Large Scale Imaging of the Retina

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SCIPP, UC Santa Cruz
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1. The Retina – a Biological Pixel Detector

2. Probing the Retina

⇒ understand the language used by the eye to send information about the visual world to the brain
⇒ use techniques and expertise from silicon microstrip detector development

3. Some First Results

4. Summary and Additional Applications
Collaborators

- **UC Santa Cruz:**
  A. Grillo, M. Grivich, S. Kachiguine, D. Petrusca, A. Sher
- **AGH U. of Science and Technology, Krakow (IC design):**
  W. Dabrowski, P. Hottowy
- **U. Glasgow (high density electrode array fabrication):**
  D. Gunning, K. Mathieson
- **The Salk Institute (neurobiology):**
  E. J. Chichilnisky, G. Field, J. Gauthier, J. Shlens
The Eye

- Sclera
- Choroid
- Retina
- Fovea
- Optic nerve
- Cornea
- Pupil
- Lens
- Iris
- Ciliary body
The Retina: an Advanced Pixel Detector

- thickness: $\approx 300 \ \mu m$
- active area: $10 \ \text{cm}^2$
- number of pixels: $10^8$
- number of output channels: $10^6$
- compression factor (# input channels/# output channels): 100:1
- output signal width: $\approx 1\text{ms}$
- output format: analog, encoded by the frequency of digital signals
- spatial resolution: down to $2 \ \mu m$
- 3D (depth perception): stereoscopic vision
- radiation hardness: non-rad-hard
- technology: mature, reliable, and in wide-spread use
The Retina

~300 µm

RODS AND CONES

HORIZONTAL CELLS

BIPOLAR CELLS

AMACRINE CELLS

GANGLION CELLS

OPTIC NERVE

↑↑↑↑↑
light
The Retina

- photoreceptors
- inner nuclear layer (horizontal, bipolar, amacrine cell bodies)
- ganglion cell layer
- nerve fiber layer
- outer plexiform layer
- inner plexiform 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
Probing the Retina

• **Goal:** understand how the retina processes and encodes dynamic visual images
• **Method:** record the patterns of electrical activity generated by hundreds of retinal output neurons in response to a movie focused on the input neurons
• **Technology:** based on silicon microstrip detector techniques and expertise developed for high energy physics experiments – an example of the application of expertise in HEP instrumentation to neurobiology
Experimental Technique
(based on work by Meister, Pine and Baylor)
Species?
Guinea Pig, Monkey, Mouse

Scale?
• Record from a population of neurons approaching a scale of interest for neural computation
• Order-of-magnitude improvement in state-of-the-art

⇒ Record simultaneously from hundreds to thousands of retinal ganglion cells in a single preparation
System Specifications

**Spatial resolution:** 30-60 µm electrode spacing
- efficiently detect/image RGCs with small active regions
- electrode density ≥ cell density

**Time/ampl. resolution:** 50 µs (20 kHz sampling freq.)/12 bits
- characterize spike waveforms well enough to identify the signals from individual neurons (time coincidence)

**Sensitive area:** 2 - 8 mm²
- detect # of RGCs comparable to # of inputs used in the visual cortex for neural computation (~hundreds to thousands)
- collect sufficient statistics, even for quite rare RGC classes
- characterize tiling of visual field

**Data collection time/experiment:** ~24 hours
**Implications**

⇒ Large number of channels (512–2048)
⇒ High data collection rate (15-60 MB/s)
⇒ Vast amount of data (1.2 – 4.8 TB per one-day experiment)

**Compare to “traditional” neurophysiology:**

- Number of channels = 1
- Data collection rate = 30 kB/s
- Data/day = 2.5 MB

**System Ingredients**

- High density/large area electrode arrays
- Multichannel VLSI analog/digital circuitry for readout
- High speed data acquisition and processing
- Automated (or at least semi-automated) data analysis
Electrode Array Geometries

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

**1 electrode:**
- "traditional"
  - Input region for monkey MT neuron
  - 0.17 mm²
  - 60 µm

**61 electrodes:**
- previous state-of-the-art
  - 7.1 mm²
  - 120 µm

**512 electrodes (32x16):**
- current system
  - 1.7 mm²
  - 60 µm

**519 electrodes:**
- high density
  - (recently fabricated)
  - 0.43 mm²
  - 30 µm

**519 electrodes:**
- large area
  - (to be fabricated)
  - 7.1 mm²
  - 120 µm

**2053 electrodes:**
- futuristic
  - 7.1 mm²
  - 60 µm
Section of 512-electrode Array (32x16)

Electrode diameter = 5 µm
Section of 512-electrode “Neuroboard”
512-electrode “Neuroboard”

- 512-electrode array
- 64-channel Neurochip
- 64-channel Platchip
- Fan-in
- chamber
- line driver
- 512-electrode array
Salamander retina on 512-electrode array

Slice of hippocampal tissue on 512-electrode array
Spikes on electrodes ⇒ spikes from identified neurons
Neuron Identification
(signals on electrodes \(\Rightarrow\) spikes from identified neurons)

Multiple electrodes

\[
\begin{array}{cccc}
1 & 2 & 3 & 4 \\
5 & 6 & 7 & \end{array}
\]

7x26=182 measurements

1.3 ms

• Principal Components Analysis
Find \(~5\) most significant variables that are linear combinations of the 182 measurements

• Multidimensional Clustering
\(\Rightarrow\) Identified Neurons

Software by D. Petrusca, SCIPP
Electrophysiological Imaging

Superimposed images of 4 monkey RGCs

1000 µV

1.6 m/s

2 ms
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

Spike Train

Stimulus

1 s

Spike-triggered average
Monkey Retinal Ganglion Cell

ON Cell

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<tr>
<th>Time (ms)</th>
<th>Image</th>
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<tbody>
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Time wrt spike
Spike-triggered average image at time of maximum absolute intensity

- Time before spike (ms)
- Spike rate
- Filter $\otimes$ image signal

900 $\mu$m
Monkey Retinal Ganglion Cell

**OFF Cell**

<table>
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<tr>
<th>Time (ms)</th>
<th>Image 1</th>
<th>Image 2</th>
<th>Image 3</th>
<th>Image 4</th>
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900 µm
Some first (preliminary) results with monkey retina

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

[Diagram showing light-sensitive regions with dimensions 3.2 mm x 1.6 mm]
Spatial/temporal response properties of individual neurons ("spike-triggered average")

ON-parasol

OFF-parasol

ON-midget

OFF-midget

Blue-ON

(8.3 ms/frame)
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.

D. Petrusca et al., Soc. for Neuroscience annual meeting (2005)
What about the guinea pig retinal ganglion cells?
Not well studied, anatomically or physiologically. Much better studied is the rabbit retina. It is known that there are at least 13 distinct anatomical types and ~11-15 physiological classes of rabbit RGCs (only ~8 anatomical types have been well-matched with physiological classes)

Rabbit RGC 13 anatomical types

Rockhill et al., J. Neuroscience 22 (2002) 3831
Guinea Pig Retinal Ganglion Cells: OFF cells

RF mosaic for 311 OFF cells

Direction selectivity for drifting sinusoidal gratings

RF mosaics for clusters 1-4
Neural activity recorded with 512-electrode system as image of vertical moving bar is focused on a section of guinea pig retina

(Animation repeats after 2 sweeps)
Guinea Pig Retinal Ganglion Cells: ON cells

RF mosaic for 169 ON cells

Direction selectivity for drifting sinusoidal gratings

RF mosaics for clusters 1-3
Guinea Pig Retinal Ganglion Cells: Non-Direction Selective

There are at least 30 distinct and highly specialized guinea pig retinal ganglion cell types

M. Grivich et al., Society for Neuroscience annual meeting, 2005
Summary and Additional Applications

• We have developed a multielectrode system for the large scale recording of retinal ganglion cell activity.
• Experimental data has been obtained with live guinea pig and monkey retinas.
• For the first time, it has become possible to study image processing and encoding by the retina in terms of the correlated activity of hundreds of neurons.
• There are numerous physiological classes of 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.
• Additional applications include studies of:
  ⇒ retinal development
  ⇒ retinal stimulation for retinal prosthesis
  ⇒ dynamical neural network activity in slices of brain tissue
  ⇒ brain activity in awake, naturally-behaving animals