Introduction to Systems Neuroscience

Methodologies used to study brain systems

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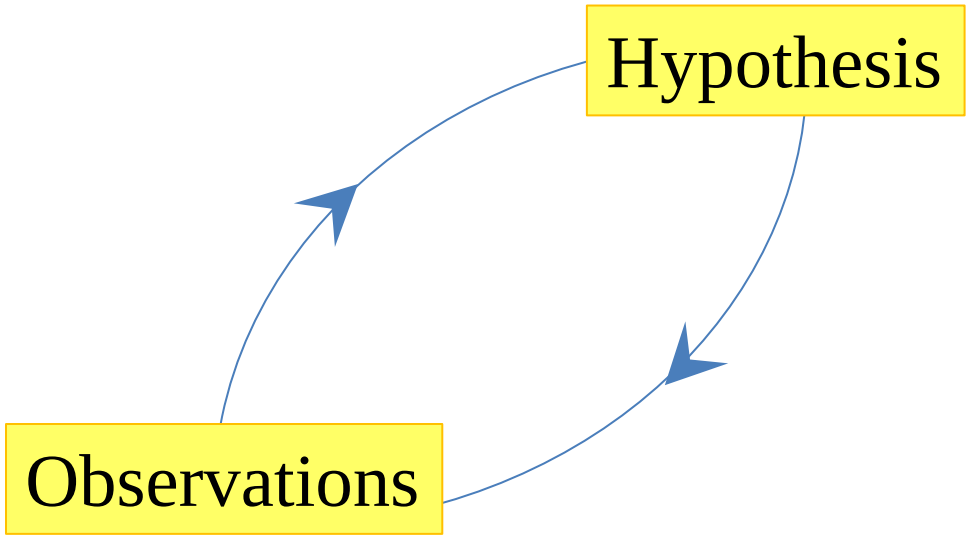
Introduction

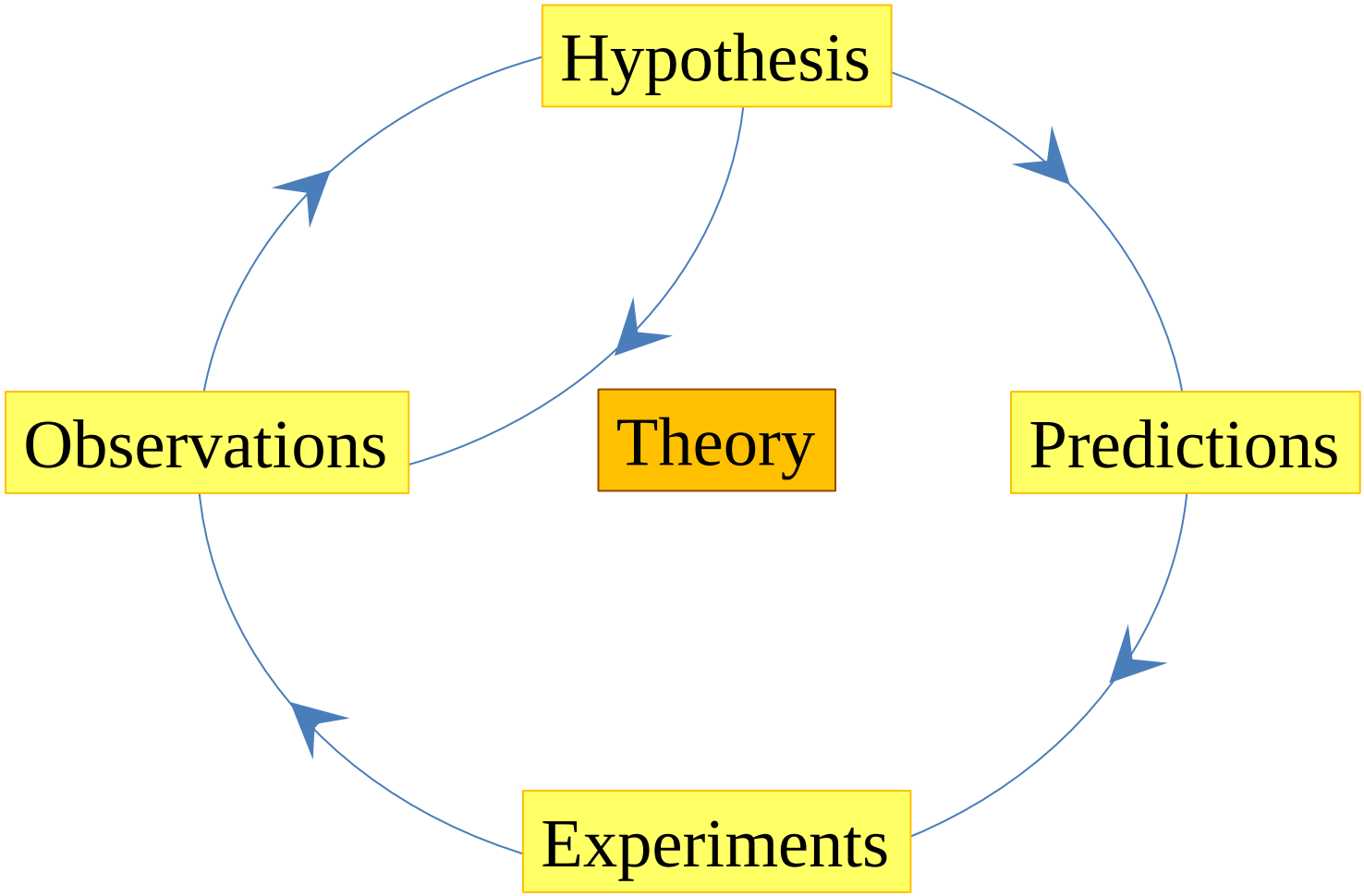
- In natural sciences, unlike in mathematics, there is ***no set of pre-defined axioms*** from which other definitions can be derived and proved.
- What can be proven in natural sciences are only ***consistencies***; hypotheses are considered valid as long as they are consistent with actual observations *(e.g., gravity is considered valid as long as the movement patterns it entails are consistent with actual movements of bodies).*
- The methods are thus developed to test consistencies of observations with assumptions

Introduction



Introduction

The scientific method



Research approaches

- **Observational:** we collect enough evidence to compose a hypothesis
- **Correlational:** we compare 2 variables the values of which have been collected without direct intervention. No causal relationships can be directly concluded.
- **Experimental:** an “independent” variable is systematically manipulated and the effects of this on a “dependent” variable are measured. Considered as the best approach for revealing causal relationships, although…

Introduction

General guiding assumptions

- All mental functions are carried out in the brain
- All brain components are relevant
- Brain processes obey the rules of physics

Examples of common working hypotheses

- “The basic component of processing is a single neuron”
- “Neural processing is mediated by neurotransmitters, brain states are controlled by neuromodulators”
- “Memories are stored in synapses”

Introduction

Major difficulties

- Complexity
  - many levels (ions, … , neurons, …, systems)
  - many components (e.g., 10^{11} neurons, 10^{15} synapses)
  - many variables (physical, chemical, mechanical, electrical, physiological, psychological)
- Small sizes (neurons ~ 10 μm, synapse <1 μm)
- Closed loops
- Self organization
- plasticity

} Cannot repeat an experiment twice

Introduction

Major difficulties – Methods to address them

- Complexity
  - many levels (ions, … , ne ??? systems) …
  - many components (e.g rons, 10^{15} synapses)
  - many variables (physical, chemical, mechanical, electrical, physiological, psychological)
- Small sizes (neurons
- Closed loops
- Self organization
- plasticity

Small tools, magnifications, genetic tools

Opening the loops by anesthesia, flashed stimuli, cuts

Cannot repeat an experiment twice

Using statistics

Introduction

About this lecture

- Review of popular methods for experiments in systems neuroscience
- Focus on resolutions and potential power. Less on limitations
- Not all methods will be covered. You are invited to complete the picture through the web or text books
- Slide numbers – appear in MOST of the slides, not in all…

Methods table

Measuring neural activity

	Spatial resolution				Temporal resolution			
	device	signal	neurons	heuristic	device	signal	spikes	heuristic
behavior	brain	brain	> 10^{11}	station	1 ms	10 ms	10	
2DG, c-fos	10 μm	10 μm	1		NA	30 min	> 10^{6}	
fMRI	> 1 mm	> 200 μm	> 10^{5}		1 s	> 1 s	1000	10 ms
EEG	10 mm	100 mm	> 10^{9}	station	< 1 ms	50 ms	50	1 ms
MEG	10 mm	100 mm	> 10^{9}		1 ms	50 ms	50	
ECoG	10 mm	1-10 mm	> $10^{4\text{-}6}$		< 1 ms	10 ms	10	
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Intracell elec	10 μm	10 μm	< 1		< 1 ms	< 1 ms	< 1	
Ca imaging	1 μm	1 μm	< 1		1 ms	> 100 ms	> 100	

Methods table

Stimulating / perturbing neural activity

	Spatial resolution				Temporal resolution			
	device	signal	neurons	heuristic	device	signal	spikes	heuristic
sensory	modality	modality	> 10^9	station	< 1 ms	< 1 ms	< 1	
TMS	100 mm	10 mm	> 10^9	station	< 1 ms	100 ms	100	
µStim	< 10 µm	> 100 µm	> 50		< 1 ms	10 ms	10	
µPharmac	< 10 µm	> 100 µm	> 50		1 ms	> 10 s	10	
single cell	< 10 µm	< 10 µm	1		< 1 ms	< 1 ms	< 1	
sub-cell	>100 µm	< 1 µm	> 50		< 1 ms	< 1 ms	< 1	

Methods table

Measuring structure (anatomy)

	Spatial resolution					Temporal resolution			
	device	signal	neurons	heuristic		device	signal	spikes	heuristic
cell density									
receptor density									
transmitter density									
tract tracing									
single-cell									

Methods table

Manipulating structure

	Spatial resolution					Temporal resolution			
	device	signal	neurons	heuristic		device	signal	spikes	heuristic
Neuropsychology		> 10 mm	> 10^7				months		
lesions	> 100 μm	> 100 μm	> 50			> 1 s	> 1 min		

Methods table

Measuring neural activity

VSD Imaging	10 μm	30 μm	< 100			< 1 ms	10 ms	< 1	
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μPharmac	< 10 μm	> 100 μm	> 50			1 ms	> 10 s	10	

Measuring neural activity

Behavior

- Present sensory stimuli
- Measure response accuracy, threshold, speed
- Infer related neural activity

Example: odor sensitivity

- Present mixture of 2 odors
- Ask if the mixture is more similar to A or B (2AFC)
- Response accuracy: randomize stimuli and compute a psychometric curve (% correct as a function of ΔO)
- Discrimination threshold: use staircase paradigm and wait for stabilization
- Perceptual speed: measure reaction time
- Infer relevant neuronal pathways and stations by known constrains

heuristics ⟹ Station resolution by known functional anatomy

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Measuring neural activity

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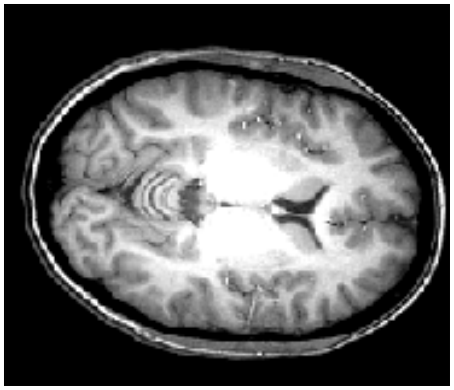
MRI vs. fMRI

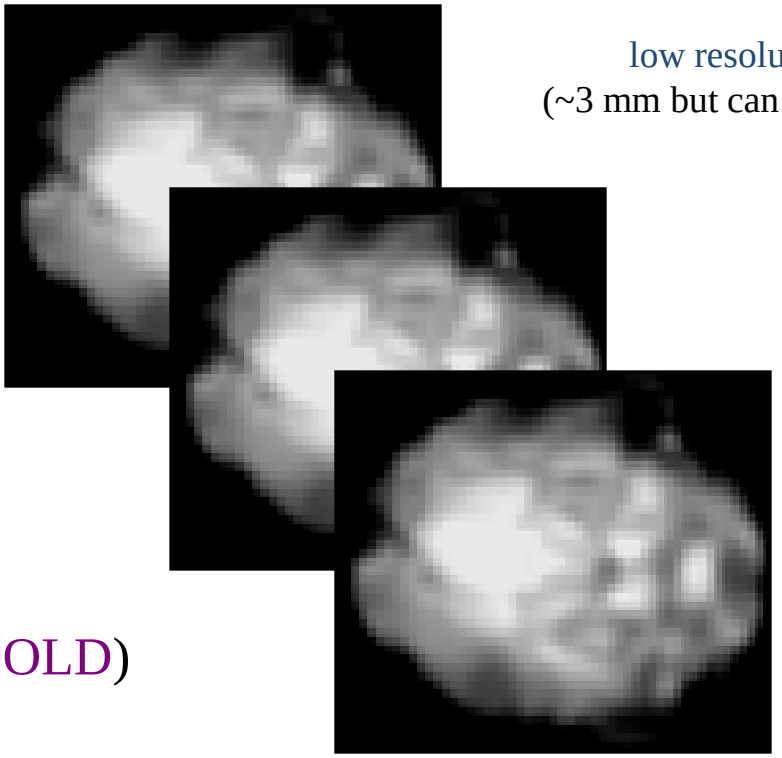
MRI

fMRI

high resolution (1 mm)



one image



low resolution (~3 mm but can be better)

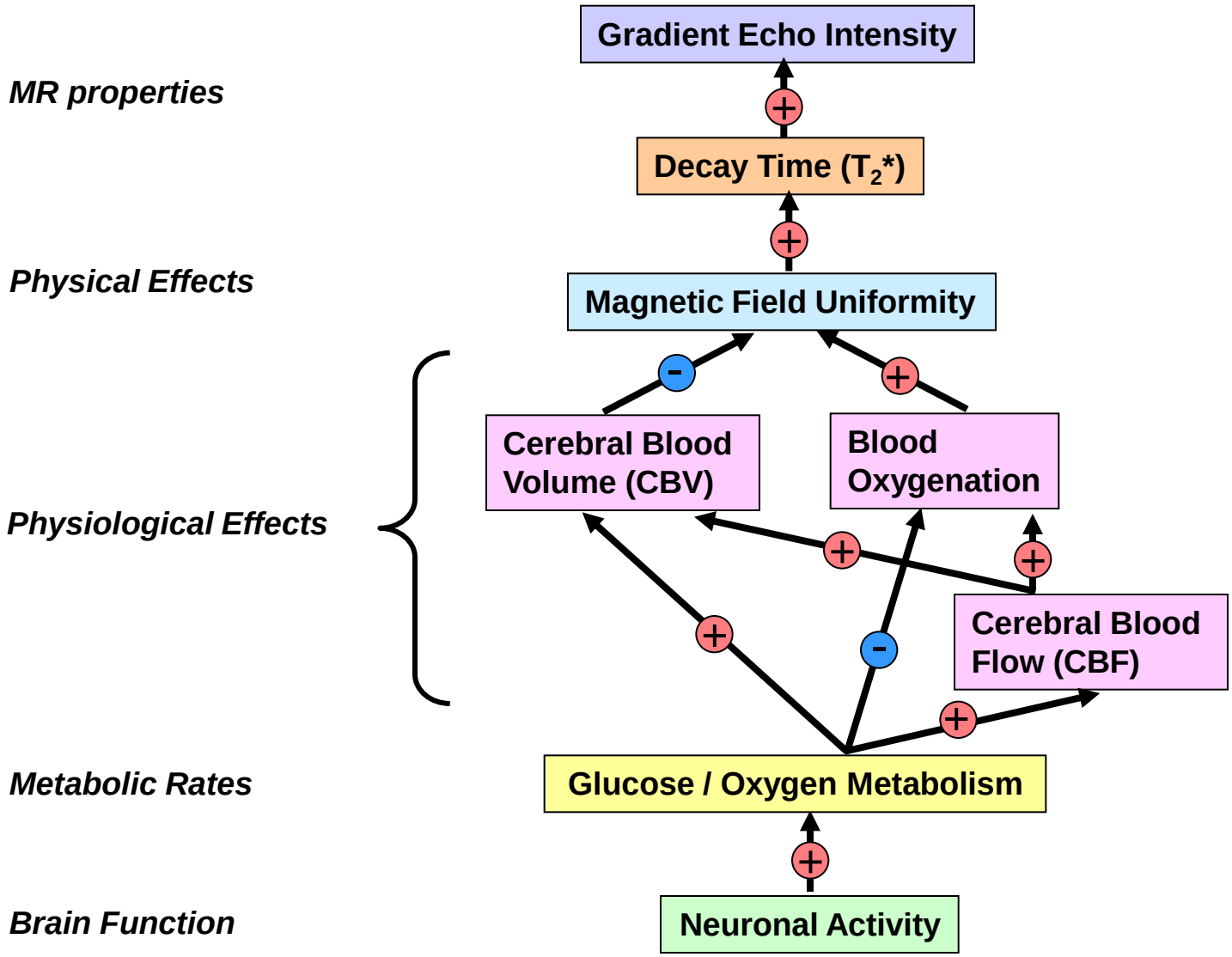
many images (e.g., every 2 sec for 5 mins)

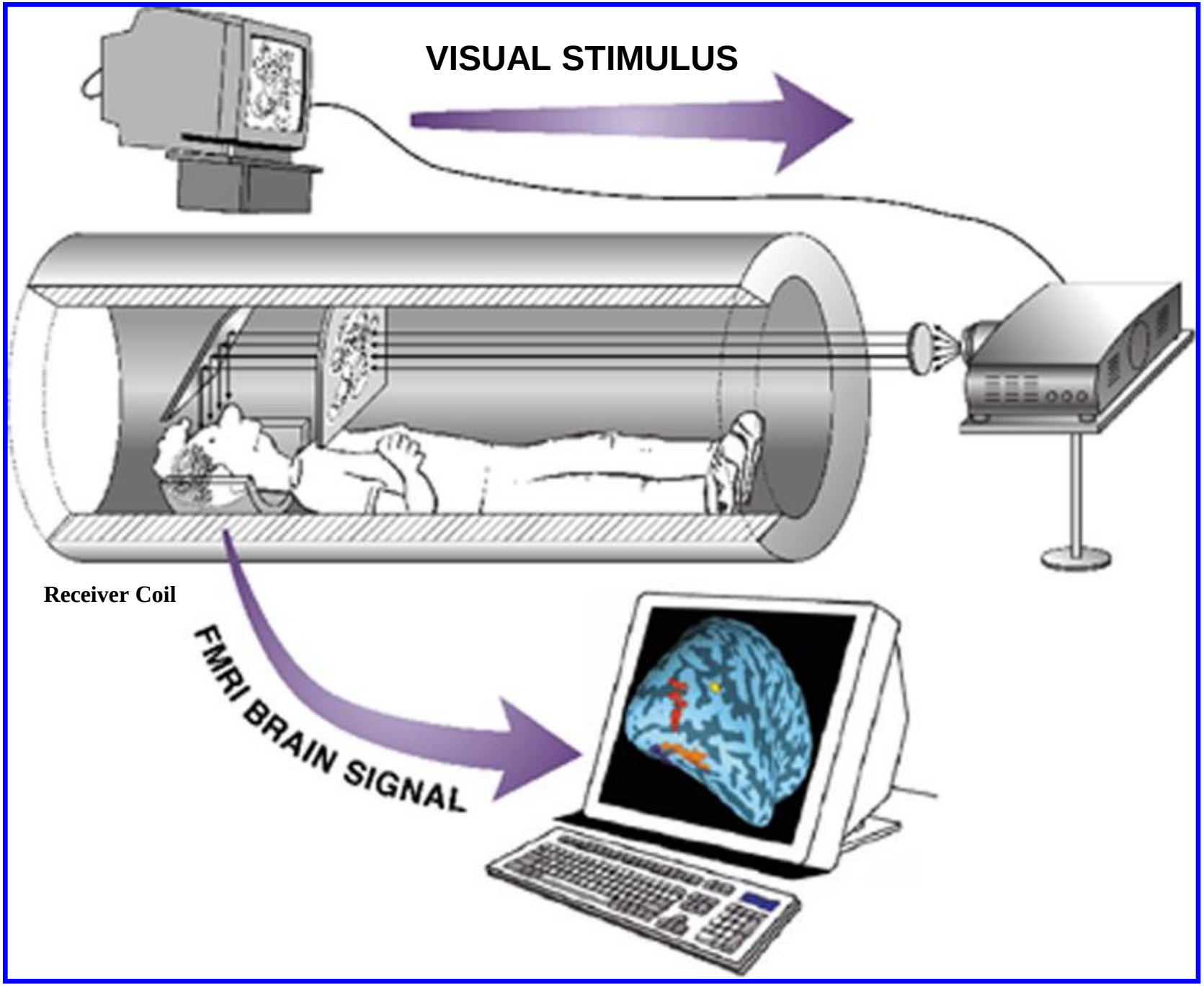
fMRI

Blood Oxygenation Level Dependent (BOLD) signal indirect measure of neural activity

↑ neural activity ➔ ↑ blood oxygen ➔ ↑ fMRI signal

fMRI using BOLD

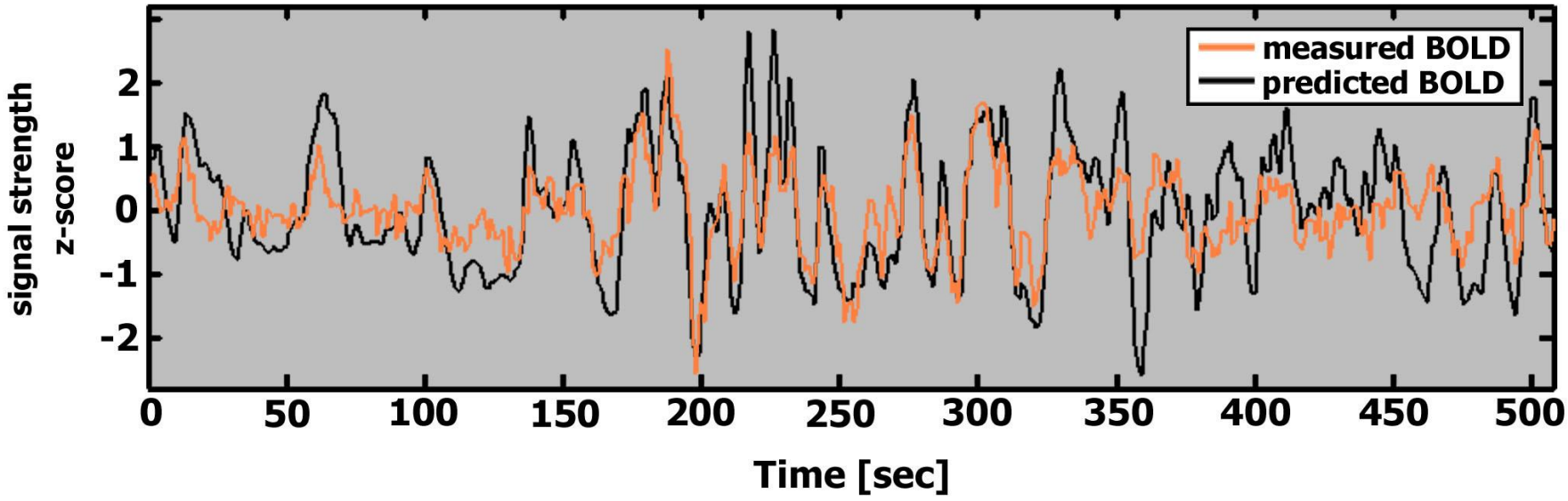



VISUAL STIMULUS
Receiver Coil
FMRI BRAIN SIGNAL

Predicting fMRI BOLD signal in one subject from spike activity in another subject during the same movie

Patient 1 predictor

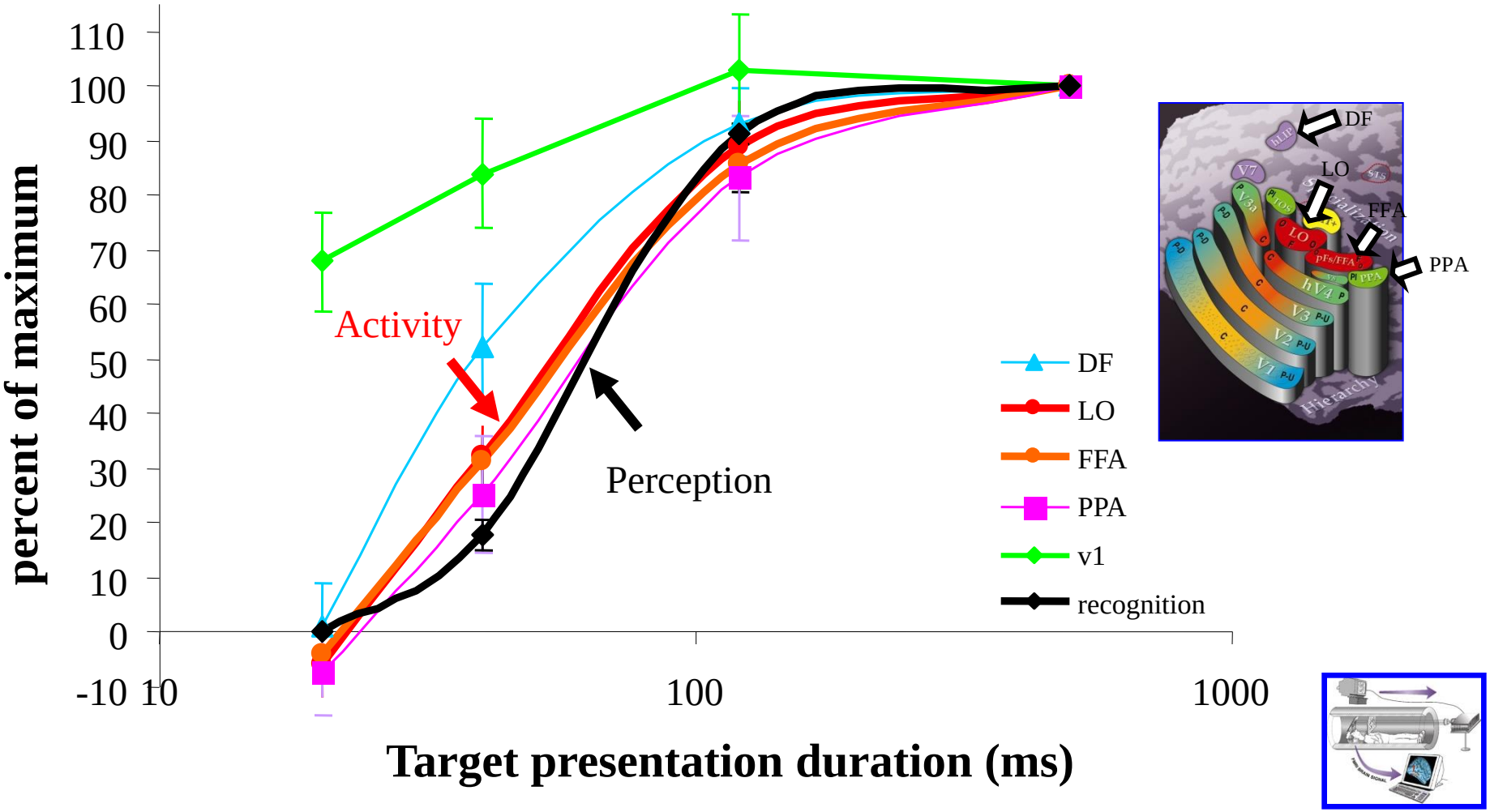
n = 6



Correlation = 0.73, p«0.001

From: Mukamel et al., 2005

Non-linear amplification revealed in fMRI signals



Backward masking

G Avidan, K. Grill-Spector

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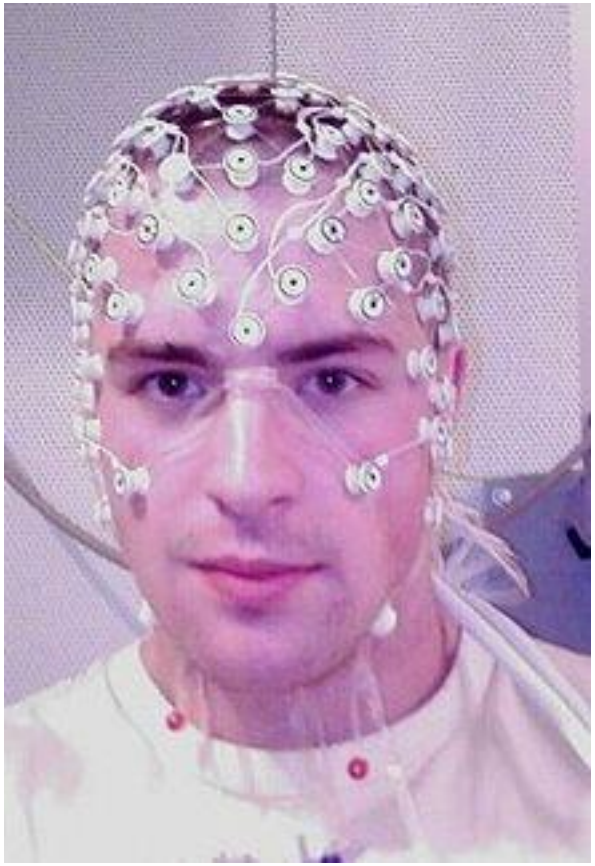
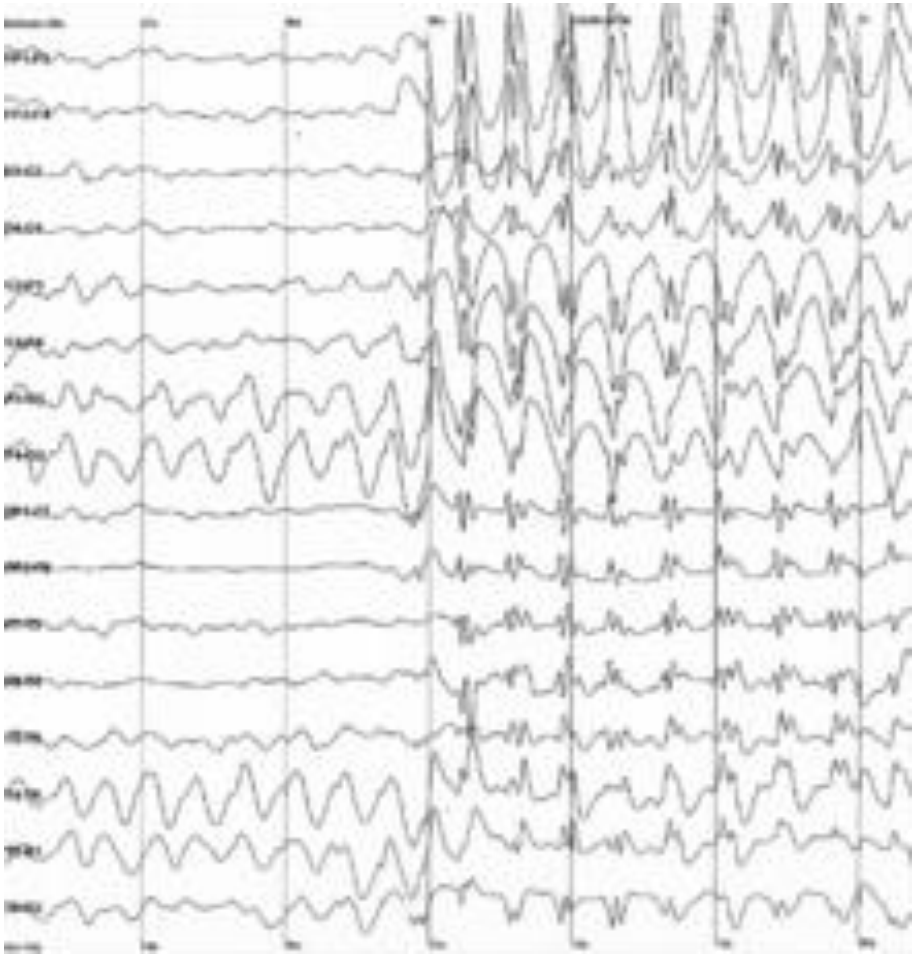
EEG recording

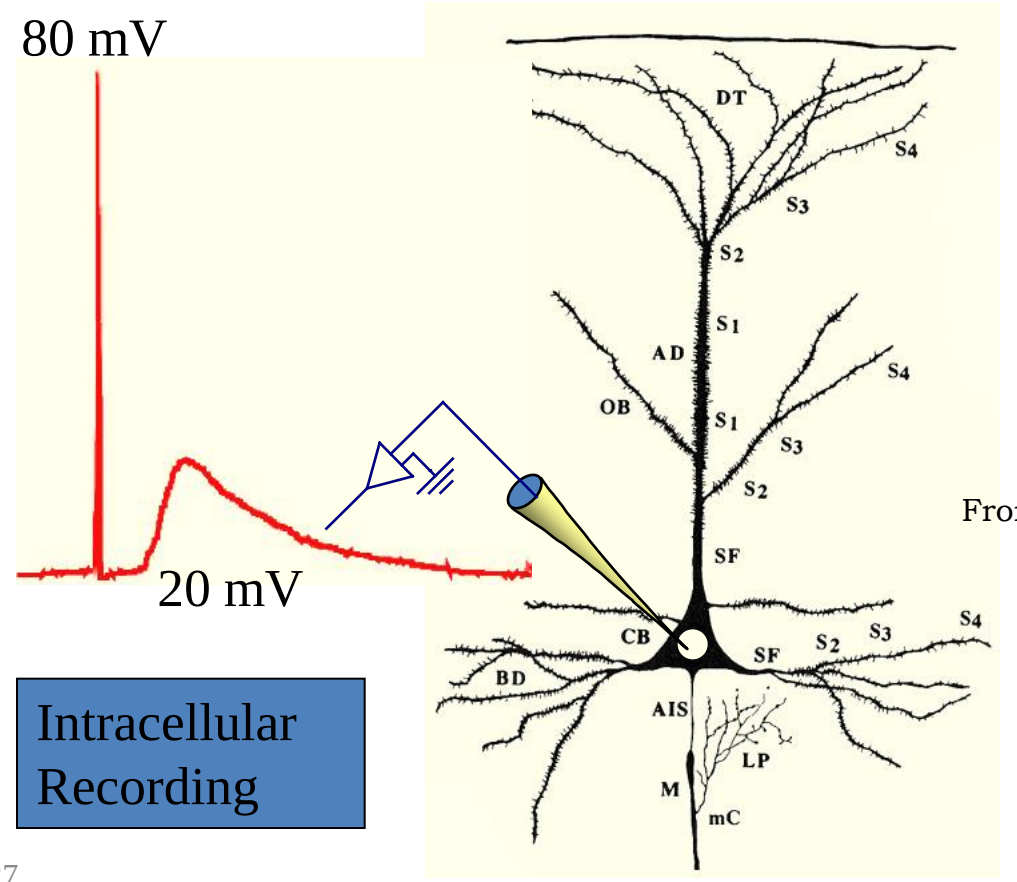




Epileptic spike and wave discharges monitored with EEG.

EEG recording

Dipole Formation in EEG Potentials

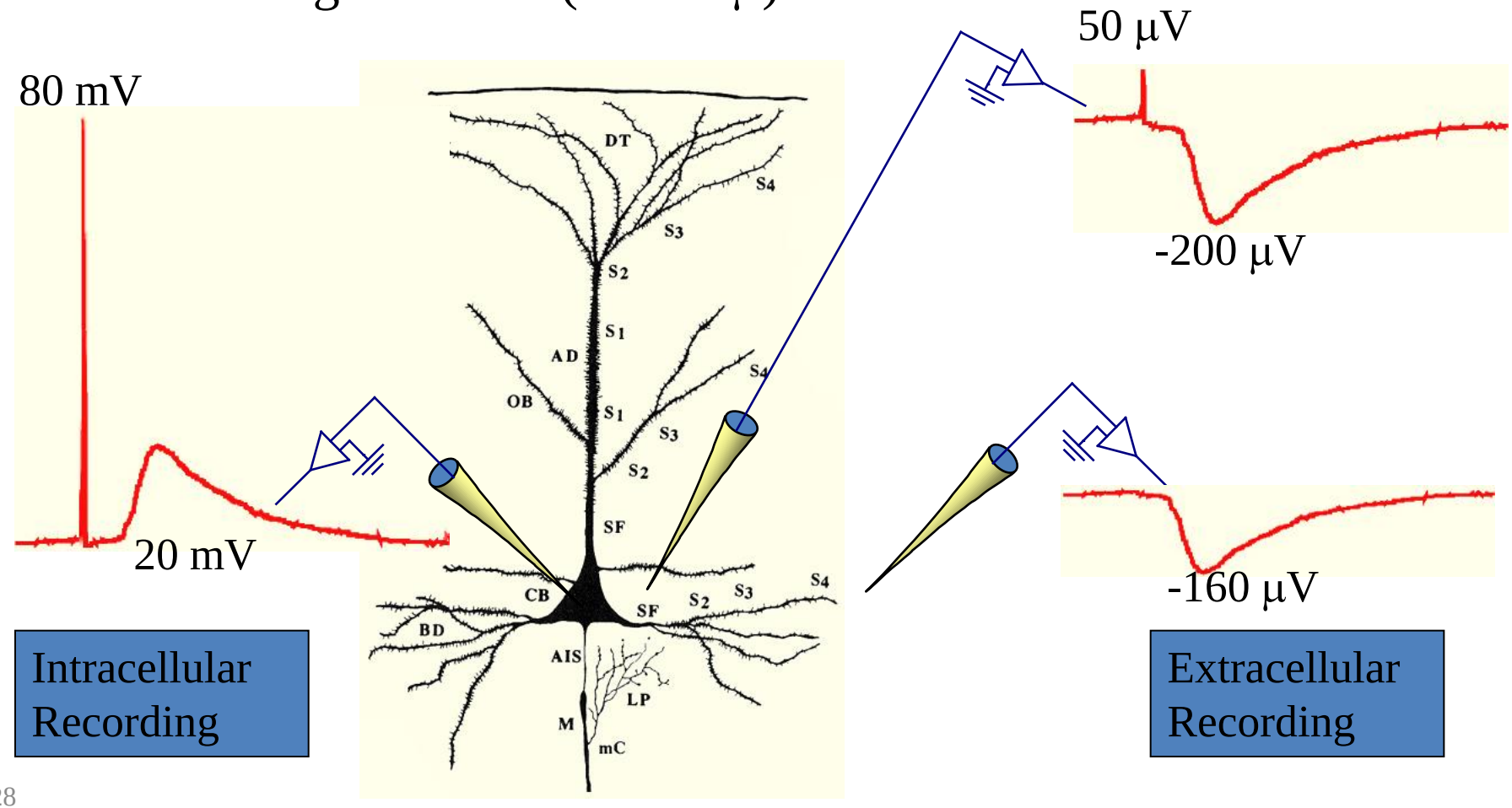


From: DeFelipe and Farinas, 1992

EEG recording

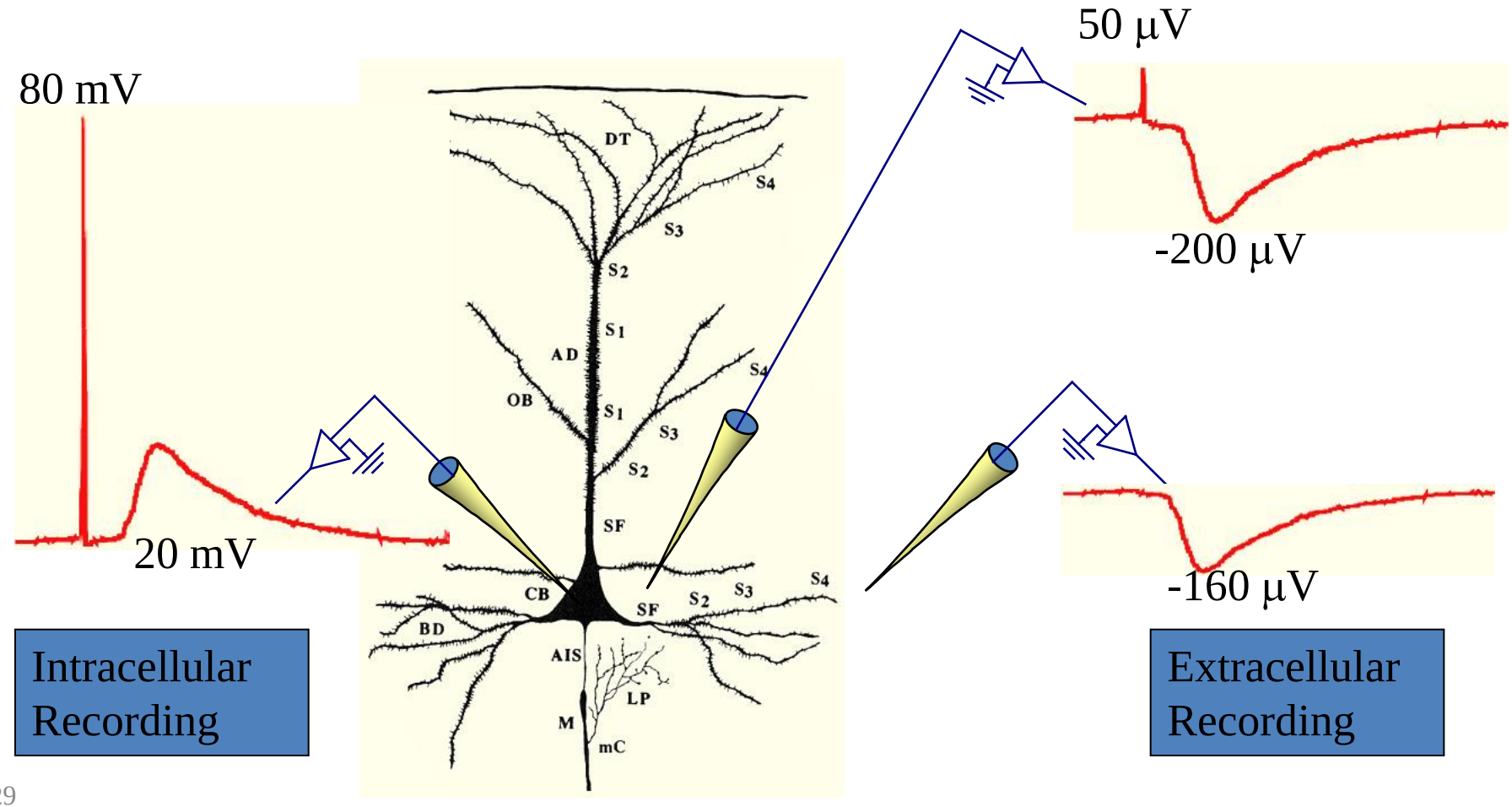
PRINCIPLES of VOLUME CONDUCTION

The amplitude of spikes strongly decay, and therefore, they do not summate to levels that is detectable by electrodes not located in their immediate neighborhood (30-100μ).

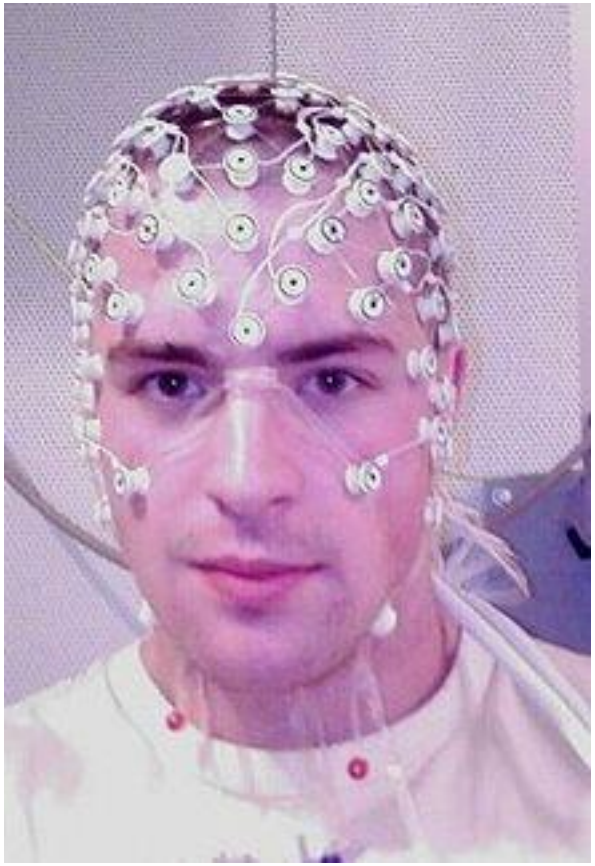


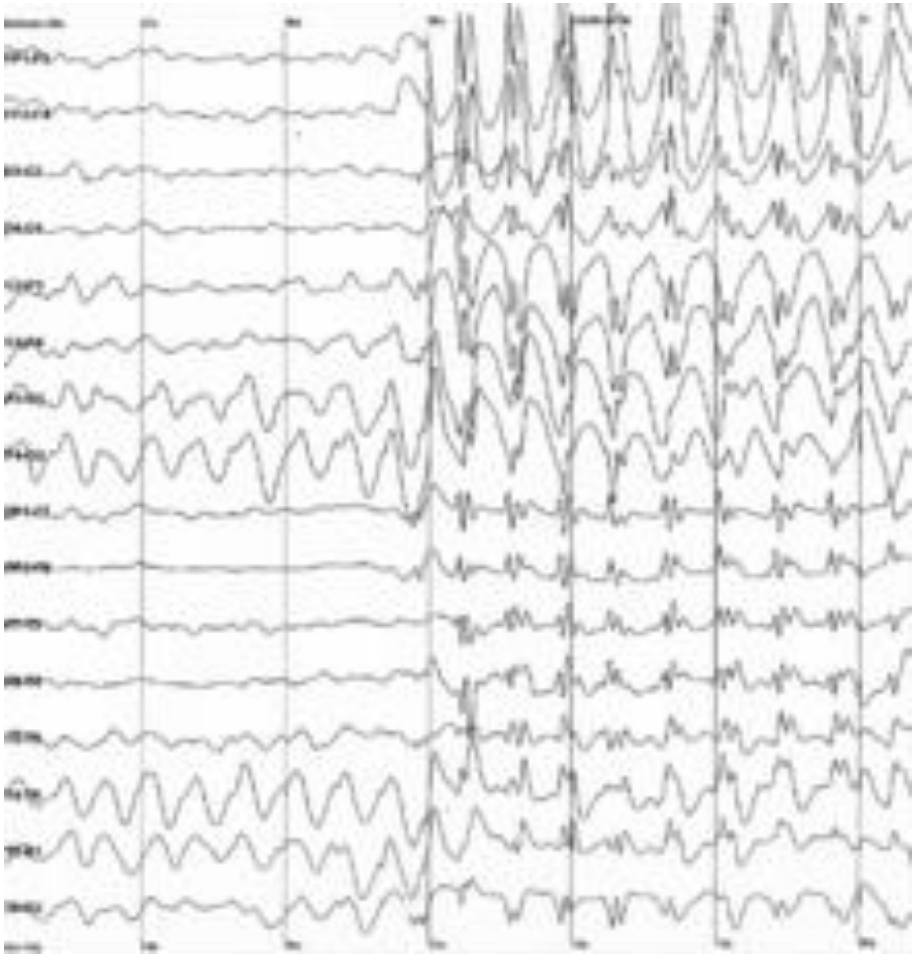
EEG recording

EEG signals reflect synchronous waves of dendritic activities



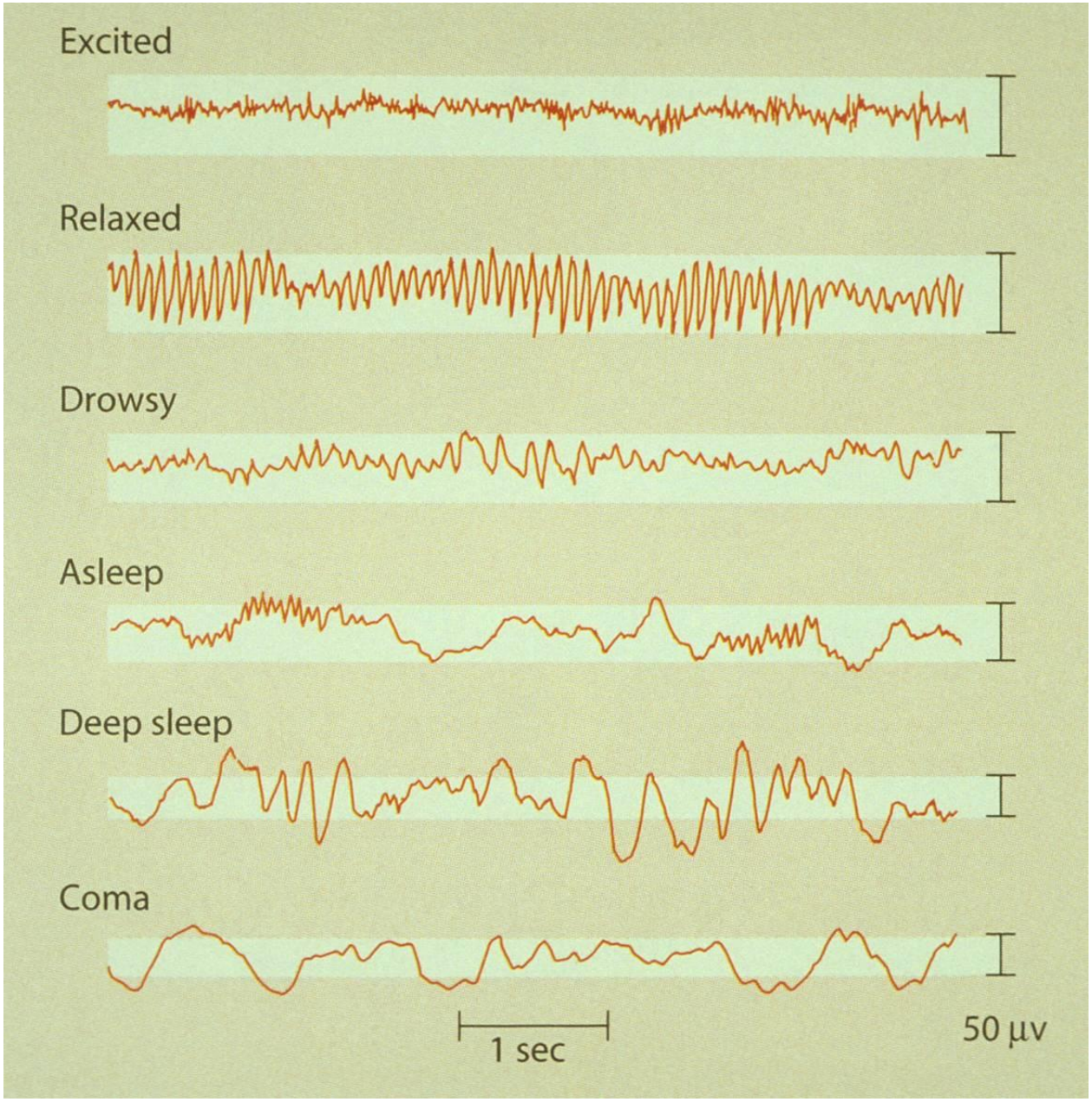
EEG recording





Epileptic spike and wave discharges monitored with EEG.

EEG & brain states



From: Jasper, 1956

EEG recording

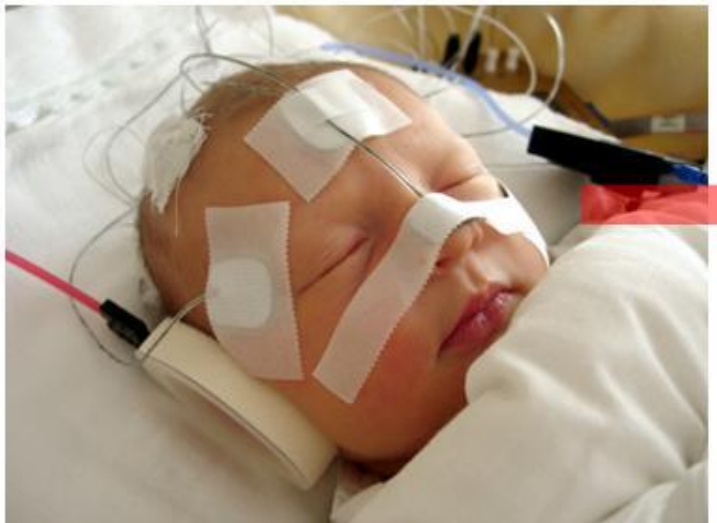
ERP – Event Related Potentials

When an event is repeated tens or hundreds of times, and the time-related EEG signals are averaged, the resulting signal is considered as the average potential evoked by the event

EEG recording

ERP – Event Related Potentials

Left ear



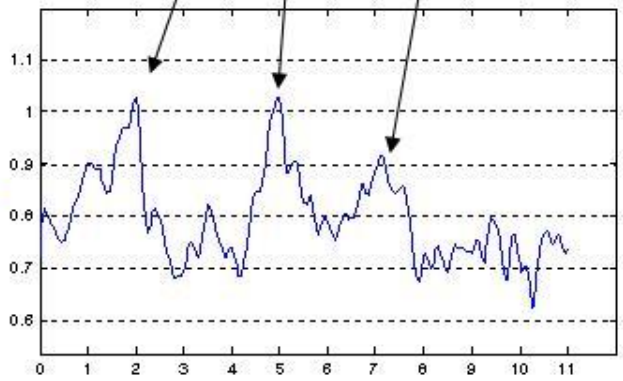
Right ear

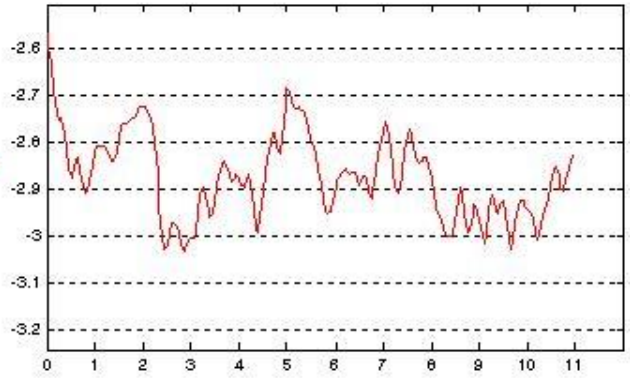
heuristics

Peak I. - distal cochlear nerve

Peak III. - cochlear nucleus

Peak V. - lateral lemniscus





Time (ms)

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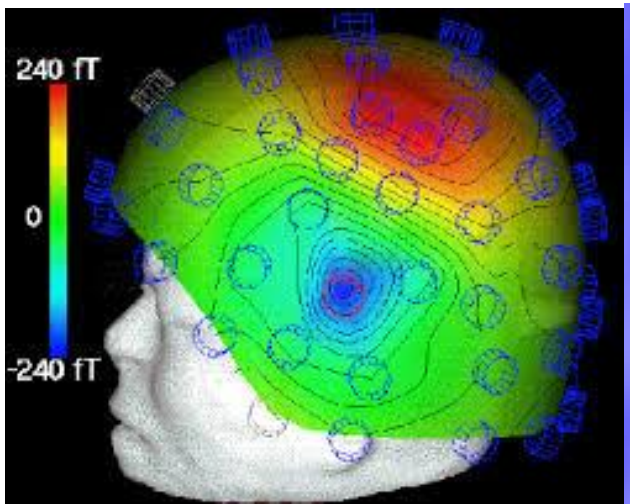
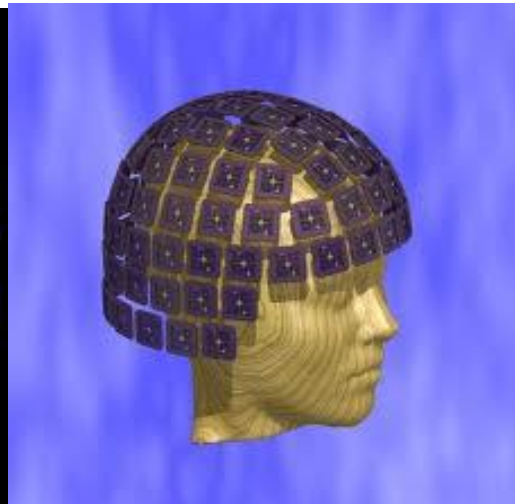
MEG recording

- MEG picks up magnetic fields generated by ionic currents in the brain
- Like EEG, it will show meaningful signals only for synchronized and coordinated currents.
- Like EEG, the major source of these signals are synaptic currents in cortical pyramidal neurons
- Unlike EEG, MEG is sensitive to the direction of the summed current

- As a result, comparison of MEG and EEG can increase the resolution of source localization
- So far, source localization is very limited.





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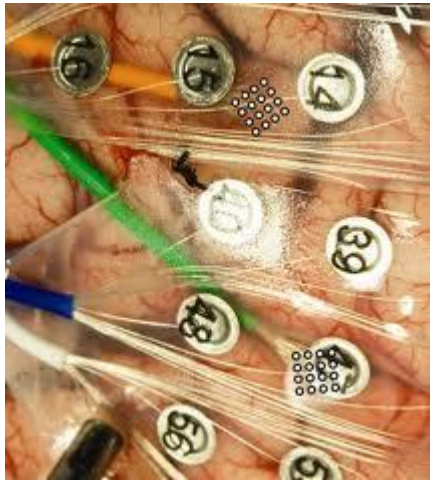
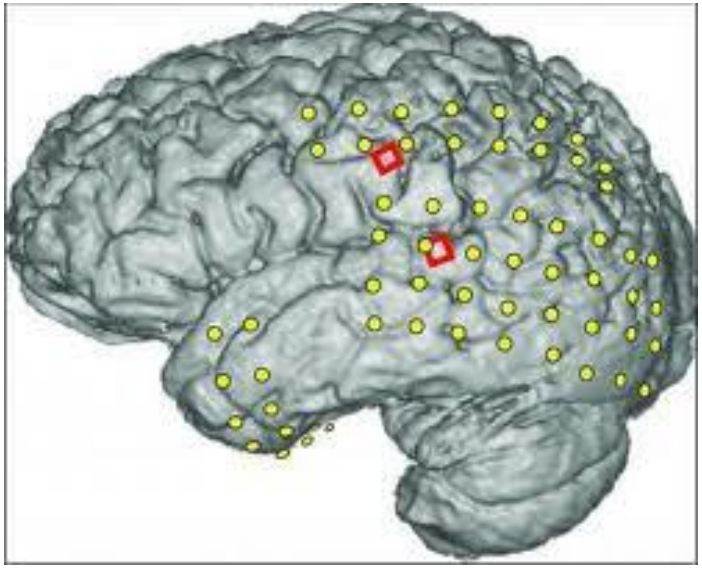
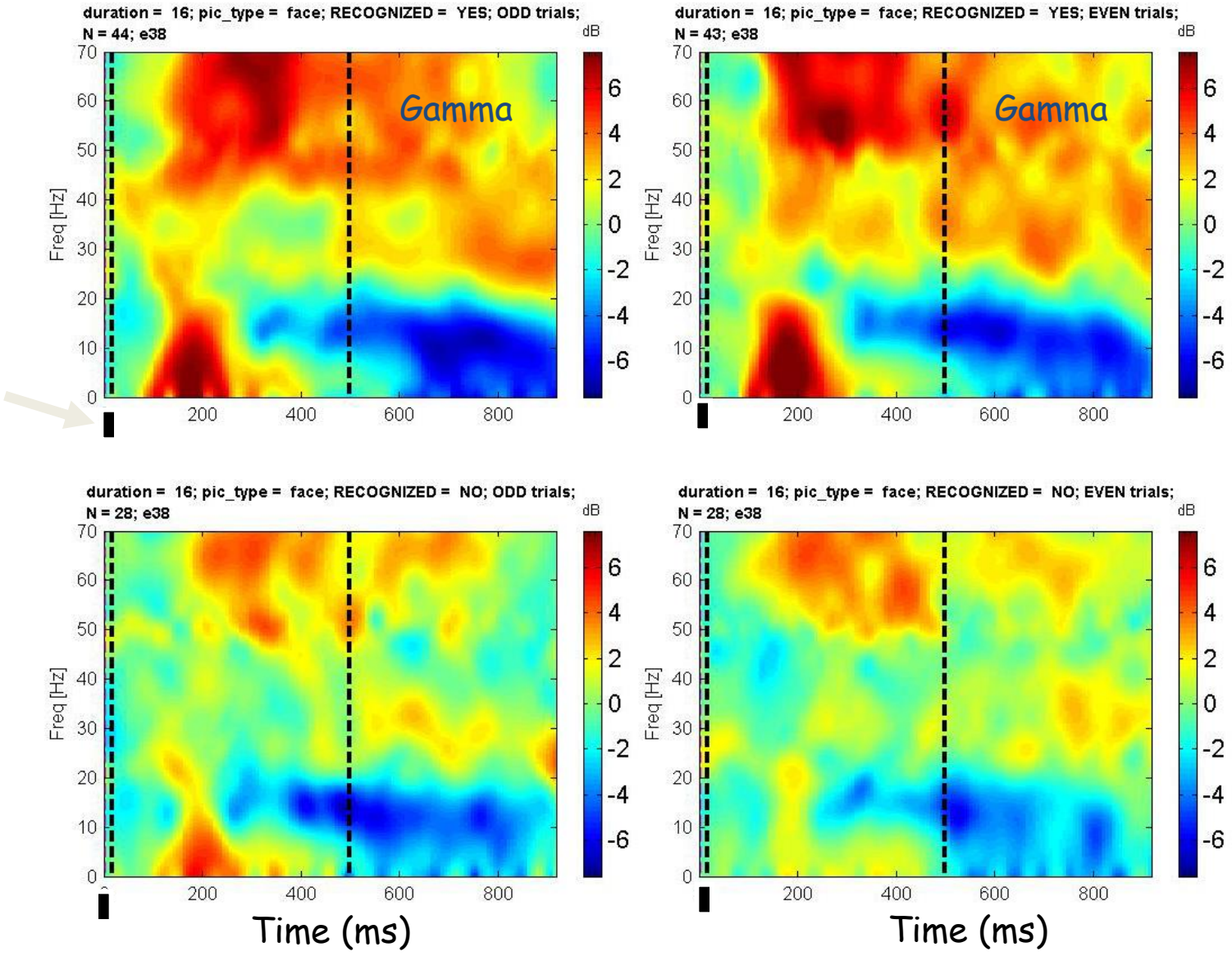
ECoG recording

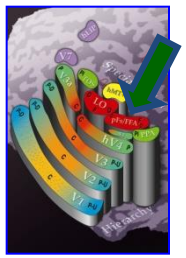
- To increase resolution one has to invade the brain
- The first step inside is with Electro-cortico-graphy (ECoG) – using electrodes that are placed on the surface of the brain, above or below the dura mater.
- The method is used mainly in the treatment of epilepsy, but also used to collect experimental data





ECoG recording





Rafi Malach

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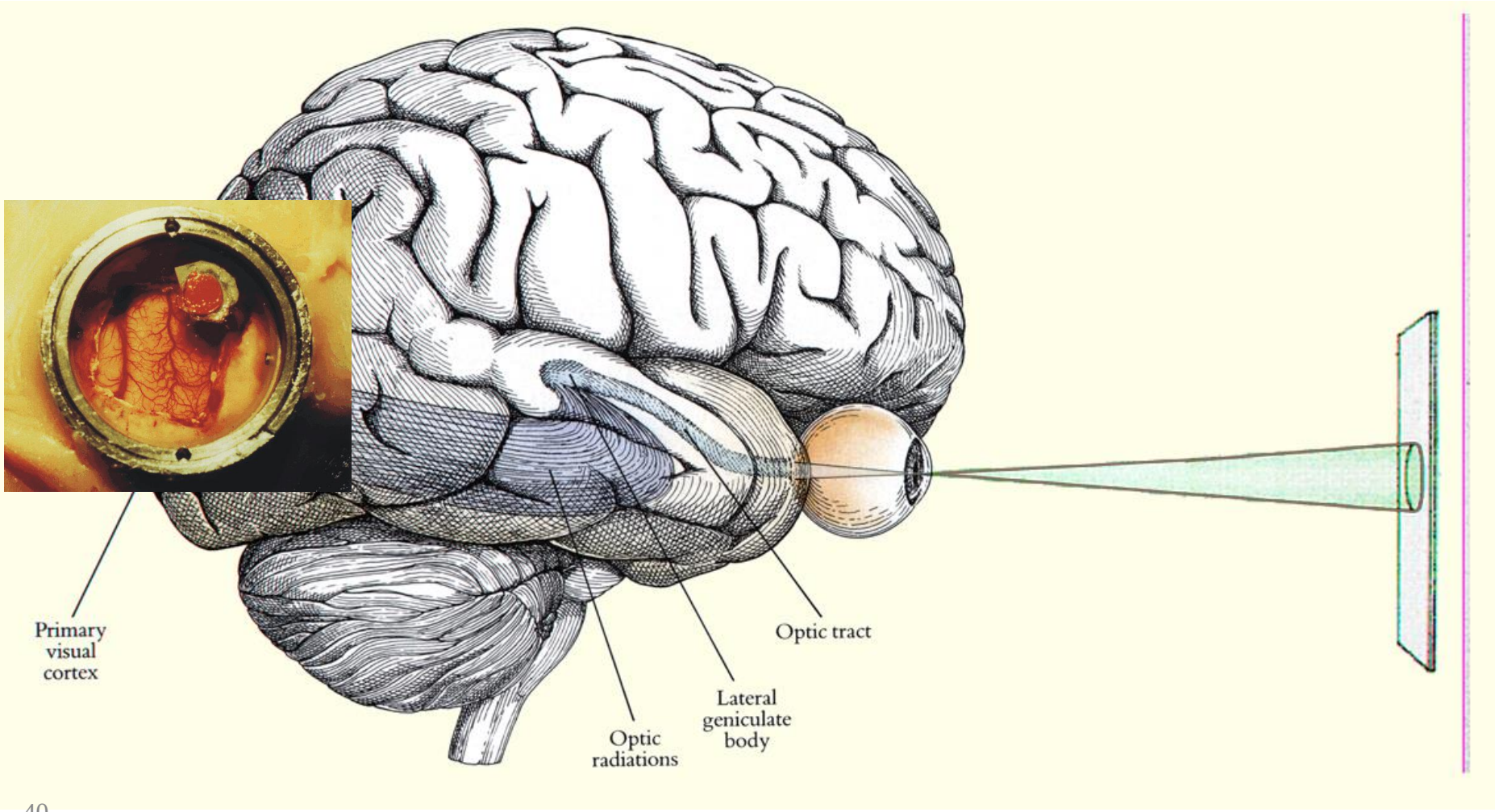
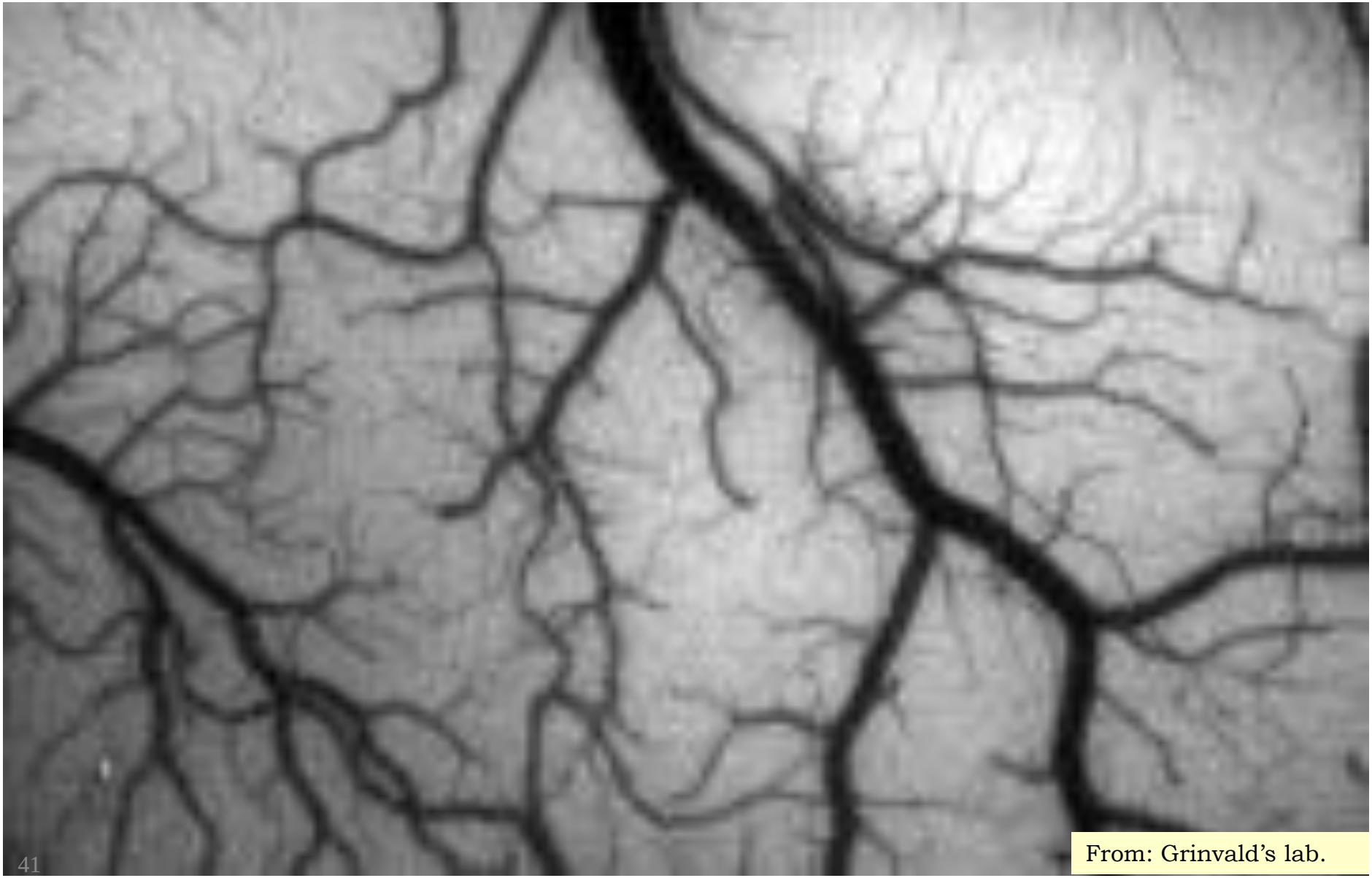
fMRI	> 1 mm	> 200 μm	> 10^{5}		1 s	> 1 s	1000	10 ms
EEG	10 mm	100 mm	> 10^{9}	station	< 1 ms	50 ms	50	1 ms
MEG	10 mm	100 mm	> 10^{9}		1 ms	50 ms	50	
ECoG	10 mm	1-10 mm	> 10^{4-6}		< 1 ms	10 ms	10	
Intr. Signal	10 μm	200 μm	> 1000		< 1 ms	1 s	1000	10 ms
VSD Imaging	10 μm	30 μm	< 100		< 1 ms	10 ms	< 1	
μElec	50 μm	10 μm	1	10 μm	< 1 ms	1 ms	1	
μDialysis	< 1 mm	100 μm	> 10^{4}		> 1 min	100 ms	> 10^{6}	
Intracell elec	10 μm	10 μm	< 1		< 1 ms	< 1 ms	< 1	
Ca imaging	1 μm	1 μm	< 1		1 ms	> 100 ms	> 100	

Intrinsic signals recording

The Cranial Window

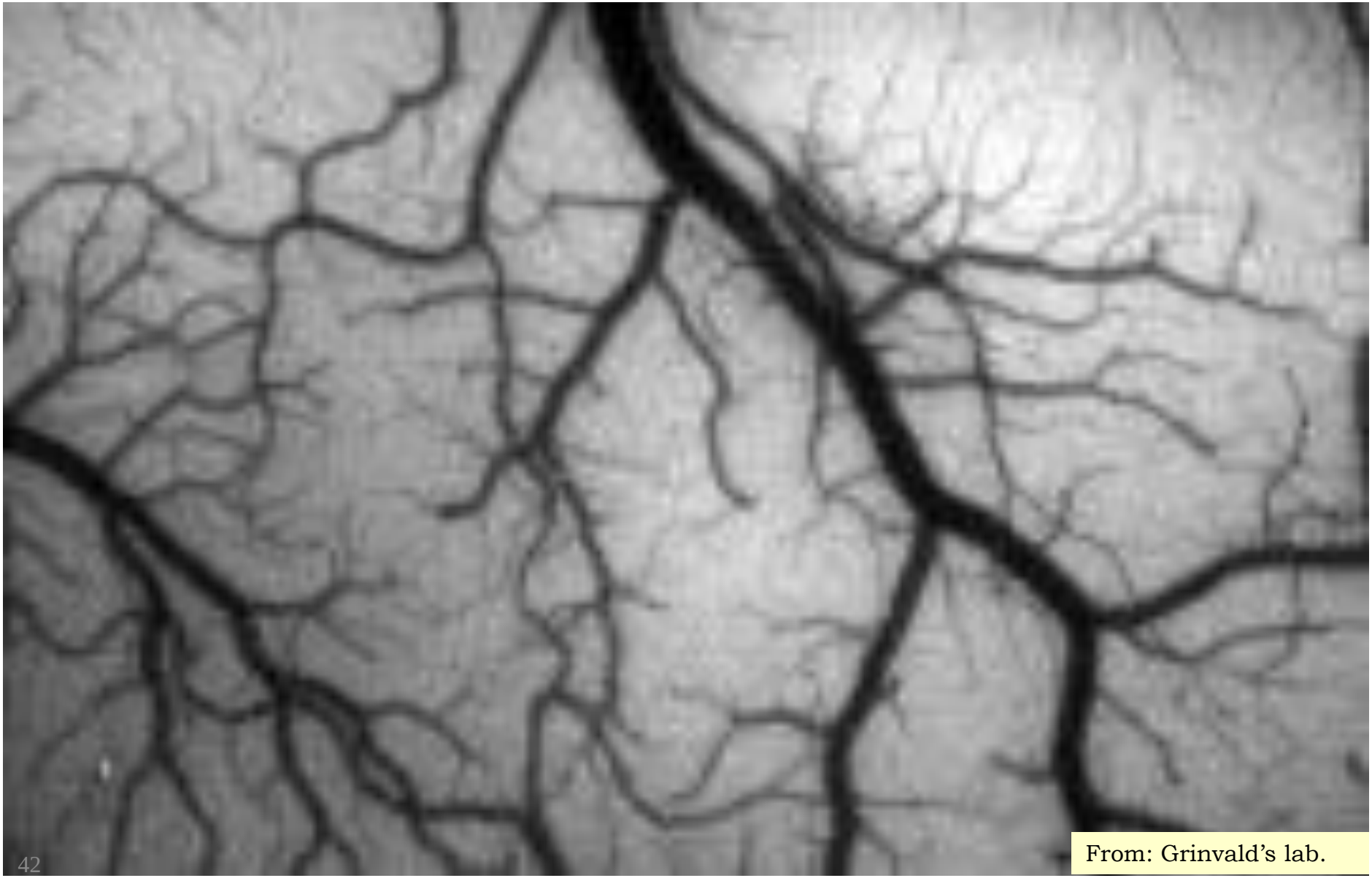


Left eyes was stimulated



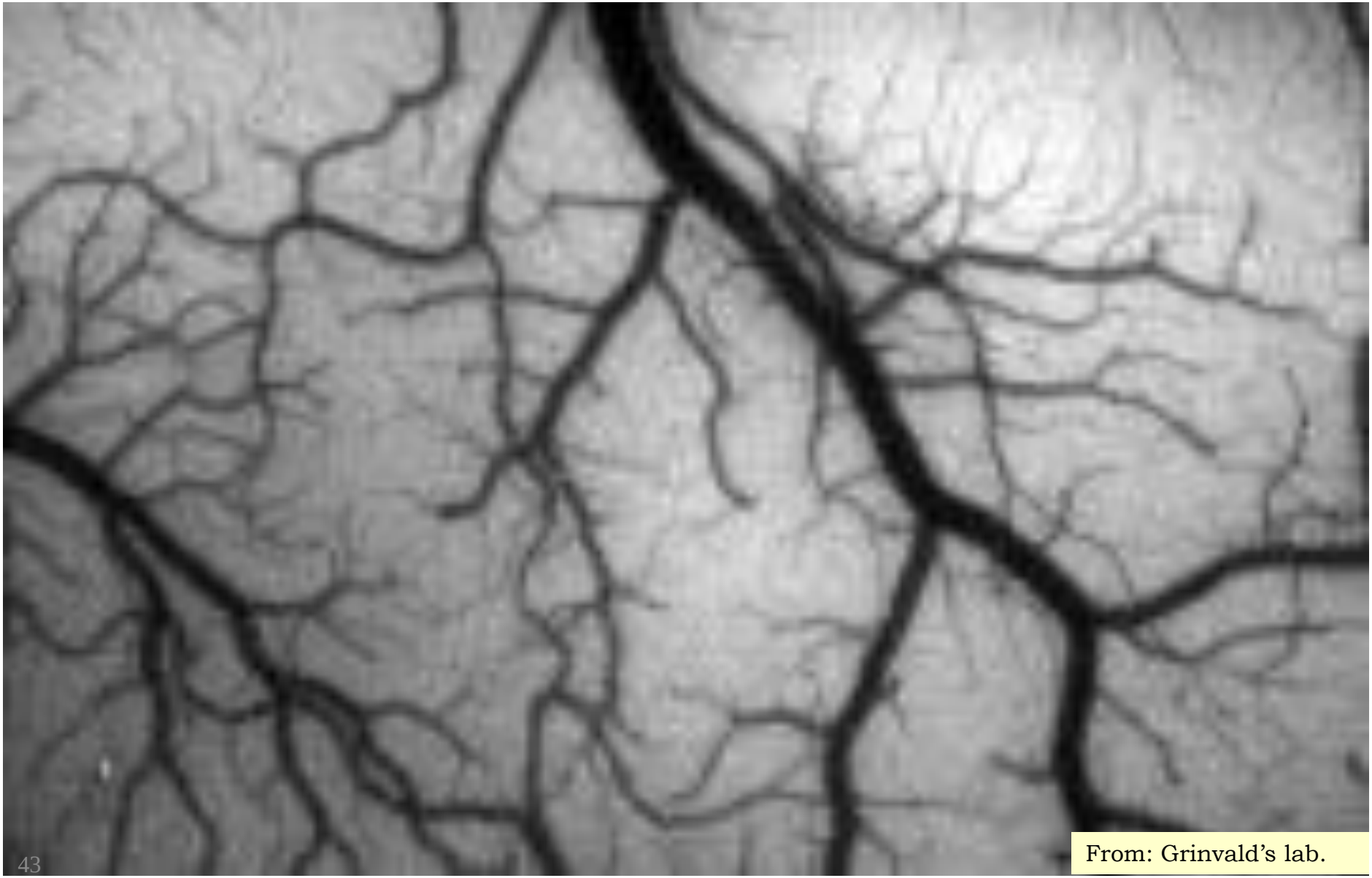
From: Grinvald's lab.

Right eyes was stimulated



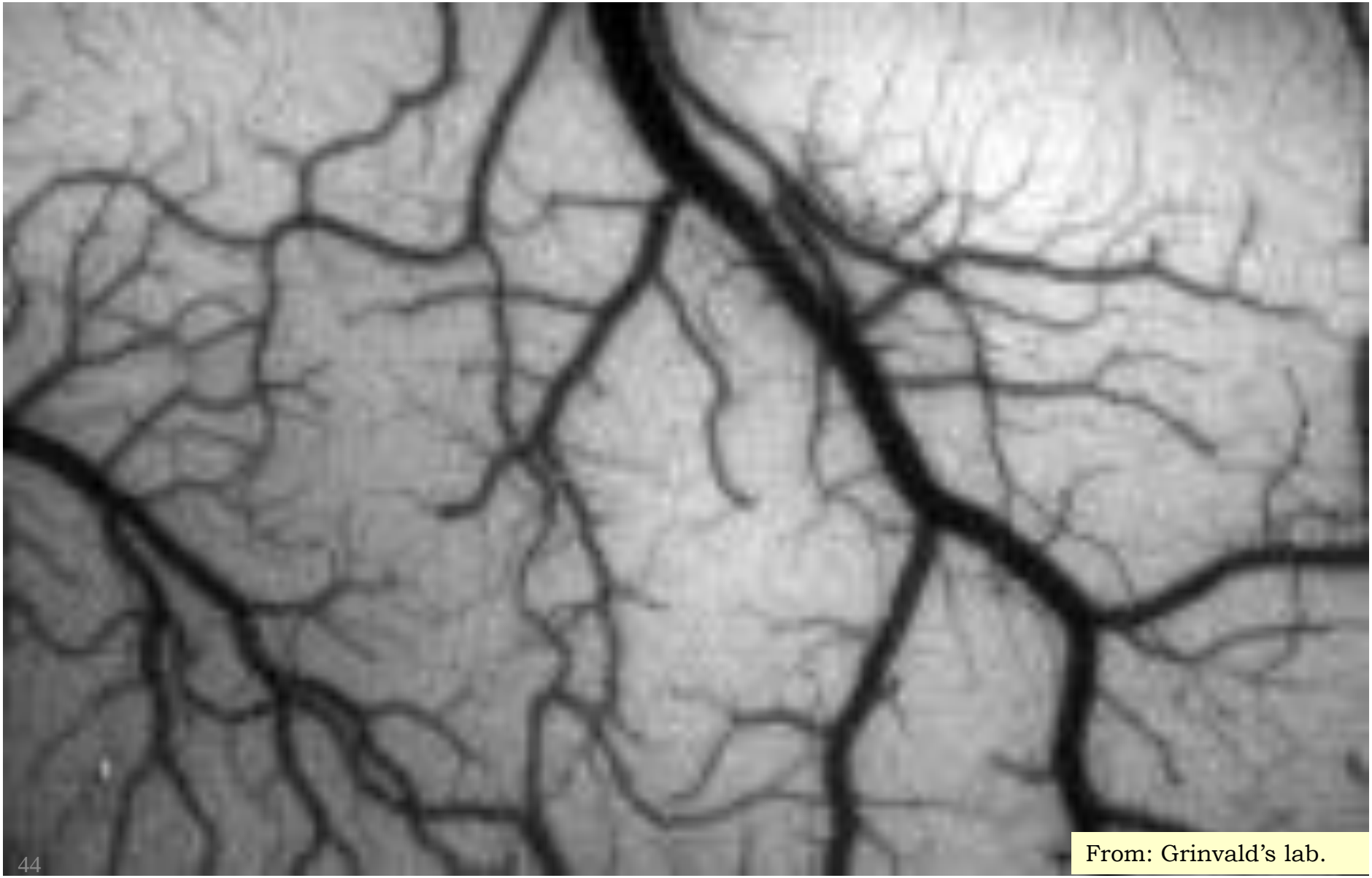
From: Grinvald's lab.

Left eyes was stimulated

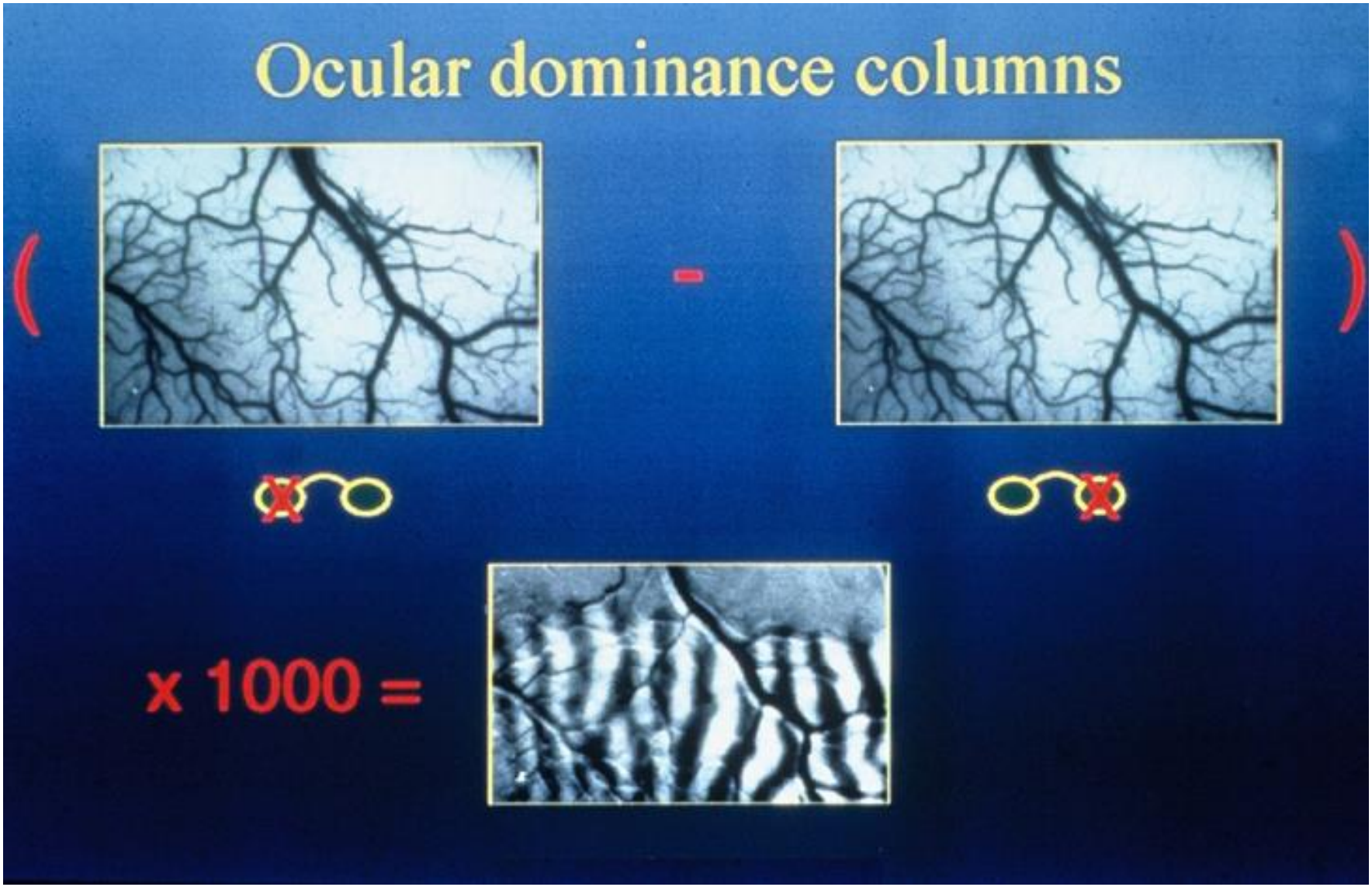


From: Grinvald's lab.

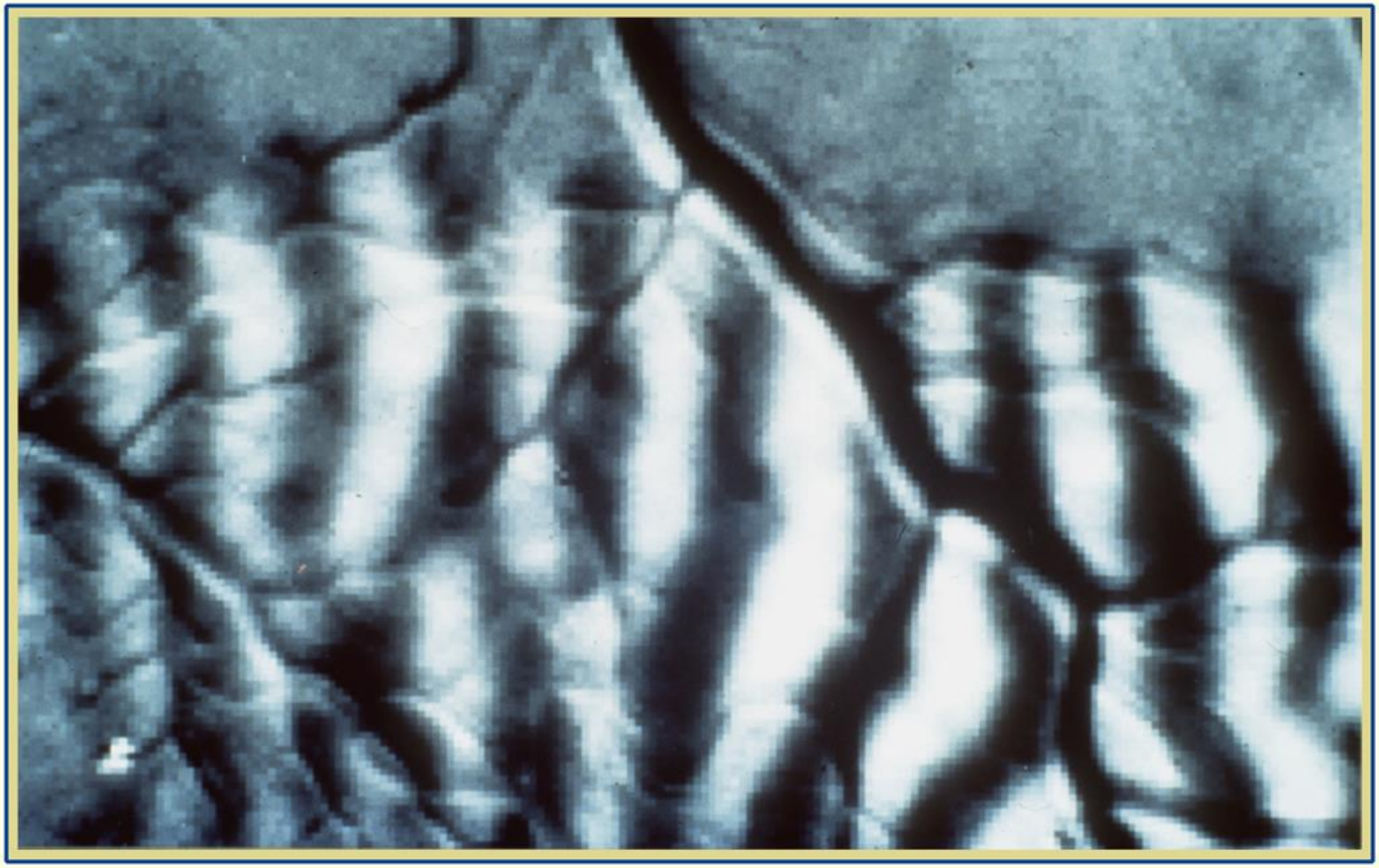
Right eyes was stimulated



From: Grinvald's lab.



From: Grinvald's lab.



From: Grinvald's lab.

SOURCES OF INTRINSIC SIGNALS

- **Changes in absorption (similar to BOLD) due to:**

  - Changes in oxygenation

  - Changes in blood volume

  - Changes in blood flow

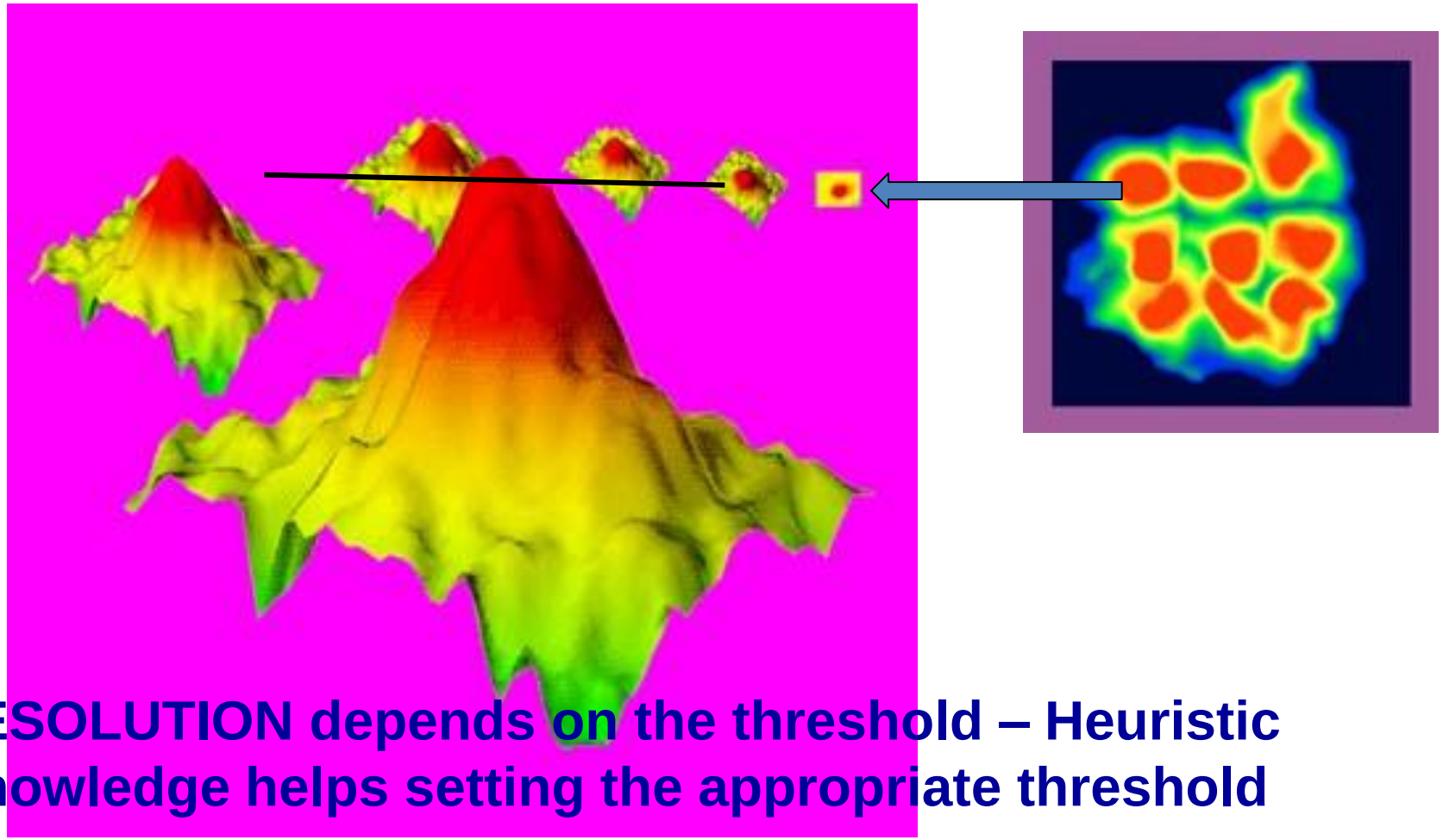
- **Changes in Light scattering due to:**

Ion movement; water movement; shrinkage or expansion of the extracellular space; transmitter release; Volume changes due to capillaries dilation

From: Grinvald's lab.

RESOLUTION OF INTRINSIC (and BOLD) SIGNALS

Full 3-D view (no threshold) of a single WFR



RESOLUTION depends on the threshold – Heuristic knowledge helps setting the appropriate threshold

Barrel area ~ 0.15 mm^2; WFR area ~ 15 mm^2

R. Frostig, 2010

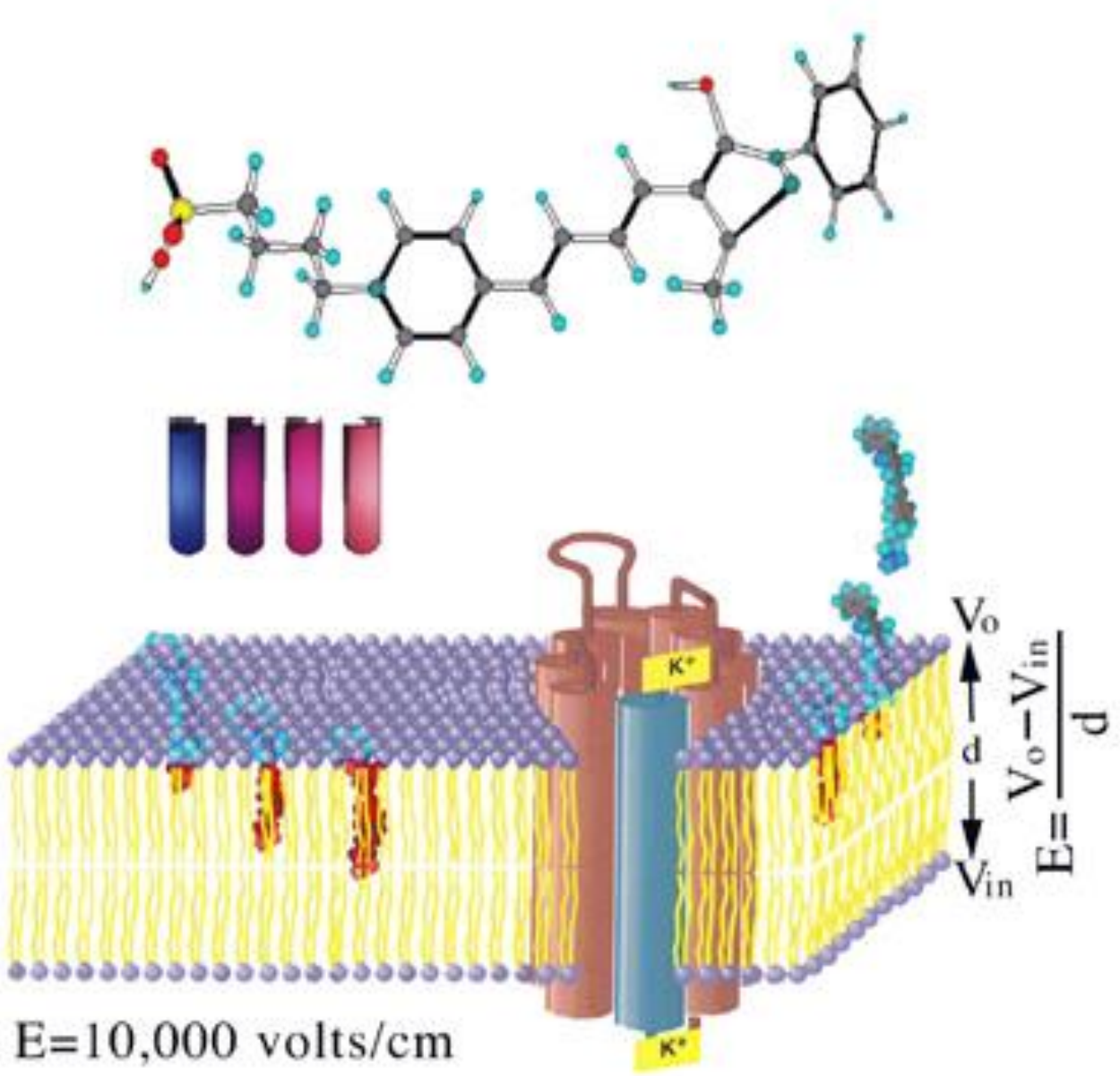
Methods table

Measuring neural activity

	Spatial resolution				Temporal resolution			
	device	signal	neurons	heuristic	device	signal	spikes	heuristic
behavior	brain	brain	> 10^{11}	station	1 ms	10 ms	10	
2DG, c-fos	10 μm	10 μm	1		NA	30 min	> 10^{6}	
fMRI	> 1 mm	> 200 μm	> 10^{5}		1 s	> 1 s	1000	10 ms
EEG	10 mm	100 mm	> 10^{9}	station	< 1 ms	50 ms	50	1 ms
MEG	10 mm	100 mm	> 10^{9}		1 ms	50 ms	50	
ECoG	10 mm	1-10 mm	> $10^{4\text{-}6}$		< 1 ms	10 ms	10	
Intr. Signal	10 μm	200 μm	> 1000		< 1 ms	1 s	1000	10 ms
VSD Imaging	10 μm	30 μm	< 100		< 1 ms	10 ms	< 1	
μElec	50 μm	10 μm	1	10 μm	< 1 ms	1 ms	1	
μDialysis	< 1 mm	100 μm	> 10^{4}		> 1 min	100 ms	> 10^{6}	
Intracell elec	10 μm	10 μm	< 1		< 1 ms	< 1 ms	< 1	
Ca imaging	1 μm	1 μm	< 1		1 ms	> 100 ms	> 100	

Voltage Sensitive Dye (VSD)

Merocyanine
Dye RH-890



Voltage Sensitive Dye (VSD)

MEASURES:

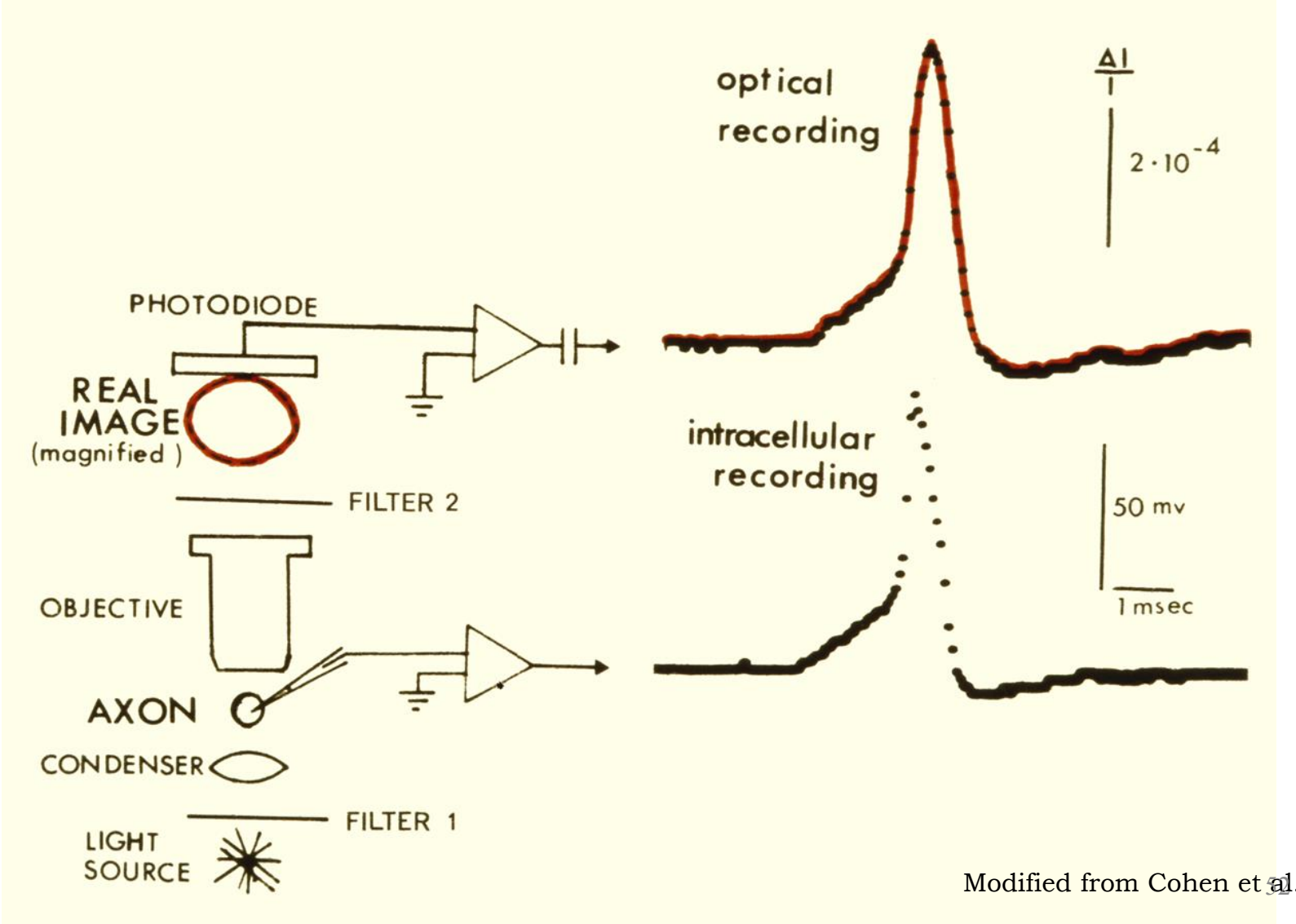
the weighted sum of membrane-potential changes in neuronal somata, dendritic and axonal arbors, and often glia

the dye signal is restricted to the site of the electrical activity

This signal mainly reflects the synaptic potentials in the dendrites

Voltage Sensitive Dye (VSD)

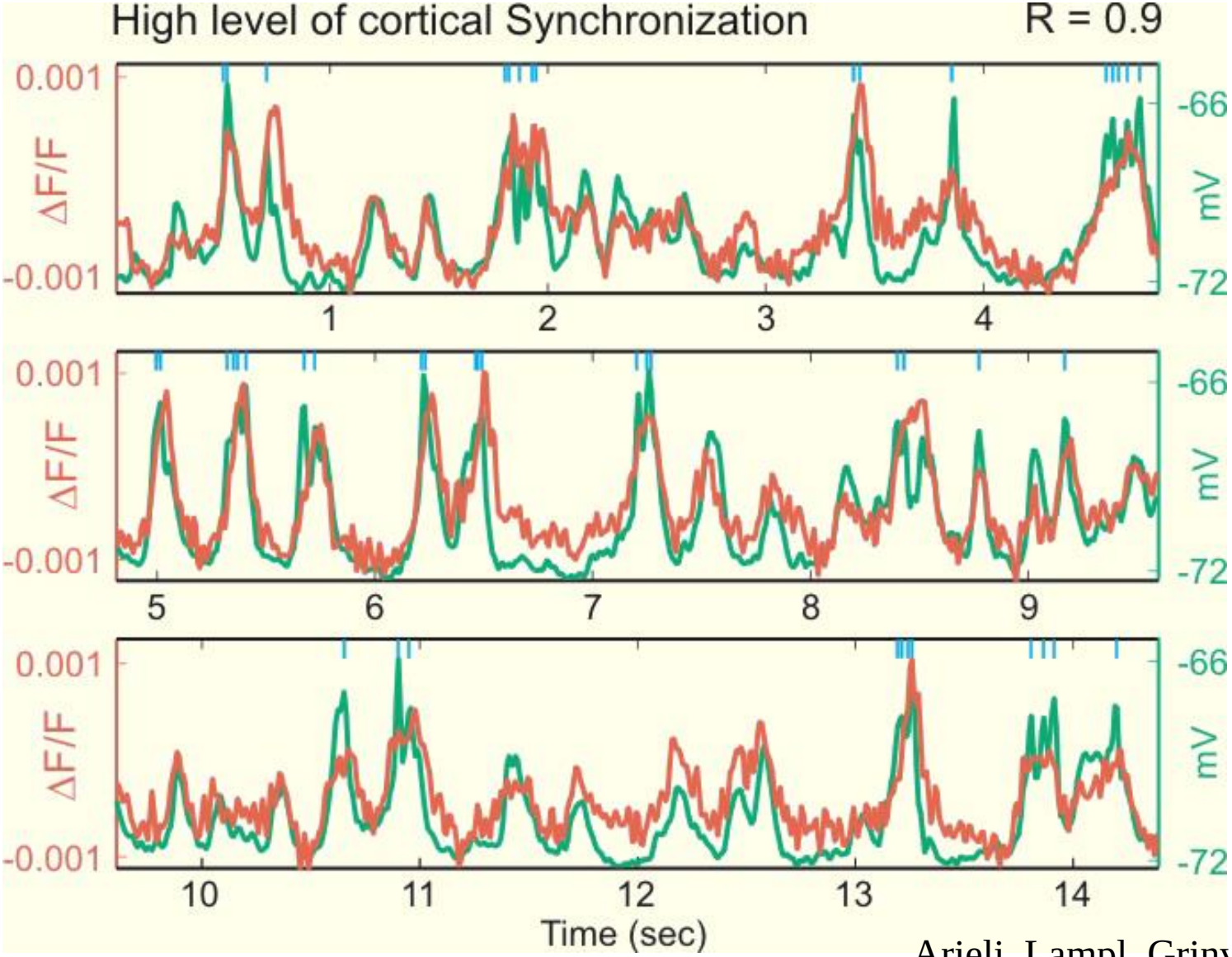
In-vitro:



Modified from Cohen et al., 1972

Voltage Sensitive Dye (VSD)

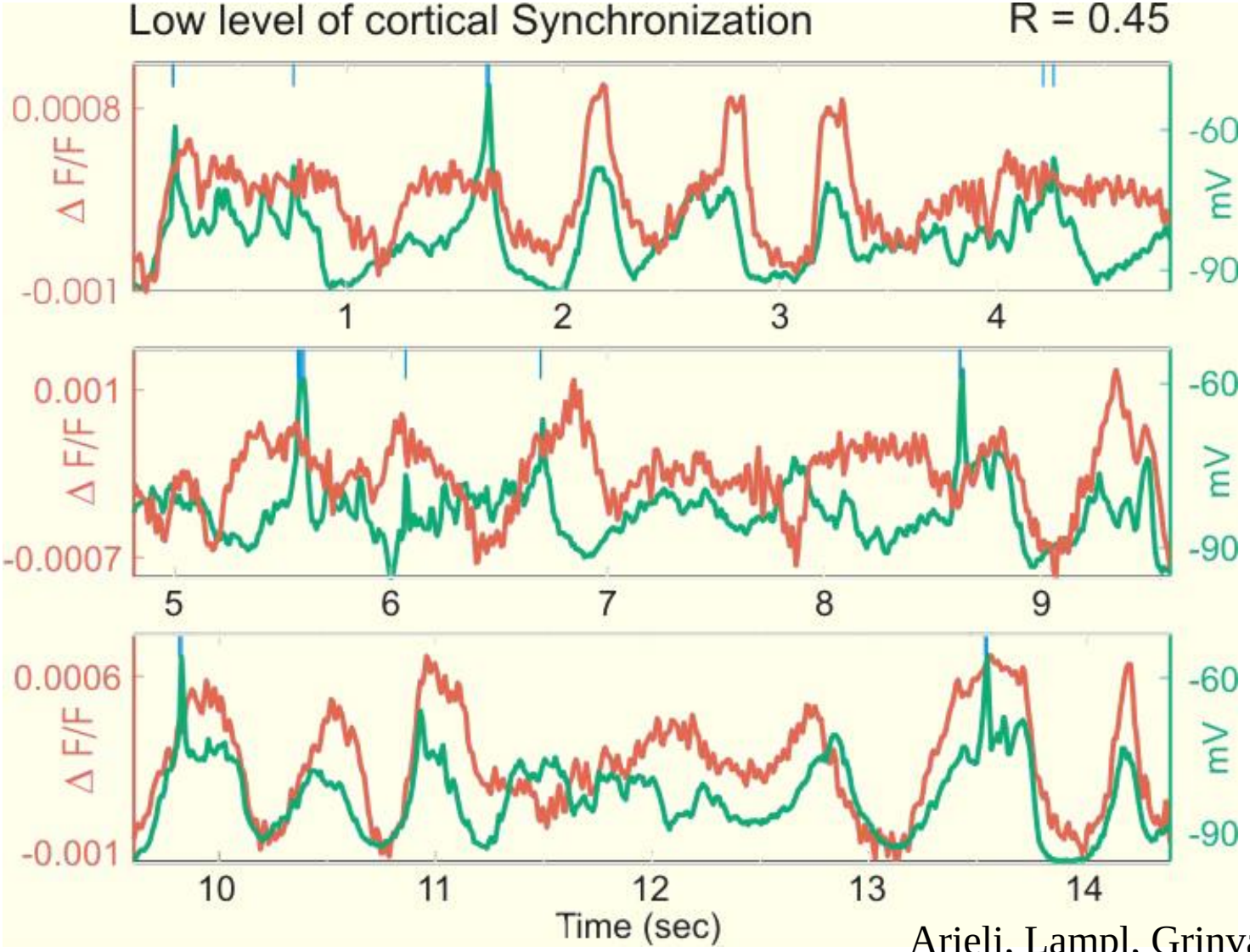
In-vivo:



Arieli, Lampl, Grinvald

Voltage Sensitive Dye (VSD)

In-vivo:

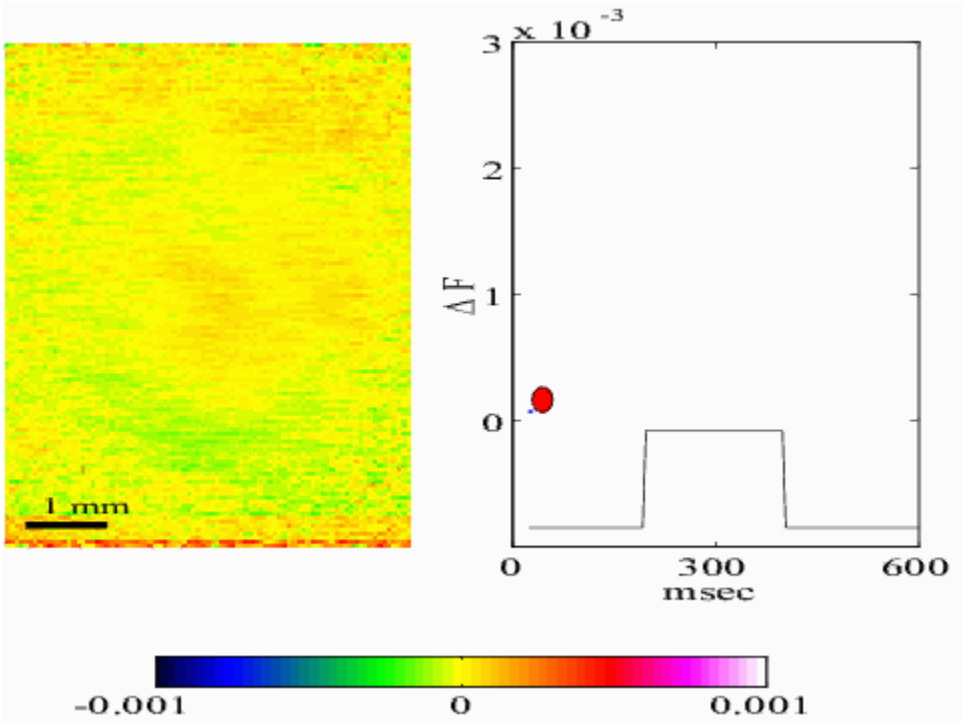


Arieli, Lampl, Grinvald

Voltage Sensitive Dye (VSD)

In-vivo:

Example: Surround Inhibition in the Rat Barrel Cortex



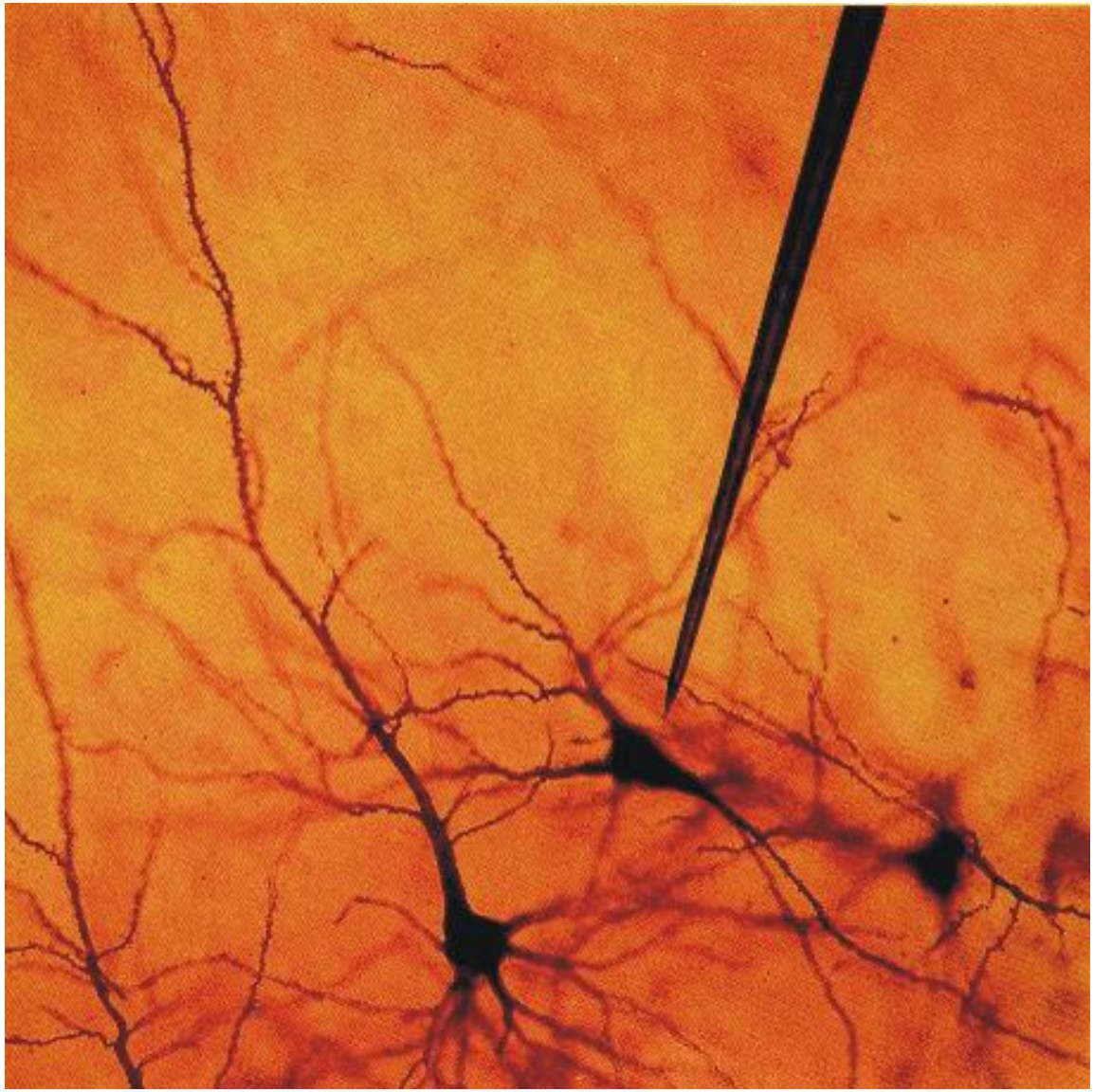
Dori Derdikman

Methods table

Measuring neural activity

	Spatial resolution				Temporal resolution			
	device	signal	neurons	heuristic	device	signal	spikes	heuristic
behavior	brain	brain	> 10^{11}	station	1 ms	10 ms	10	
2DG, c-fos	10 μm	10 μm	1		NA	30 min	> 10^{6}	
fMRI	> 1 mm	> 200 μm	> 10^{5}		1 s	> 1 s	1000	10 ms
EEG	10 mm	100 mm	> 10^{9}	station	< 1 ms	50 ms	50	1 ms
MEG	10 mm	100 mm	> 10^{9}		1 ms	50 ms	50	
ECoG	10 mm	1-10 mm	> $10^{4\text{-}6}$		< 1 ms	10 ms	10	
Intr. Signal	10 μm	200 μm	> 1000		< 1 ms	1 s	1000	10 ms
VSD Imaging	10 μm	30 μm	< 100		< 1 ms	10 ms	< 1	
μElec	50 μm	10 μm	1	10 μm	< 1 ms	1 ms	1	
μDialysis	< 1 mm	100 μm	> 10^{4}		> 1 min	100 ms	> 10^{6}	
Intracell elec	10 μm	10 μm	< 1		< 1 ms	< 1 ms	< 1	
Ca imaging	1 μm	1 μm	< 1		1 ms	> 100 ms	> 100	

Micro-electrode recordings



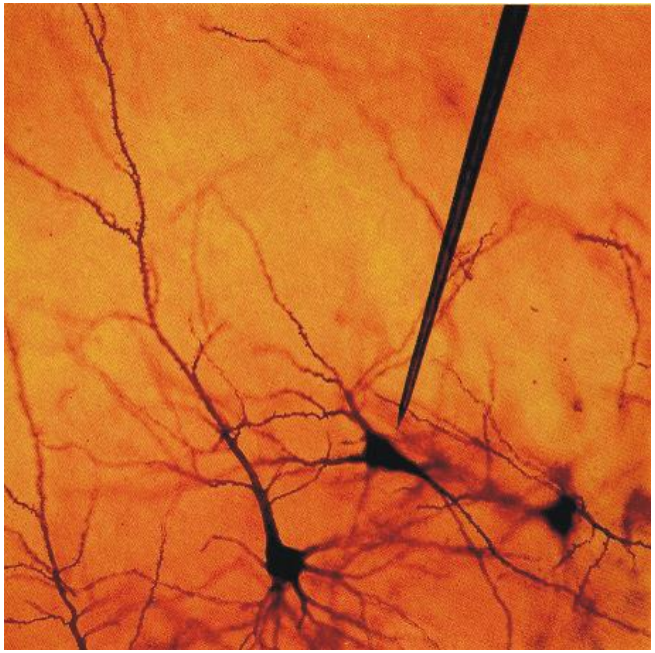
Micro-electrode recordings

From the tip of the microelectrode one can record:

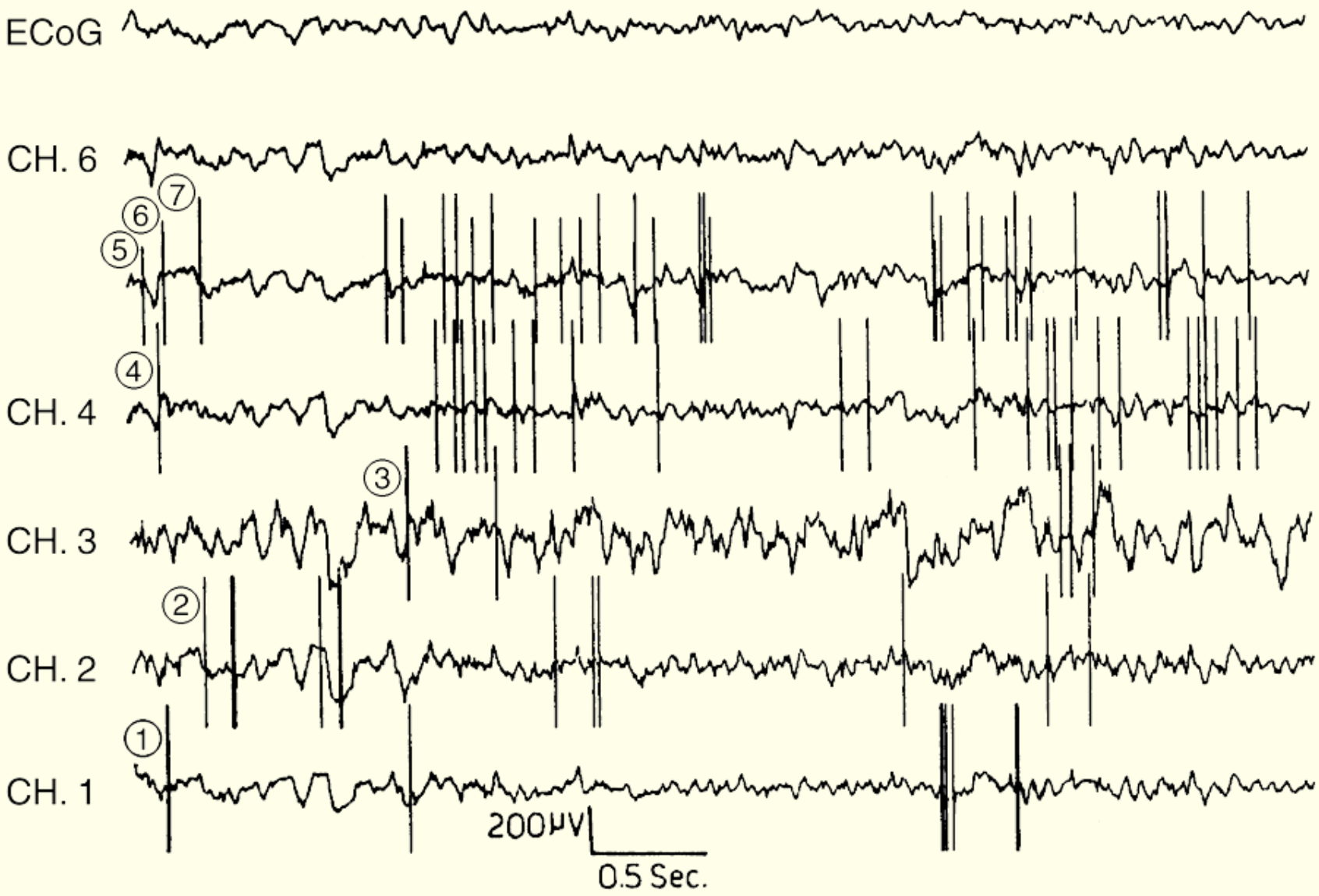
LFP – local field potential

MUA – multi-unit activity

SUA – single-unit activity (using spike sorting)



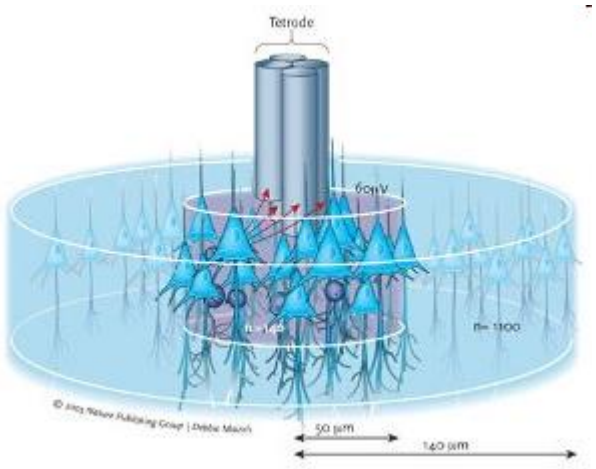
Spikes, LFP and ECoG



Arieli, 1992

Micro-electrode recordings

Multiple single-unit recordings using tetrodes

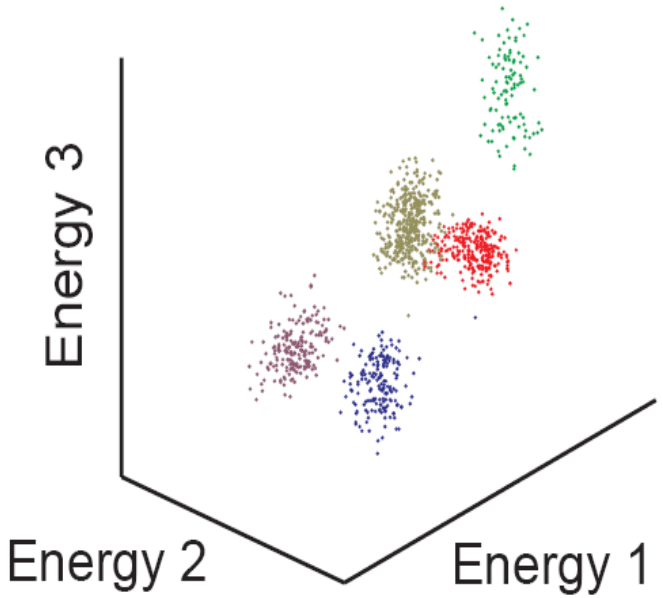


- Neurons are spike-sorted based on relative amplitudes on the 4 tetrode channels (amplitude differences are caused by physical proximity of neurons to different tetrode wires)
- Up to 25 cell can be well-separated per tetrode
- More typically: 5 – 10 cells per tetrode
- Can record > 100 neurons overall (in 10-20 tetrodes) in a freely behaving, freely moving animal

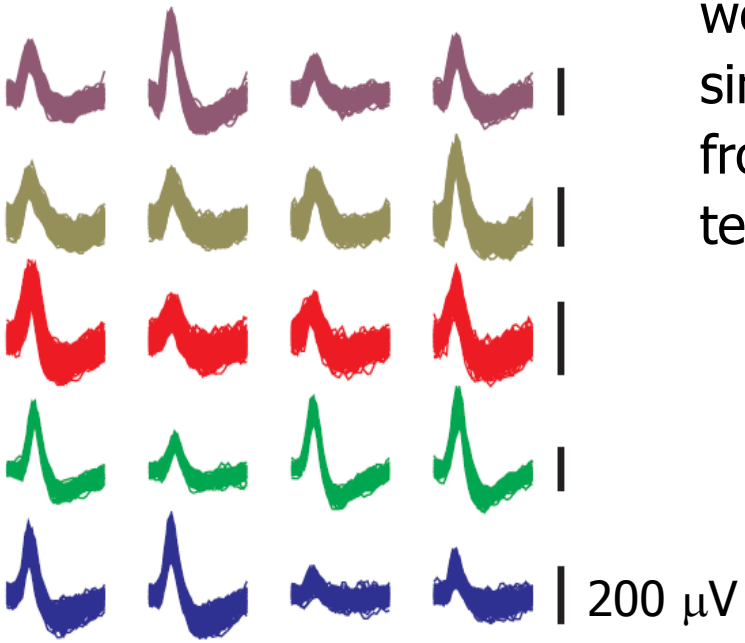
Micro-electrode recordings

Multiple single-unit recordings using tetrodes

Spike energies



Waveforms



- Here, 5 neurons were recorded simultaneously from one tetrode

Nachum Ulanovsky

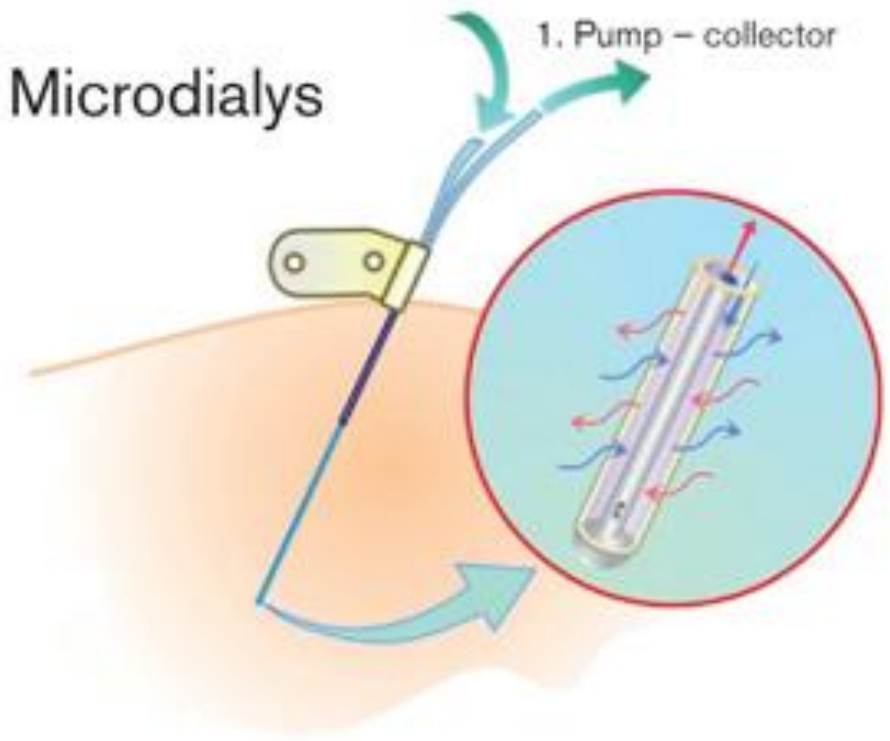
Methods table

Measuring neural activity

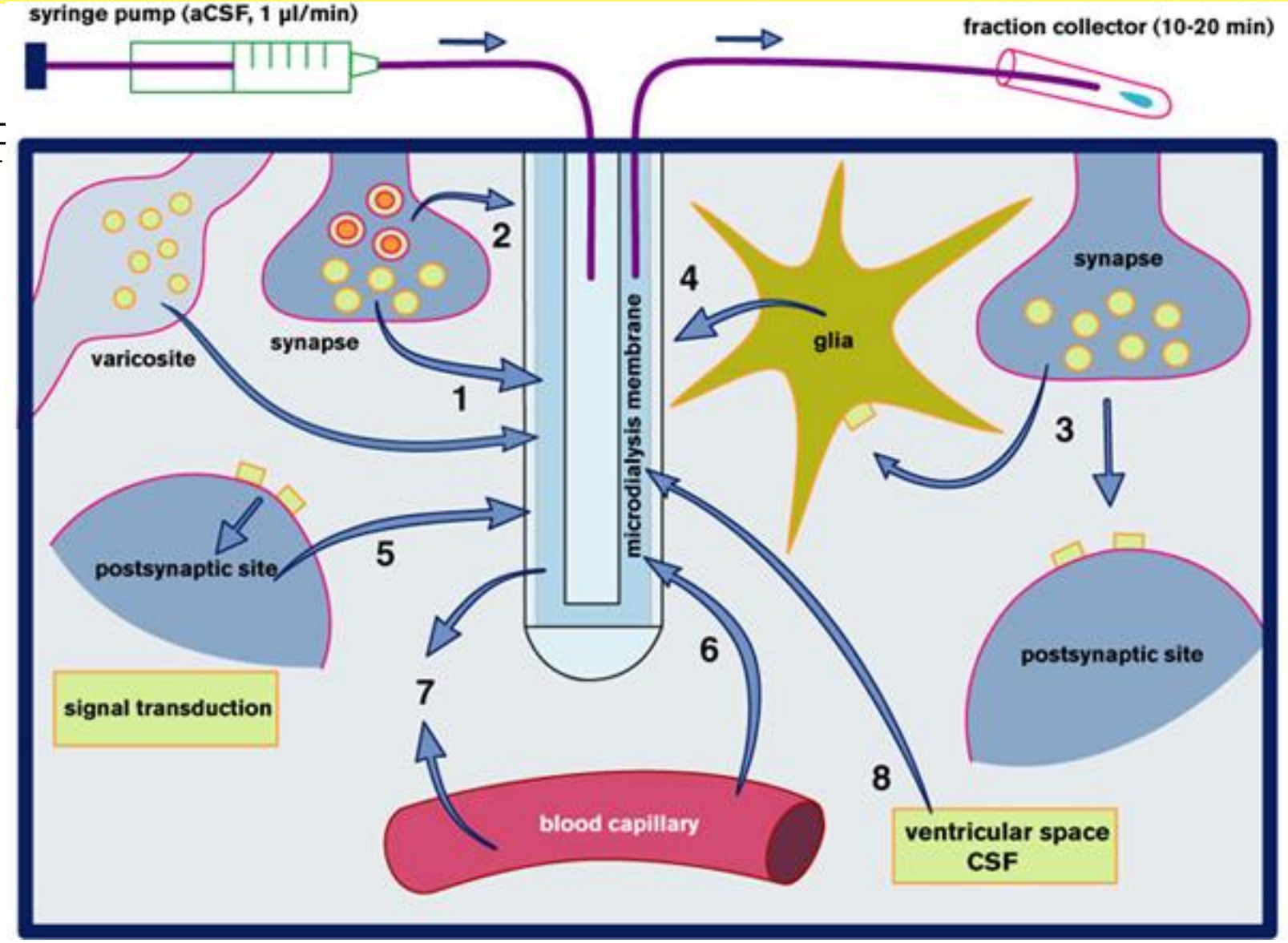
	Spatial resolution				Temporal resolution			
	device	signal	neurons	heuristic	device	signal	spikes	heuristic
behavior	brain	brain	> 10^{11}	station	1 ms	10 ms	10	
2DG, c-fos	10 μm	10 μm	1		NA	30 min	> 10^{6}	
fMRI	> 1 mm	> 200 μm	> 10^{5}		1 s	> 1 s	1000	10 ms
EEG	10 mm	100 mm	> 10^{9}	station	< 1 ms	50 ms	50	1 ms
MEG	10 mm	100 mm	> 10^{9}		1 ms	50 ms	50	
ECoG	10 mm	1-10 mm	> $10^{4\text{-}6}$		< 1 ms	10 ms	10	
Intr. Signal	10 μm	200 μm	> 1000		< 1 ms	1 s	1000	10 ms
VSD Imaging	10 μm	30 μm	< 100		< 1 ms	10 ms	< 1	
μElec	50 μm	10 μm	1	10 μm	< 1 ms	1 ms	1	
μDialysis	< 1 mm	100 μm	> 10^{4}		> 1 min	100 ms	> 10^{6}	
Intracell elec	10 μm	10 μm	< 1		< 1 ms	< 1 ms	< 1	
Ca imaging	1 μm	1 μm	< 1		1 ms	> 100 ms	> 100	

Micro-dialysis recordings

From the tip of the micro-dialysis probe one can record concentrations of chemicals



Micro-dialysis recordings



Methods table

Measuring neural activity

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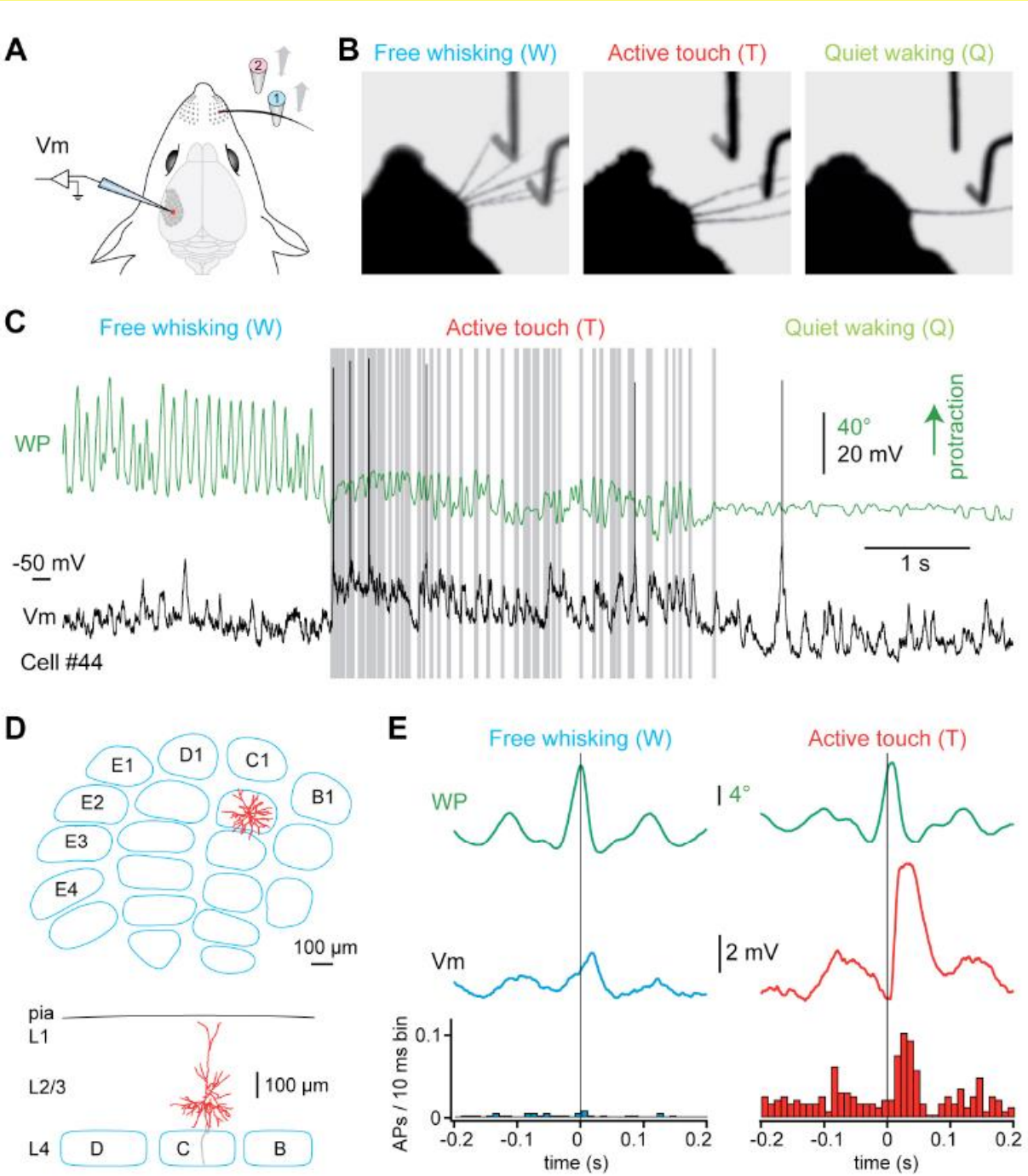
EEG	10 mm	100 mm	> 10^{9}	station	< 1 ms	50 ms	50	1 ms
MEG	10 mm	100 mm	> 10^{9}		1 ms	50 ms	50	
ECoG	10 mm	1-10 mm	> $10^{4\text{-}6}$		< 1 ms	10 ms	10	
Intr. Signal	10 μm	200 μm	> 1000		< 1 ms	1 s	1000	10 ms
VSD Imaging	10 μm	30 μm	< 100		< 1 ms	10 ms	< 1	
μElec	50 μm	10 μm	1	10 μm	< 1 ms	1 ms	1	
μDialysis	< 1 mm	100 μm	> 10^{4}		> 1 min	100 ms	> 10^{6}	
Intracell elec	10 μm	10 μm	< 1		< 1 ms	< 1 ms	< 1	
Ca imaging	1 μm	1 μm	< 1		1 ms	> 100 ms	> 100	

Intra-cellular recordings

In awake behaving mouse

- Identifying cell type
- Recording the inputs (reflecting individual inputs, network state, network activity)
- revealing mechanisms (ex-inh, …)

C. Petersen and colleagues, 2010



Methods table

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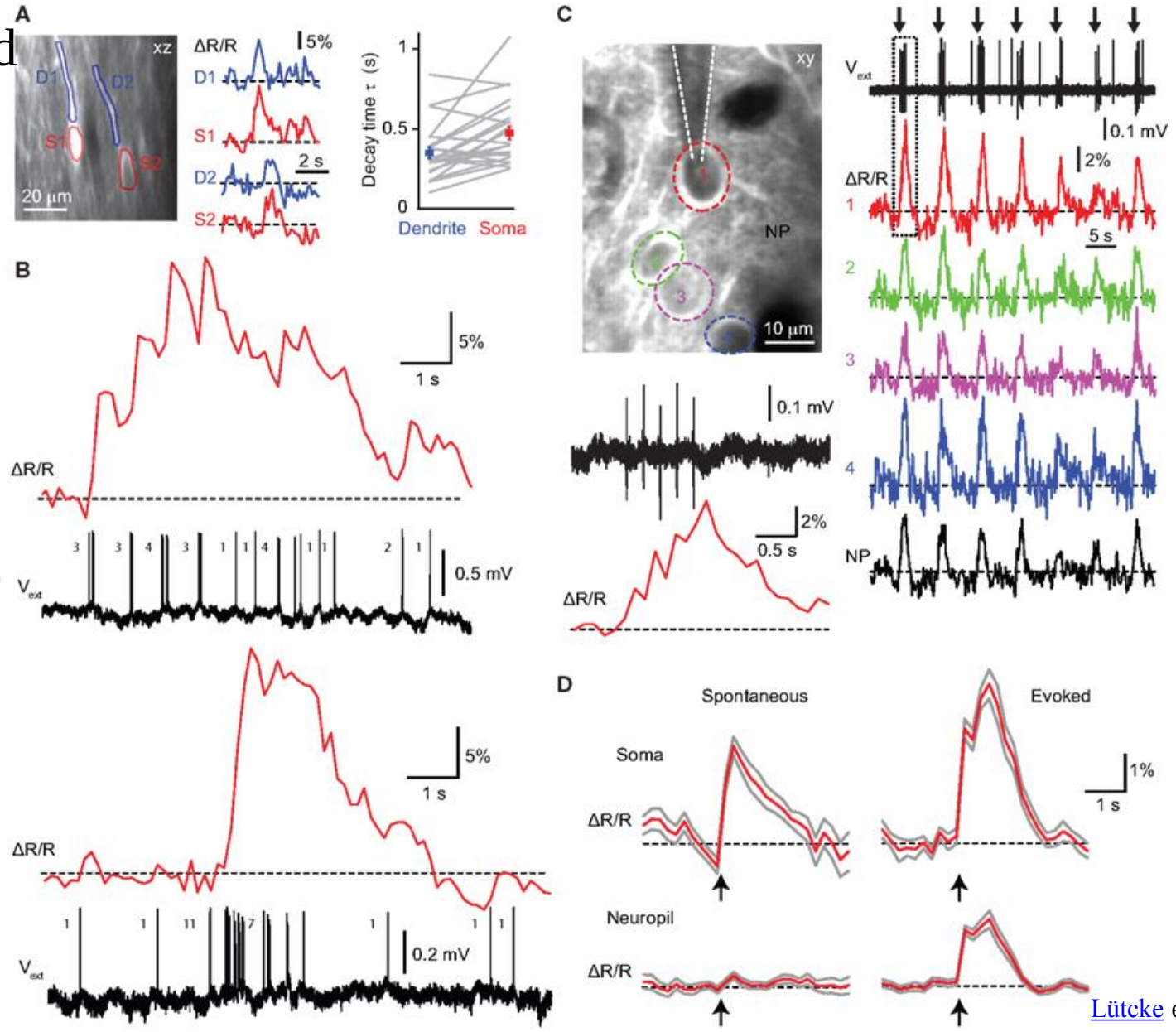
fMRI	> 1 mm	> 200 μm	> 10^{5}		1 s	> 1 s	1000	10 ms
EEG	10 mm	100 mm	> 10^{9}	station	< 1 ms	50 ms	50	1 ms
MEG	10 mm	100 mm	> 10^{9}		1 ms	50 ms	50	
ECoG	10 mm	1-10 mm	> $10^{4\text{-}6}$		< 1 ms	10 ms	10	
Intr. Signal	10 μm	200 μm	> 1000		< 1 ms	1 s	1000	10 ms
VSD Imaging	10 μm	30 μm	< 100		< 1 ms	10 ms	< 1	
μElec	50 μm	10 μm	1	10 μm	< 1 ms	1 ms	1	
μDialysis	< 1 mm	100 μm	> 10^{4}		> 1 min	100 ms	> 10^{6}	
Intracell elec	10 μm	10 μm	< 1		< 1 ms	< 1 ms	< 1	
Ca imaging	1 μm	1 μm	< 1		1 ms	> 100 ms	> 100	

Ca imaging using 2-photon microscopy

Anesthetized mice

Ca

APs (juxtacell)

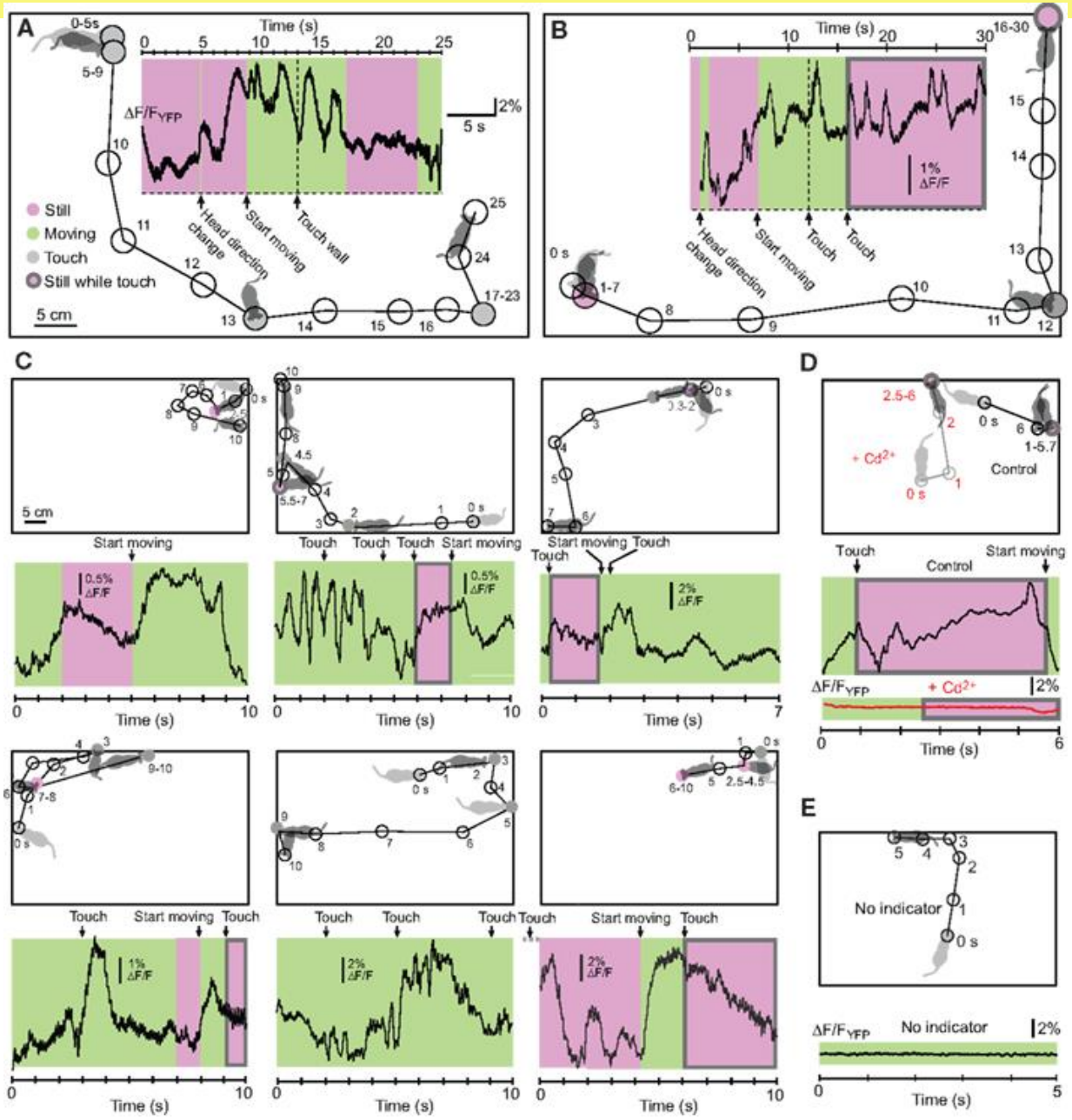


Lütcke et al 2010

- **Figure 5. Single-cell and population YC3.60 Ca^{2+} signals in L2/3 of barrel cortex. (A)** Simultaneous two-photon Ca^{2+} imaging in soma and dendrites of L2/3 neurons using vertical (xz-)imaging. Examples of spontaneous somatic (S, red) and apical dendritic (D, blue) YC3.60 Ca^{2+} transients for the cells depicted in the left image. Right: Mean decay times in dendrites compared to somata for 23 measurements (gray lines; mean ± SEM). **(B)** Simultaneous juxtacellular voltage recording and two photon Ca^{2+} imaging from a neuron showing rare events of sustained and high-frequency AP firing that are accompanied by large YC3.60 Ca^{2+} transients with peak amplitudes of up to 30% $\Delta R/R$. Top: Sustained AP firing leads to prolonged elevation of the fluorescence ratio. Bottom: A short burst of 11 APs is accompanied by a fast Ca^{2+} transient, which returns to baseline following a stereotypical exponential decay. **(C)** Two-photon Ca^{2+} imaging of a small population of neurons during sensory stimulation (seven times five air-puffs to contra-lateral whiskers at 5 Hz). Large Ca^{2+} transients in cell 1 (red trace) correlated with the spiking activity observed in the simultaneous juxtacellular voltage recording. Concomitant Ca^{2+} transients were also evoked in neighboring neuronal somata and in the nearby neuropil (NP). The response to the first stimulation episode (dashed box) is shown on expanded scale in the lower left, indicating that YC3.60 resolves the individual steps in the accumulated Ca^{2+} response. **(D)** Event-triggered average Ca^{2+} traces from somata and adjacent neuropil for spontaneous (n = 37 events of 1–3 APs) and evoked (n = 32 events of 1–5 APs) action potentials. Multi-whisker air puff evoked Ca^{2+} transients in somata were significantly larger than those in the neuropil while spontaneous spikes were accompanied by somatic but no neuropil transients. Errors are shown as SEM.

Ca imaging using 2-photon microscopy

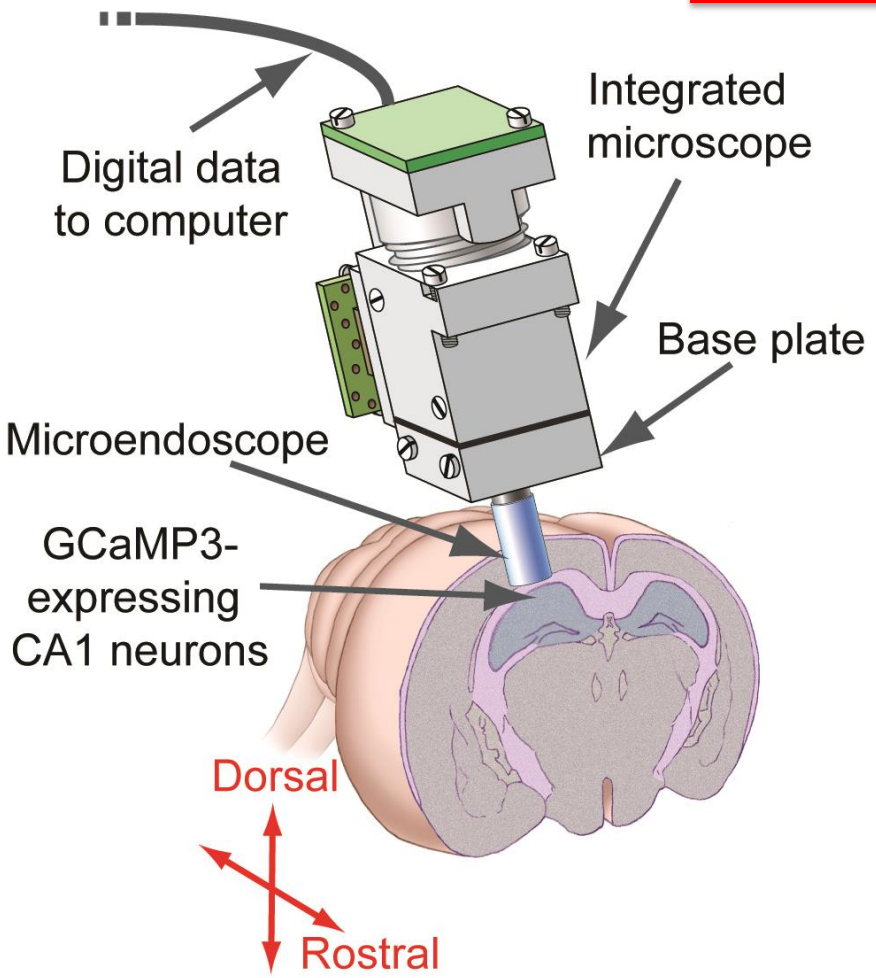
Freely moving mice

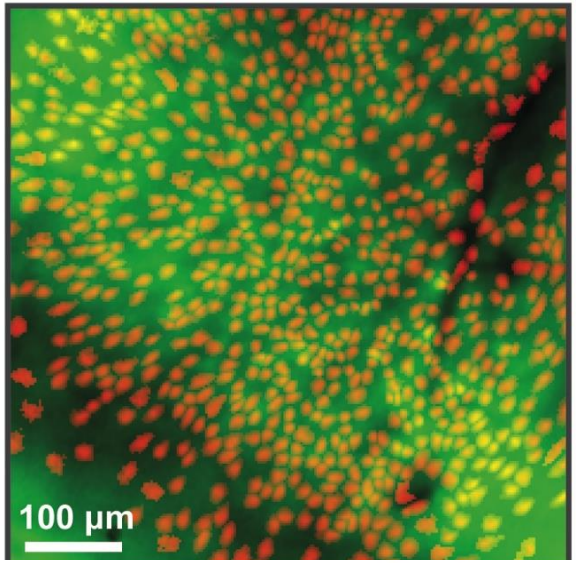


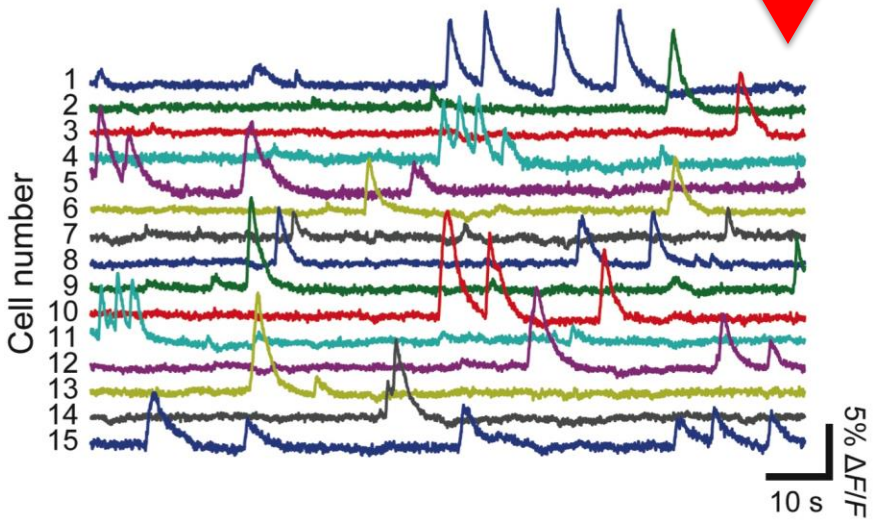
Lütcke et al 2010

- **Figure 7. Fiber-optic recording of brain area activity in freely moving mice using YC3.60. (A,B)** Two examples of fiber-optic recording of YC3.60 signals in awake, freely moving mice. Bulk Ca^{2+} signals indicating neuronal activity were recorded in somatosensory cortex through a single-core optical fiber as shown in Figure 6C. Fluorescence changes in the YFP-channel are shown during 25–30 s periods together with the position of the mouse in an open field box. Animal behavior (sitting still, moving, touches, or having contact to the wall) is indicated by background colors. The trajectory of the animals' movement is indicated with selected time stamps. **(C)** Six more examples of Ca^{2+} imaging from three mice, together with corresponding behavioral observations. Changes of the animal's behavioral state (e.g., start of movement) were frequently associated with marked discontinuities in the fluorescence trace, indicating complex underlying Ca^{2+} dynamics. **(D)** Control experiment showing that Ca^{2+} signals are blocked by local perfusion of the cortical region with Cd^{2+}. **(E)** Control experiment demonstrating that a flat fluorescence trace is observed in the absence of YC3.60 expression.

A system for chronic imaging of neuronal ensemble activity in the hippocampus of freely behaving mice



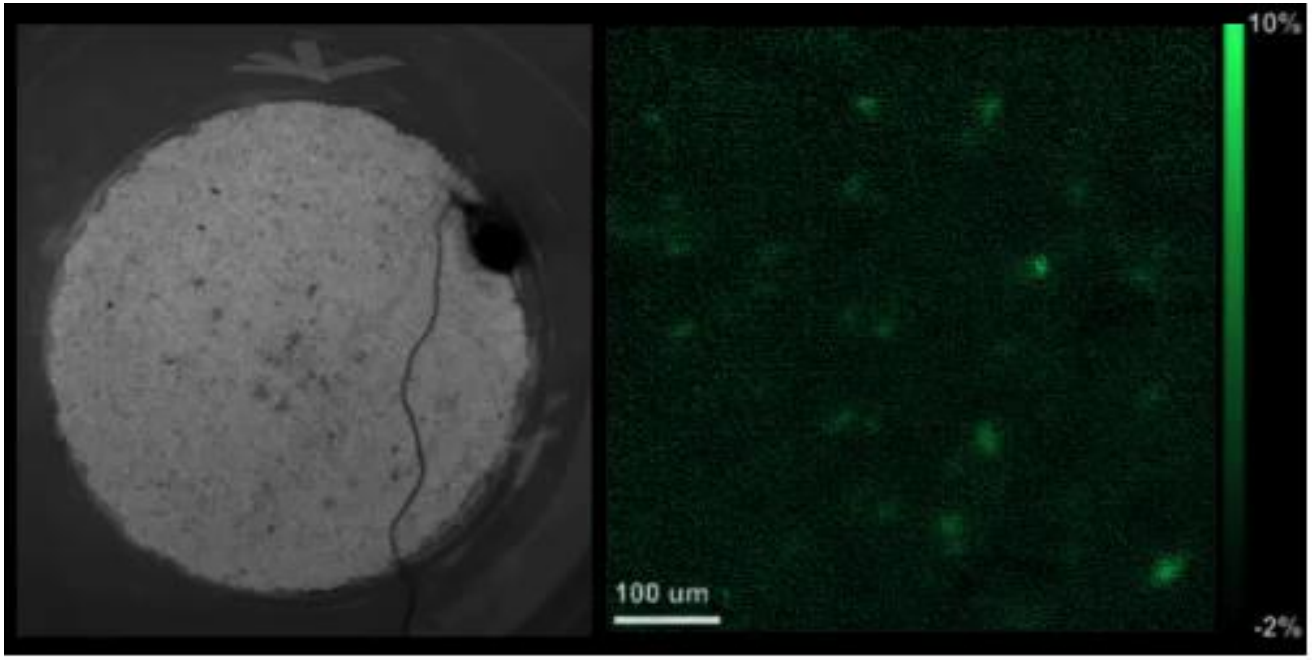




Yaniv Ziv

Ziv *et al*, *Nature Neuroscience* 2013

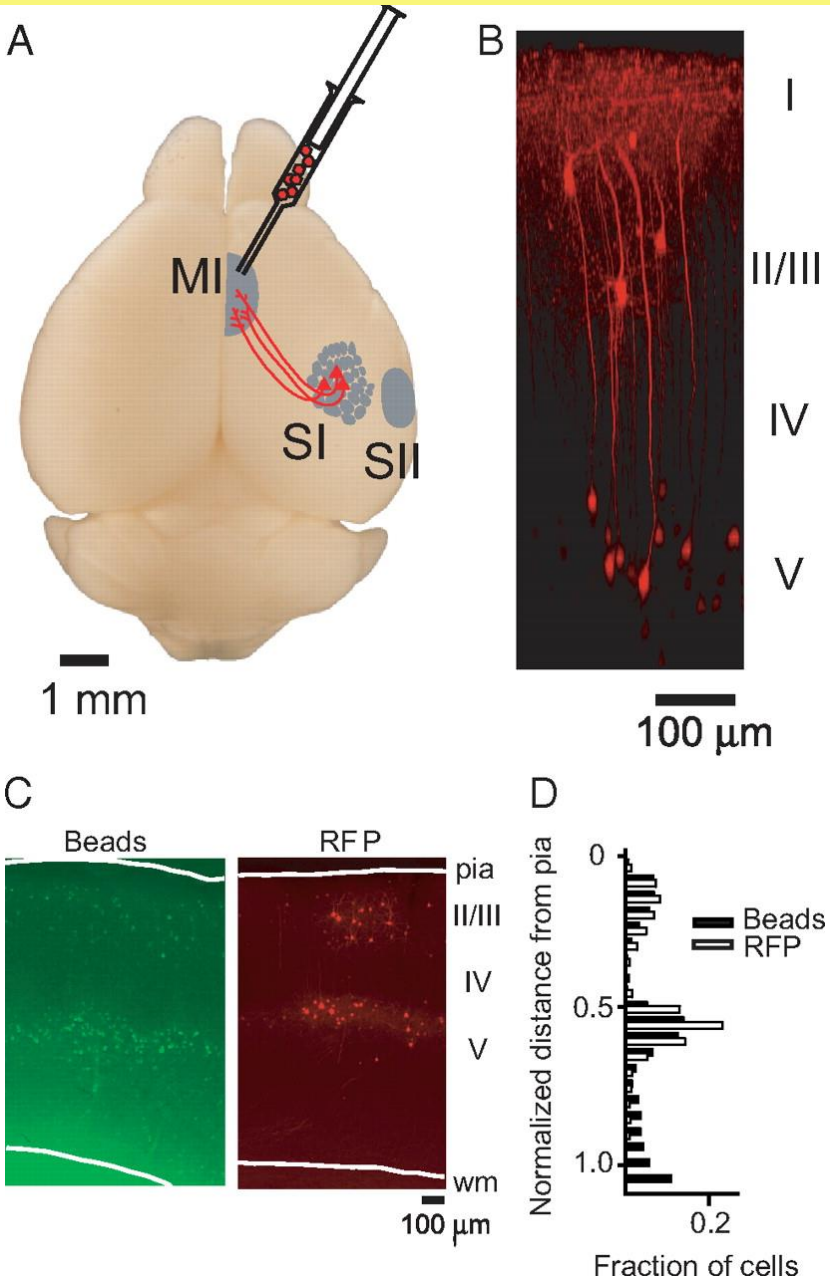
Imaging of Ca^{2+} dynamics in CA1 neurons of freely behaving mice



Ziv *et al*, *Nature Neuroscience* 2013

Ca imaging using 2-photon microscopy

Selecting only projecting neurons

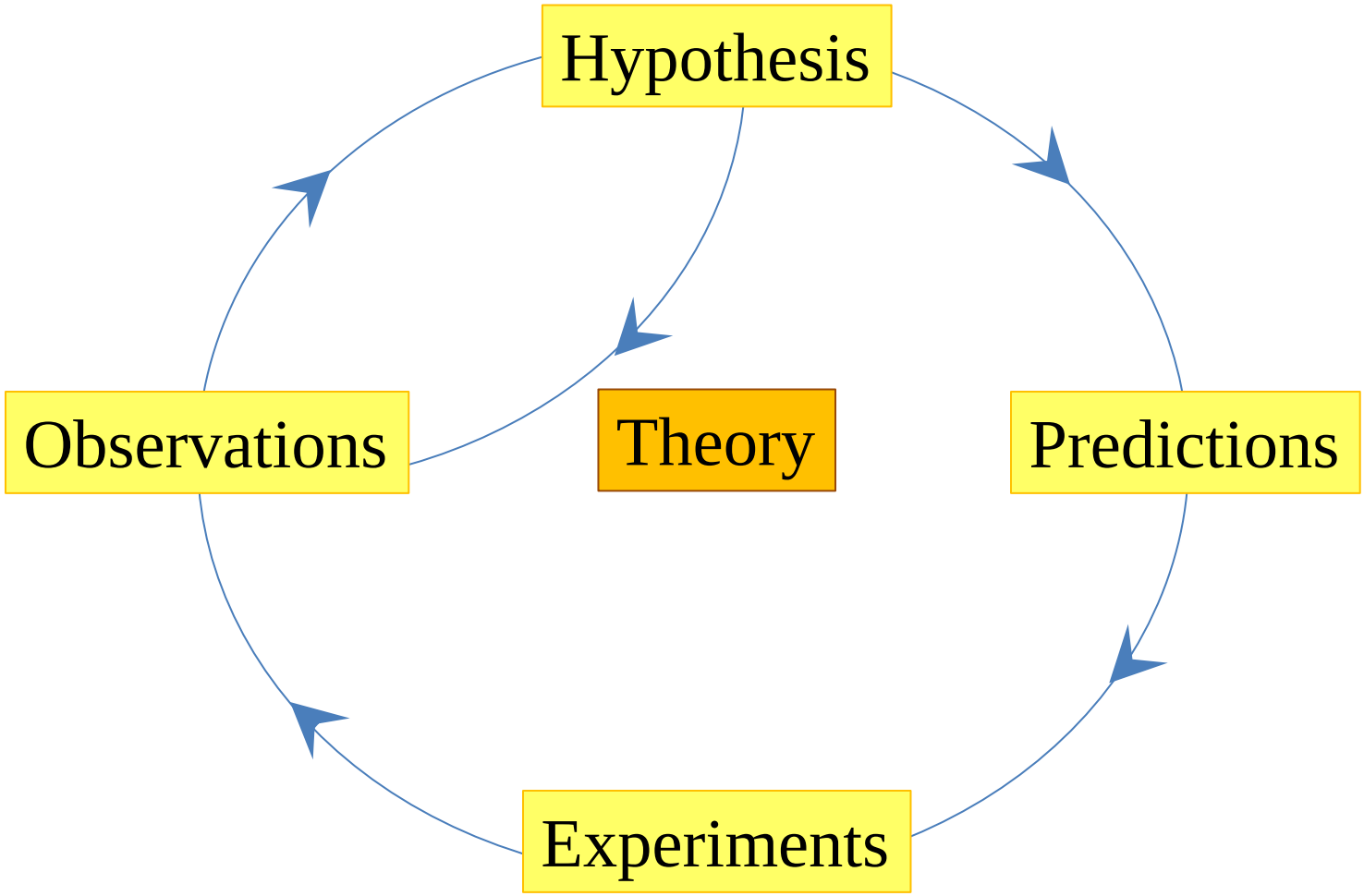


Sato & Svoboda, 2010

- **Figure 1.** Retrograde labeling with a virus expressing a red fluorescent protein. ***A***, Schematic showing retrograde labeling of neurons in SI by injection of the retrograde virus HSV1 into MI. ***B***, *In vivo* image of RFP+ neurons (maximum-intensity side projection of an image stack of RFP+ neurons; 512 x 128 x 96; section spacing, 8 μm). ***C***, Distribution of labeled neurons in SI barrel cortex after bead (left) or virus (right) injection into MI. The white lines indicate the pia and the border between the cortex and the white matter. ***D***, Normalized distribution of labeled neurons after bead (black, 1293 neurons) and HSV (white, 808 neurons) injection into MI.

Introduction

The scientific method



Methods table

Stimulating / perturbing neural activity

	Spatial resolution				Temporal resolution			
	device	signal	neurons	heuristic	device	signal	spikes	heuristic
sensory	modality	modality	> 10^9	station	< 1 ms	< 1 ms	< 1	
TMS	100 mm	10 mm	> 10^9	station	< 1 ms	100 ms	100	
µStim	< 10 µm	> 100 µm	> 50		< 1 ms	10 ms	10	
µPharmac	< 10 µm	> 100 µm	> 50		1 ms	> 10 s	10	
single cell	< 10 µm	< 10 µm	1		< 1 ms	< 1 ms	< 1	
sub-cell	>100 µm	< 1 µm	> 50		< 1 ms	< 1 ms	< 1	

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Stimulating / perturbing neural activity

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μPharmac	< 10 μm	> 100 μm	> 50		1 ms	> 10 s	10	
single cell	< 10 μm	< 10 μm	1		< 1 ms	< 1 ms	< 1	
sub-cell	>100 μm	< 1 μm	> 50		< 1 ms	< 1 ms	< 1	

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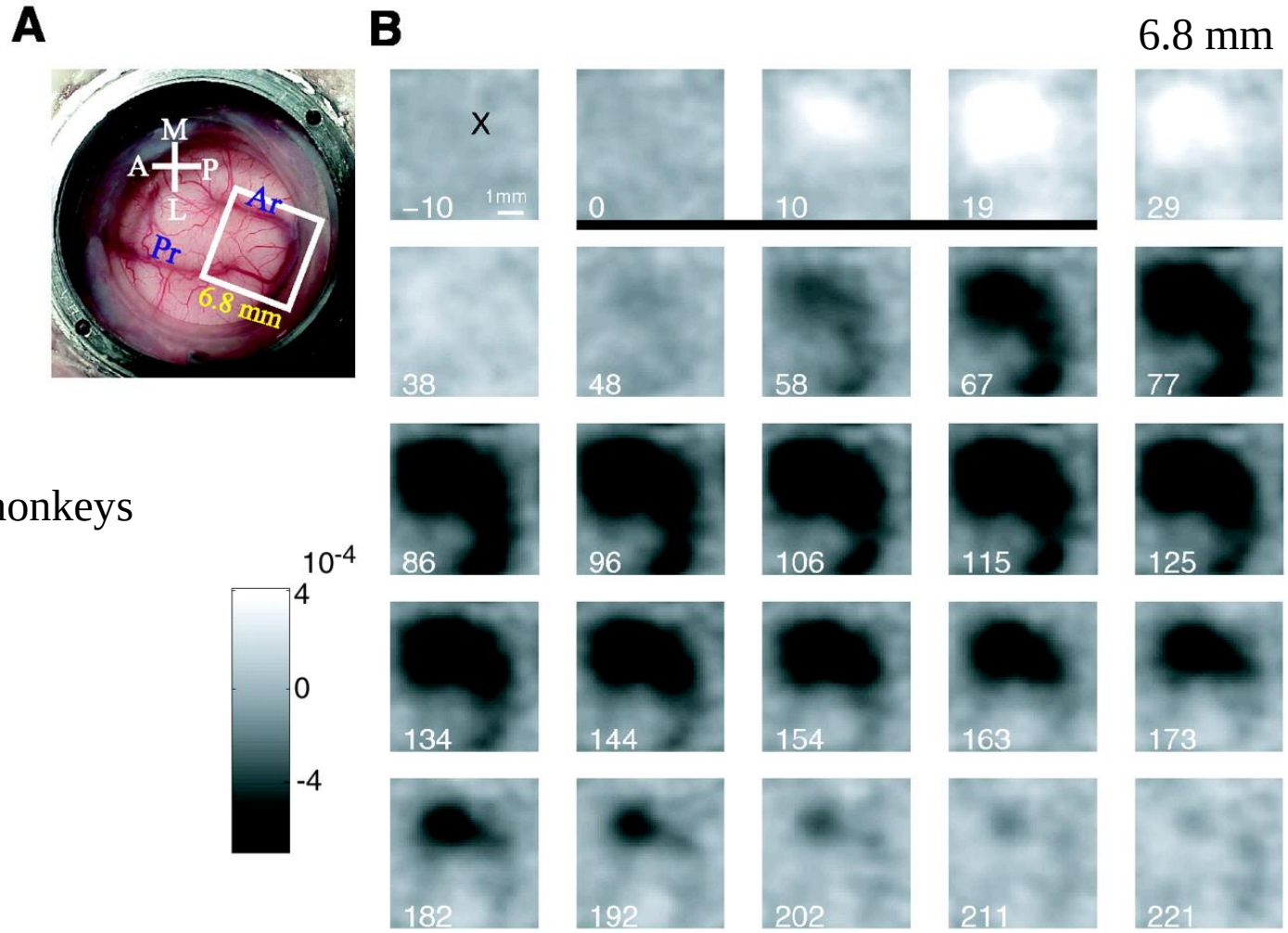
TMS	100 mm	10 mm	> 10^9	station	< 1 ms	100 ms	100	
µStim	< 10 µm	> 100 µm	> 50		< 1 ms	10 ms	10	
µPharmac	< 10 µm	> 100 µm	> 50		1 ms	> 10 s	10	
single cell	< 10 µm	< 10 µm	1		< 1 ms	< 1 ms	< 1	
sub-cell	>100 µm	< 1 µm	> 50		< 1 ms	< 1 ms	< 1	

Micro-stimulation



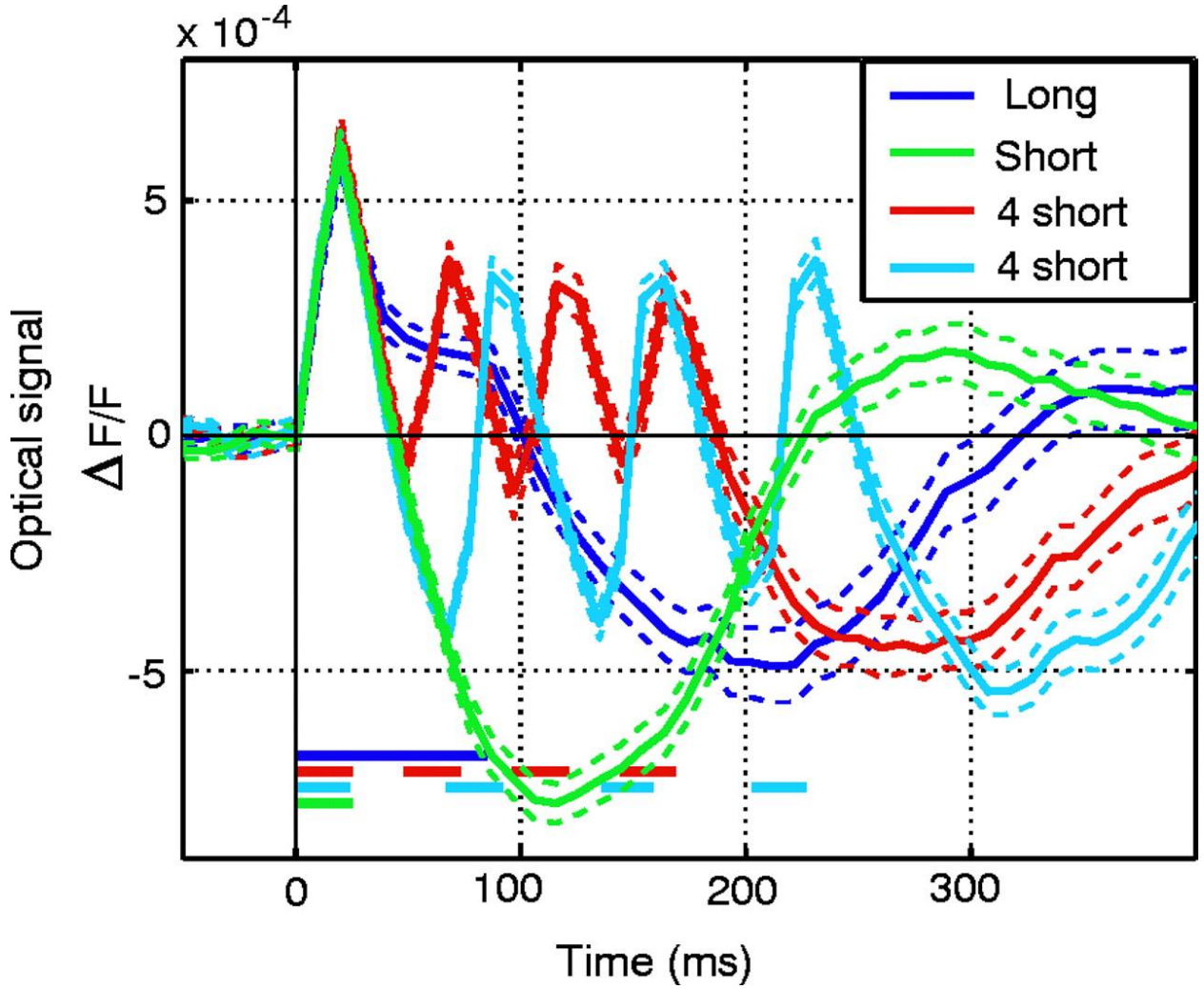
Awake monkeys

E Seidemann et al. Science 2002;295:862-865

Figure 1 Spatiotemporal dynamics of microstimulation-evoked activity.

Published by AAAS

Micro-stimulation



E Seidemann et al. Science 2002;295:862-865

Figure 2 Time course of the response to microstimulation.

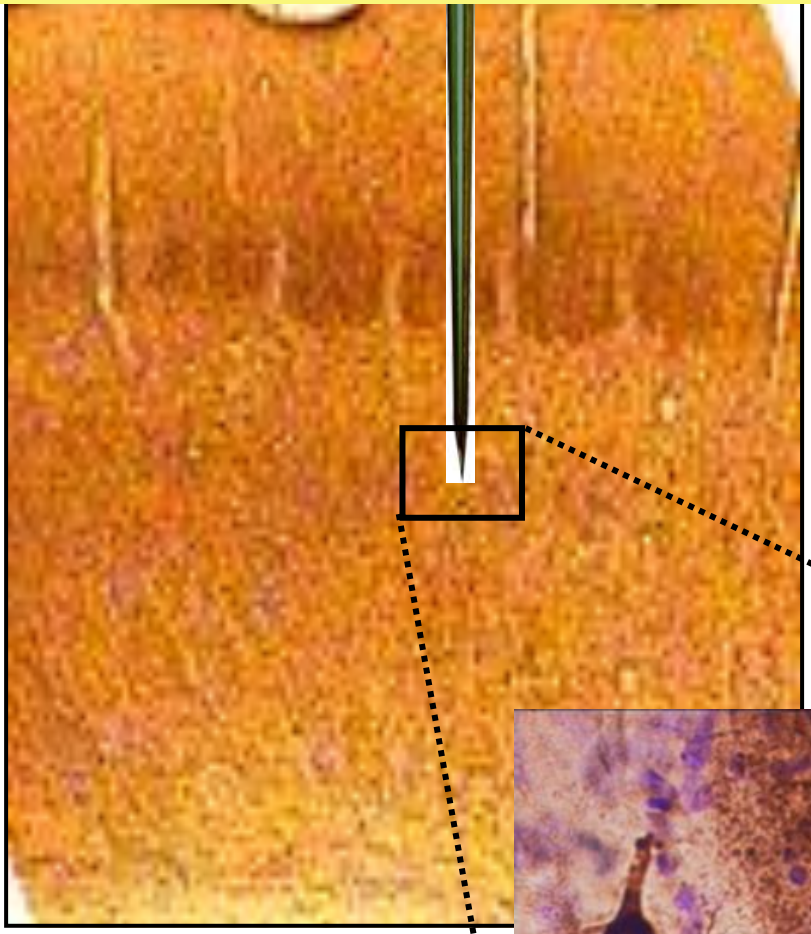
Published by AAAS

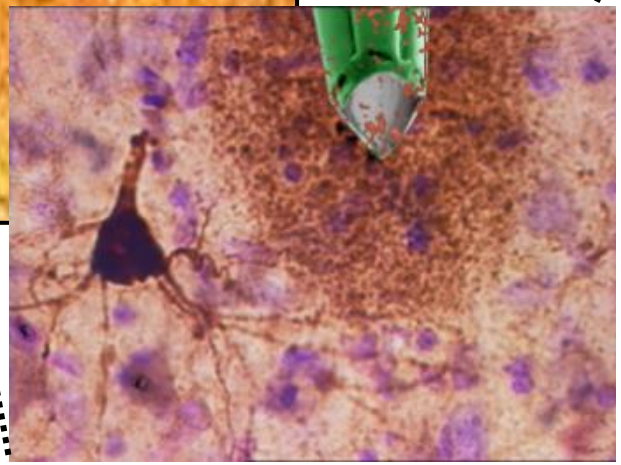
Methods table

Stimulating / perturbing neural activity

	Spatial resolution				Temporal resolution			
	device	signal	neurons	heuristic	device	signal	spikes	heuristic
sensory	modality	modality	> 10^9	station	< 1 ms	< 1 ms	< 1	
TMS	100 mm	10 mm	> 10^9	station	< 1 ms	100 ms	100	
µStim	< 10 µm	> 100 µm	> 50		< 1 ms	10 ms	10	
µPharmac	< 10 µm	> 100 µm	> 50		1 ms	> 10 s	10	
single cell	< 10 µm	< 10 µm	1		< 1 ms	< 1 ms	< 1	
sub-cell	>100 µm	< 1 µm	> 50		< 1 ms	< 1 ms	< 1	

Micro-pharmacology





Haidarliu, et al*1995*

combined electrode:

Extracellular recording &
drug application

Inject
- charged chemicals using iontophoresis
- uncharged chemicals using pressure

Methods table

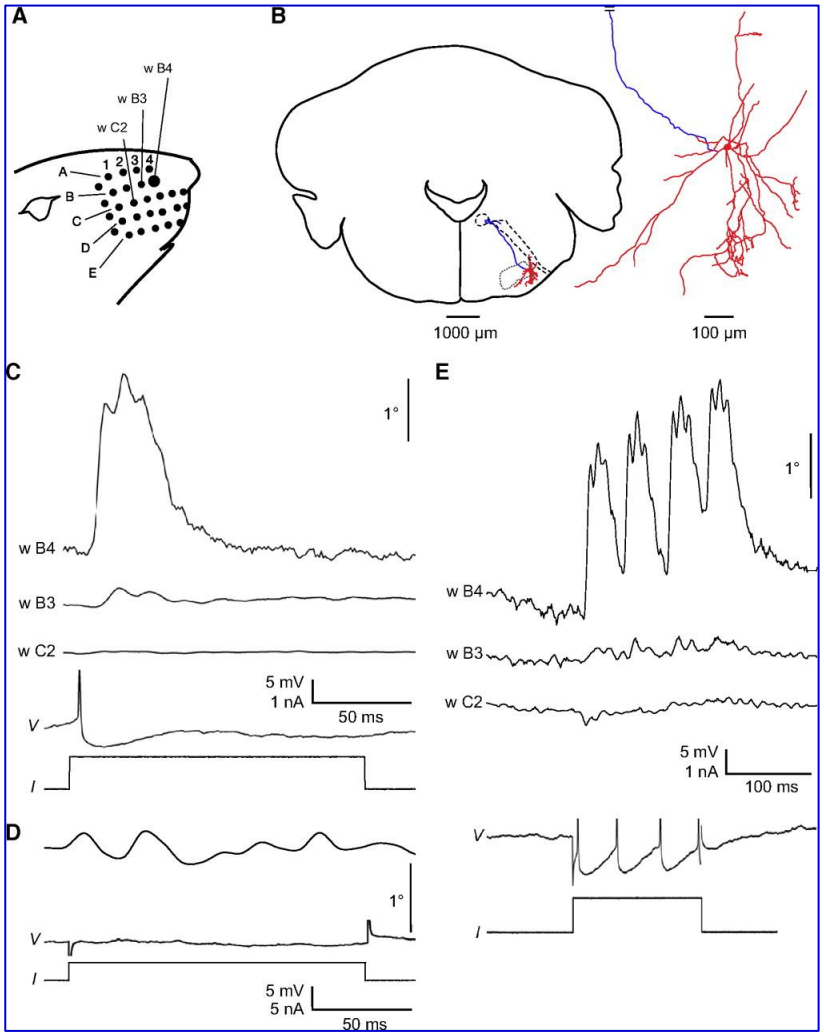
Stimulating / perturbing neural activity

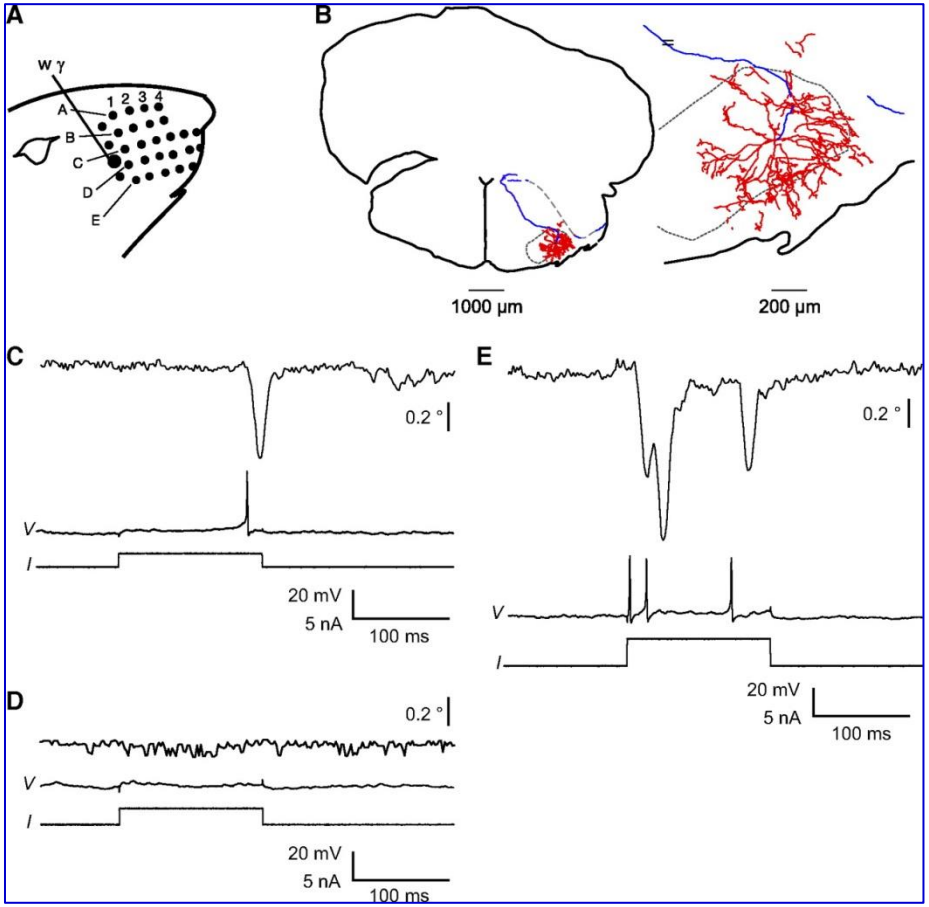
	Spatial resolution				Temporal resolution			
	device	signal	neurons	heuristic	device	signal	spikes	heuristic
sensory	modality	modality	> 10^9	station	< 1 ms	< 1 ms	< 1	
TMS	100 mm	10 mm	> 10^9	station	< 1 ms	100 ms	100	
µStim	< 10 µm	> 100 µm	> 50		< 1 ms	10 ms	10	
µPharmac	< 10 µm	> 100 µm	> 50		1 ms	> 10 s	10	
single cell	< 10 µm	< 10 µm	1		< 1 ms	< 1 ms	< 1	
sub-cell	>100 µm	< 1 µm	> 50		< 1 ms	< 1 ms	< 1	

Nano-stimulation

Examples of whisker protraction and retraction caused by single and multiple spikes

Anesthetized rats

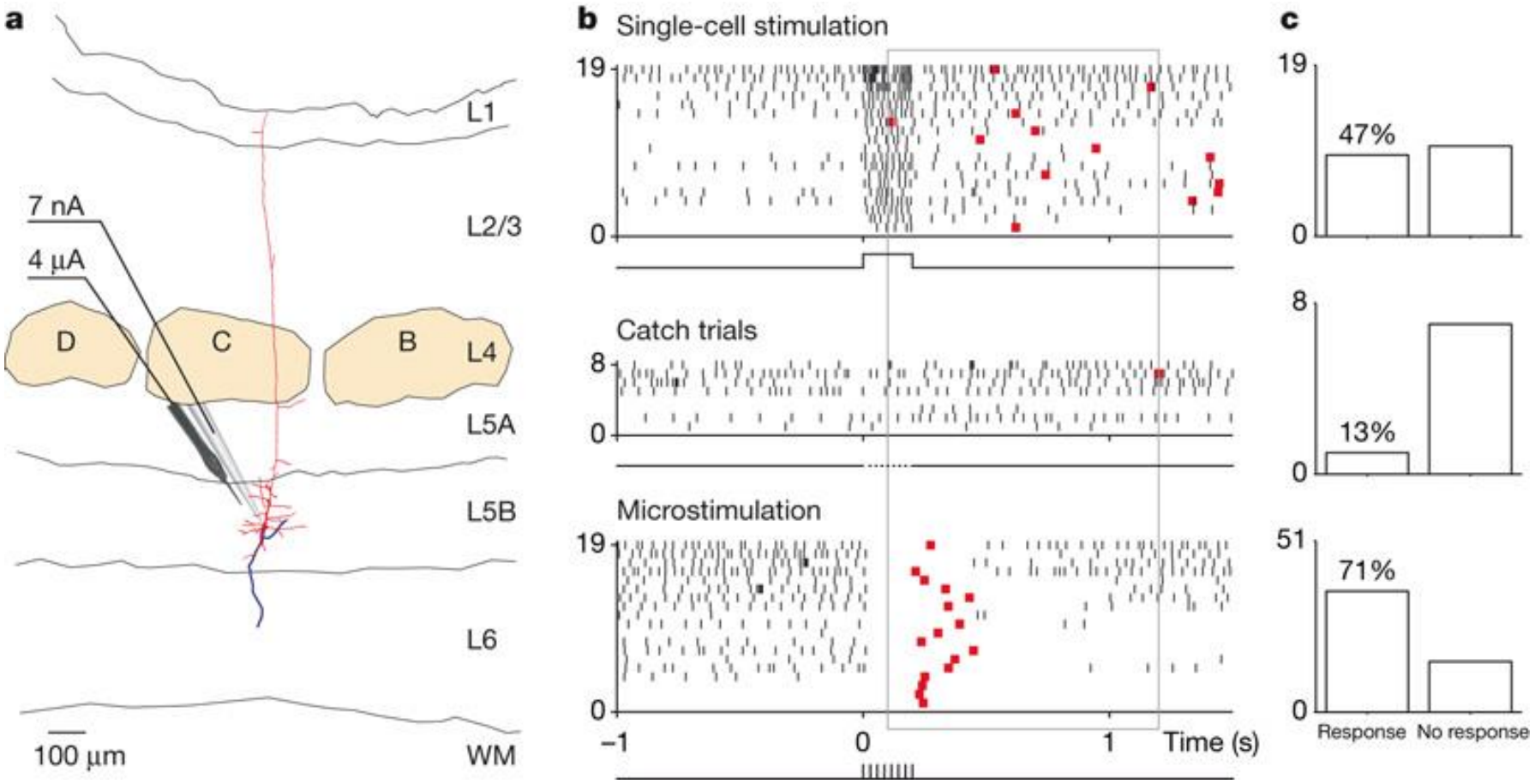




Herfst, L. J. et al. J Neurophysiol 99: 2821-2832 2008; doi:10.1152/jn.01014.2007

Nano-stimulation

Behaving rats



Houweling & Michael Brecht, Nature 2008

Methods table

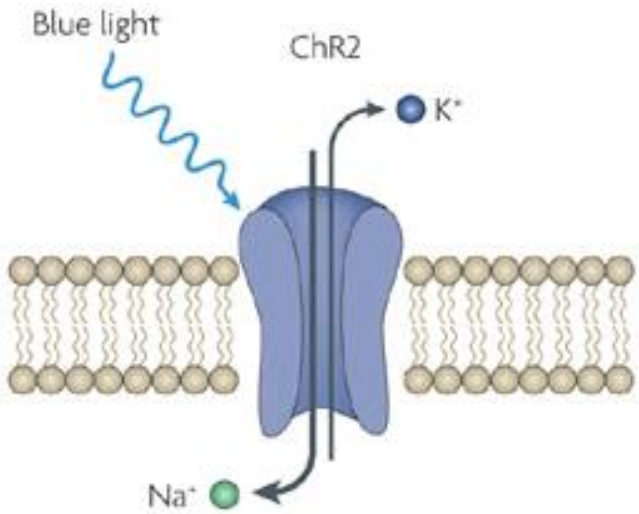
Stimulating / perturbing neural activity

	Spatial resolution				Temporal resolution			
	device	signal	neurons	heuristic	device	signal	spikes	heuristic
sensory	modality	modality	> 10^9	station	< 1 ms	< 1 ms	< 1	
TMS	100 mm	10 mm	> 10^9	station	< 1 ms	100 ms	100	
μStim	< 10 μm	> 100 μm	> 50		< 1 ms	10 ms	10	
μPharmac	< 10 μm	> 100 μm	> 50		1 ms	> 10 s	10	
single cell	< 10 μm	< 10 μm	1		< 1 ms	< 1 ms	< 1	
sub-cell	>100 μm	< 1 μm	> 50		< 1 ms	< 1 ms	< 1	

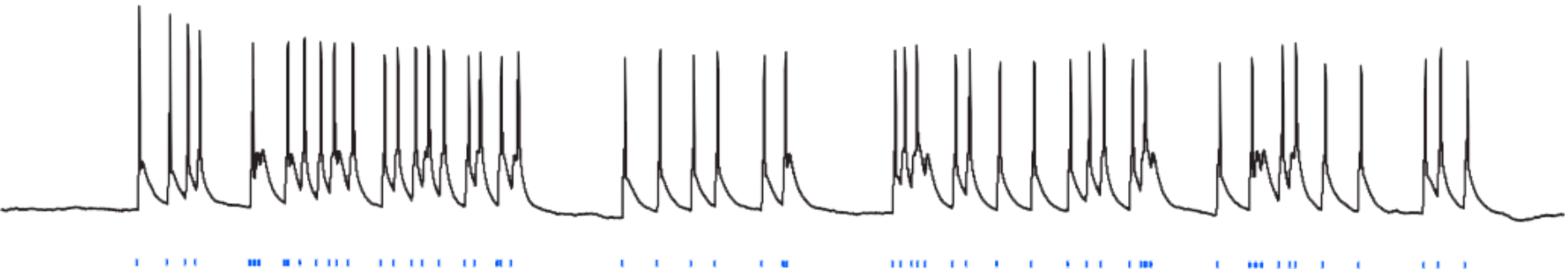
Optogenetic-stimulation

Channelrhodopsins (ChR1,2)

- Chrs function as light-gated ion channels.
- They serve as sensory photoreceptors in unicellular green algae, controlling phototaxis.
- Expressed in cells of other organisms, they enable the use of light to control electrical excitability
- All known Chrs are nonspecific cation channels, conducting H^+, Na^+, K^+, and Ca^{2+} ions.



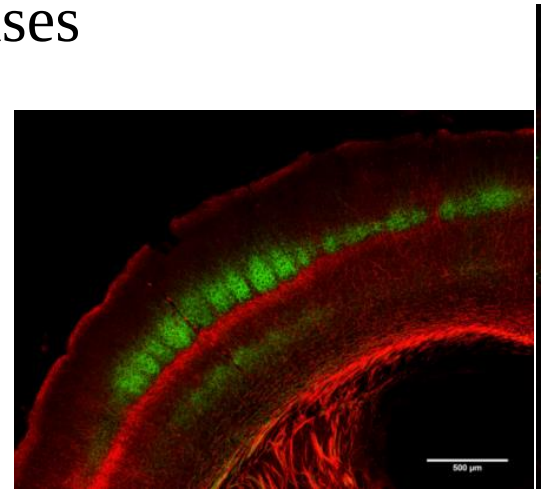
Cultured neuron expressing ChR2-eYFP stimulated with pulses of blue light

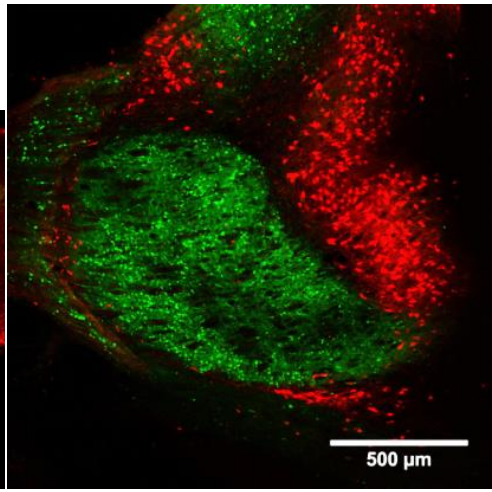


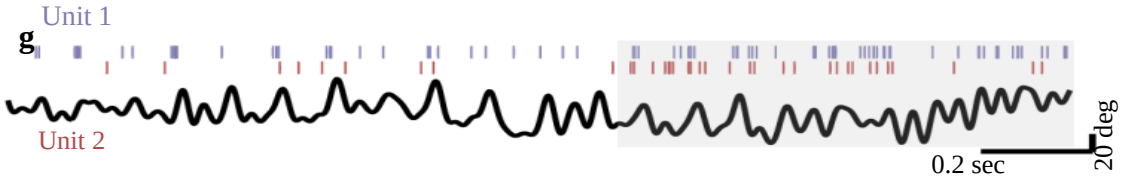
Ofer Yizhar

Optogenetic-stimulation

- Variety of excitatory channels: ON short duration, ON long duration, ON/OFF, ON subliminal, …
- Inhibitory channels
- Linking to identified promoters to be functional in indentified neurons
- Localized infection using viruses
- Combined with dye reporters





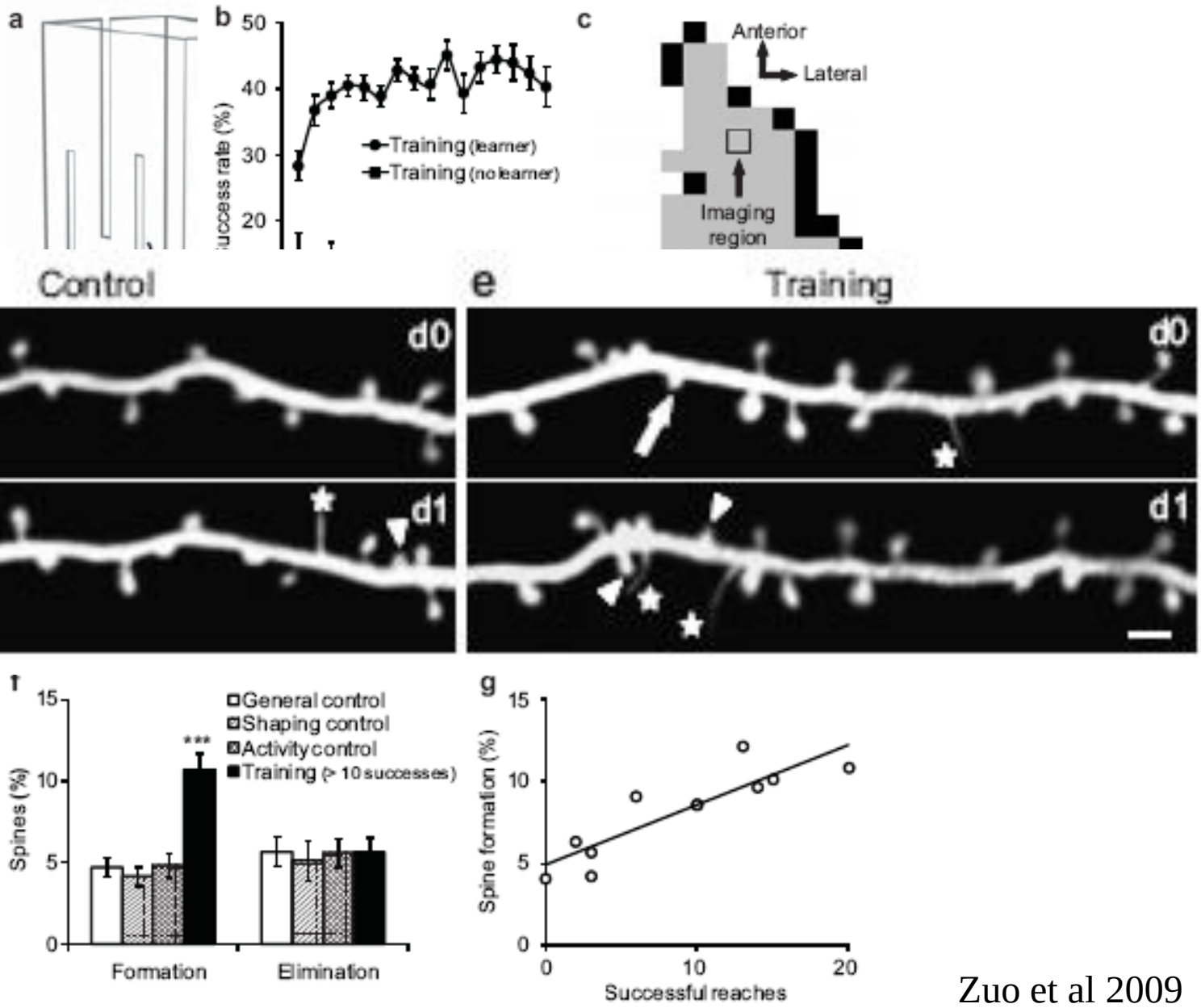


Methods table

Measuring structure

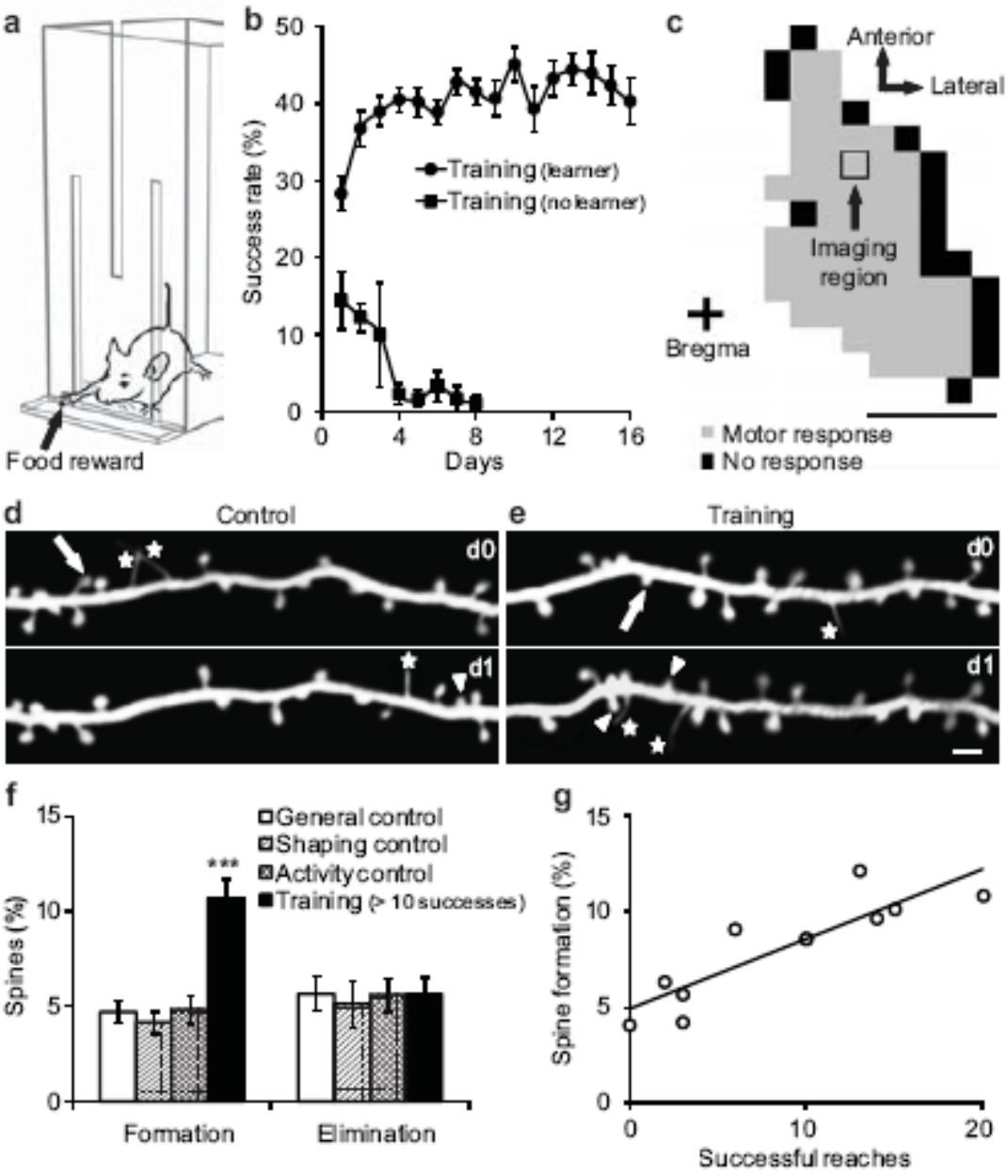
	Spatial resolution					Temporal resolution			
	device	signal	neurons	heuristic		device	signal	spikes	heuristic
cell density									
receptor density									
transmitter density									
tract tracing									
single-cell									
single-spine	< 1 μm	< 1 μm	< 1			< 1 s	hours		

Single-spine monitoring



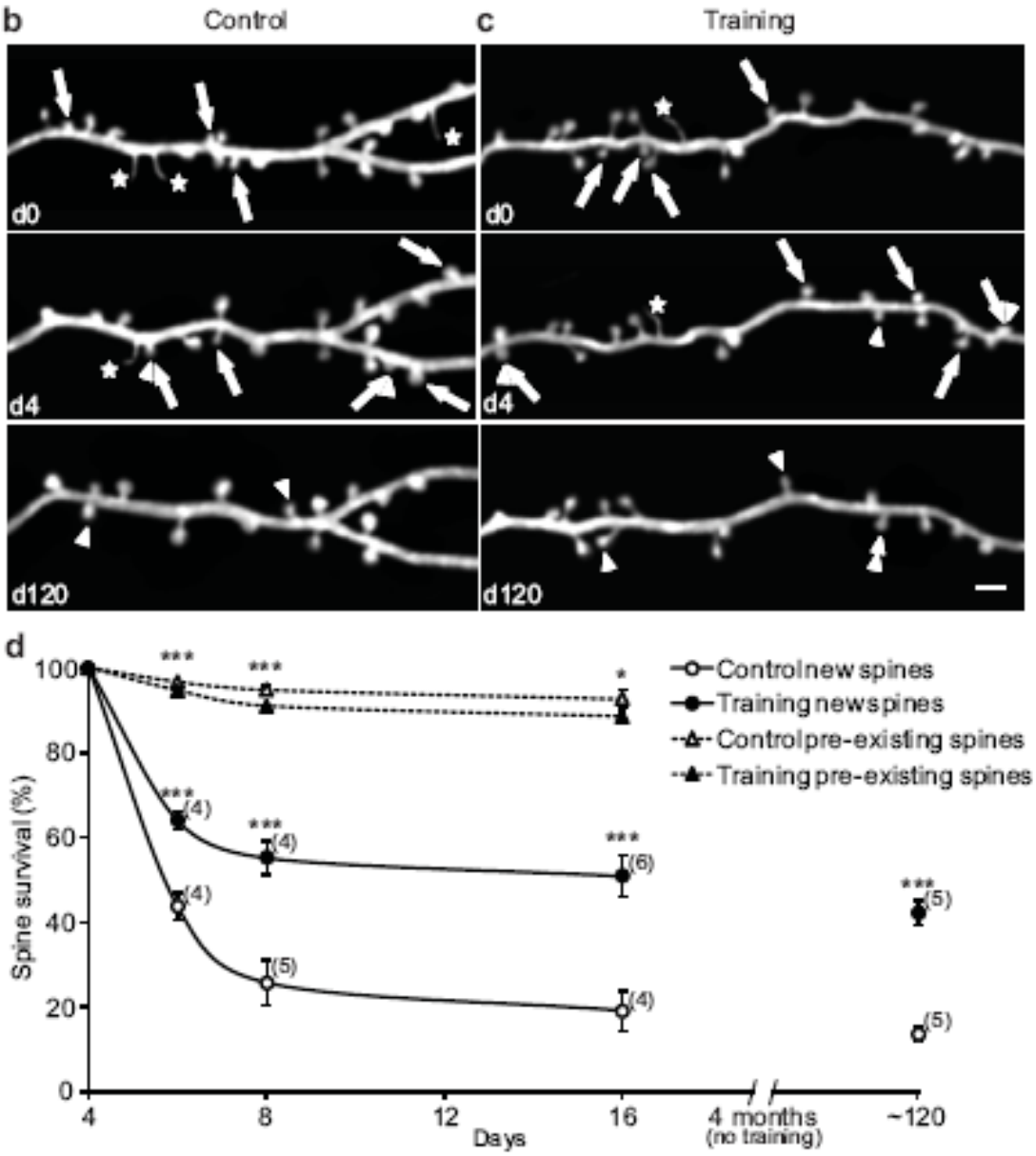
Zuo et al 2009

Single-spine monitoring



Zuo et al 2009

Single-spine monitoring



Zuo et al 2009

Methods table

Manipulating structure

	Spatial resolution					Temporal resolution			
	device	signal	neurons	heuristic		device	signal	spikes	heuristic
Neuropsychology		> 10 mm	$> 10^7$				months		
lesions	> 100 μm	> 100 μm	> 50			> 1 s	> 1 min		