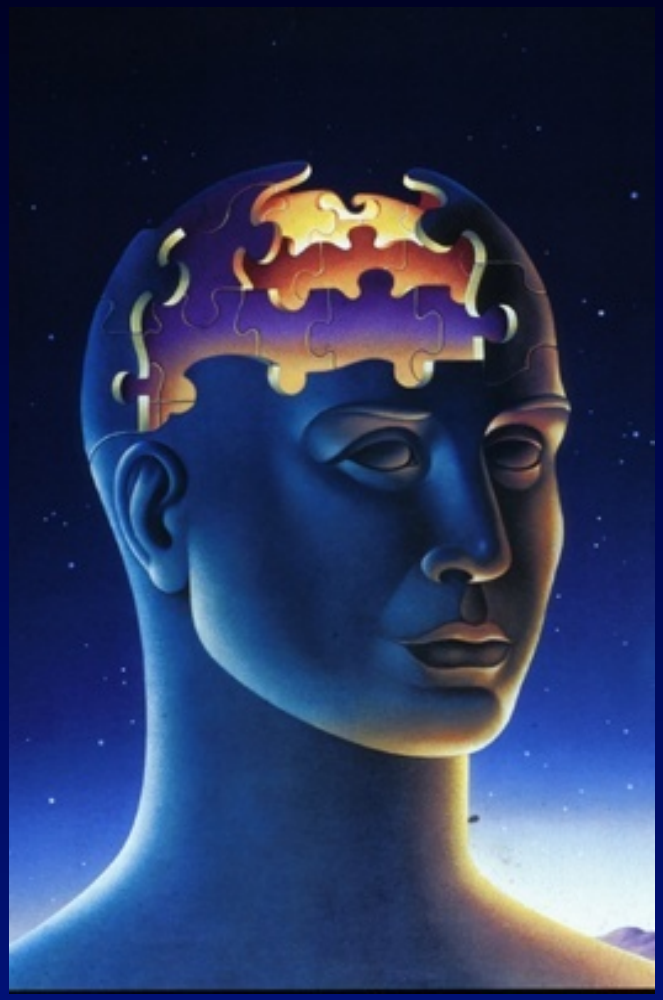
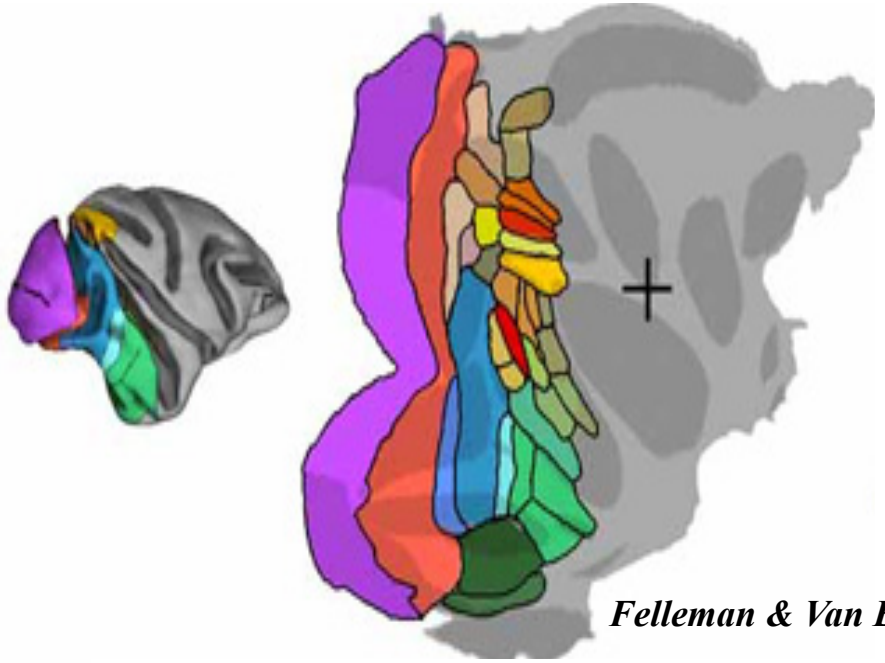
Interrogating neural circuits with optical methods

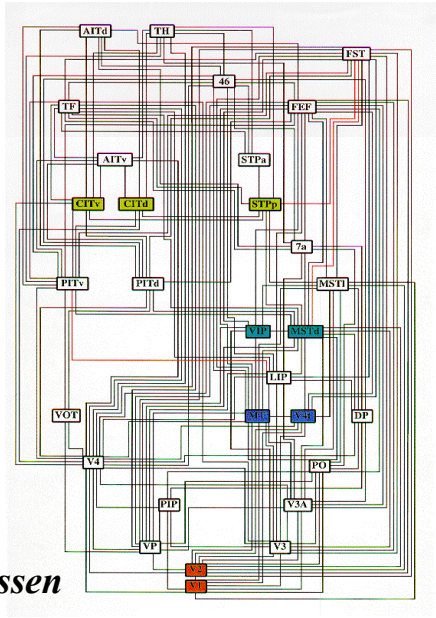
Stelios M. Smirnakis, M.D., Ph.D.
Associate Professor of Neurology
Harvard Medical School



Our lab is interested in neural circuit function at two levels:

Inter-area interactions (fMRI)

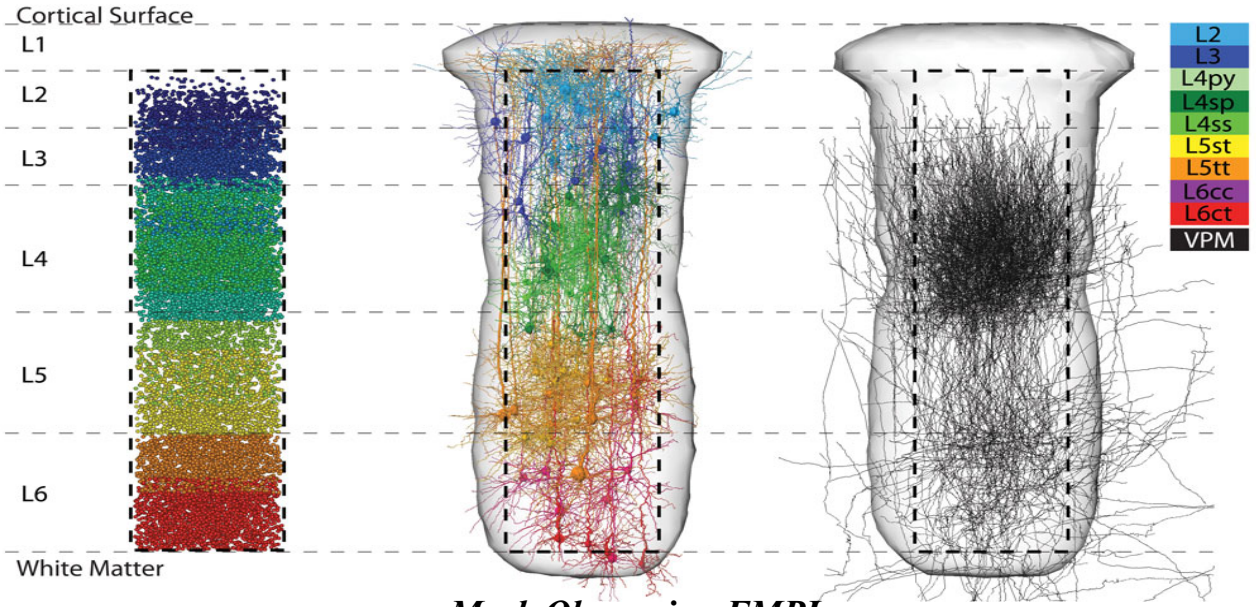




Felleman & Van Essen

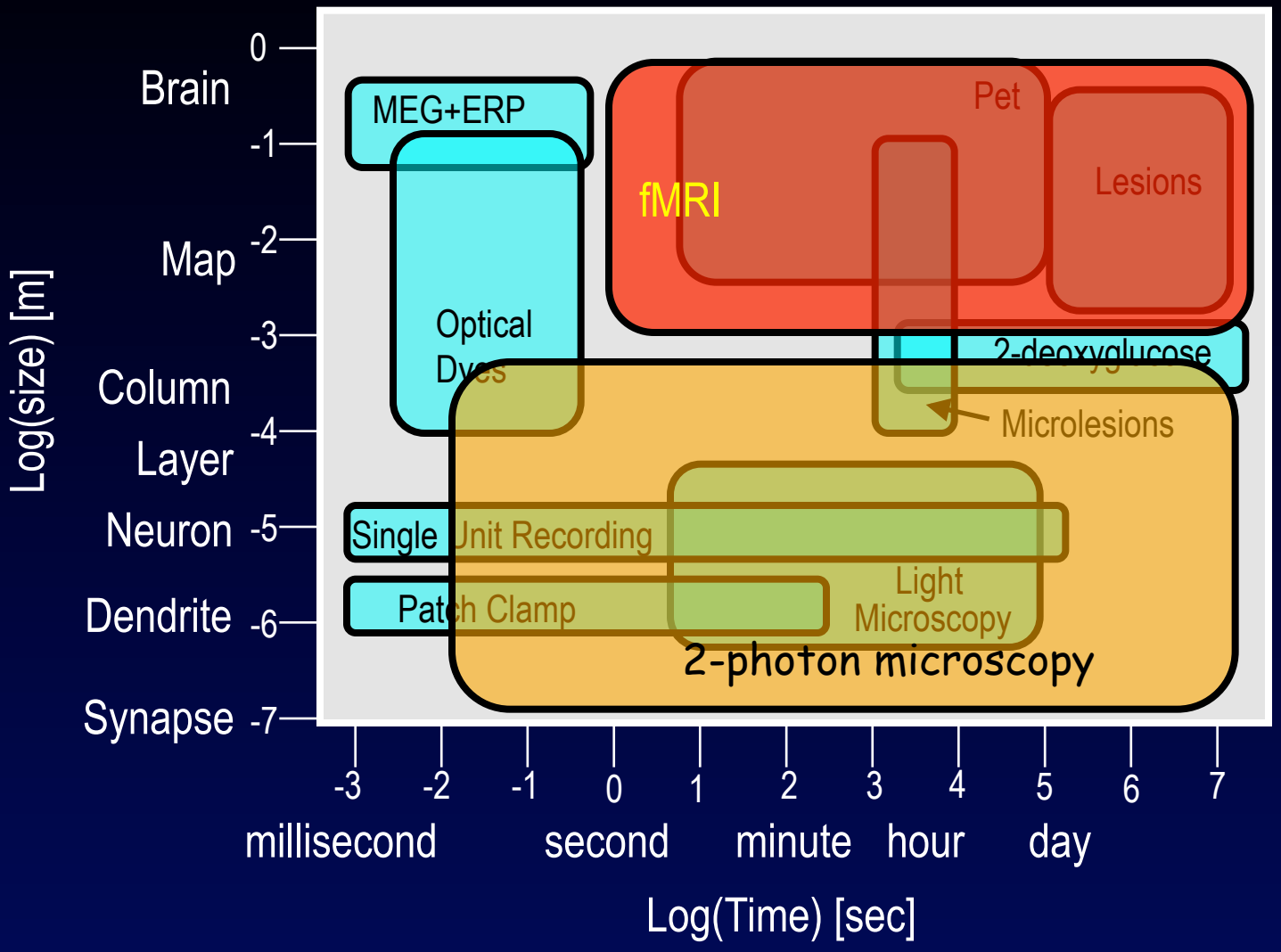
Microcircuit (~1.8mm x 500μm)

(Electrophysiology 2-photon imaging)



Mark Obermeier, FMPI

Spatial and Temporal Resolution of Techniques for Studying Brain Function



Adapted from Churchland and Sejnowski, 1988

What information is missing ?

- More information accumulates daily about the molecular basis of neural circuit function and malfunction.
- Behavioral phenotypes of disease processes are also generally well characterized.
- Mid-level information about the function of cortical circuits is however conspicuously lacking.
- i.e. it is not well understood how molecular defects affect neural circuit structure and function in vivo; nor is it understood how the resulting aberrant computational properties of affected circuits manifest in the expected behavioral phenotypes.

Understanding neocortical micro-circuit function requires

- Simultaneous monitoring and characterization of the dynamic patterns of activity generated by multiple neuronal units
- Chronically monitoring and characterizing structural elements of the cortical micro-circuitry (neuronal types, axonal-dendritic morphology)
- Correlating structure with function at the cortical microcircuit level, relating these measurements to behavioral phenotypes.
- Establishing causality by manipulating cortical network activity

It is important to be able to make these characterizations

in vivo

In vivo 2-photon approaches

- Can monitor simultaneously the activity of all units in the supra-granular layers across a region of cortex ~500μm.
- Can monitor chronically axonal and dendritic anatomy including dendritic spine motility and dendritic tree branching
- In conjunction with channel opsins and other engineered photo-activatable channels, optical methods can be used to obtain exquisite control of neocortical circuitry.
- Are amenable to studies of cerebral circulation, and can be used to manipulate and study the neurovascular unit.
- They promise to be the tool of the future for dissecting local cortical circuit function, as well as neural repair processes.

Studying both large and small animal models is critical

- Genetic Tools To Dissect Cortical Circuit Function
- Large Numbers / Low Cost / Effort per Animal

- Vascular/Cortical Physiology Similar to Human
- Better Control of Behavior gives us the ability to study questions not possible to study in mice.

- Less applicable to Human Physiology
- Poor Control of Behavior
- Cortical Maps are Coarse and Scale of Cortical Connectivity Different.

- High Cost/Effort per Animal
- Small Numbers
- As yet, limited genetic tools, but…

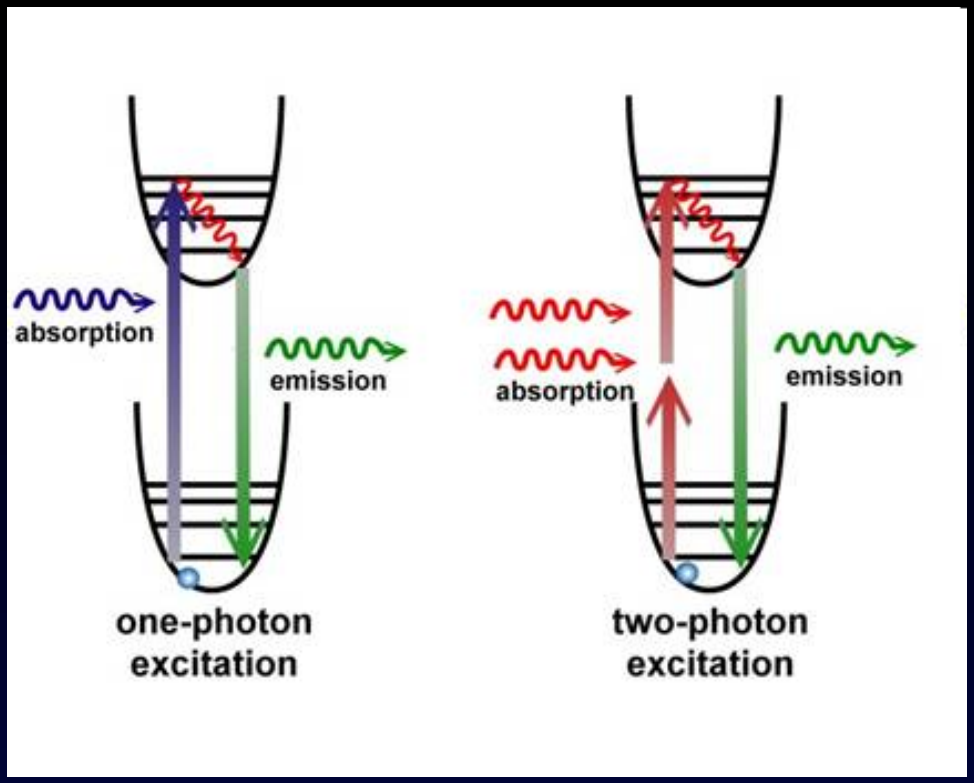
Today's lecture will proceed as follows:

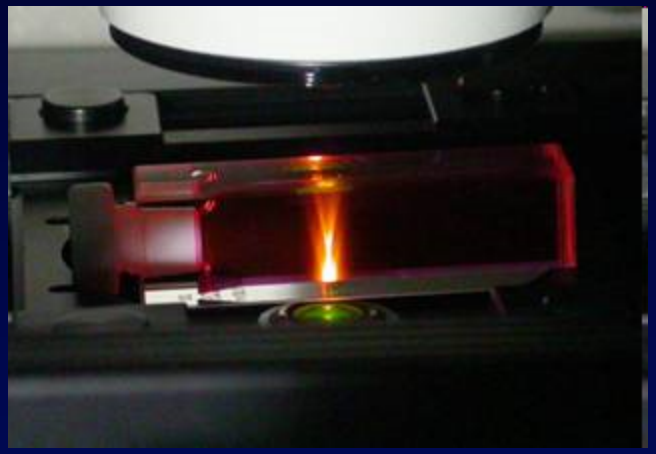
- Brief Introduction to 2-photon imaging
- Discuss an example of 2-photon application: Study of how spontaneous neocortical activity patterns evolve during postnatal development
- Further illustrate the promise of optical imaging methods by discussing a few examples of how they might be used to study disease processes.

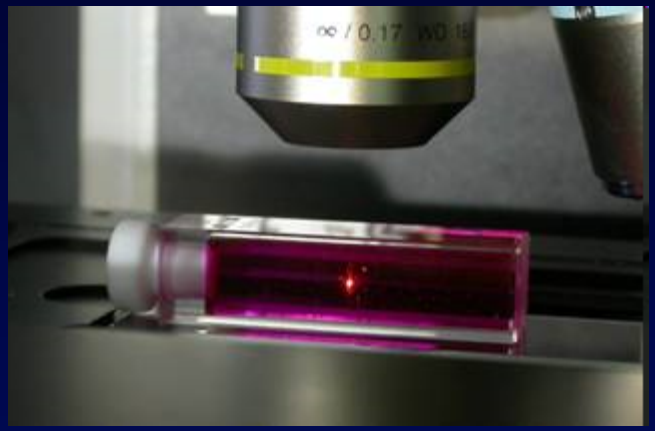
Brief Introduction to 2-photon methods

Two-photon microscopy (TPM)

- Two low energy photons need to be absorbed to excite a fluorophore to emit a photon .
- As a result the fluorescence profile is proportional to the square of the incident photon flux, resulting in the sharp drop of emitted fluoresence outside the focal point (little out-of-focus blur).
- Long wavelength excitation -> less scattering and absorption, greater penetration depth
- Excitation and emission wavelengths separated by >~200 nm: full recording of emission spectrum



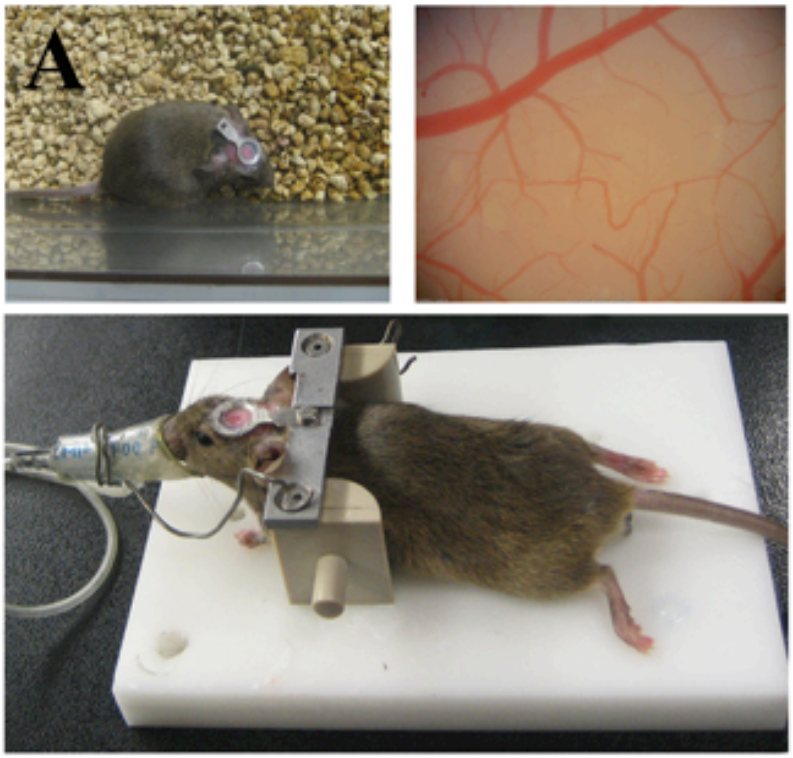


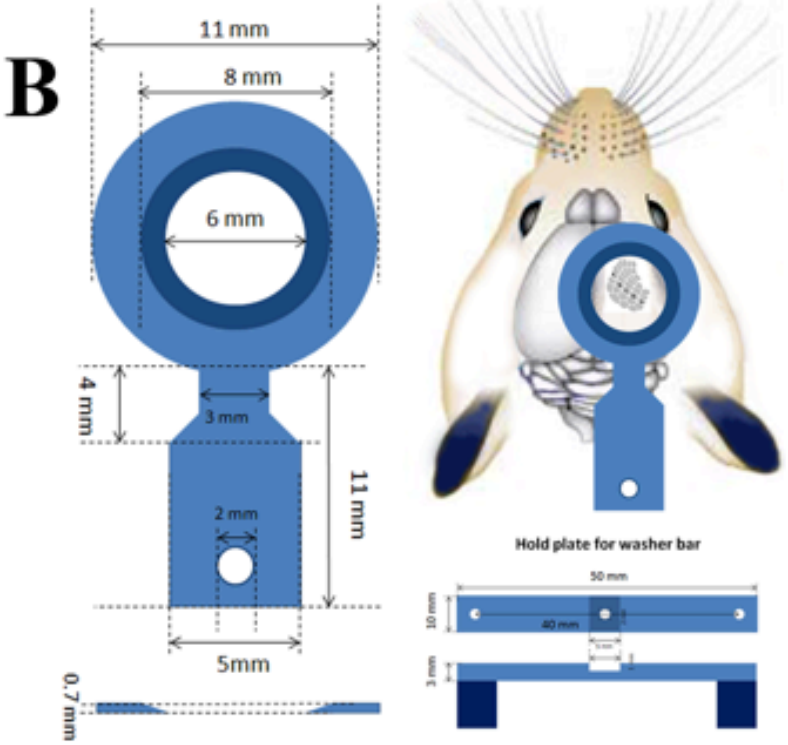


Fluorescence is proportional to the square of intensity

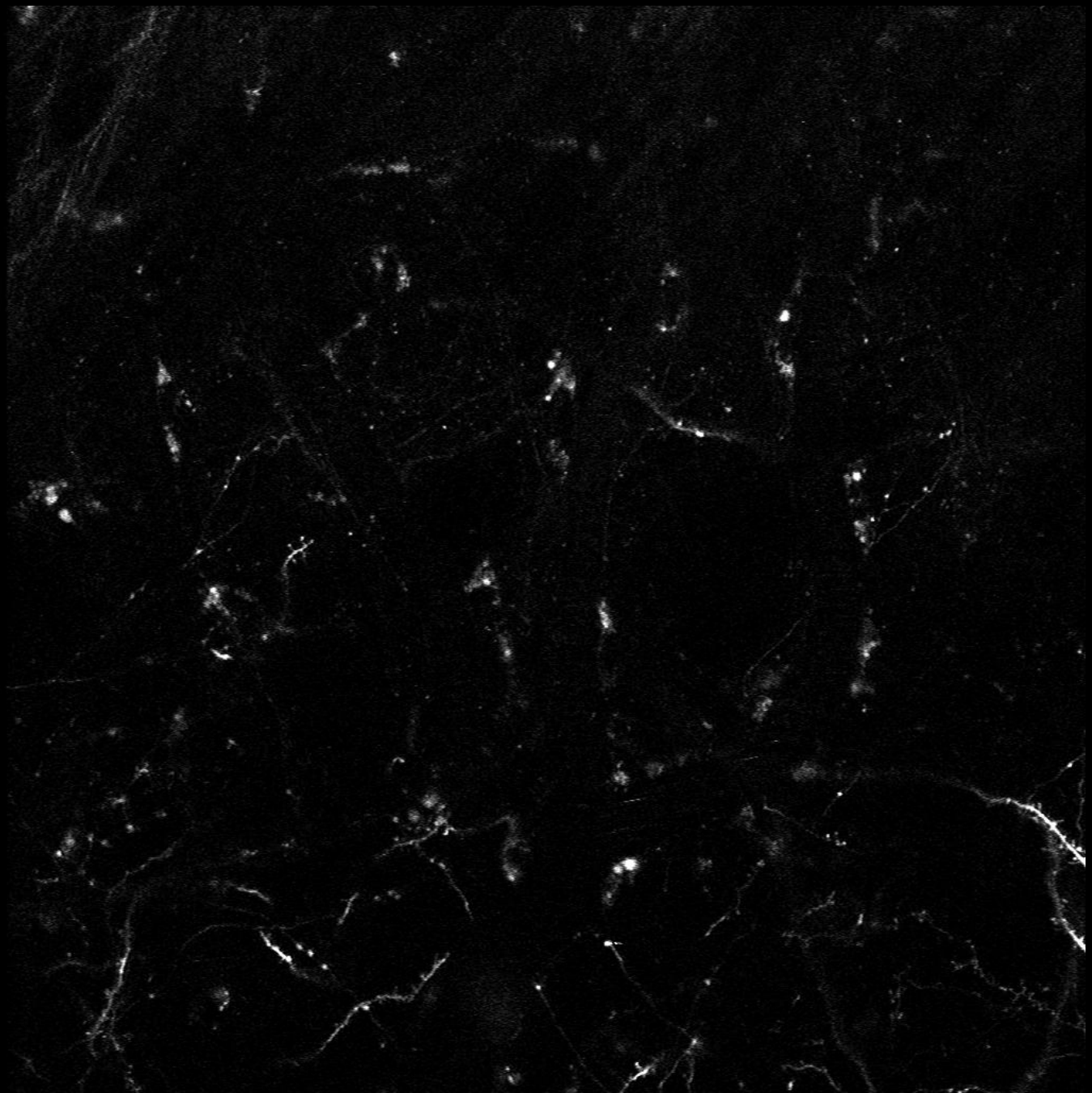
The advent of cleverly engineered molecular dyes allows the use of this method in live tissue (Tsien et al.)

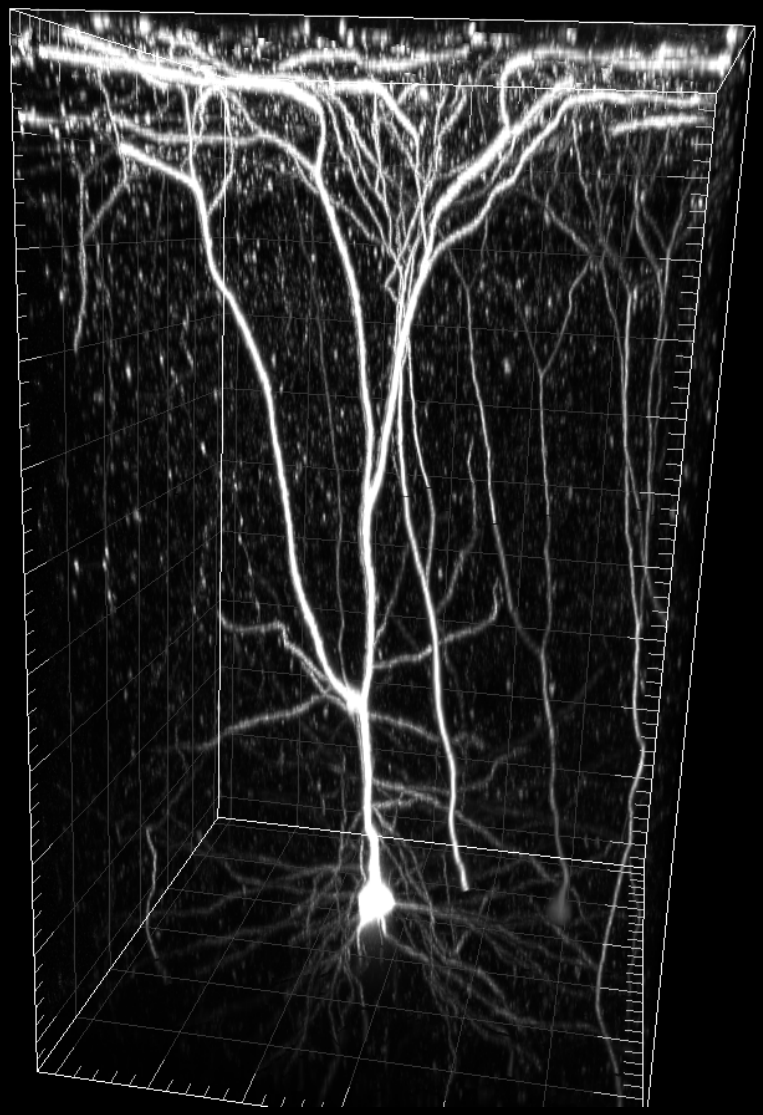
L5 Barrel Cortex Neurons
MecP2-Tg1 X Thy-1 GFP line M





Z stack – Series of images attained at increasing cortical depths (dura to 600 um)




70 um

Dendritic arbor analysis

Apical dendritic tufts
Terminal Branches

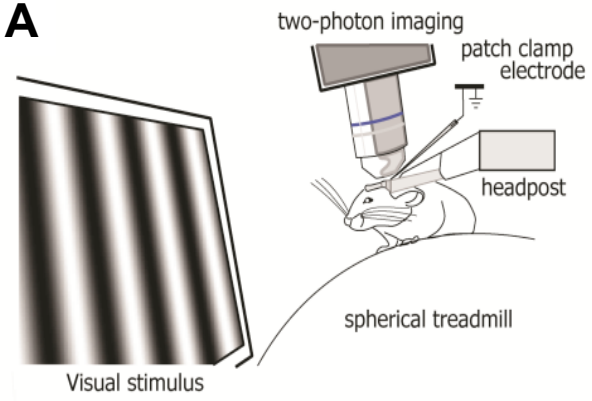
Oblique dendrites

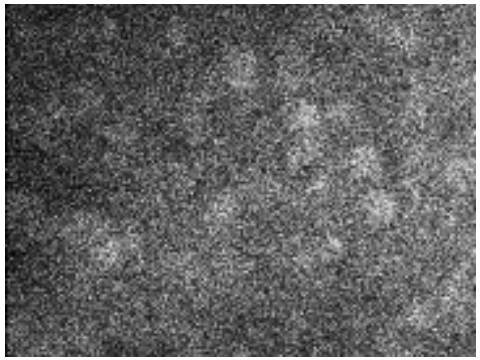
Basal dendrites

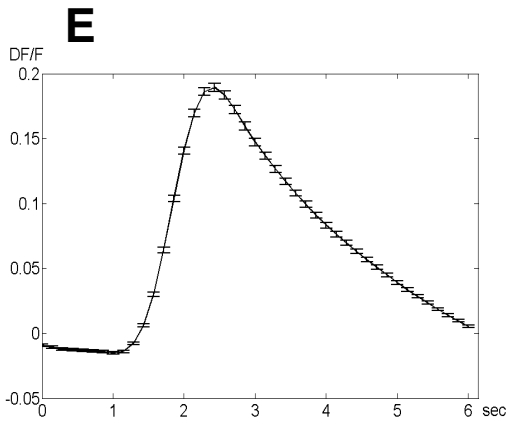
So how do you monitor activity?

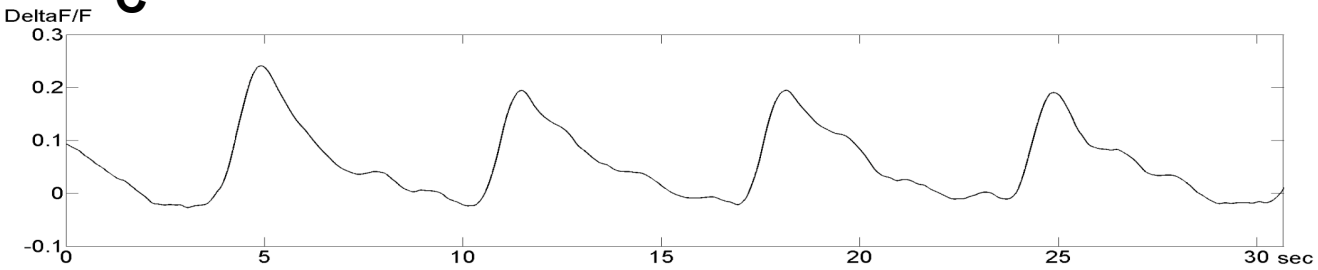
Single Cell Stimulation Mapping of Cortical Connectivity in vivo

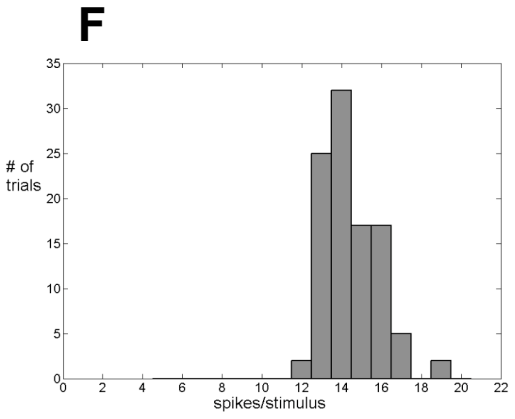
J Meyer, P Golshani, S Smirnakis

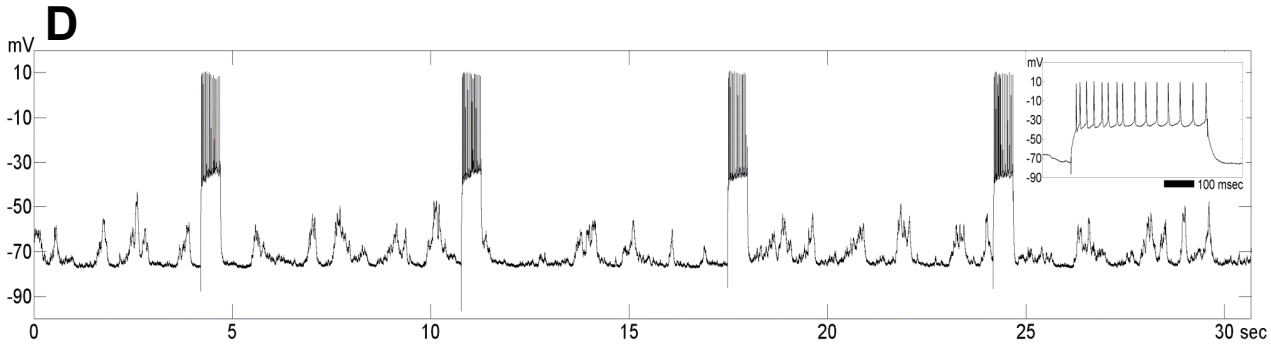


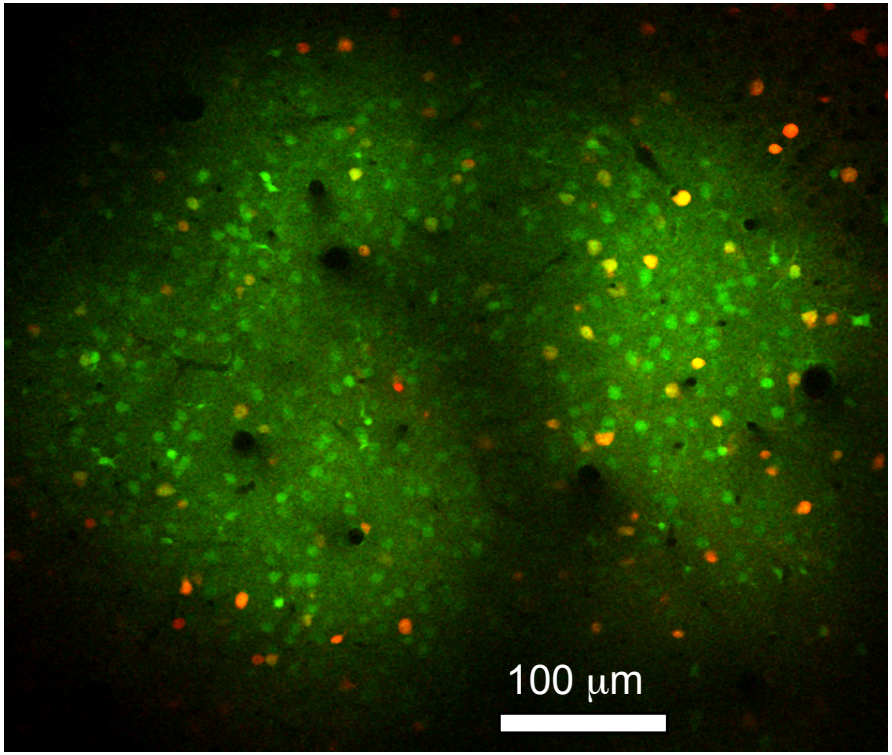


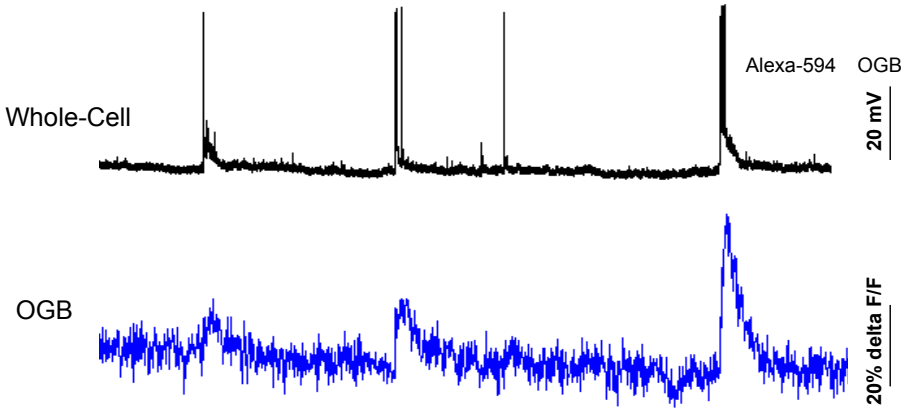






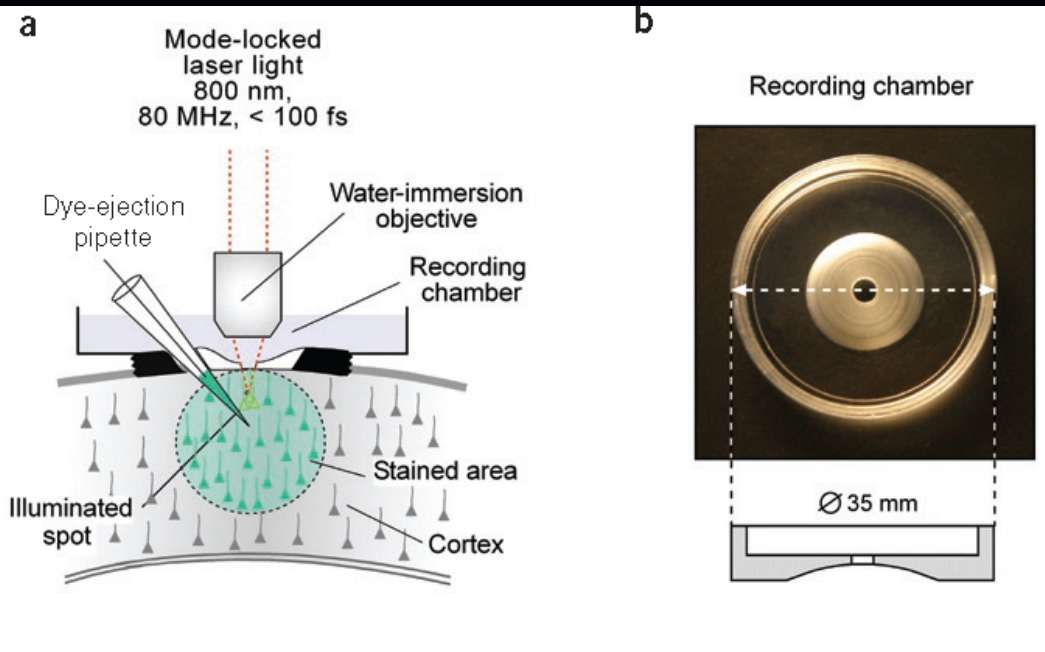






L 2/3 field of view in V1 of a DLX5/6 mouse expressing Td tomato (red) in 60% of GABA-ergic interneurons, after OGB injection (green).

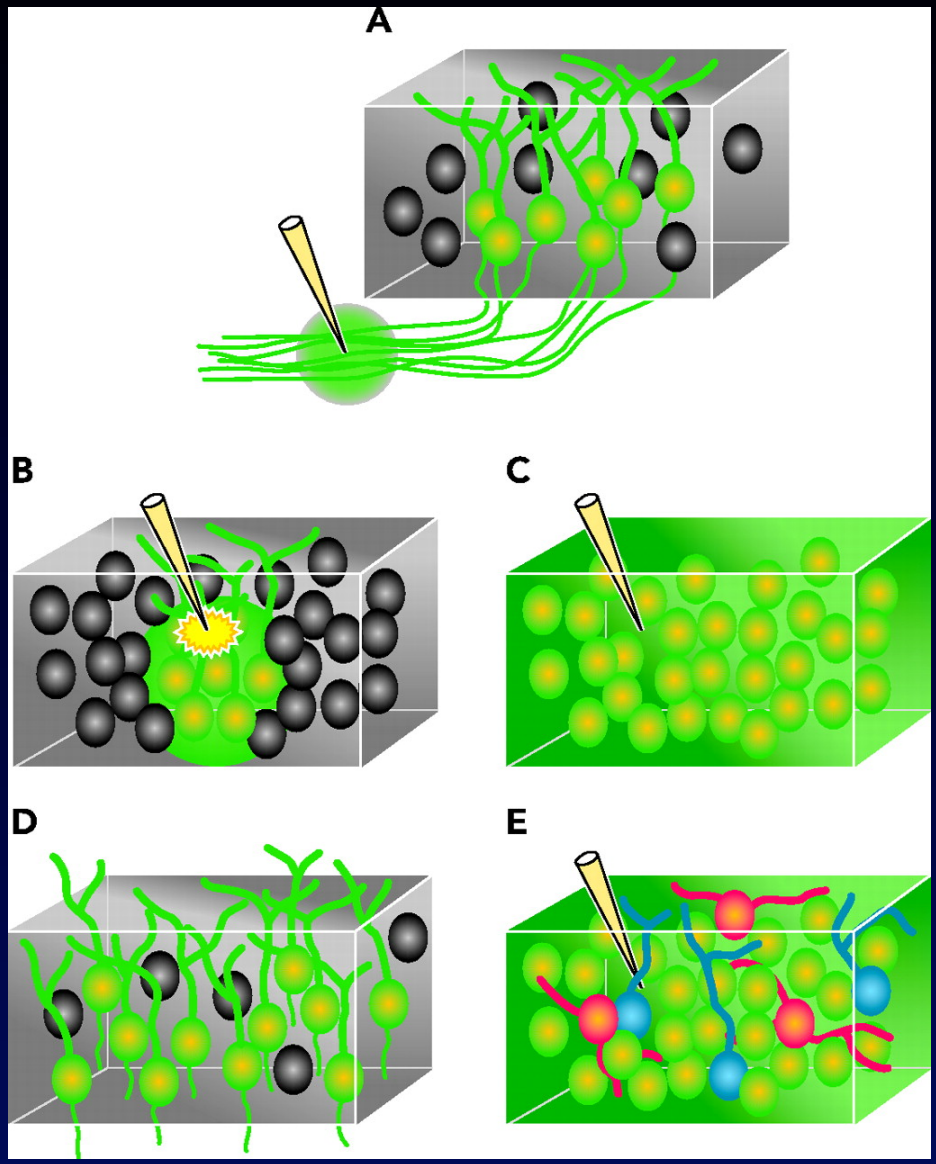
Figure 1 | Experimental arrangement for *in vivo* staining of neuronal populations with Ca^{2+}-indicator dyes. (**a**) A custom-made recording chamber is glued to the skull to allow head fixation and the use of a water-immersion objective. The chamber is perfused with warm (37 °C) standard external saline. Two small craniotomies are performed: one for the insertion of the staining-patch micropipette (obligatory) and one above the field of view for improvement of the imaging depth (optional). Cells, stained using MCBL, are shown in green. The stained area has a diameter of 200–400 μm. Modified from ref. 2. (**b**) Photograph of the recording chamber (upper panel) and schematic drawing of its cross-section (lower panel). Note that a circular region around the perforation is thinned to fit the curvature of the animal's skull.



Garashuk et al., Nature Protocols, 2006

Labeling the neurons

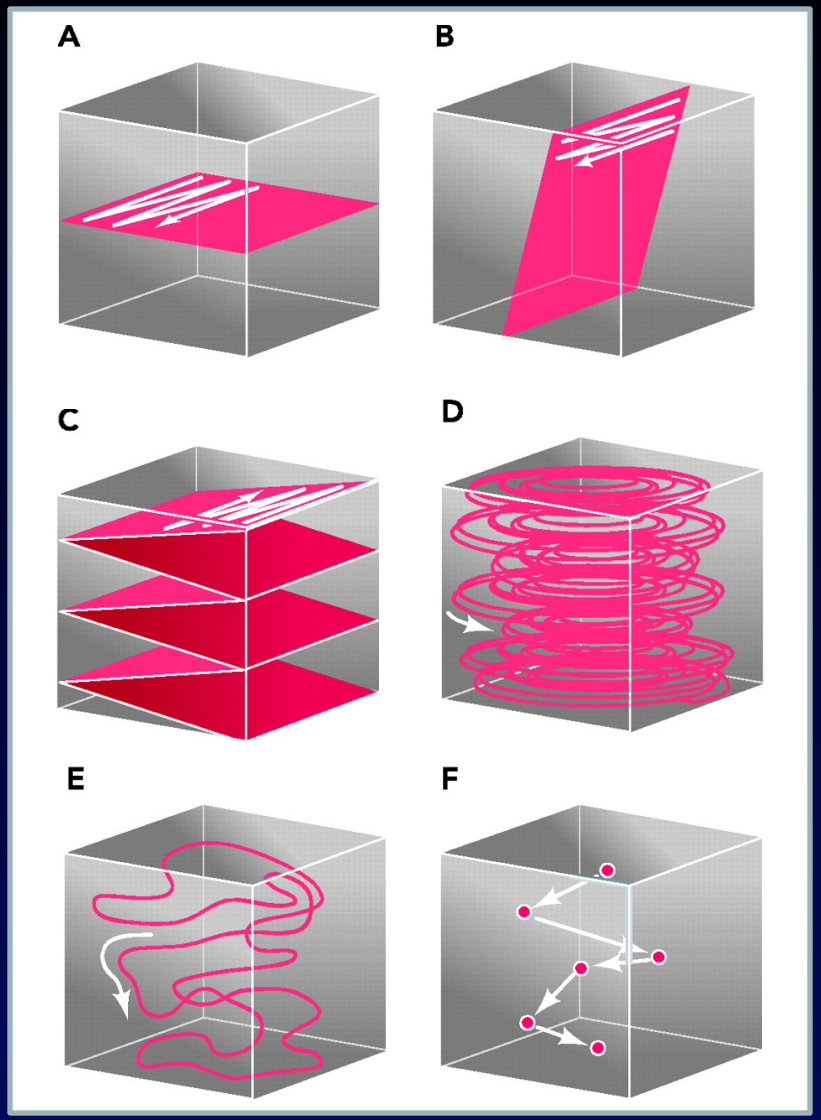
- A: Retrograde Dextran-conjugated dye injection into axons
- B: Electroporation (targeted, local)
- C: AM ester indicators, "trapped" after cleavage by cytosolic esterases, most common method
- D: Genetically encoded indicators (GECIs)
- E: Bulk loaded tissue with counterstained astrocytes or transgenically encoded neuronal subtypes



Göbel, Helmchen, Physiology 22:358-365, 2007

Network scanning approaches

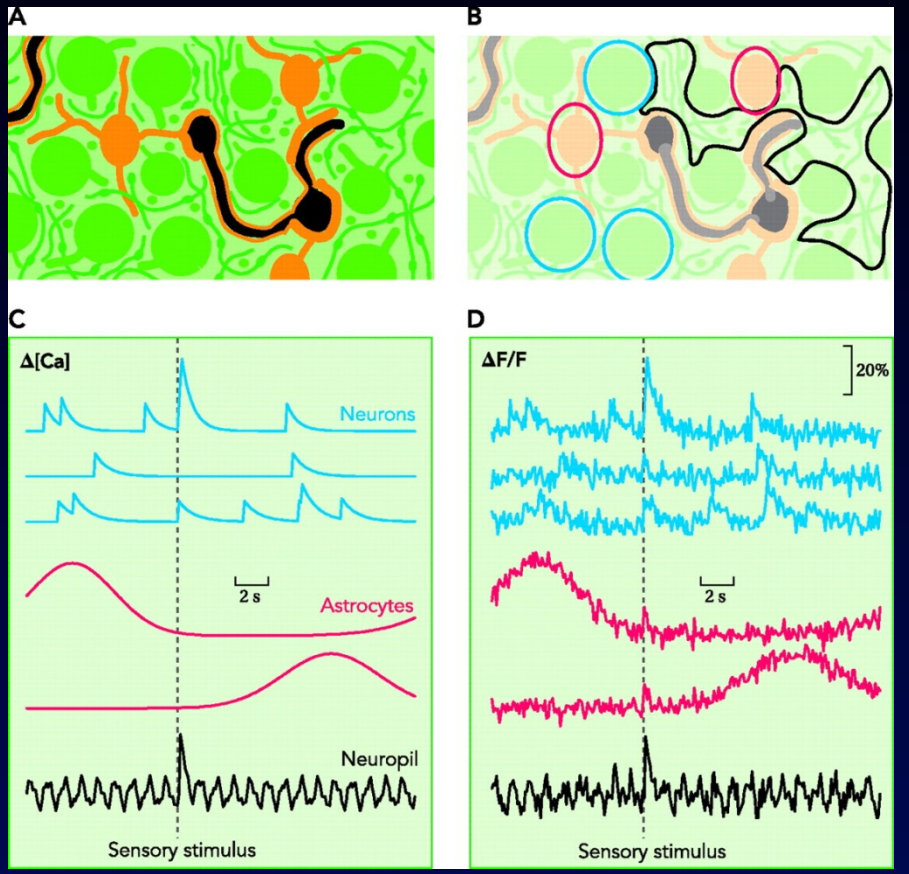
- A: In plane raster mode: slower
- B: Rotation of image plane using piezoelectric focusing
- C: 2D raster while moving objective
- D: 3D line scan on closed trajectory and piezoelectric z-movement (~10 Hz)
- E: 3D scan along points of interest
- F: deflect beam discontinuously (with AODs), theoretically capable of imaging 1,000 cells @ ~100 Hz



Göbel, Helmchen, Physiology 22:358-365, 2007

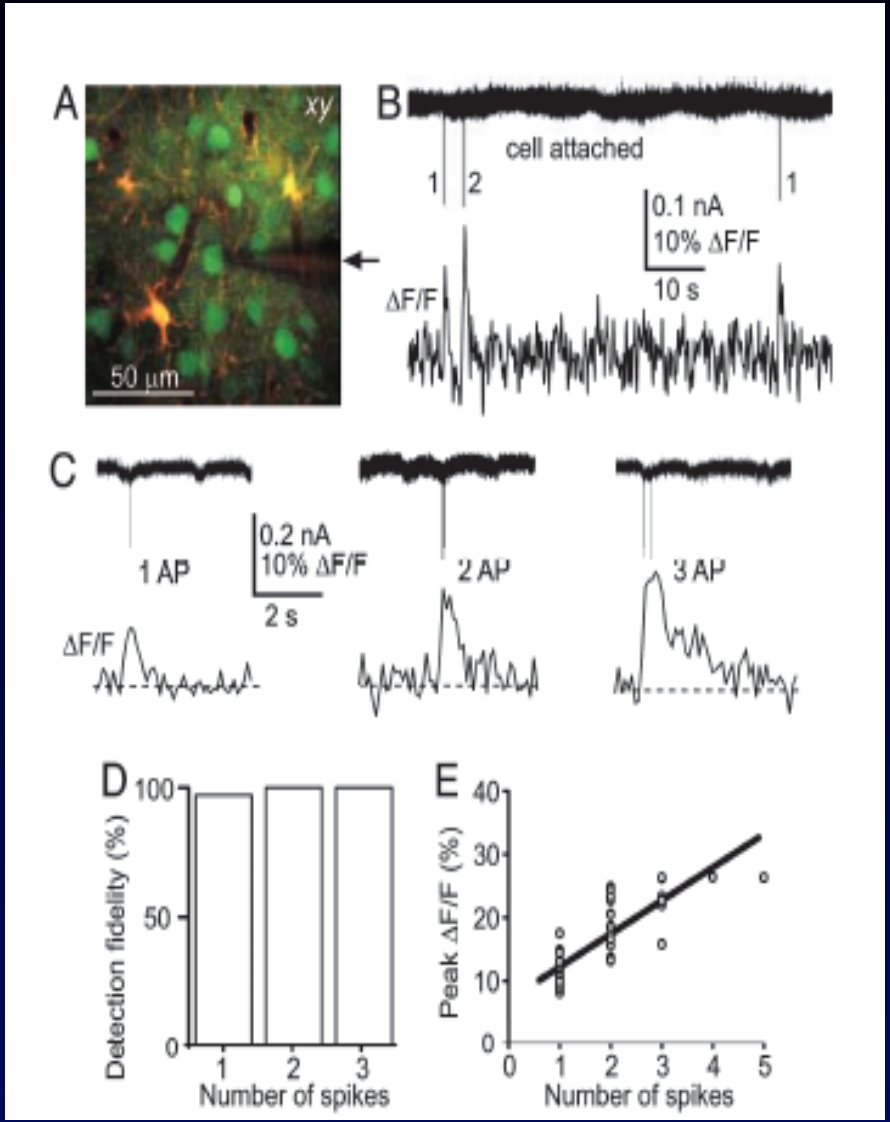
Interpreting calcium signals

- A: Multicell bolus loading – with Calcium indicator dye
- B: Selecting regions of interest (ROIs)
- C: Typical (schematic) Calcium transient in various structures
- D: Fractional fluorescence signals: shot noise, electronics noise, tissue movement



Göbel, Helmchen, Physiology 22:358-365, 2007

Calcium imaging can be used to detect single action potentials in-vivo.



Channel Opsins For Cortical Circuit Control

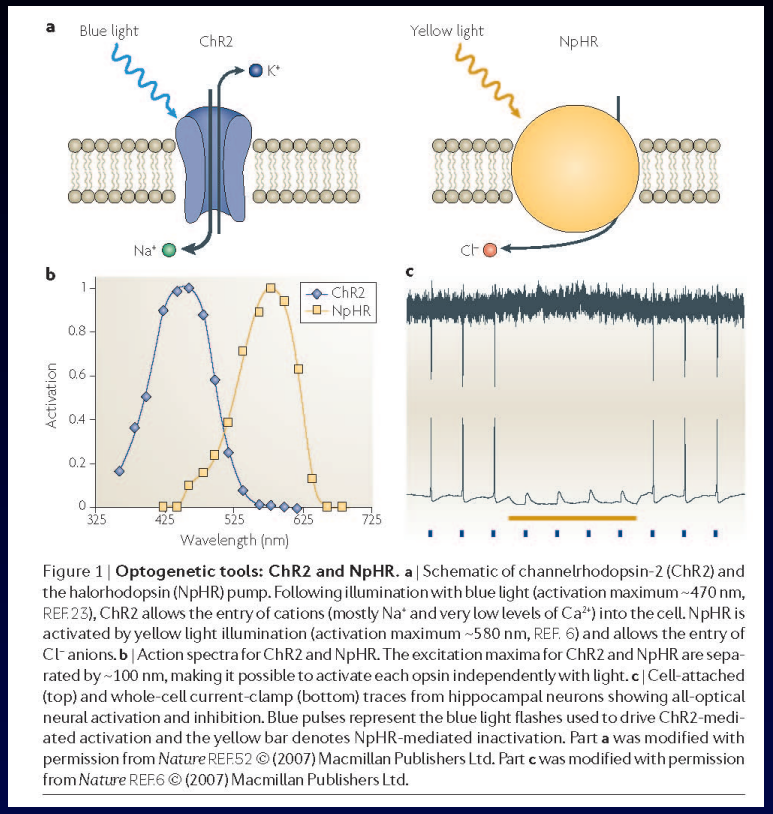
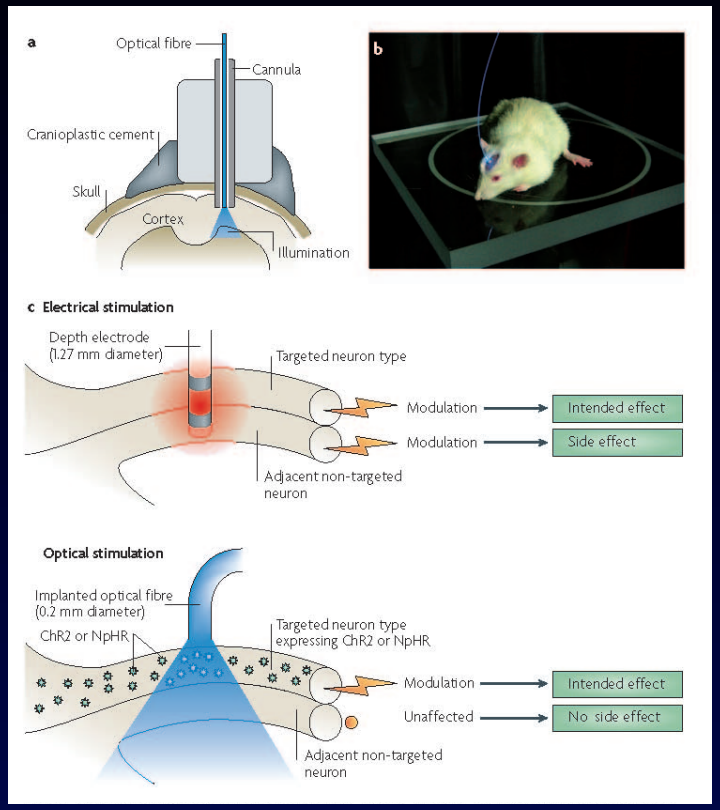


Figure 1 | **Optogenetic tools: ChR2 and NpHR. a** | Schematic of channelrhodopsin-2 (ChR2) and the halorhodopsin (NpHR) pump. Following illumination with blue light (activation maximum ~470 nm, REF 23), ChR2 allows the entry of cations (mostly Na^+ and very low levels of Ca^{2+}) into the cell. NpHR is activated by yellow light illumination (activation maximum ~580 nm, REF. 6) and allows the entry of Cl^- anions. **b** | Action spectra for ChR2 and NpHR. The excitation maxima for ChR2 and NpHR are separated by ~100 nm, making it possible to activate each opsin independently with light. **c** | Cell-attached (top) and whole-cell current-clamp (bottom) traces from hippocampal neurons showing all-optical neural activation and inhibition. Blue pulses represent the blue light flashes used to drive ChR2-mediated activation and the yellow bar denotes NpHR-mediated inactivation. Part **a** was modified with permission from *Nature* REF 52 © (2007) Macmillan Publishers Ltd. Part **c** was modified with permission from *Nature* REF 6 © (2007) Macmillan Publishers Ltd.



Zhang, Aravanis, Adamantidis, de Lecea, Deisseroth. Nature Neuroscience, 2005

Boyden, Zhang, Bamberg, Nagel, Deisseroth. Nature Reviews Neuroscience, 2007

Limitations of in vivo 2-photon imaging:

- Needs exceptional measures to penetrate beyond 500 mm, and really it is not yet possible to image deeper than ~900 mm to 1mm, unless one uses invasive fiber optic probes (optodes) which cause damage and have limited field of view.

- Current calcium imaging dyes have limited ability to temporally follow the pattern of underlying spiking activity; however this will eventually be overcome with a new generation voltage sensitive dyes under development.

Even with these limitations in vivo 2-photon imaging remains a "dream-come-true" for systems electro-physiology

- All supra-granular neurons can be simultaneously monitored
- Chronic imaging; following single unit structure over months opens a window for understanding how function affects structure in processes like cortical learning e.t.c
- Genetic dissection and precise manipulation of cortical circuitry is now possible

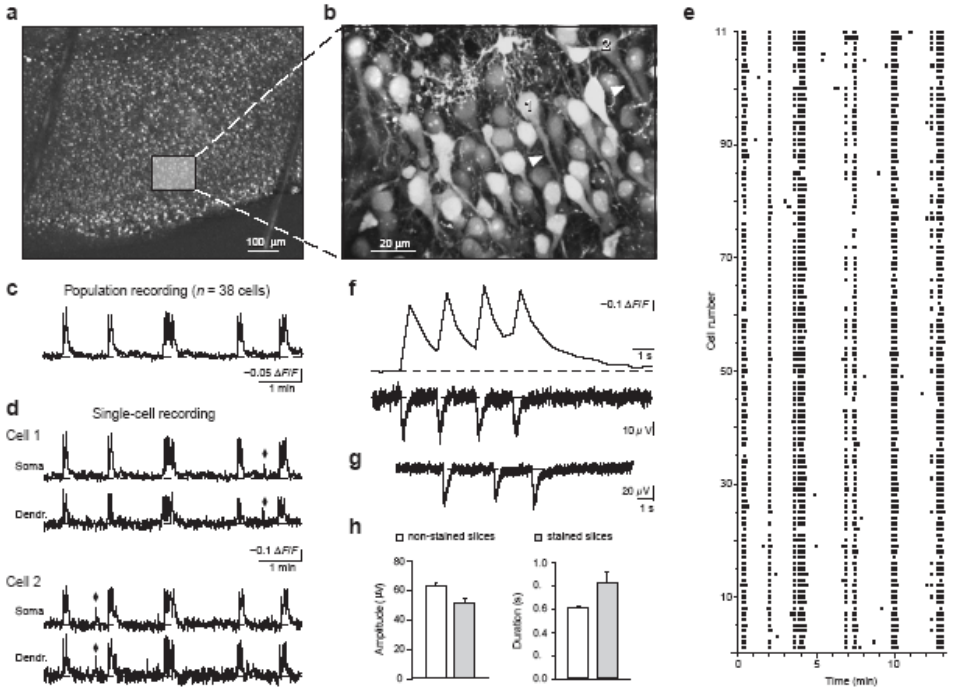
Monitoring neuronal network dynamics during development, in vivo

Background

- A number of studies show synchronized waves of activity propagating through retina, spinal cord, hippocampus, and cerebral cortex during late embryonic and early postnatal life.
- Mature patterns of cortical activity are much more decorrelated
- No study has documented how early patterns of activity evolves into more mature patterns of network activity in the cerebral cortex.
- The mechanisms that govern this important transition are unknown.

Large-scale oscillatory calcium waves in the immature cortex

Olga Garaschuk[1,2], Jennifer Linn[2], Jens Eilers[2,3] and Arthur Konnerth[1,2]



Early motor activity drives spindle bursts in the developing somatosensory cortex

Rustem Khazipov[1,2,3]*, Anton Sirota[2]*, Xavier Leinekugel[1,2,4]*, Gregory L. Holmes[3], Yehezkel Ben-Ari[1] & György Buzsáki[2]

[1]*INMED, INSERM U29, Avenue de Luminy, B.P. 13, 13273 Marseille, France*
[2]*CMBN, Rutgers University, 197 University Avenue, Newark, New Jersey 07102, USA*

P0-P4 rats
Fura 2AM

Network Mechanisms of Spindle-Burst Oscillations in the Neonatal Rat Barrel Cortex In Vivo

Marat Minlebaev, Yehezkel Ben-Ari, and Rustem Khazipov
The Mediterranean Institute of Neurobiology, Institut National de la Santé et de la Recherche Médicale U29, Université Méditerrenéen, Marseille, France

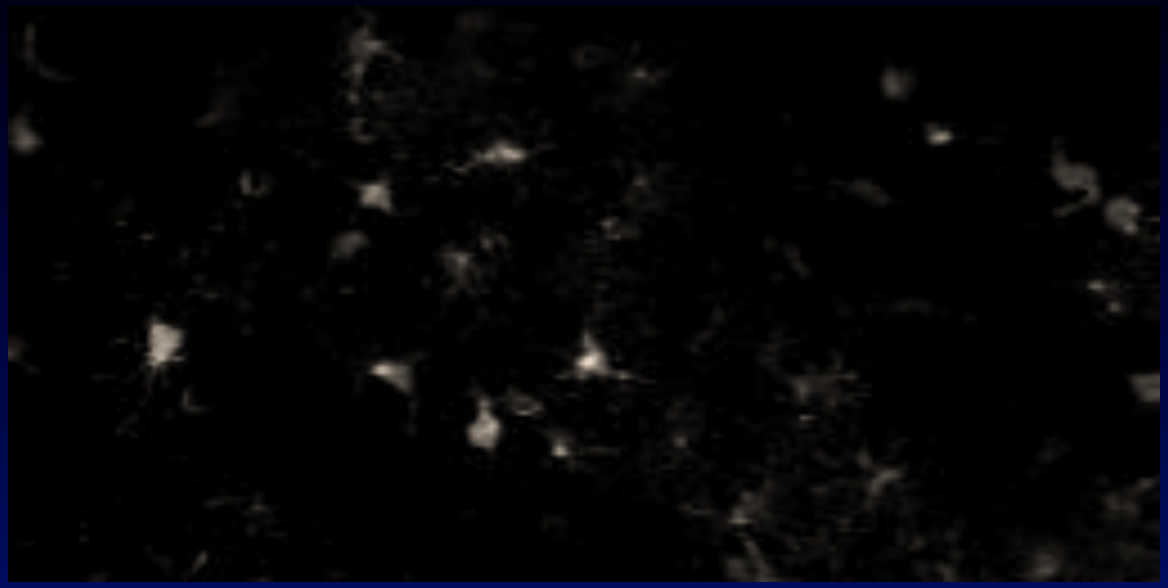
Methods:

- P5 to Adult C57/Bl6 mice have a 1-2 mm craniotomy is placed over the barrel cortex.
- Oregon-Green BAPTA-AM or Fluo-4 (calcium indicators) was injected into the cortex under a glass coverslip.
- Sulforhodamine 101 – the astrocytic marker is also injected.
- 2-photon imaging is performed in mice that are awake or under light isoflurane anesthesia (0.5-0.8 L/min) .
- 10 three minute movies at (2-14 Hz) were made in areas as large as 800X400 microns using a 20 X objective. Line Scans were also performed at 500 Hz for improved temporal resolution.
- Images were analyzed using custom-made programs written in Matlab.
- In-vivo patch clamp recordings of cortical neurons were performed at times synchronously with imaging to validate the calcium measure of neuronal activity.

P10

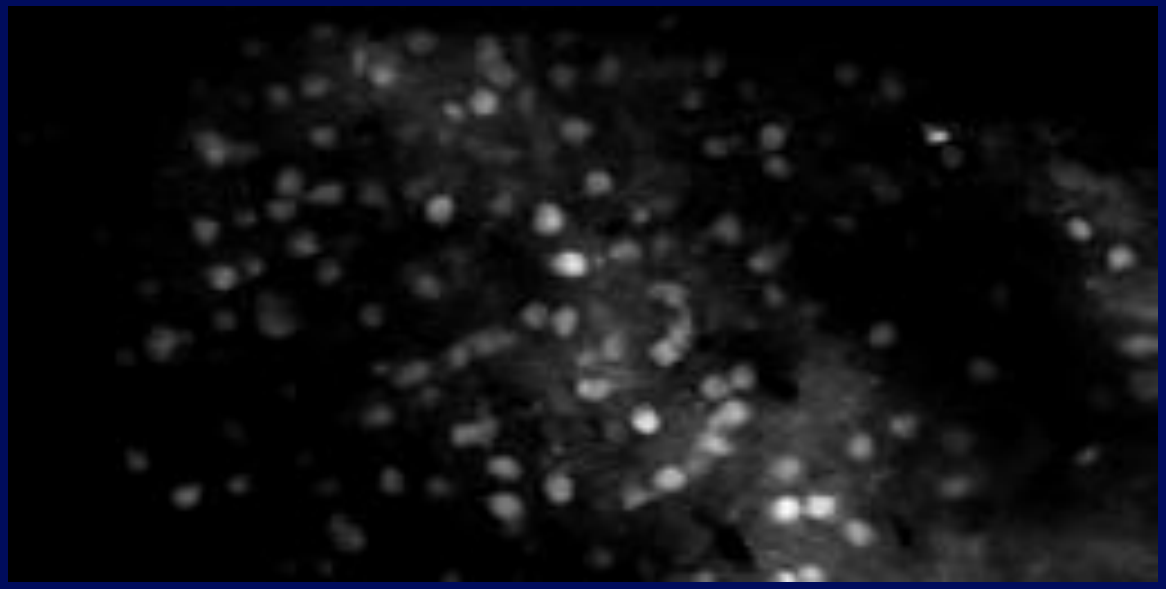
SR-101

(astrocytes)

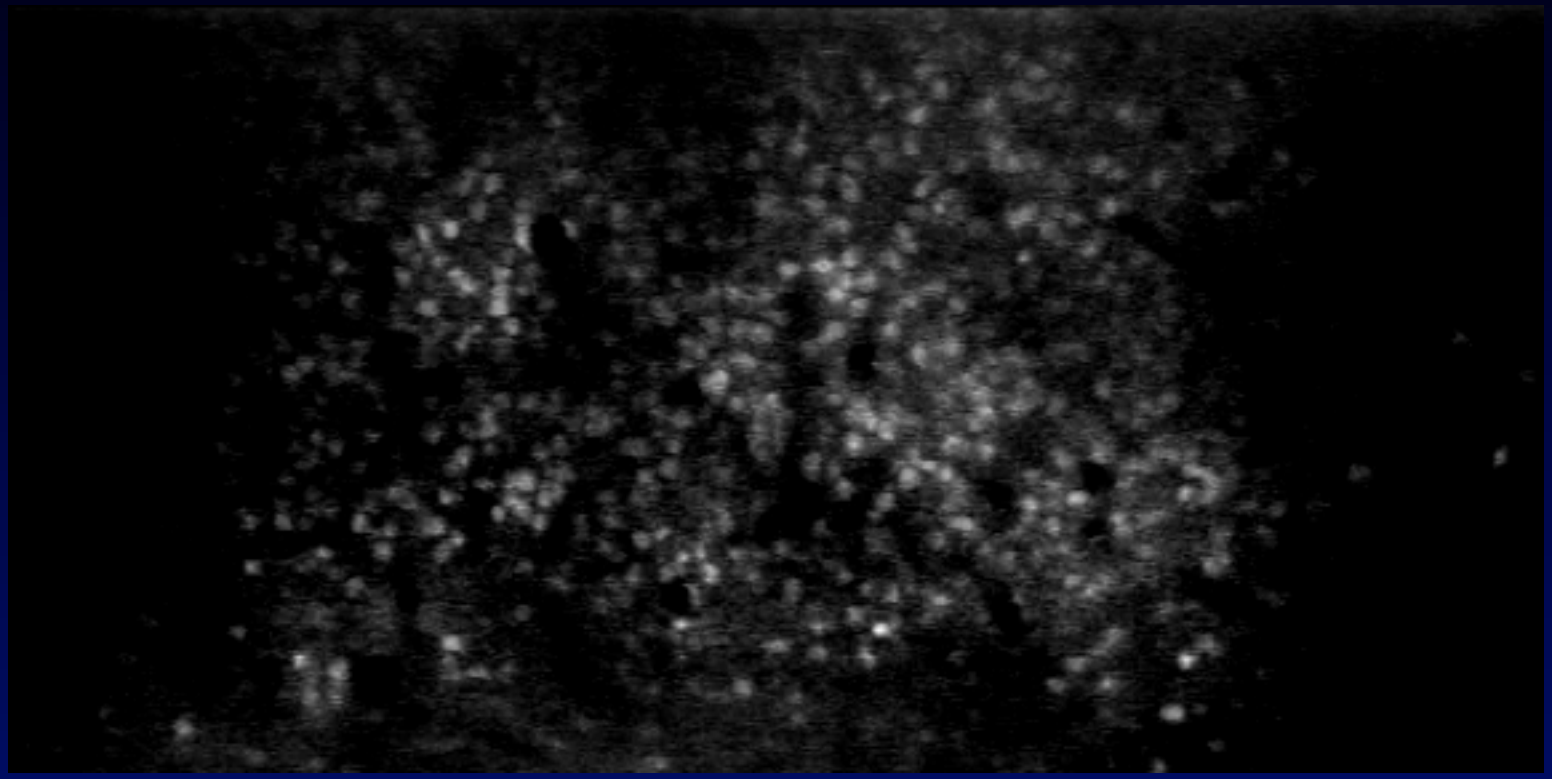


OGB

Neurons
+ astrocytes

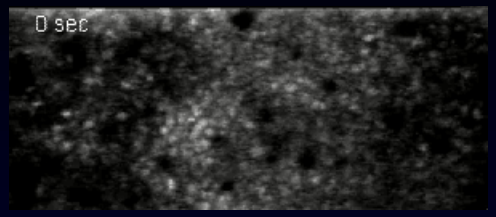


Hundreds of Neurons can be monitored simultaneously, and higher order patterns can be detected

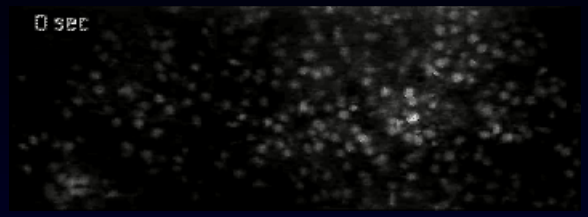


Golshani P, Gonçalves JT, Khoshkhoo S, Mostany R, Smirnakis SM, Portera-Cailliau C Rapid desynchronization of network activity in developing neocortex is internally mediated (J Neurosci, 2009).

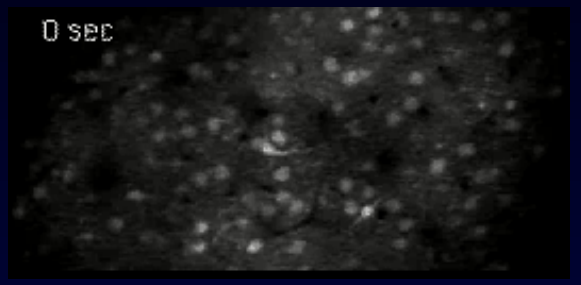
Early (P5)



P9

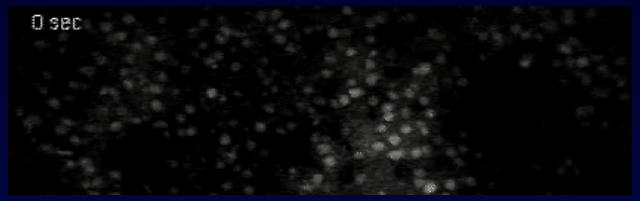


P26



Early (P6)

P13



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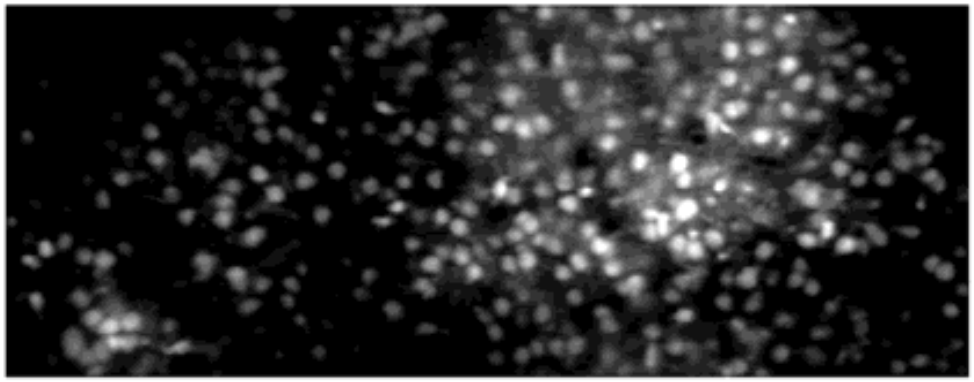

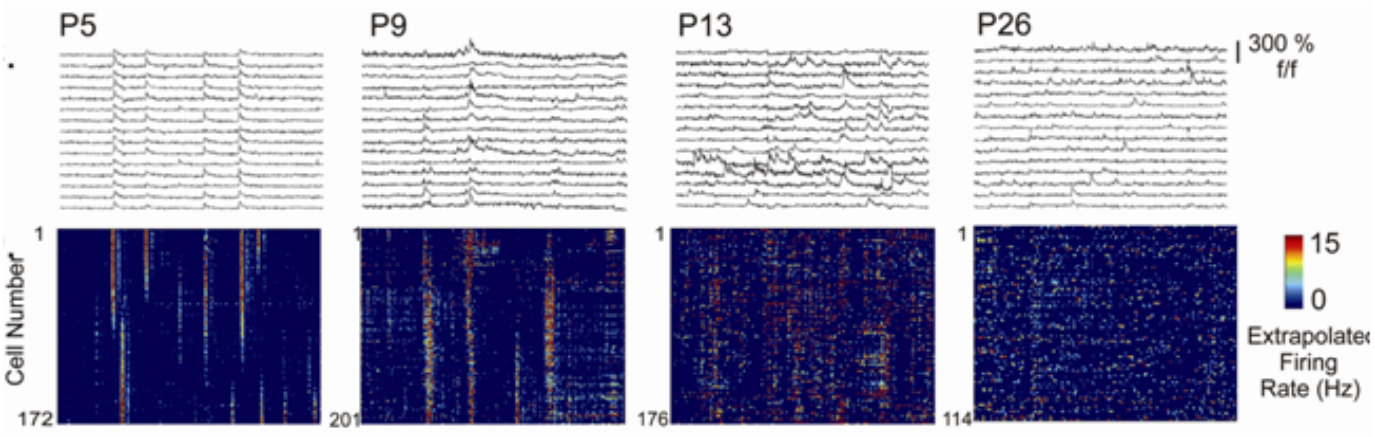

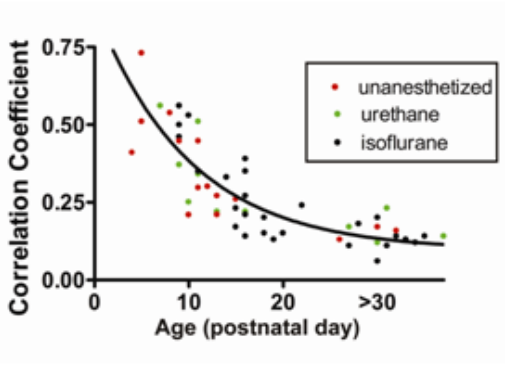

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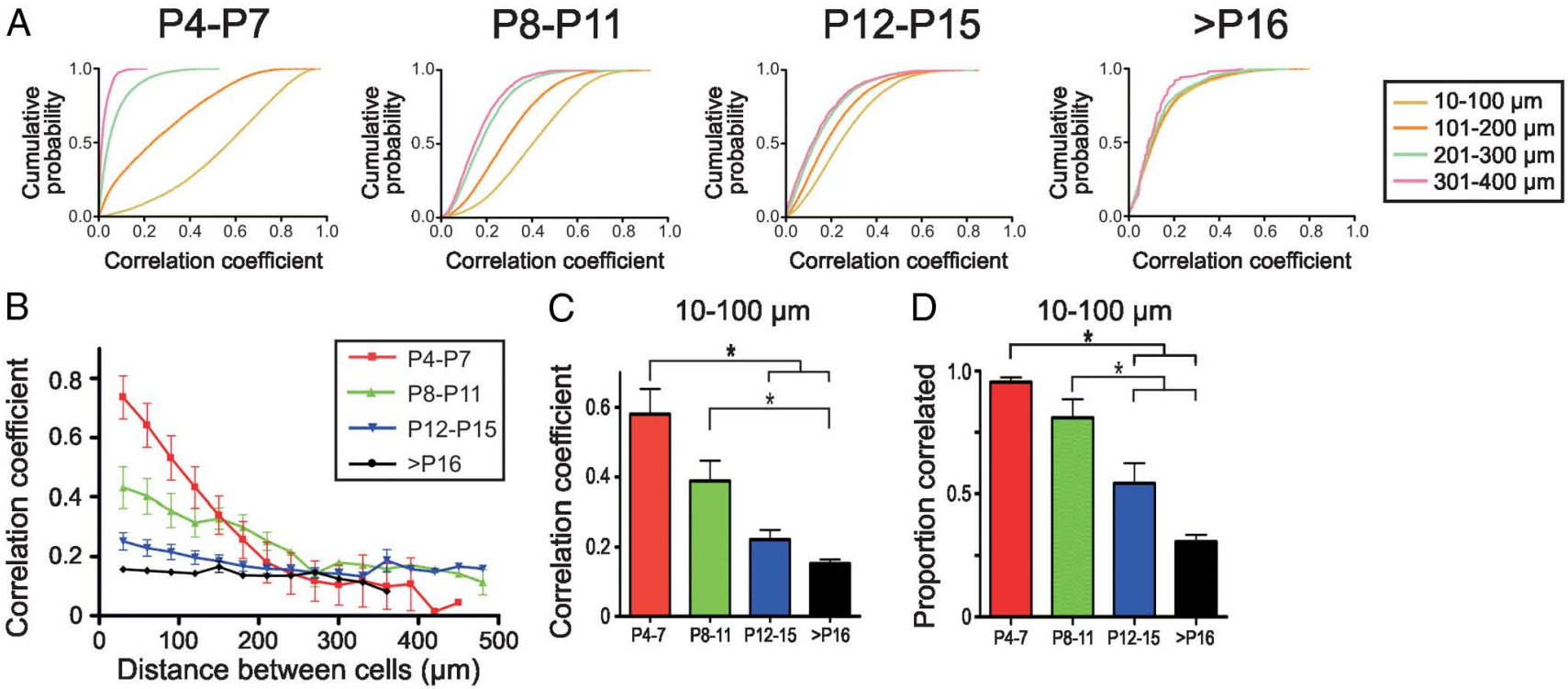
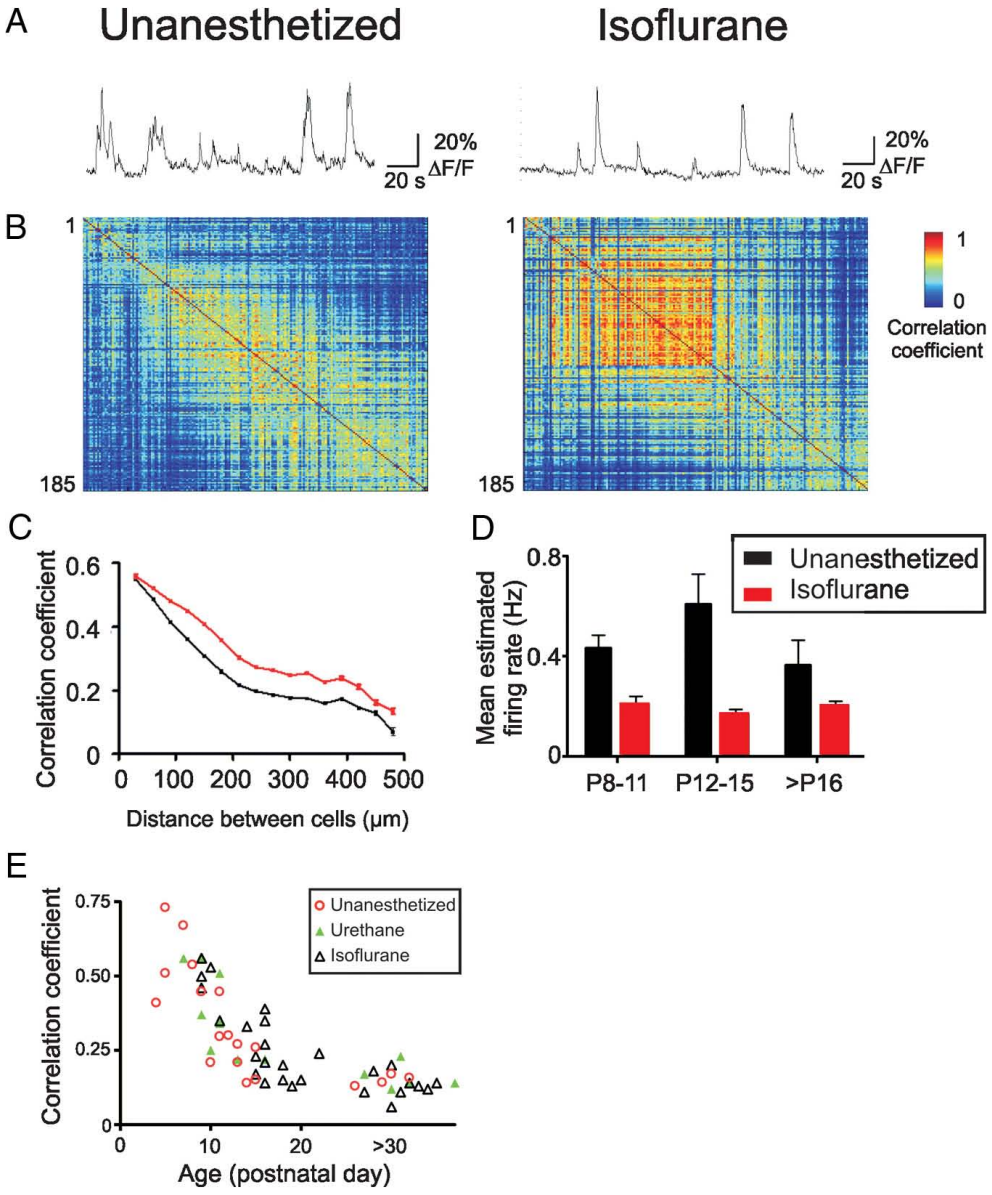

A
P4-P7
P8-P11
P12-P15
>P16
Cumulative probability
Correlation coefficient
10-100 µm
101-200 µm
201-300 µm
301-400 µm
B
Correlation coefficient
Distance between cells (µm)
P4-P7
P8-P11
P12-P15
>P16
C
10-100 µm
Correlation coefficient
P4-7
P8-11
P12-15
>P16
D
10-100 µm
Proportion correlated
P4-7
P8-11
P12-15
>P16


A
Unanesthetized
Isoflurane
20%
ΔF/F
20 s
20%
ΔF/F
20 s
B
1
185
1
185
1
0
Correlation coefficient
C
Correlation coefficient
0.6
0.4
0.2
0
0
100
200
300
400
500
Distance between cells (μm)
D
Mean estimated firing rate (Hz)
0.8
0.4
0
Unanesthetized
Isoflurane
P8-11
P12-15
>P16
E
Correlation coefficient
0.75
0.50
0.25
0
Unanesthetized
Urethane
Isoflurane
0
10
20
>30
Age (postnatal day)

In other words: Neocortical activity transitions from a pattern that promotes developmental circuit connectivity to a pattern that permits efficient information processing.

We believe that this transition is fundamental for the normal operation of neocortical circuits

Many questions remain. For example, what does this transition depend on?

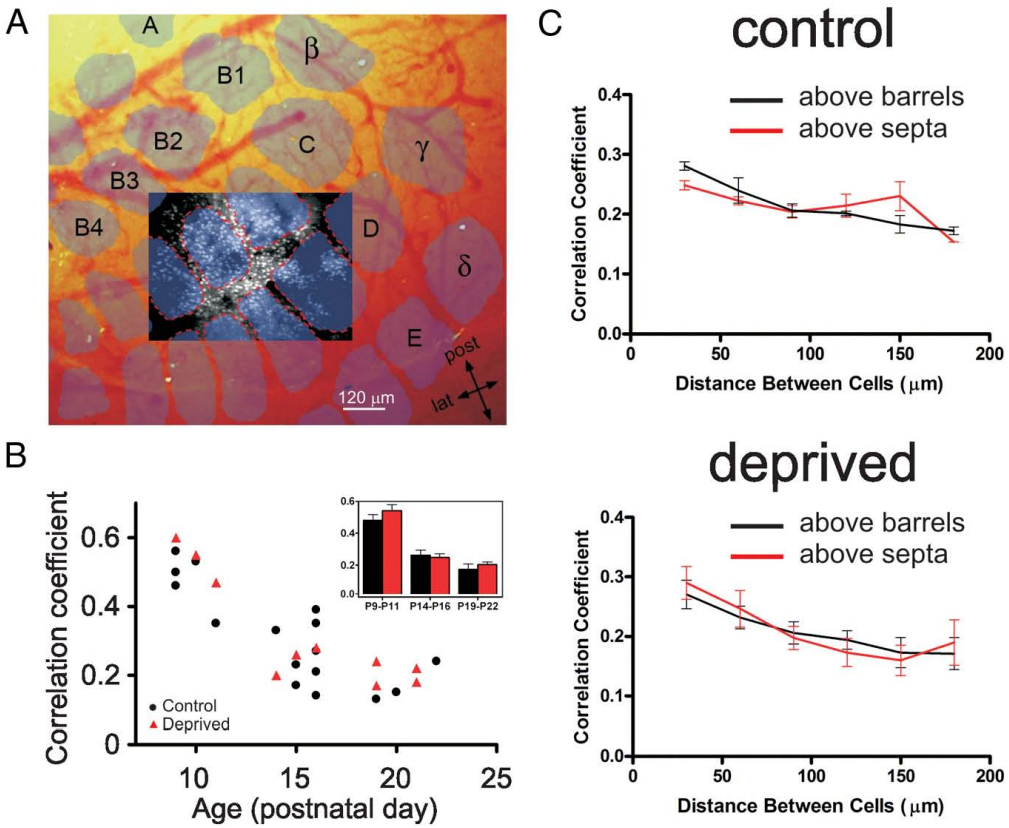
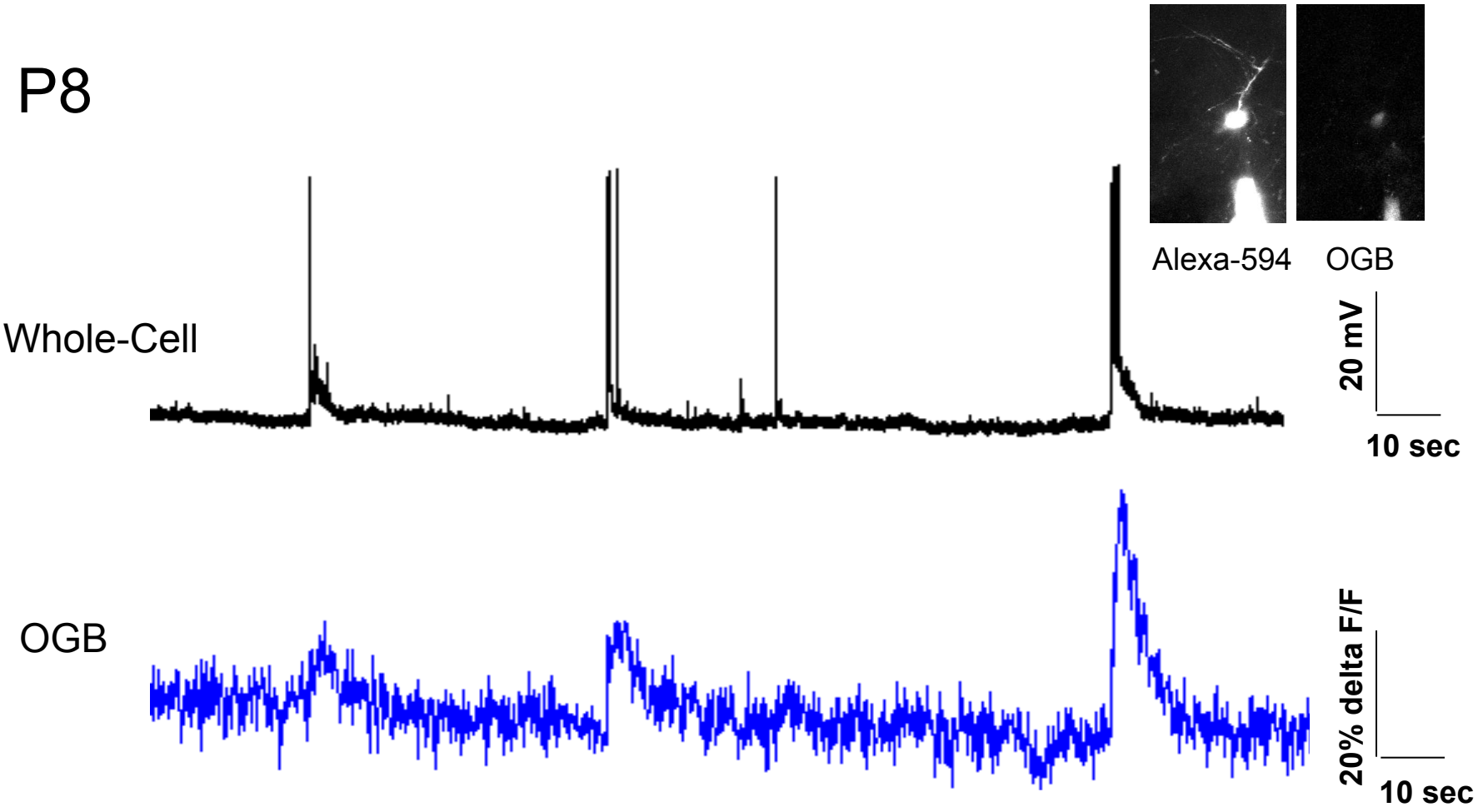
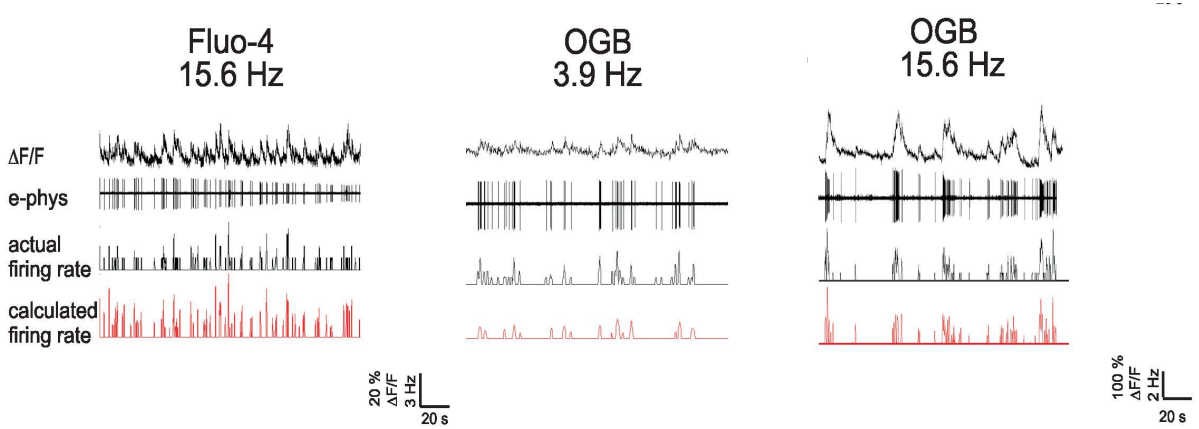


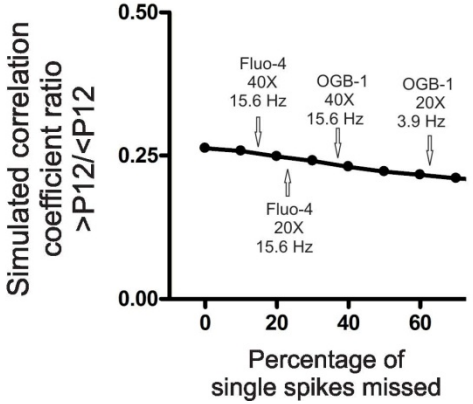
Figure 6. Sensory deprivation does not affect the decorrelation of spontaneous network activity. ***A***, *Post hoc* reconstruction of the L4 barrel cytoarchitecture (from cytochrome oxidase staining) in relationship to the OGB-stained neurons in L2/3 previously imaged with *in vivo* two-photon microscopy. The mouse was deprived of whisker input by plucking all contralateral whiskers from P2 until the day of imaging, in this case P15. ***B***, Scatter plot and bar graph (inset) of mean correlation coefficients of neuronal pairs located within 10–100 μm of each other for control (black) and deprived (red) mice at P9–P11 ($n = 3$), P14–P16 ($n = 3$), and P19–P22 ($n = 4$). Each point represents a different animal. There are no significant differences. ***C***, Mean correlation coefficient versus distance separating pairs of cells located above barrels (black) or above septae (red) at P14–P16, in control (top) or sensory-deprived (bottom) animals. Error bars reflect the SEM.

Calcium transients correlate with action potential discharges through postnatal development





	Correlation coefficient between e-phys and deconvolved trace	Percent of single spikes detected	Percent of spike doublets detected	Percent of bursts of 3 or more action potentials detected
OGB 20X 0.95 NA 3.9 Hz n = 5 cells Figs. 1,2,3,4,6	0.69 ± 0.03	38 ± 13%	80 ± 12%	98 ± 2 %
OGB 40X 0.8 NA 15.6 Hz n = 4 cells	0.83 ± 0.30	63 ± 13%	98 ± 1%	100 ± 0 %
Fluo-4 20X 0.95 NA 15.6 Hz n = 3 cells Fig. S1	0.85 ± 0.05	76 ± 20 %	97 ± 3%	100 ± 0 %
Fluo-4 40X 0.8 NA 15.6 Hz n = 2 cells	0.90 ± 0.02	87 ± 14%	100 ± 0 %	100 ± 0 %



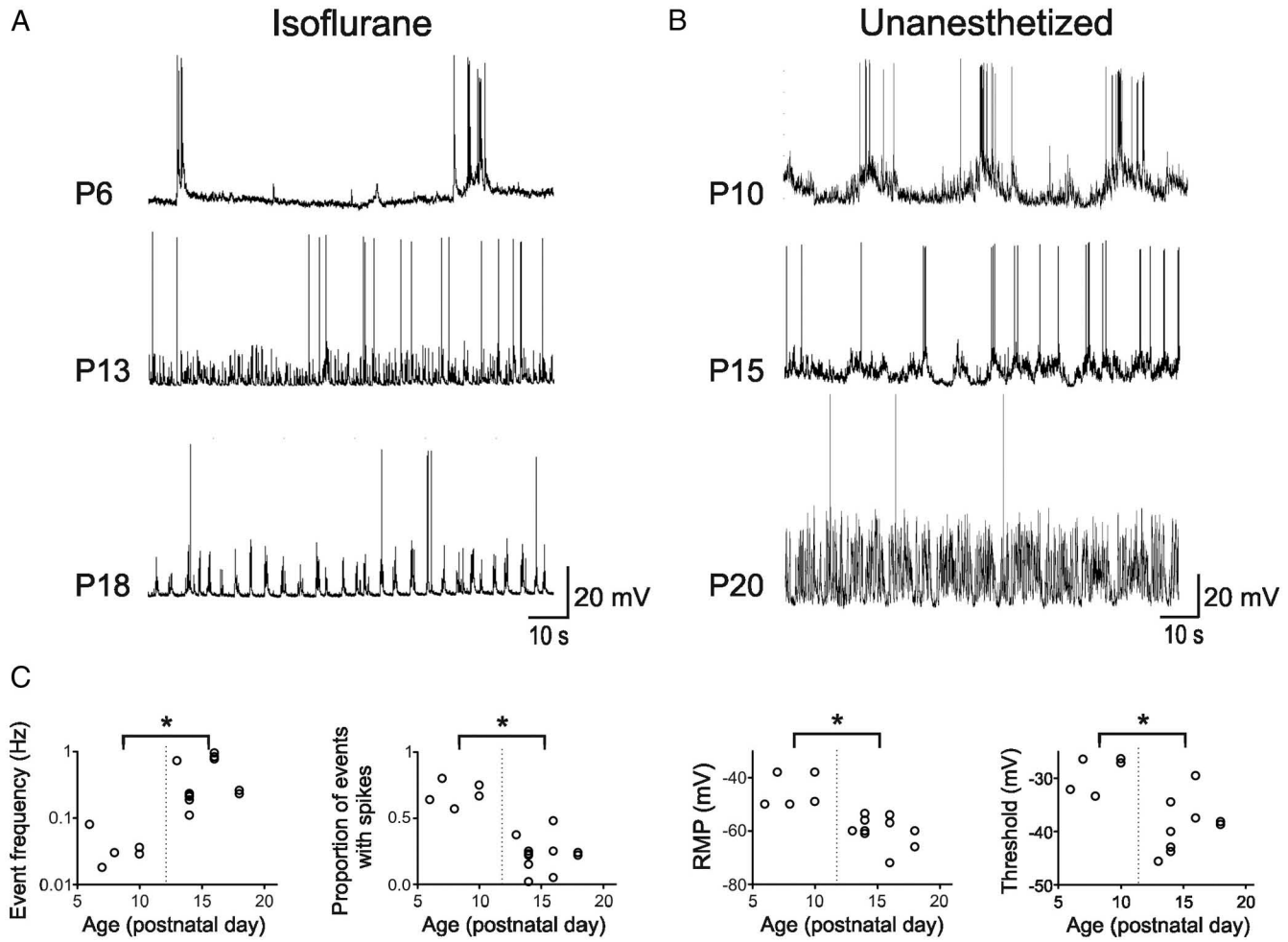


Figure 5. Lower neuronal firing probability per network event after the second postnatal week. ***A***, Representative *in vivo* whole-cell recordings from L2/3 neurons at P6, P13, and P18 under light isoflurane anesthesia (0.5–0.8%). ***B***, Representative *in vivo* whole-cell recordings from L2/3 neurons in unanesthetized mice at P10, P15, and P20. ***C***, Frequency of network events, proportion of network events resulting in the firing of action potentials, resting membrane potential, and action potential threshold across different postnatal ages, in mice under light isoflurane anesthesia. Each point represents data from a single neuron. $^*p < 0.05$.

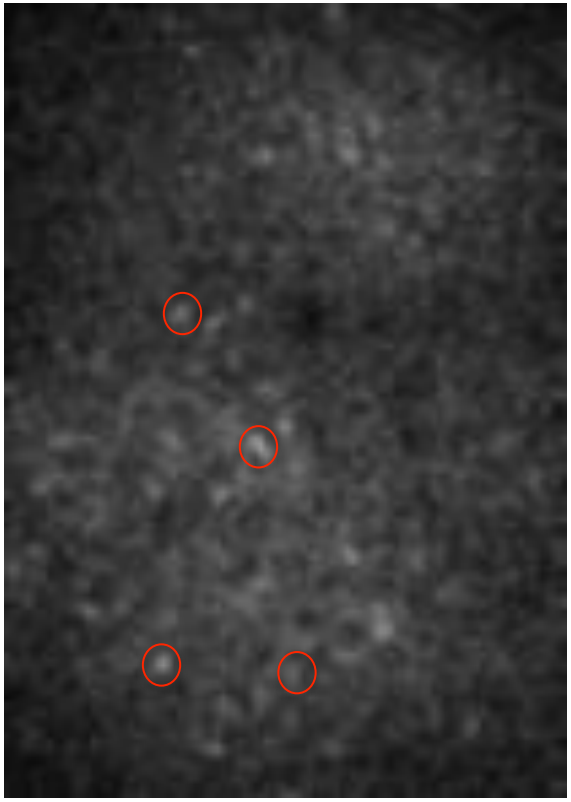
Conclusions

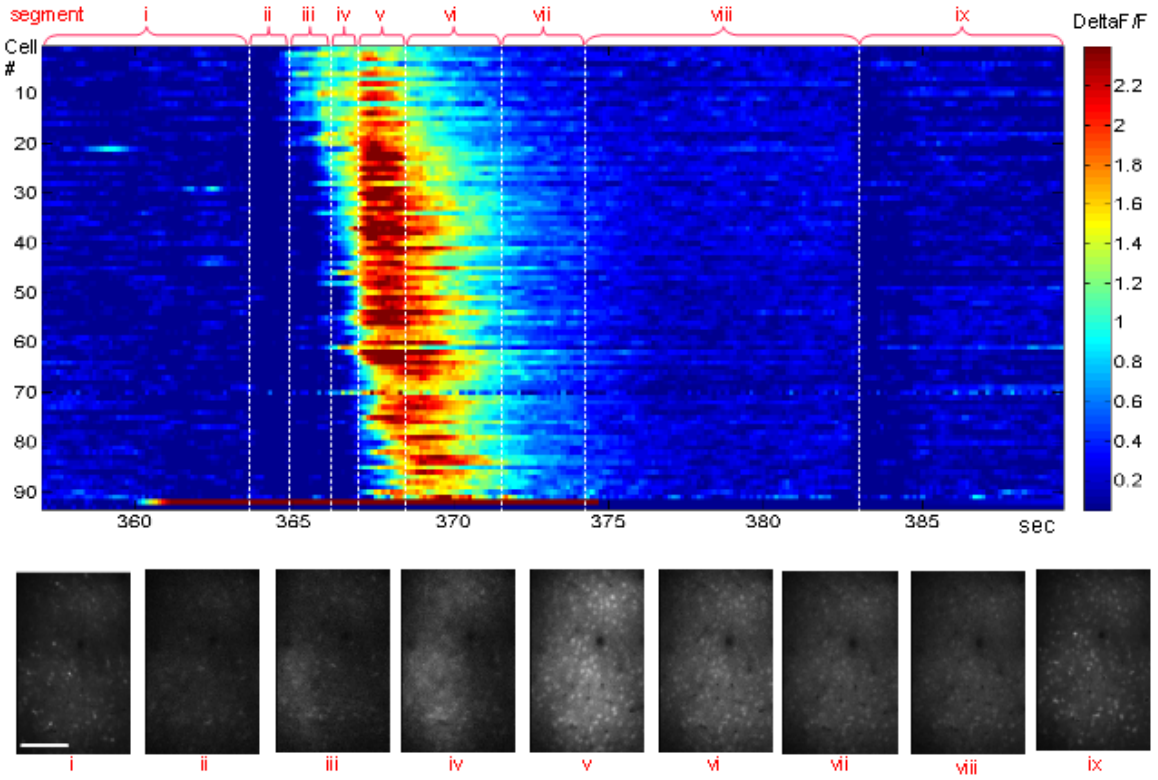
- We find a dramatic transition in the spatiotemporal structure of the internal dynamics of neocortical circuits, which occurs over a few days between P9 and P16.
- Neuronal activity, which is initially highly correlated within small domains of neurons, becomes much less correlated over the range of distances imaged.
- Surprisingly, the decorrelation of neuronal activity is not strongly dependent on sensory experience, and
- may come about via a change in membrane resistance and a shift in the balance between excitation and inhibition.
- Our findings have implications for information coding in neocortex. Subtle perturbations in this fundamental step of cortical maturation may turn out to play a role in some "information processing disorders" s.a. epilepsy, schizophrenia or autism.

Epilepsy Models

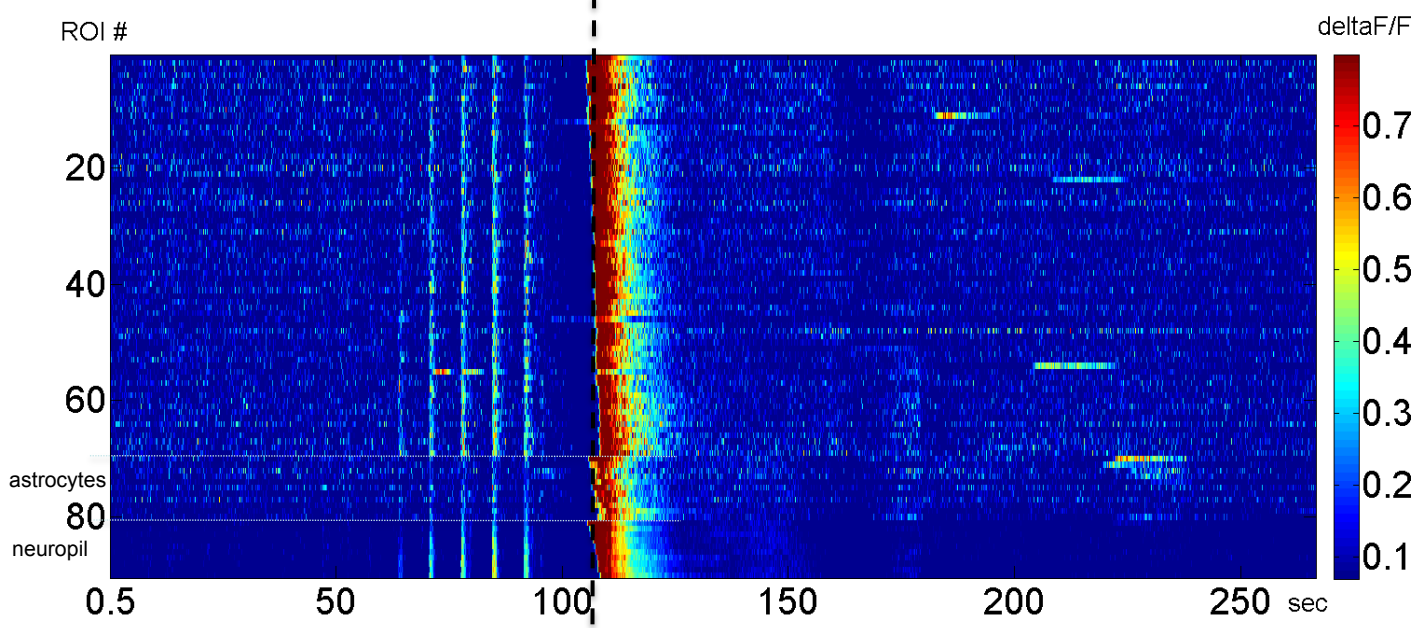
J Meyer, A Maheshwari, S Torsky, J Noebels, S Smirnakis

Stargazer Mouse Spontaneous Event

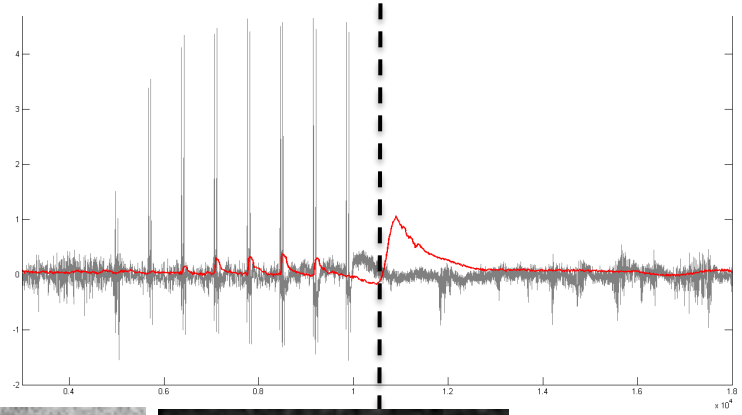




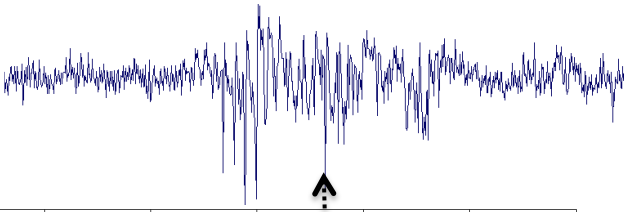
Scalebar in (i): 100 um



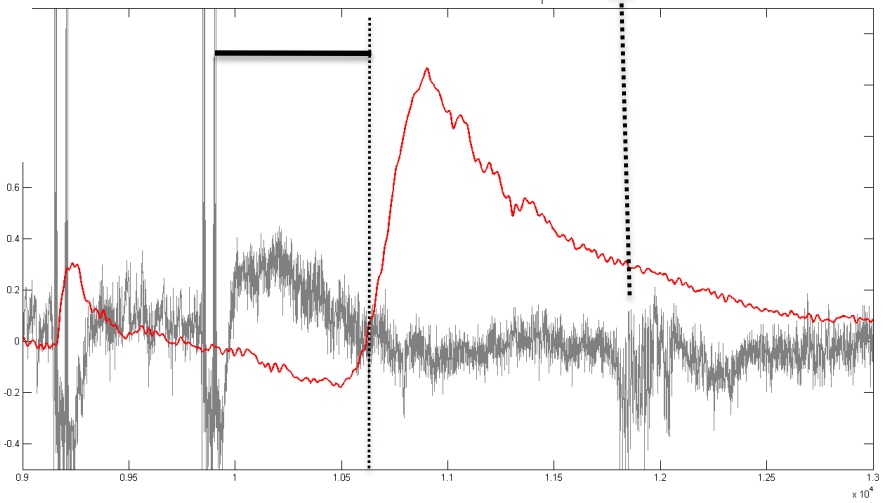
Stargazer Mouse
Evoked Event

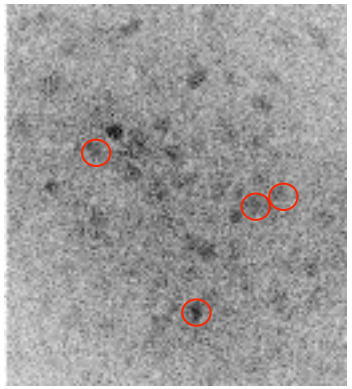


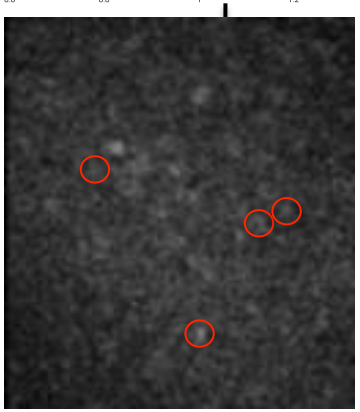
8-10Hz seizure



5 seconds







Monitoring vascular dynamics in vivo

(Microvascular Stroke Model – Kleinfeld D. et al.)

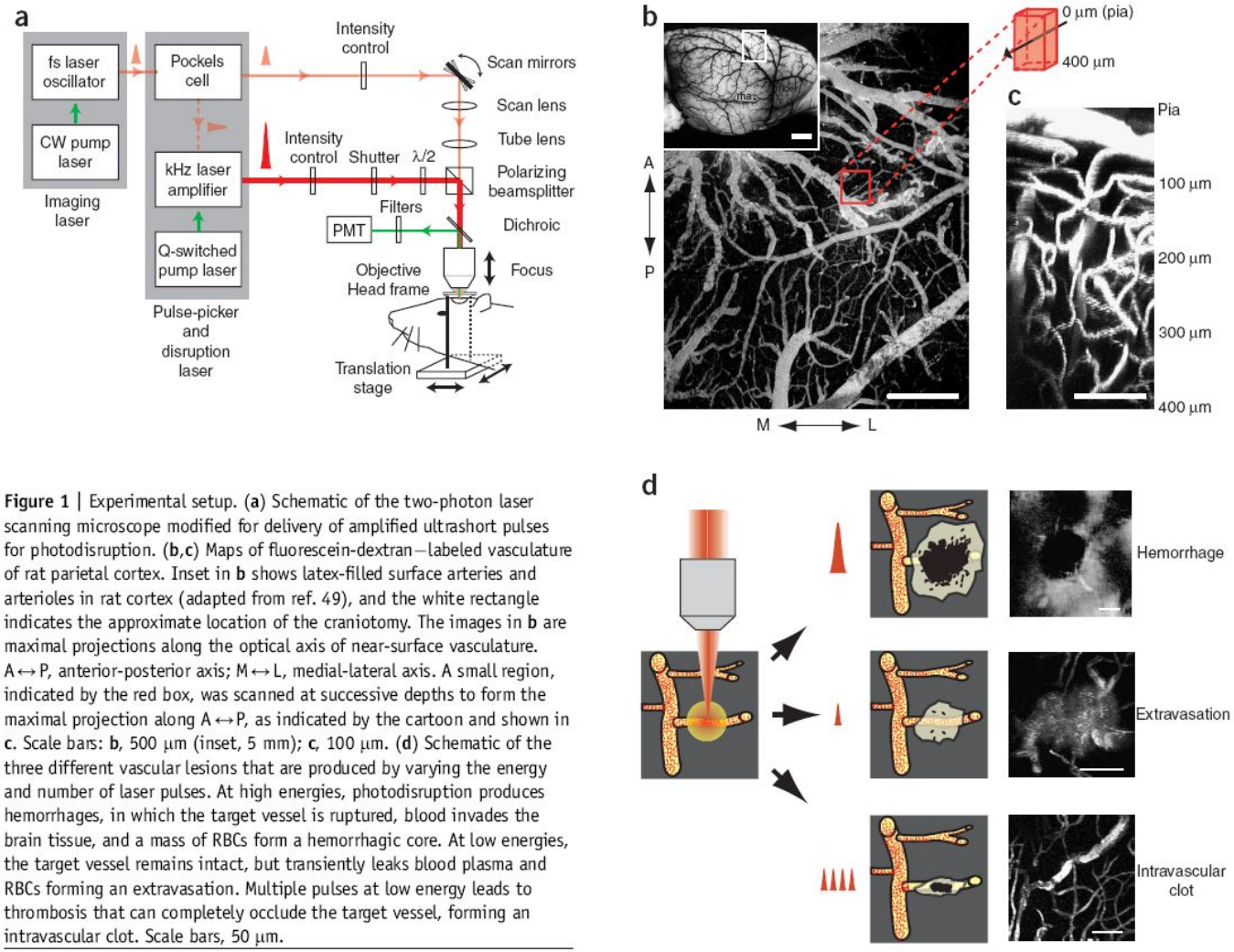
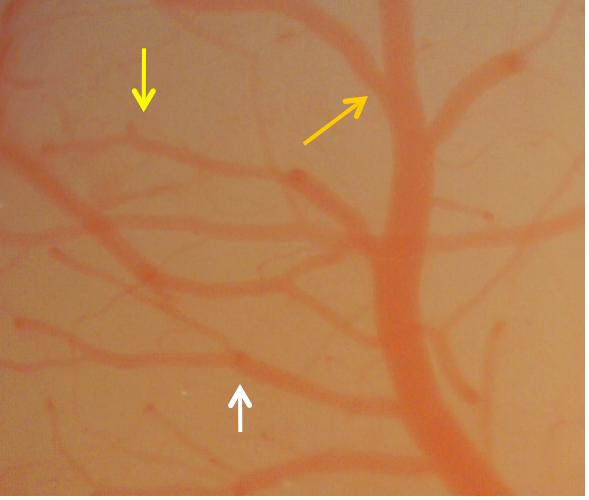


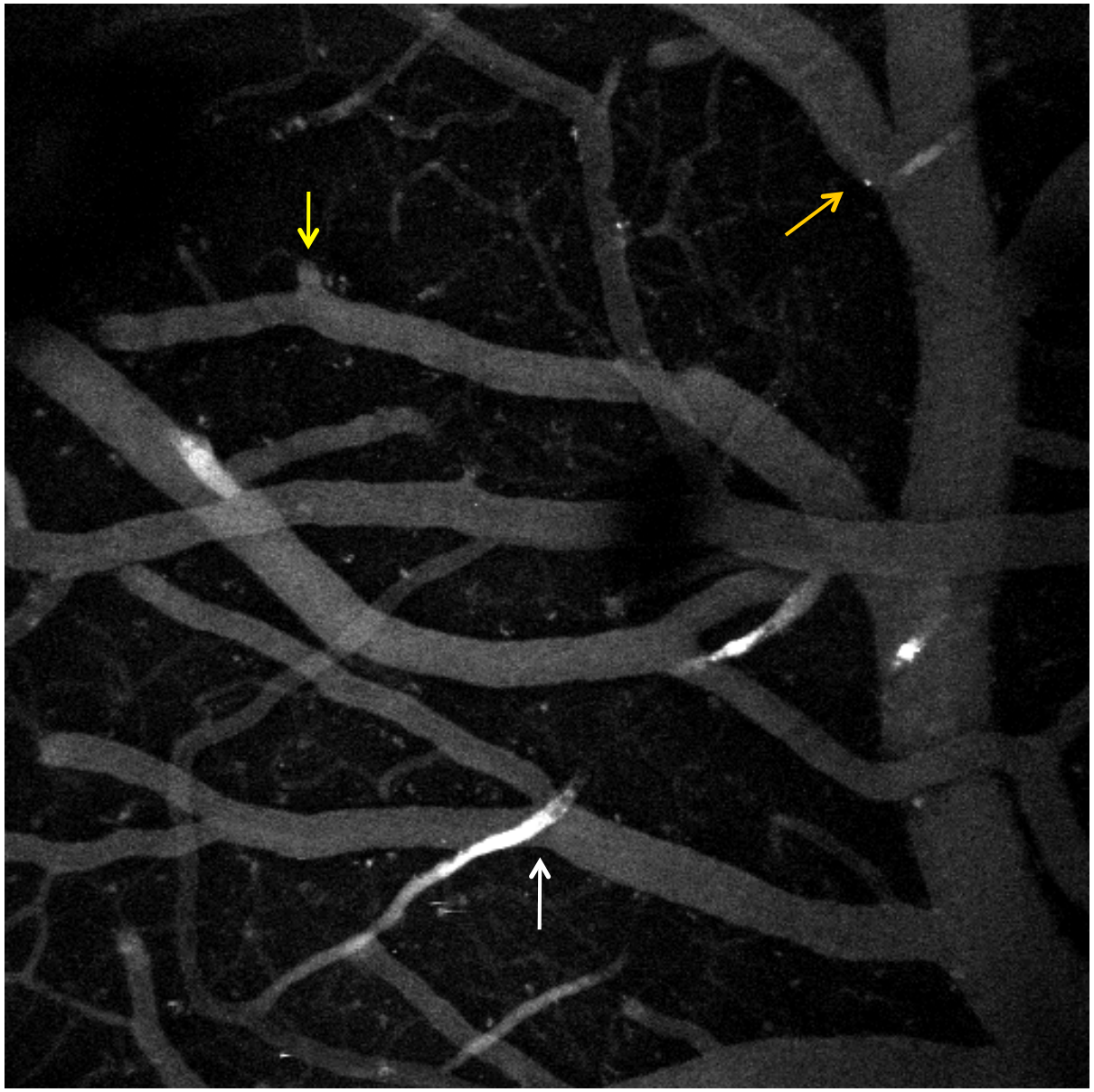
Figure 1 | Experimental setup. (**a**) Schematic of the two-photon laser scanning microscope modified for delivery of amplified ultrashort pulses for photodisruption. (**b**,**c**) Maps of fluorescein-dextran–labeled vasculature of rat parietal cortex. Inset in **b** shows latex-filled surface arteries and arterioles in rat cortex (adapted from ref. 49), and the white rectangle indicates the approximate location of the craniotomy. The images in **b** are maximal projections along the optical axis of near-surface vasculature. A↔P, anterior-posterior axis; M↔L, medial-lateral axis. A small region, indicated by the red box, was scanned at successive depths to form the maximal projection along A↔P, as indicated by the cartoon and shown in **c**. Scale bars: **b**, 500 μm (inset, 5 mm); **c**, 100 μm. (**d**) Schematic of the three different vascular lesions that are produced by varying the energy and number of laser pulses. At high energies, photodisruption produces hemorrhages, in which the target vessel is ruptured, blood invades the brain tissue, and a mass of RBCs form a hemorrhagic core. At low energies, the target vessel remains intact, but transiently leaks blood plasma and RBCs forming an extravasation. Multiple pulses at low energy leads to thrombosis that can completely occlude the target vessel, forming an intravascular clot. Scale bars, 50 μm.

Nishimura, Schaffer, Friedman, Tsai, Lyden, Kleinfeld, Nature Methods, 2006

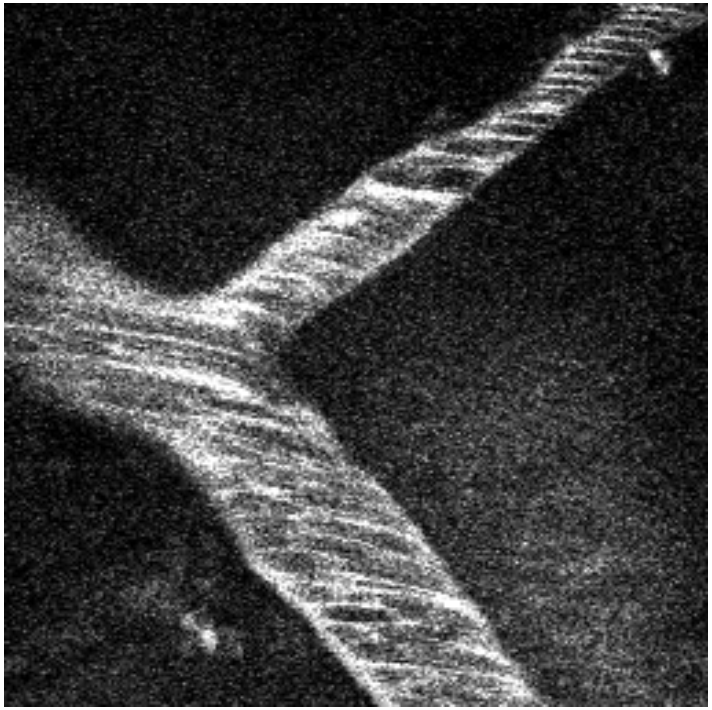


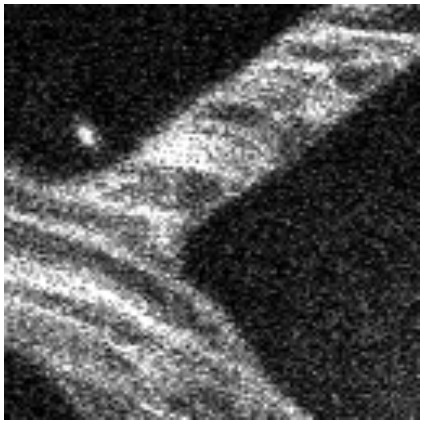
4X epi-illumination

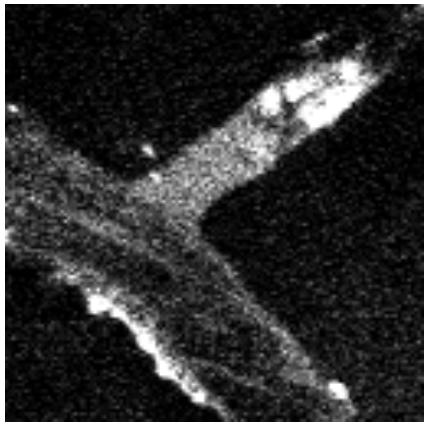
20x, NA 0.95, 2-photon



Vascular (+Blood Flow) Monitoring
Modeling Stroke







The ability to monitor the neural and the vascular components of the neurovascular unit in vivo, and to precisely control the induced vascular insult, gives us unparalleled power for studying cortical repair processes that take place after micro-vascular infarcts.

In summary,

- Understanding the principles of neural information processing in health and disease requires the detailed characterization of neural circuits, including the ability to monitor the properties of different cell types and their connections, as well as the ability to selectively manipulate the activity of these cell types, in vivo.
- Chronic, in vivo, optical imaging techniques can monitor both functional (local neuronal network dynamics) and structural (dendritic spine motility) changes, and can be used to visualize directly how neural circuits recover following injury, change during perceptual learning, or how the neuro-vascular unit responds to compromise.
- By taking advantage of genetic channel rhodopsin targeting methods optical imaging techniques can be used to obtain exquisite control over the activity of specific neuronal subtypes.
- Overall, I hope I have convinced you that these techniques are here to stay, and that they promise to lead to a revolution in our understanding of cortical circuit function in the not too distant future.

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Other laboratory members

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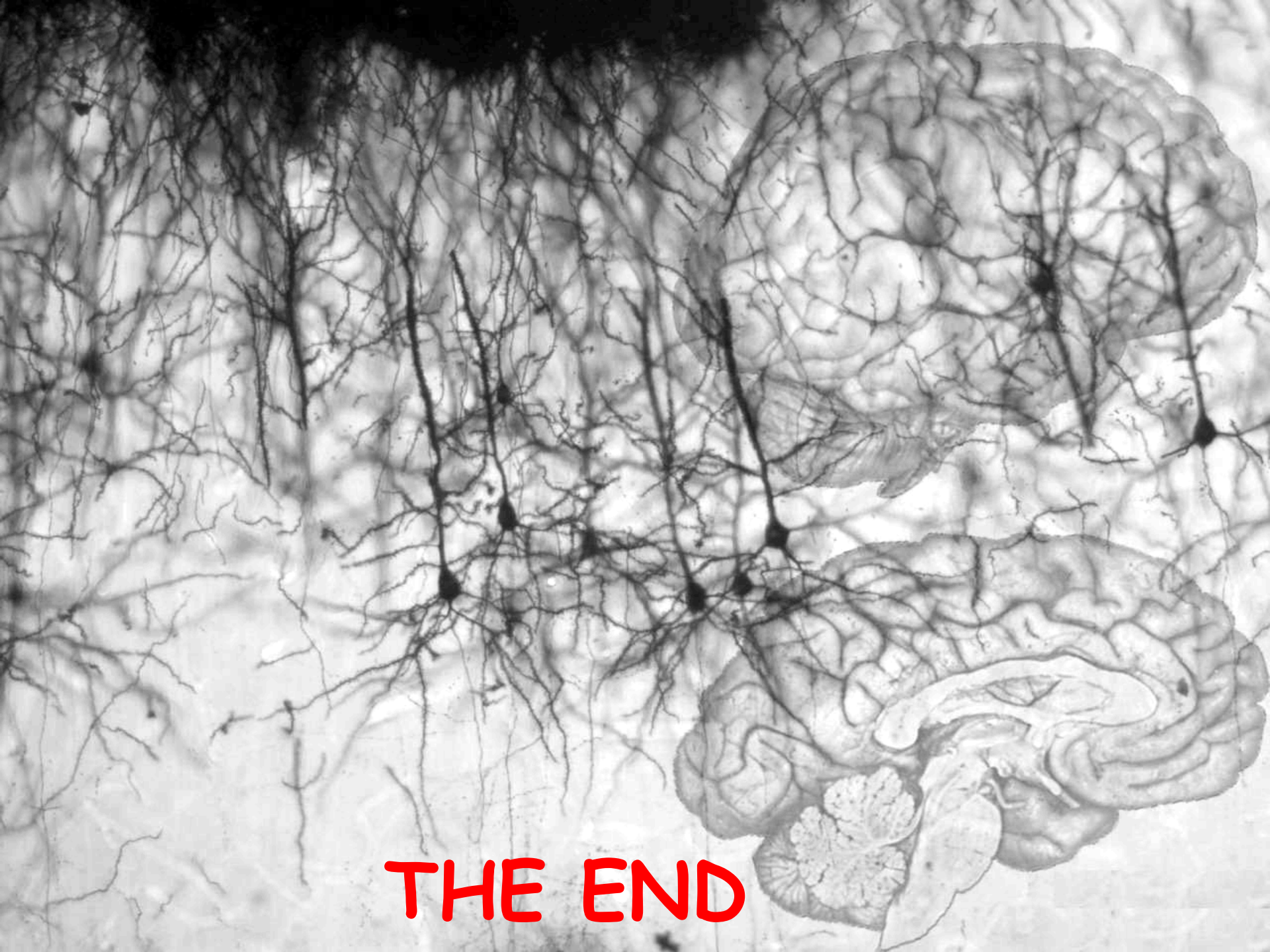
THE END