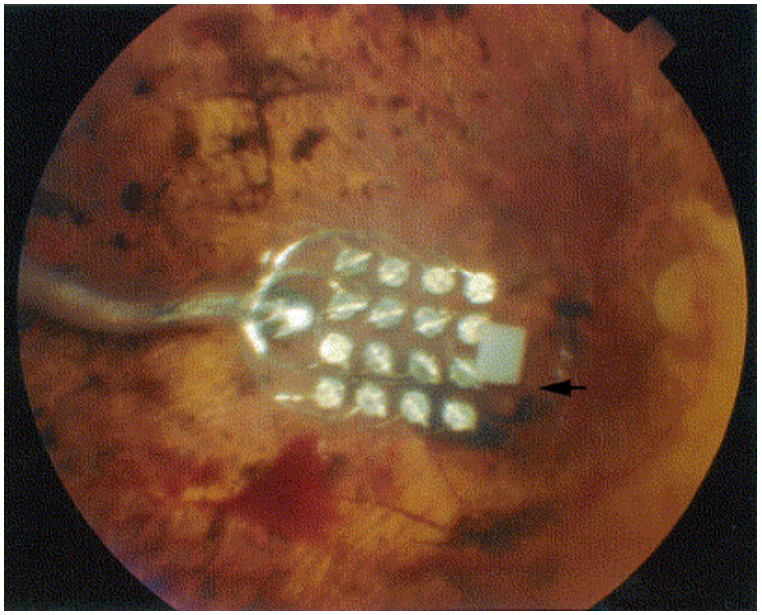
## for diseases that cause blindness due to photoreceptor degeneration

- Reitinitis pigmentosa (1 in 3500 births in US)
- Age-related macular degeneration (1.75M [2000] → 3M [2020] in US)



# Retinal Prosthesis in Blind Subject



Implanted 4 x 4 electrode array;
electrode diameter = 520 μm,
electrode spacing = 720 μm

Humayan et al., Vision Research 43 (2003) 2573.

Yanai et al., Am J Opthalmology 143 (2007) 820. (3 subjects)

# Retinal Prosthesis Studies

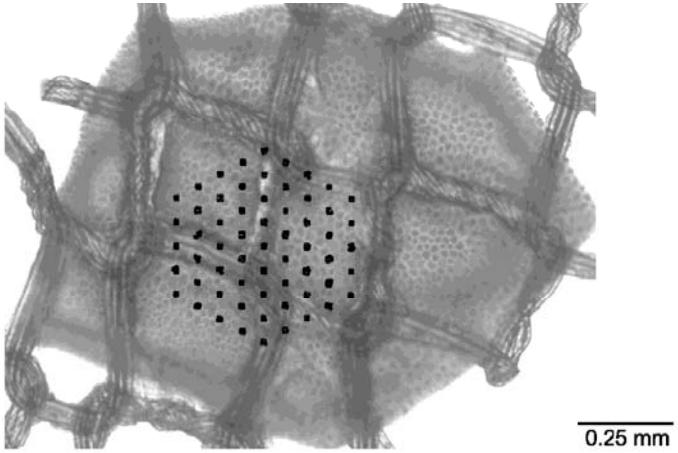
Some issues to be addressed to guide the design of a retinal prosthetic device better able to stimulate RGC activity for more normal visual functioning:

## Stimulate RGC activity:

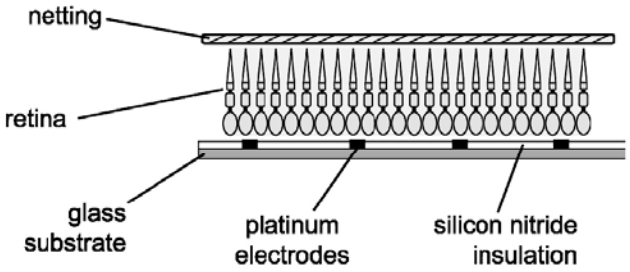
- with a high density array of small diameter electrodes
- in primate retina
- independently in individual RGCs
- in a general spatiotemporal pattern – to recreate the activity pattern expected for the visual stimulus

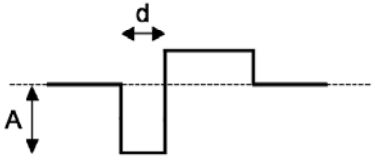
# Retinal Prosthesis Studies I

multielectrode electrical stimulation
combined with multielectrode recording;
**Use small diameter electrodes with high spatial density**




61-electrode array;
electrode diameter = 5-25 μm;
electrode spacing = 60 μm;
rat retina



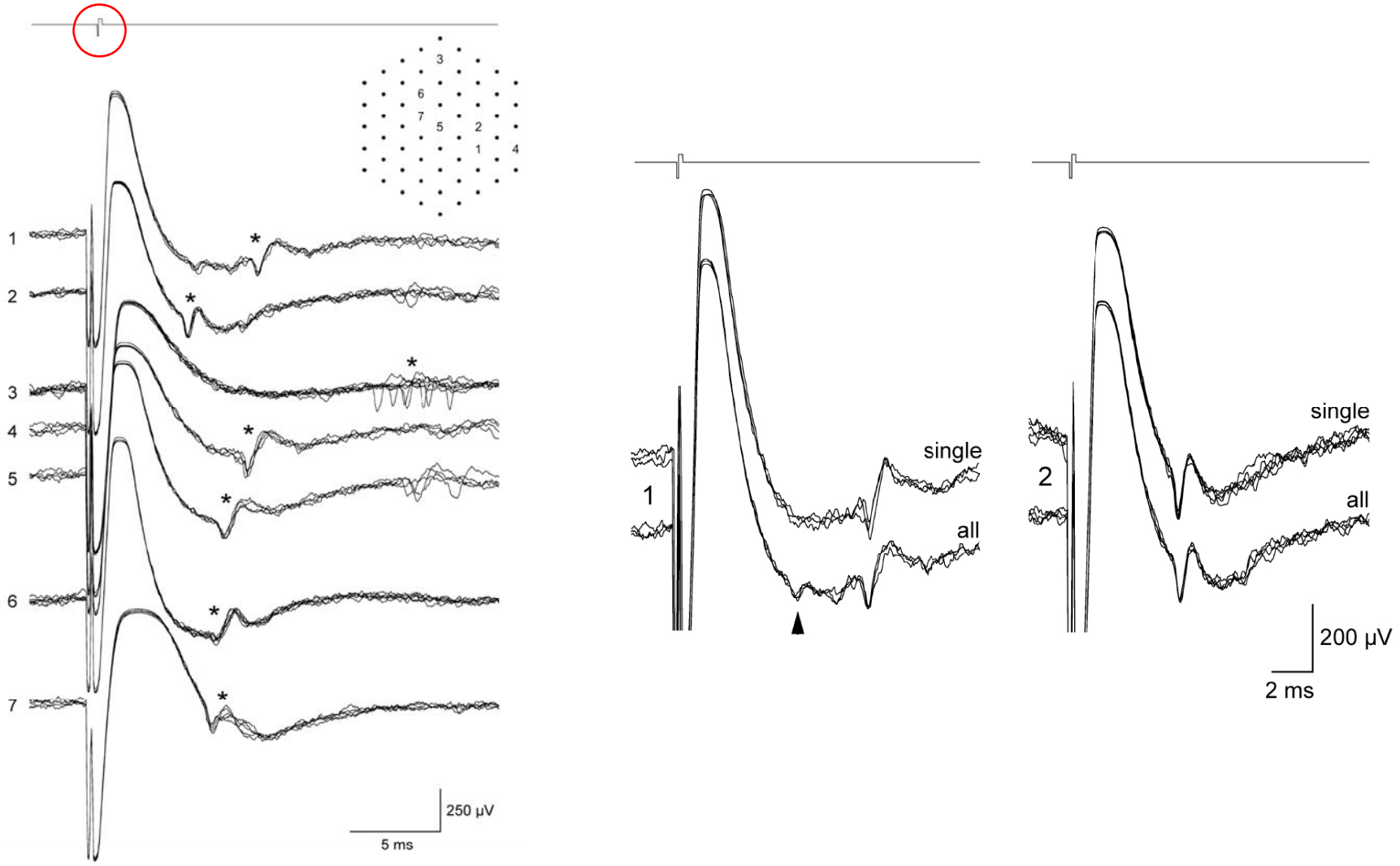




stimulation pulse
supplied by Platchip

C. Sekirnjak et al.,
J. Neurophysiol. (2006)

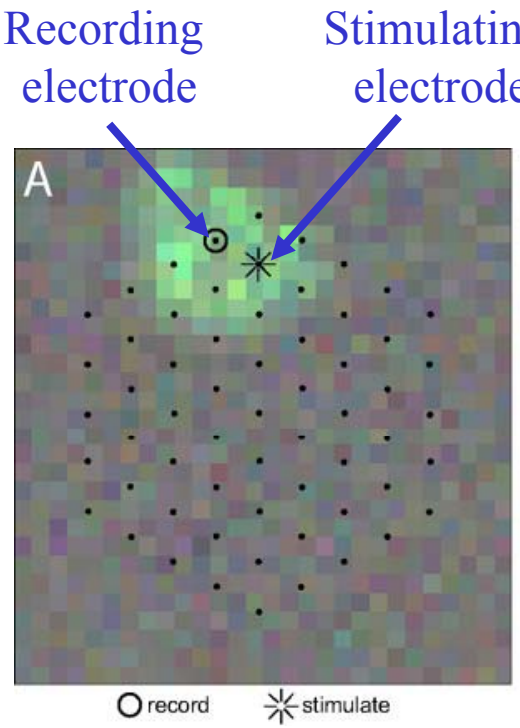
# multiple site stimulation

Stimulation pulse

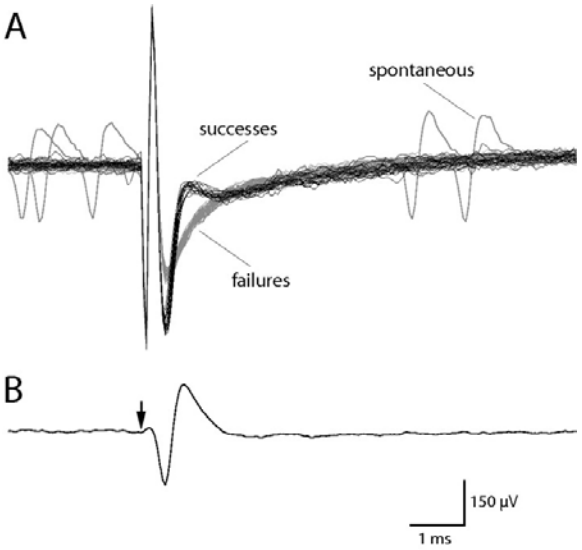



C. Sekirnjak et al., J. Neurophysiology **95** (2006) 3311

# Retinal Prosthesis Studies II
## (primate retina)



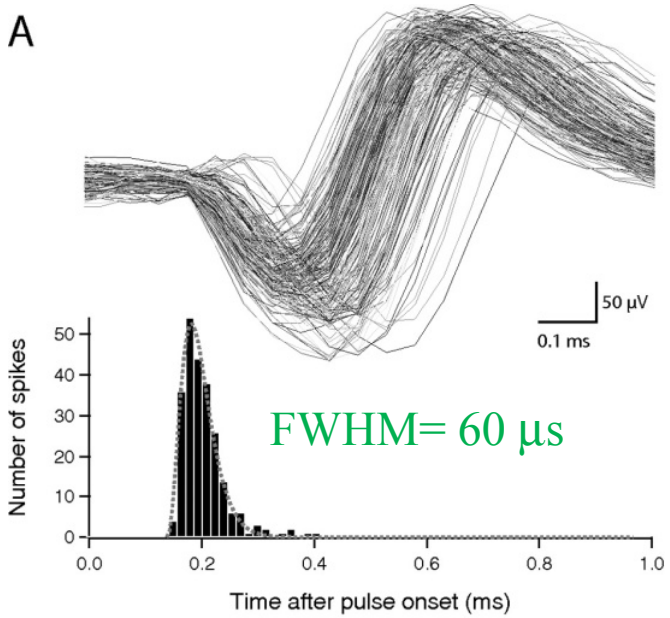

Parasol cell identified from response to white noise stimulus




Low stimulation threshold levels
(~0.05 mC/cm$^2$; factor of 3-20 below safety limit)

C. Sekirnjak et al., J. Neuroscience **28** (2008) 4446

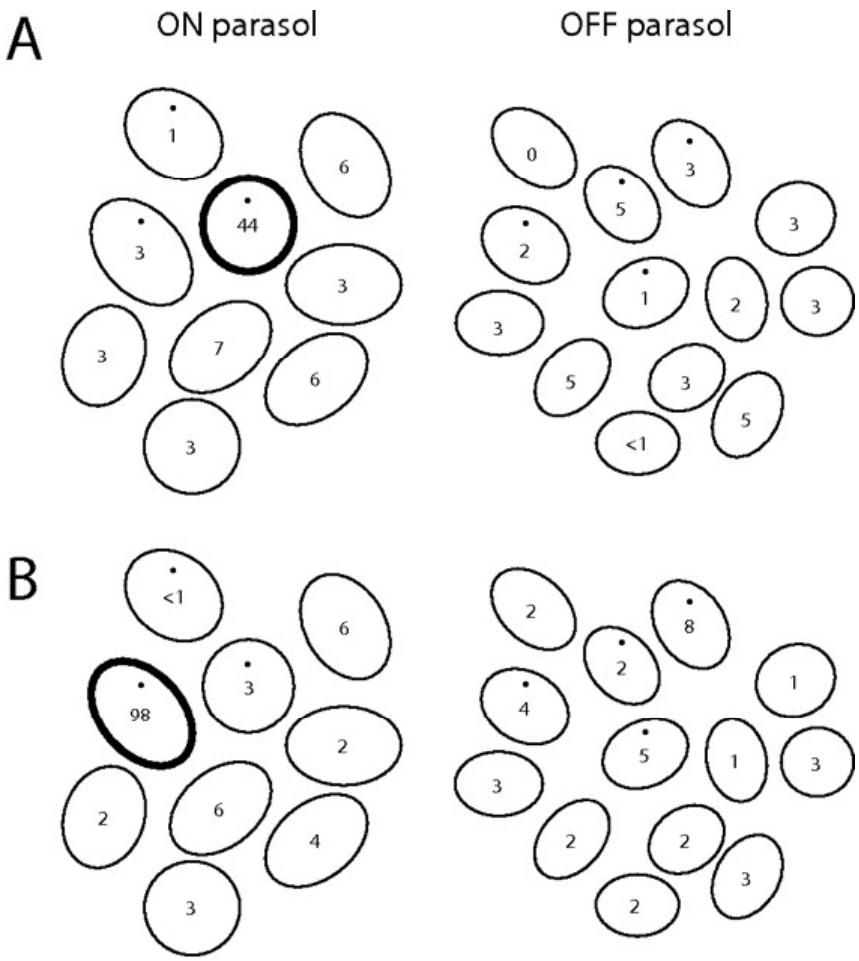
## Stimulation latency and temporal precision




FWHM= 60 µs

## Response rates to stimulation (%)

(includes spontaneous firing rates)




In these examples, the stimulating electrode was also the recording electrode for the cell outlined in bold

**Individual primate parasol RGCs can be electrically stimulated:**

- at low (safe) threshold levels
- with temporal precision
- with little activation of other parasol cells

# Retinal Prosthesis Studies III

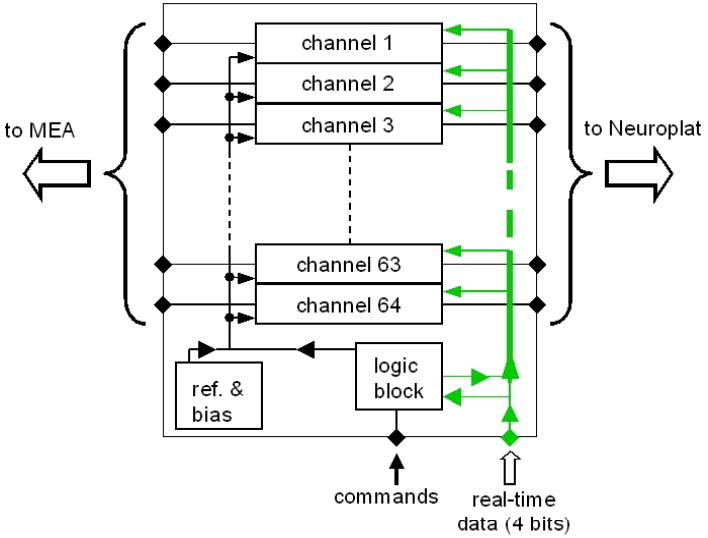
## Overcome limitations of original stimulation system:

- huge electrical artifacts; difficult to record from stimulating electrode
- cannot stimulate with arbitrary spatiotemporal patterns

$\Rightarrow$ new stimulation chip

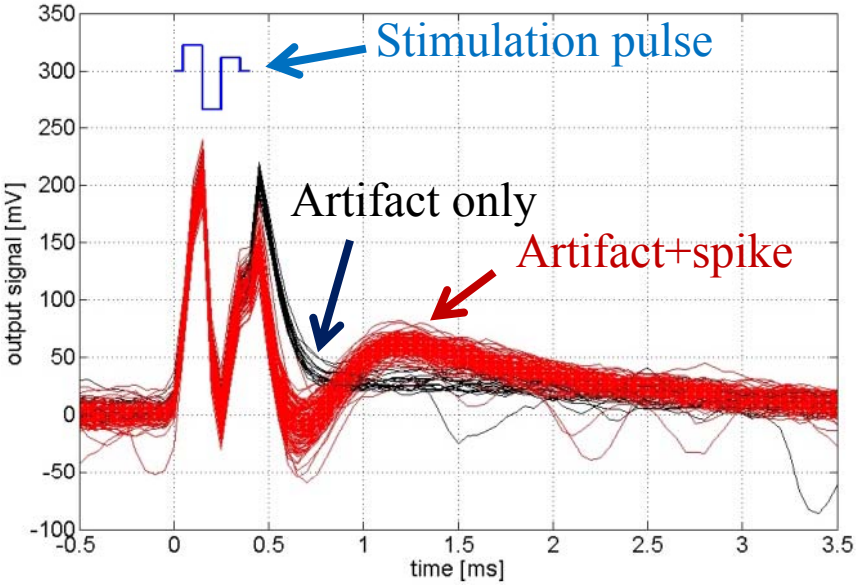
"Stimchip"

- 64 channels
- ability to generate arbitrary, independent waveforms on each channel, under software control
- stimulation in current or voltage mode
- artifact suppression for signal recording

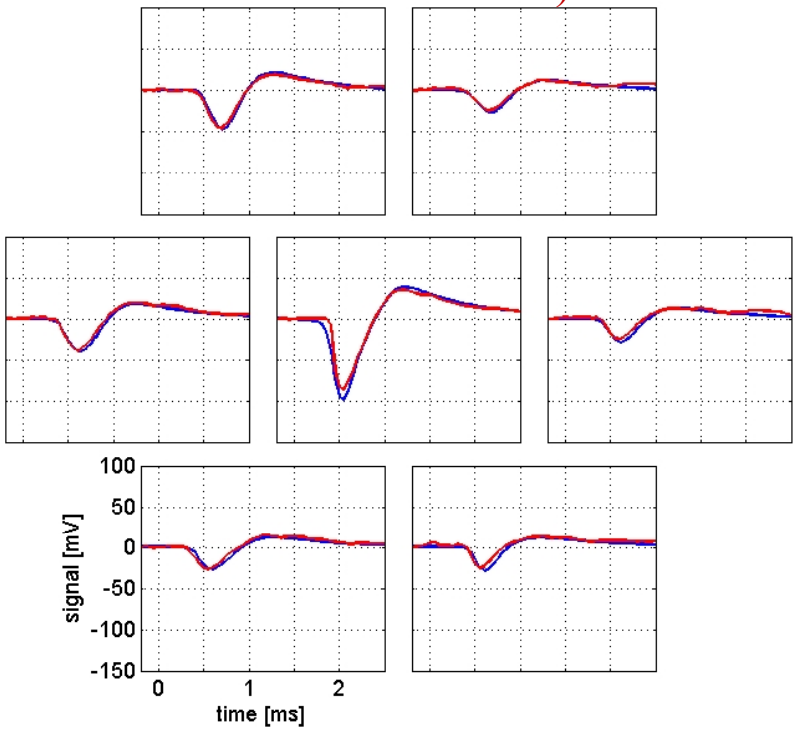



P. Hottowy et al., Analog ICs and Sig. Processing **55** (2008) 239

# Artifact suppression: stimulate and record on same electrode (mouse RGCs)



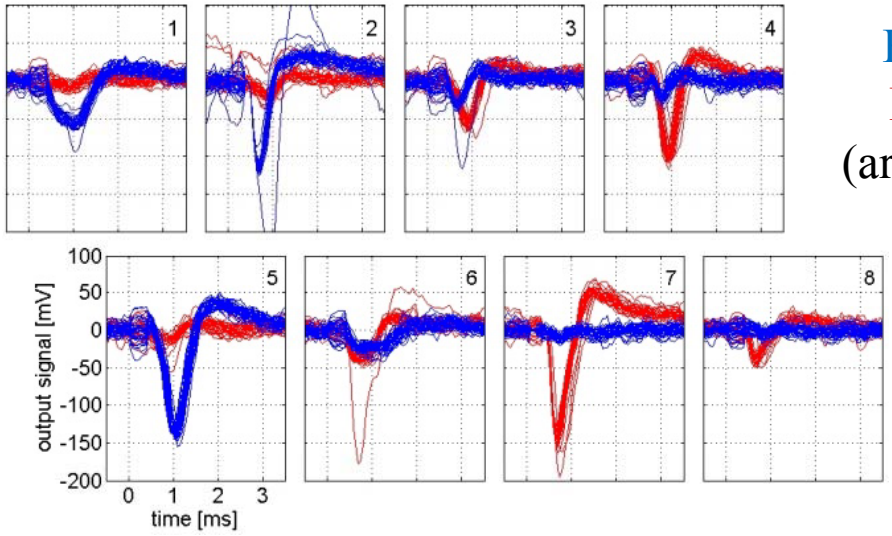

spontaneous spike
stimulated spike (with artifact subtraction)



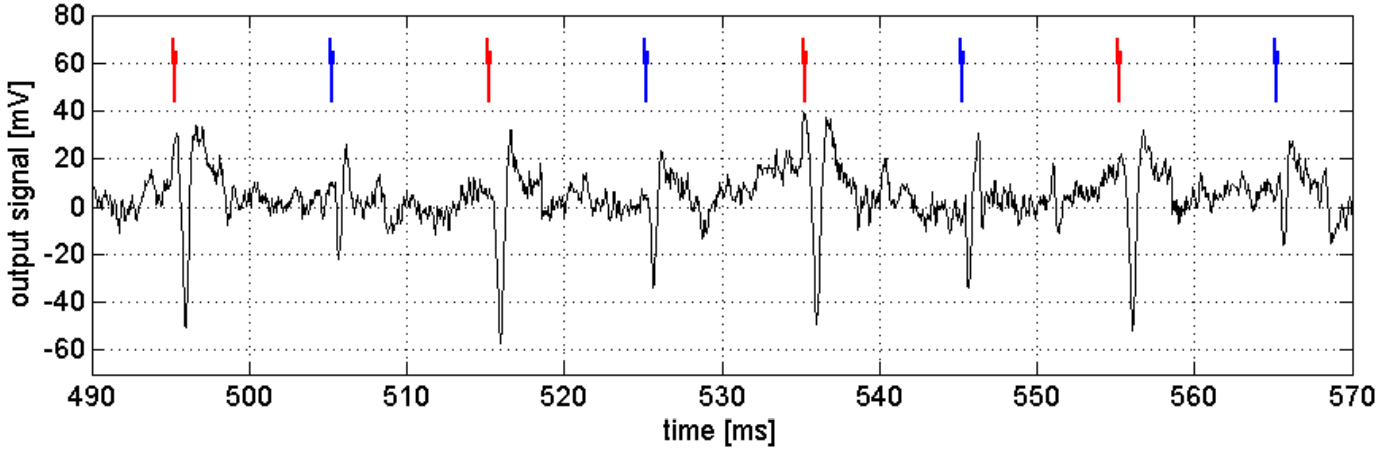

spike waveforms on stimulating electrode and 6 neighboring electrodes

P. Hottowy et al., Proceedings, MEA Meeting 2008 (Reutlingen, Germany)

# Patterned stimulation
# (with 2 independently stimulated mouse RGCs)




Blue: electrode 2 stimulation
Red: electrode 7 stimulation
(artifact subtraction on all traces)




electrode 2 stimulation
electrode 7 stimulation
recording on electrode 3

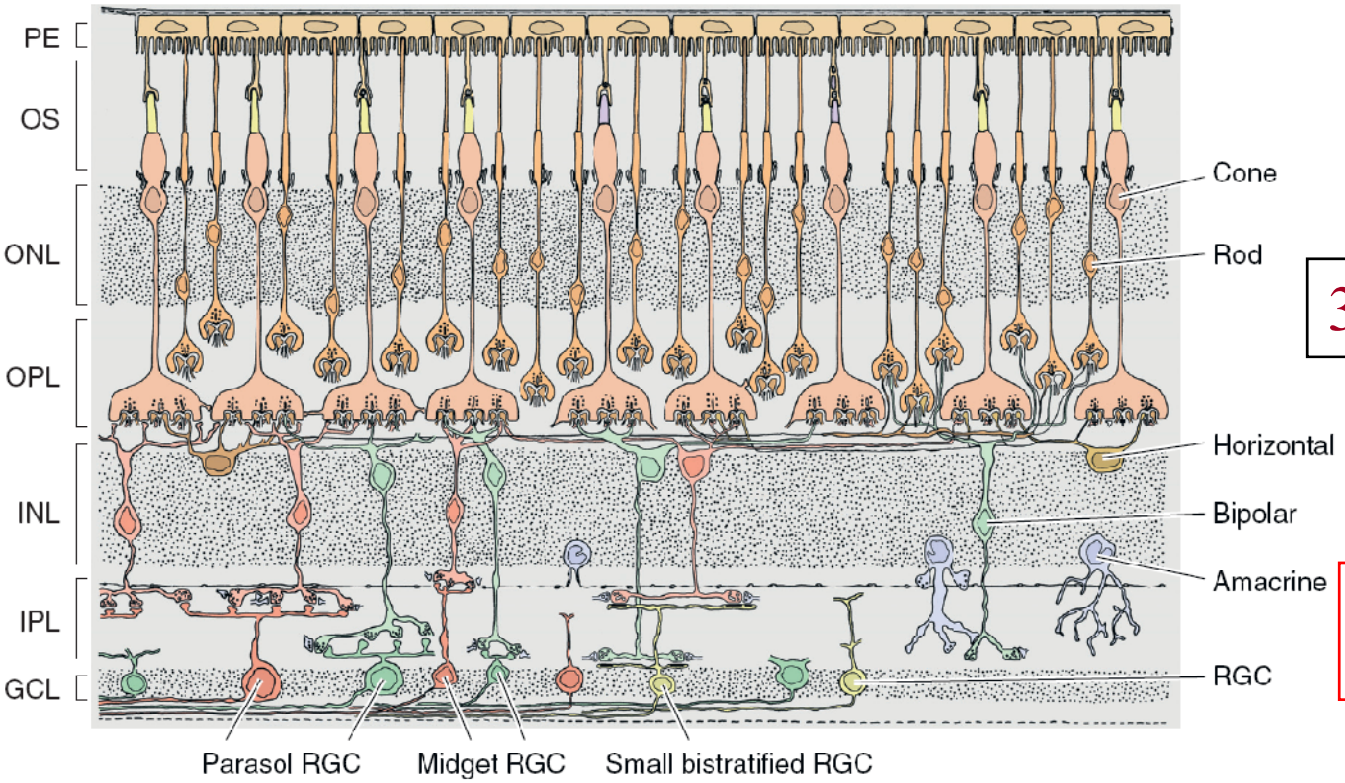
P. Hottowy et al., Proceedings, MEA Meeting 2008 (Reutlingen, Germany)

# Retinal Development

(with D. Feldheim, UCSC and M. Feller, UC Berkeley)

- How does the retina wire itself up?
- How are ~two dozen independent RGC mosaics formed?
- How are the orientations of the direction-selective RGCs formed?

## Primate Retina

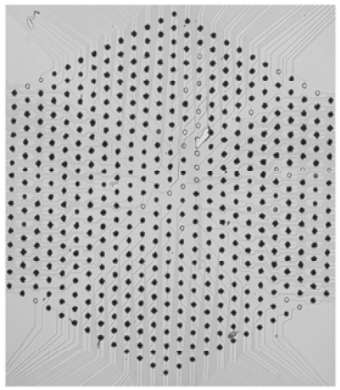



3D wiring problem!

IPL has ≥18 layers;
each layer is 1-2 μm thick

## Use Mouse Retina due to the genetic possibilities

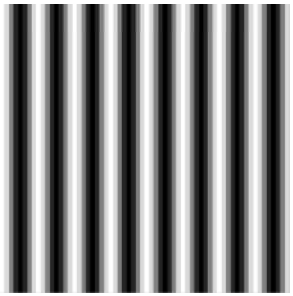
- Measure RGC functional properties and mosaics of wildtype mouse retina
- Compare with the corresponding retinal properties of
  - genetically modified mice
  - mice deprived of visual experience and/or spontaneous correlated neural activity ("retinal waves")
- to relate function with structure, match anatomical image with EI
- Develop high density electrode arrays (to better identify and image the small-sized mouse RGCs)



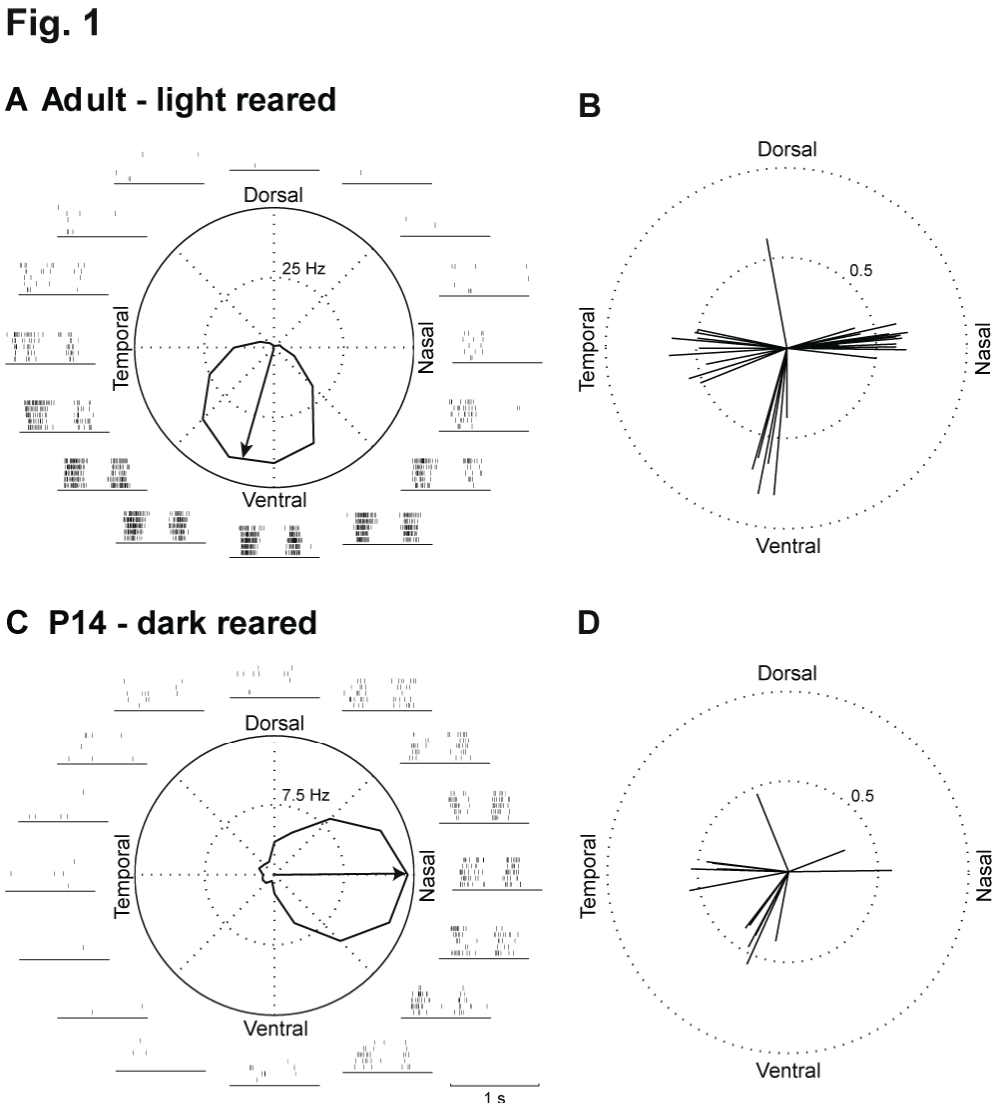
519-electrode array
with 30 μm spacing

(Developed by K. Mathieson and D. Gunning, U. Glasgow)

# Mouse On-Off Direction Selective Ganglion Cells

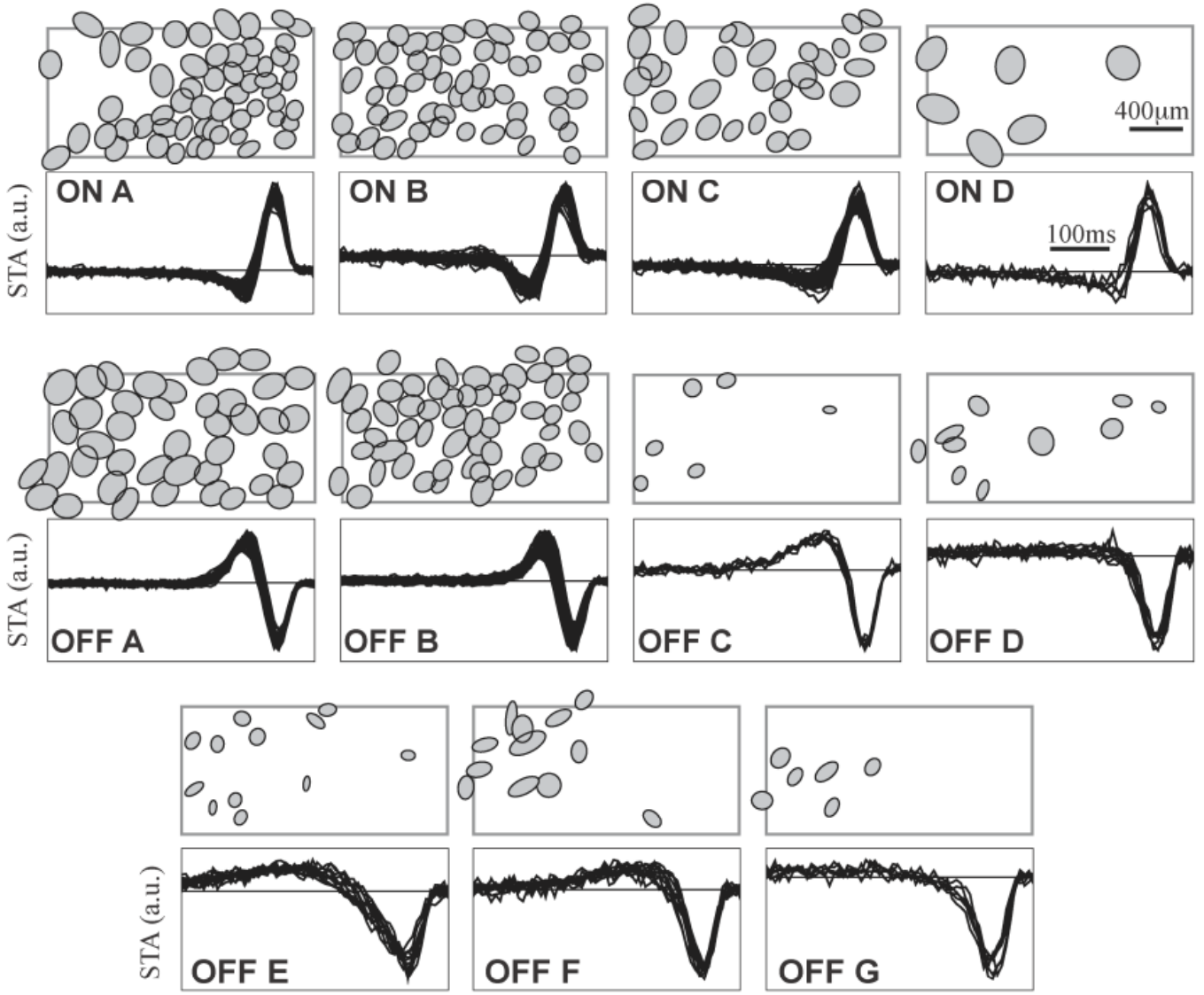


drifting sinusoidal gratings in 16 directions



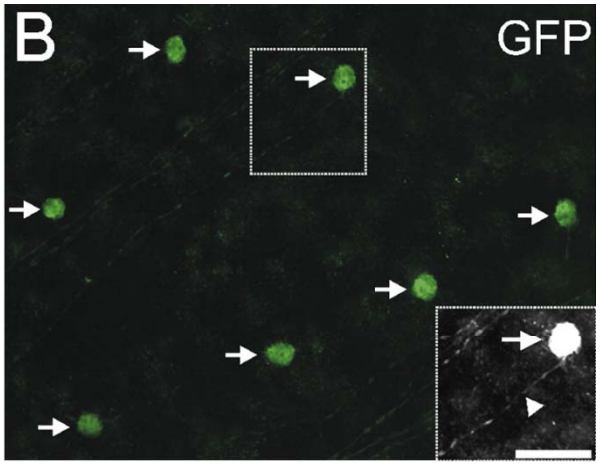

J. Elstrott et al., Neuron **58** (2008) 499

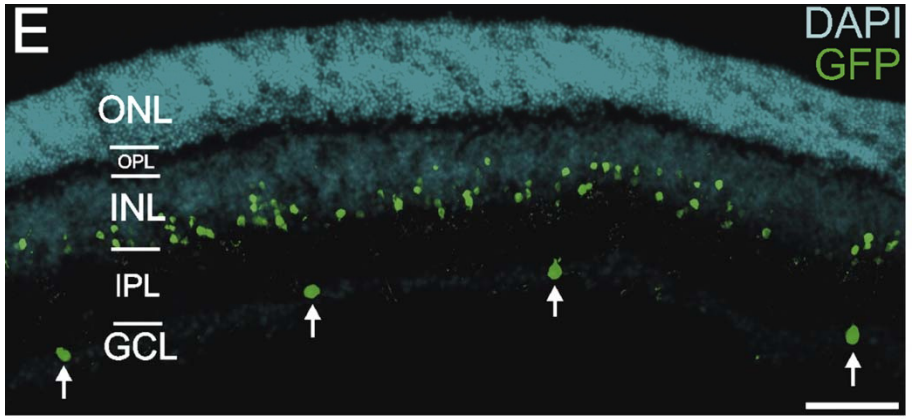
# Mosaic and functional properties of mouse RGC types

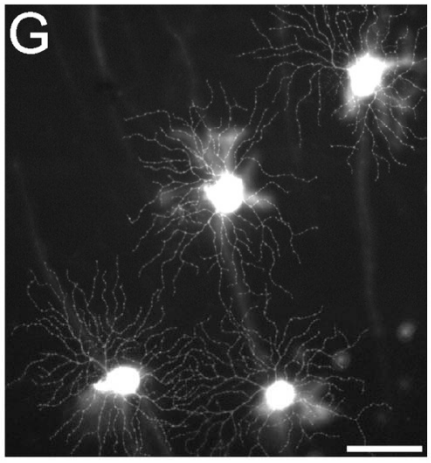


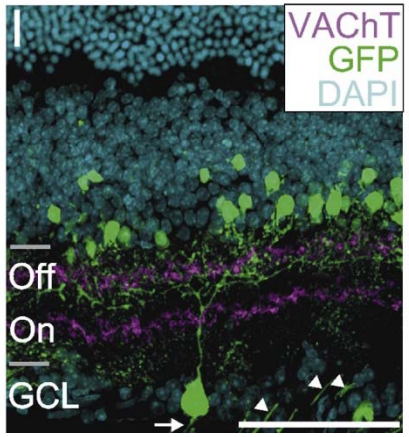

A. Sher et al., FASEB conference on Retinal Neurobiology and Visual Processing (2008)

# Mosaic of transient-OFF-α RGCs in mouse retina is genetically labeled with green fluorescent protein (GFP)




A. Huberman et al., Neuron **59** (2008) 425

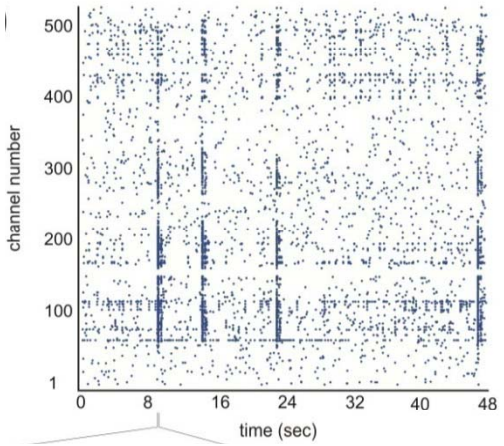
# Conclusions

- We have developed a multielectrode array system for the large scale recording and stimulation of retinal ganglion cell activity
- For the first time, it has become possible to study image processing and encoding by the retina in terms of the correlated spiking activity of hundreds of neurons
- There are at least two dozen functional types of mammalian retinal ganglion cells, each of which appears to tile the visual field, and each of which appears to send a separate “image” to the brain
- A new functional type of primate retinal ganglion cell has been found with large receptive field, highly transient light response, and non-linear spatial summation (Y-like)
- Retinal prosthesis studies indicate that a dense array of small diameter electrodes can be used to electrically stimulate retinal ganglion cells in a spatiotemporal pattern

# Outlook

- We have a variety of tools in hand (electrode arrays, ICs for neural activity recording and stimulation, data acquisition systems, software) to study how populations of neurons in a variety of neural systems process and encode information. The fun has just begun!
- Additional tools are under development:
  - Bed-of-nails arrays to study brain tissue slices; see Debbie Gunning's talk in this session
  - 512-electrode stimulation and recording system to study cortical network dynamics in brain tissue slices; establish two-way communication with a living neural system (in collaboration with J. Beggs, Indiana U. and W. Dabrowski, Krakow)
  - Wireless in vivo recording system; study brain activity in awake, naturally-behaving animals (in collaboration with M. Meister, Harvard U., and W. Dabrowski, Krakow)
- Much work remains to be done on retinal processing, retinal prosthesis and retinal development

Rat cultured cortical slice on 512-electrode array




portable, battery-operated, wireless system to record brain activity on multiple electrodes; can be carried by a rat or a flying barn owl