Cerebral Cortex, and how to study it

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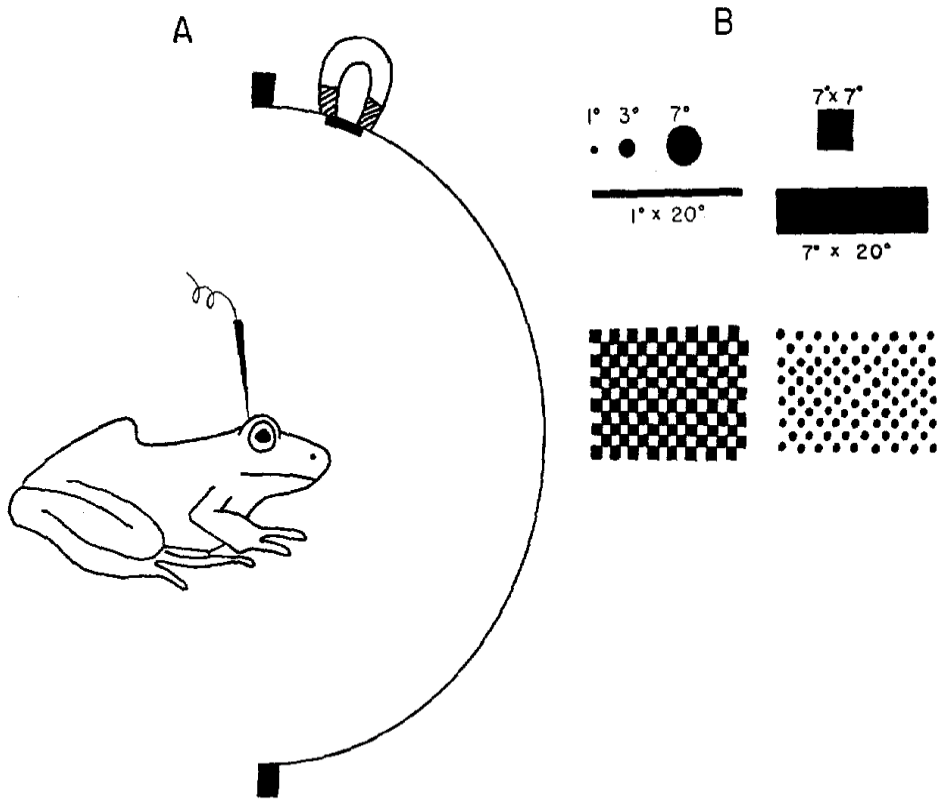
History of Visual Cortex Physiology:

The earliest evidence on the location of the primary visual cortex came from human brain lesions, from comparative anatomy and connection anatomy (with techniques now only of historical interest), and then from evoked potentials. The inferred location was correct.

After it became possible to record the activity of single neurons, the early recordings of neural activity in the mammalian visual cortex were unrevealing. Surprisingly to everyone, the neurons simply did not respond, or responded only very weakly and unreliably when you gave strong visual stimulation, such as turning the room lights on and off.

This failure to respond was all the more puzzling because the same stimuli worked well in retina and LGN, and quite elaborate and complex visual responses were found even in frogs and fish in the retina and optic tectum by the neuroethologists and by the predecessors of modern theoretical neuroscientists (the group of electrical engineers and mathematicians and physicians who worked with information theory and thought about robotics and early neural network models, eg McCulloch, Pitts, Lettvin). The classic example of this tradition is probably Lettvin et al. (*1959)* What The Frog's Eye Tells The Frog's Brain. *Proc. Inst. Radio Engr.* 47: 1940-1951, available at http://jerome.lettvin.info/WhatTheFrogsEyeTellsTheFrogsBrain.pdf

They found quite specific "detectors" for visual features important to the life of the frog: dimming detectors, net convexity ("bug") detectors, etc.



TEXT-FIGURE 1. *A*. Schematic drawing of the relations between the frog and the hemisphere that constitutes the experimental visual field.

B. Scale drawings of some of the objects used as stimuli. The degrees indicate their diameter if placed inside a hemisphere of the same radius as that represented in *A*. The actual hemisphere used was larger, 14 inches in diameter.

Numerous attempts were made at quantitative studies, by von Baumgartner, Peter Bishop and others. Stimuli used were diffuse illumination and various configurations of center-surround spots. In general, only occasional and then mostly very weak responses were observed in anesthetized cortex.

David Hubel says at the time that everyone believed that anesthesia was the problem. As a postdoc, he was made to spend a lot of time working out methods for recording from alert animals, which is difficult for the visual system because the eyes and head move so rapidly. As it turned out, anesthesia was not the problem.

The breakthrough came from the collaboration between Hubel and Wiesel and is said to be by chance, but a chance made possible by careful preparation. Receptive fields were plotted one at a time with enormous care, sometimes for hours. A second advance was that they stimulated the eyes by using a slide projector on a screen instead of by projecting lights directly onto the retina, allowing much more flexibility in creating visual stimuli.

A description from the essay in Hubel and Wiesel's book, **Brain and Visual Perception**:

“The break came one long day in which we held onto one cell for hour after hour. To find a region of retina from which our spots gave any hint of responses took many hours, but we finally found a place that gave vague hints of responses. We worked away, in shifts. Suddenly, just as we inserted one of our glass slides into the ophthalmoscope, the cell seemed to come to life and began to fire impulses like a machine gun. It took a while to discover that the firing had nothing to do with the small opaque spot—the cell was responding to the fine moving shadow cast by the edge of the glass slide as we inserted it into the slot. It took still more time and groping around to discover that the cell gave responses only when this faint line was swept slowly forward in a certain range of orientations. Even changing the stimulus orientation by a few degrees made the responses much weaker, and an orientation at right angles to the optimum produced no responses ac all. The cell completely ignored our black and white spots. When finally we could think of nothing more to do with this cell, we discovered we had worked with it for nine hours. The 1959 paper of course gives no hint of our struggle. As usual in scientific reports we presented the bare results, with little of the sense of excitement or fun.”

“With the Talbot-Kuffler head-holder-and-ophthalmoscope combination we had the problem that it was only possible to stimulate one eye at a time. Consequently we had no idea whether our cells were binocular or monocular. (It was clear from the anatomy of the visual pathway that the striate cortex was the first place where the inputs from the two eyes could combine, but no one knew whether single cells would typically be influenced from the two eyes.) To be able to work with the two eyes, we obviously had to make radical changes in our system of stimulating the eyes. (It was impossible to imagine using two Talbot-Kuffler ophthalmoscopes--one was bad enough!) ”

“… just as important as stubbornness, in getting results, was almost certainly the simplicity, the looseness, of our methods of simulation, The incredible crudeness of our first slide projectors and the projection screens that soon replaced the ophthalmoscope, and our refusal to waste time bothering with measuring intensities, rates of movement and so on, or to spend time drawing graphs or histograms, all worked in our favor.”

Neurons in the core of the LGN in cats and monkeys are very similar to ON-center of OFF-center retinal ganglion cells

Two properties of the visual responses of cells in V1 distinguished them from the LGN cells, which provide the visual input to the cortex:

- Orientation selectivity, linear rather than circularly symmetric
- Binocularly excited

The first Simple Cell, from Hubel & Wiesel (1959)

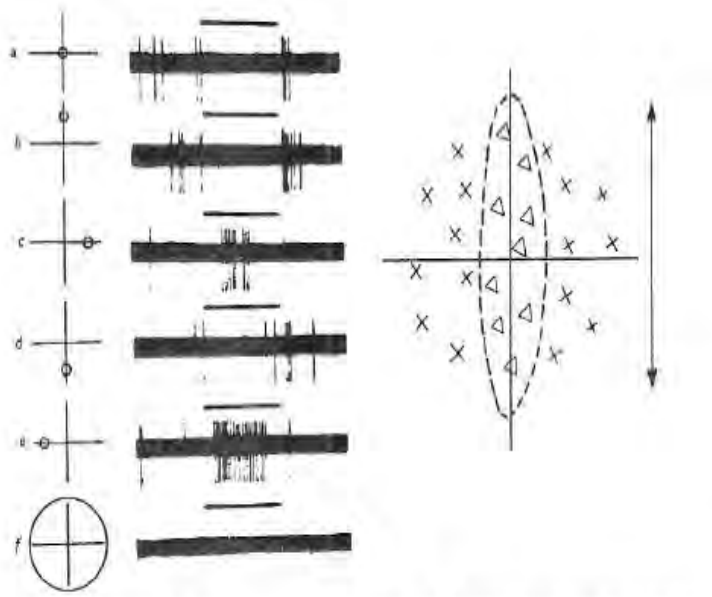


Fig. 1. Responses of a cell in the cat's striate cortex to a 1° spot of light. Receptive field located in the eye contralateral to the hemisphere from which the unit was recorded, close to and below the area centralis, just nasal to the horizontal meridian. No response evoked from the ipsilateral eye. The complete map of the receptive field is shown to the right. ×, areas giving excitation; △, areas giving inhibitory effects. Scale, 4°. Axes of this diagram are reproduced on left of each record. a, 1° (0.25 mm) spot shone in the centre of the field; b–e, 1° spot shone on four points equidistant from centre; f, 5° spot covering the entire field. Background illumination 0.17 log. m.c. Stimulus intensity 1.65 log. m.c. Duration of each stimulus 1 sec. Positive deflexions upward.

Similarity of Receptive fields in the Two Eyes, and Binocular Summation, from Hubel & Wiesel (1959)

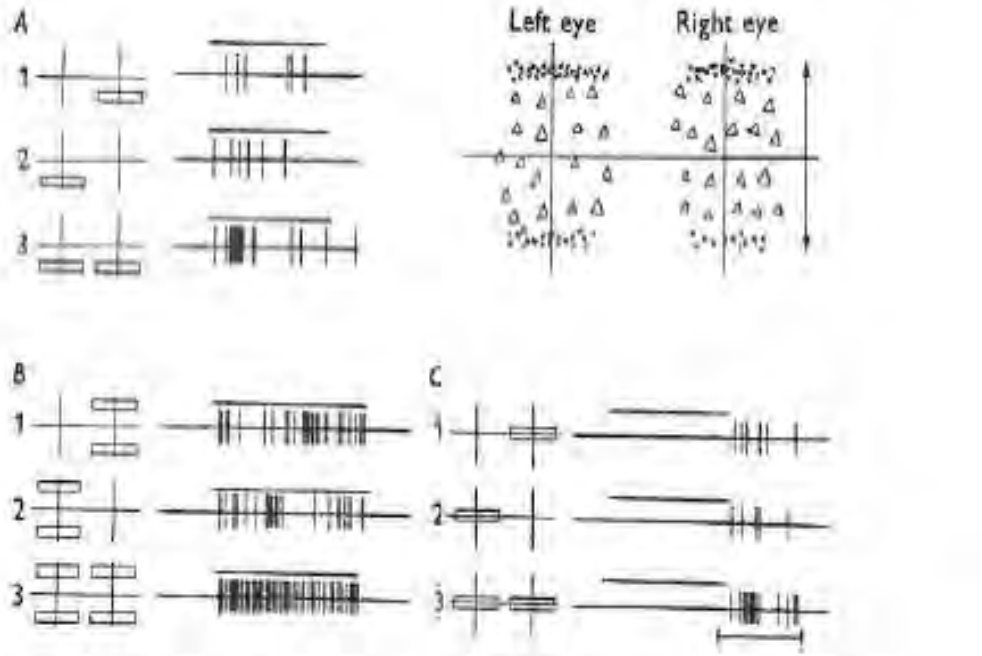


Fig. 9. This unit was activated from either eye independently. The illustration shows summation between corresponding parts of the two receptive fields. Receptive field in the contralateral eye was located just above and nasal to area centralis in the ipsilateral eye, above and temporal. Receptive fields of the two eyes were similar in form and orientation, as shown in upper right of the figure; scale 8°. The pairs of rows in the receptive field diagram are reproduced to the left of each record. Background and stimulus intensities and conventions as in Fig. 6. (Same unit as in Fig. 6.) A. 1, horizontal slit covering lower flanking region of right eye; 2, same for left eye; 3, pair of slits covering the lower flanking regions of the two eyes. B. 1, pair of horizontal slits covering both flanking regions of the right eye; 2, same for left eye; 3, simultaneous stimulation of all four flanking regions. C. 1, horizontal slit in central region of right eye; 2, same for left eye; 3, simultaneous stimulation of central regions of both eyes. Time, 1 sec.

Antagonism Between Excitatory and Inhibitory Regions of Receptive Fields in the Two Eyes, from Hubel & Wiesel (1959)

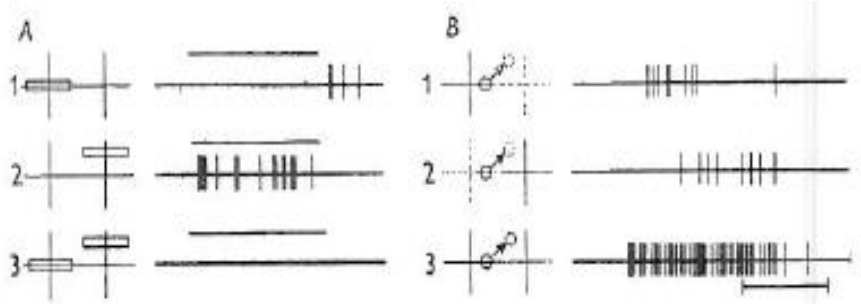
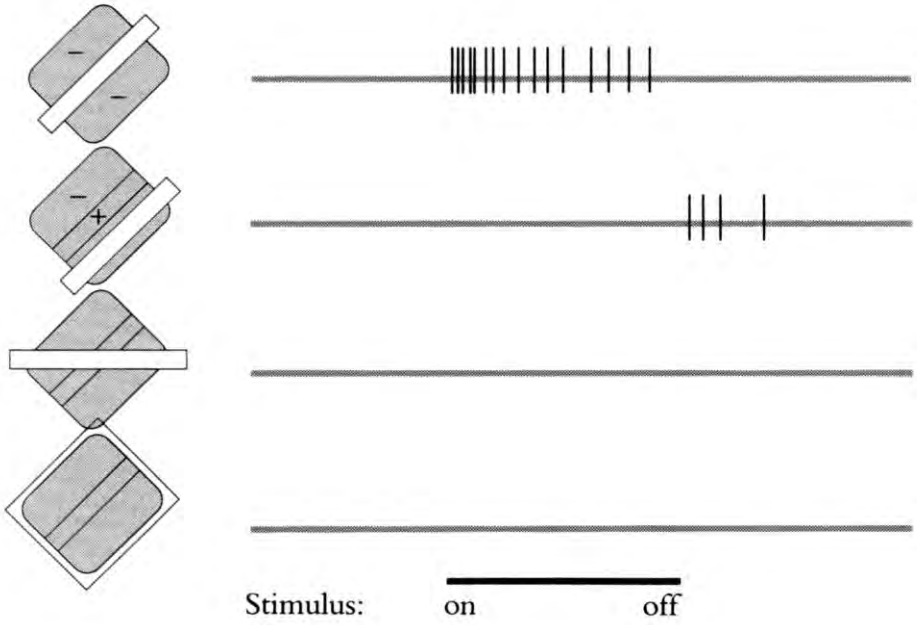
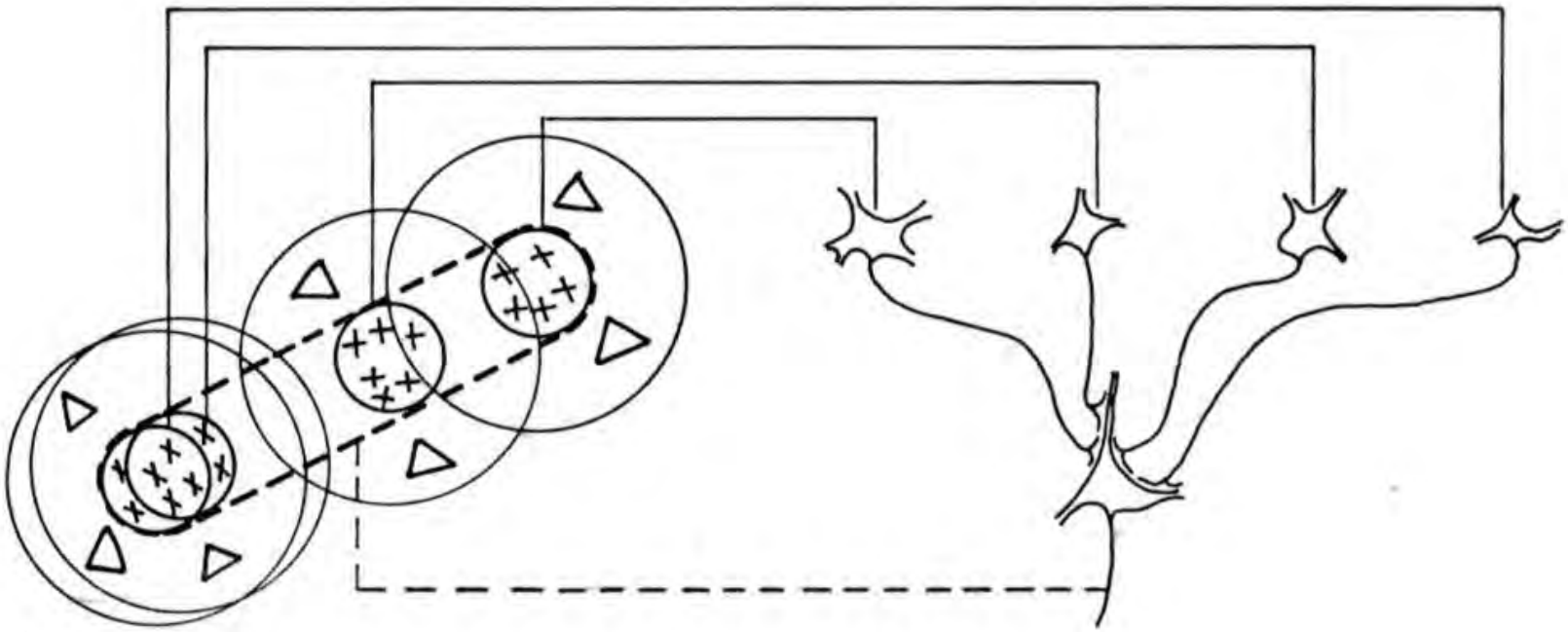
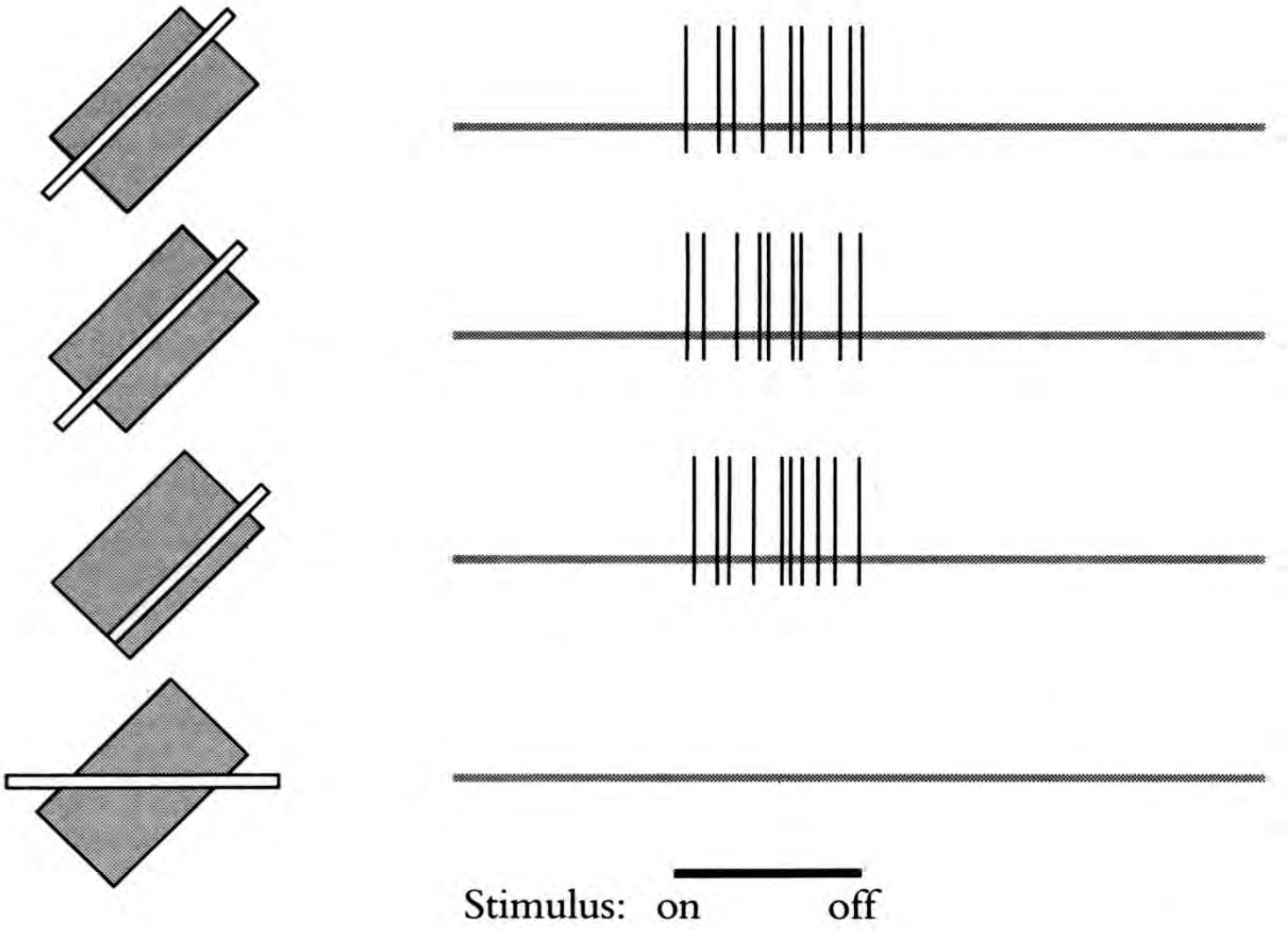


Fig. 10. Same unit as in Fig. 9. A. Antagonism between inhibitory region in the left eye and an excitatory region in the right eye; stationary spots. 1, horizontal slit in centre of left eye; 2, horizontal slit covering upper flanking region of right eye; 3, simultaneous stimulation of the regions of 1 and 2. B. Synergism between inhibitory region in left eye and an excitatory region in the right eye; moving spot of light. 1, right eye covered, spot moved from inhibitory region in left eye, producing an 'off' response; 2, left eye covered, spot moved into excitatory region in right eye, producing an 'on' response; 3, both eyes uncovered, spot moved from inhibitory region in left eye into excitatory region of right eye, producing a greatly enhanced response. Time, 1 sec.

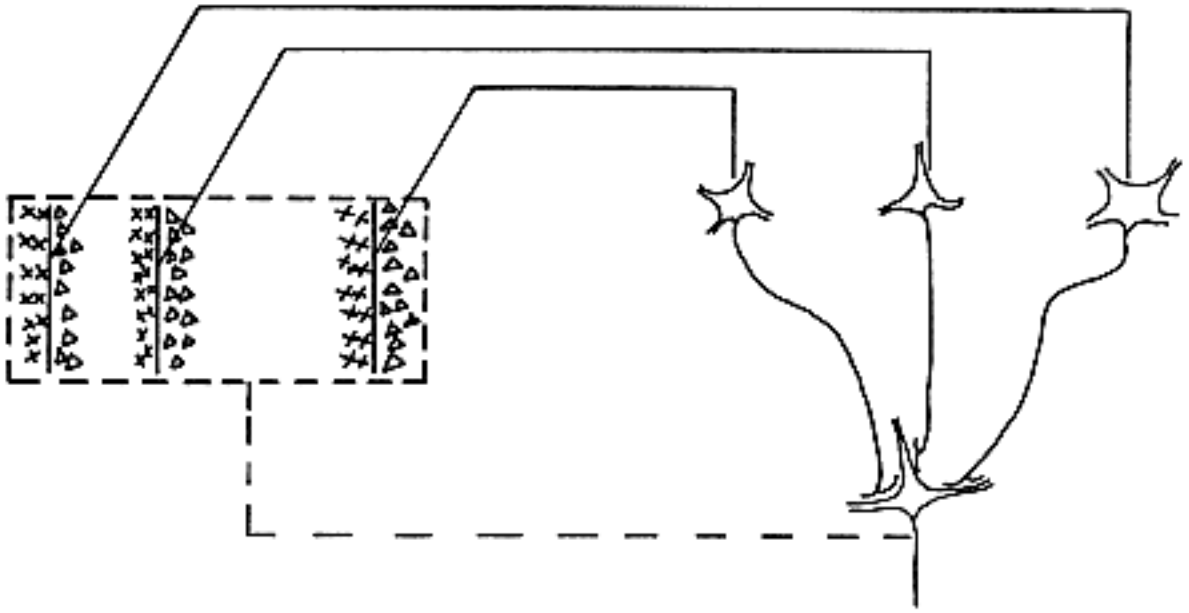

Stimulus:
on
off



Response of a Complex Cell

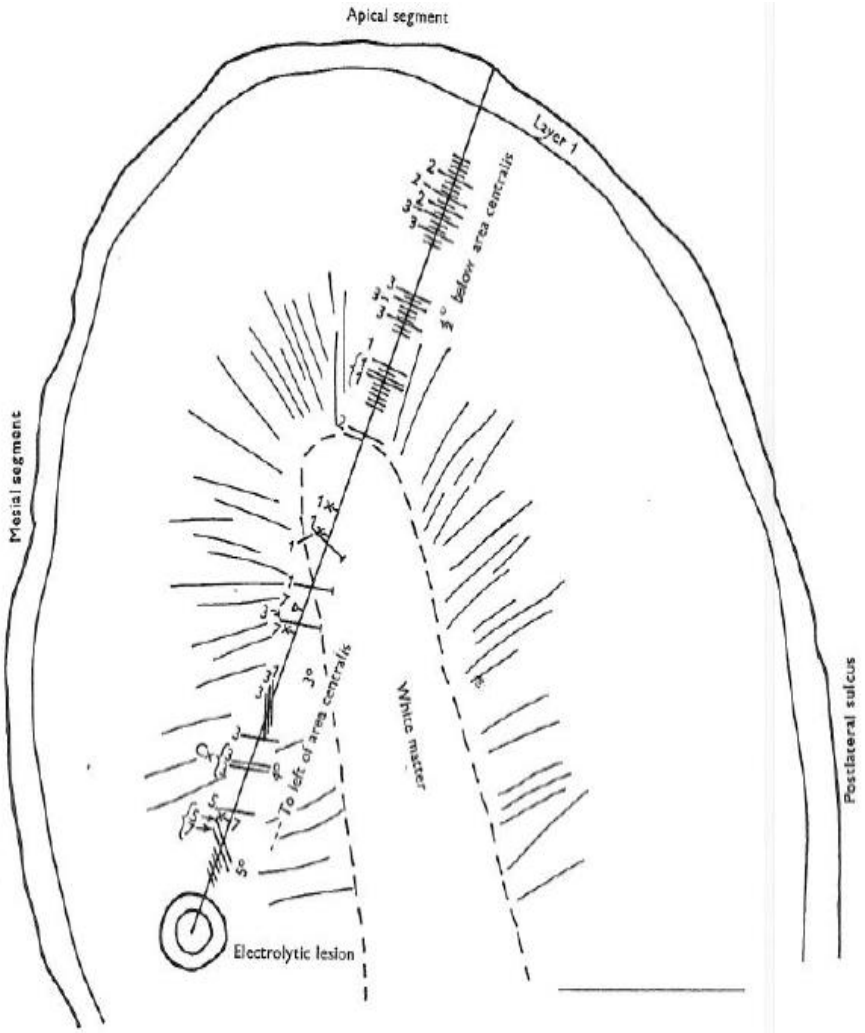


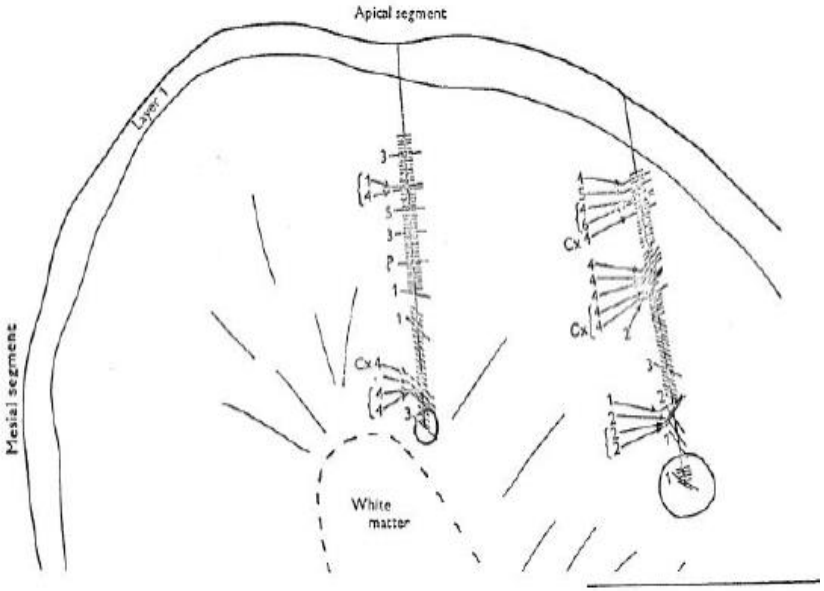
Hubel & Wiesel (1962) Model for Complex Cell.
Supported by RF properties and laminar arrangement of cell types



Text-fig. 20. Possible scheme for explaining the organization of complex receptive fields. A number of cells with simple fields, of which three are shown schematically, are imagined to project to a single cortical cell of higher order. Each projecting neurone has a receptive field arranged as shown to the left: an excitatory region to the left and an inhibitory region to the right of a vertical straight-line boundary. The boundaries of the fields are staggered within an area outlined by the interrupted lines. Any vertical-edge stimulus falling across this rectangle, regardless of its position, will excite some simple-field cells, leading to excitation of the higher-order cell.

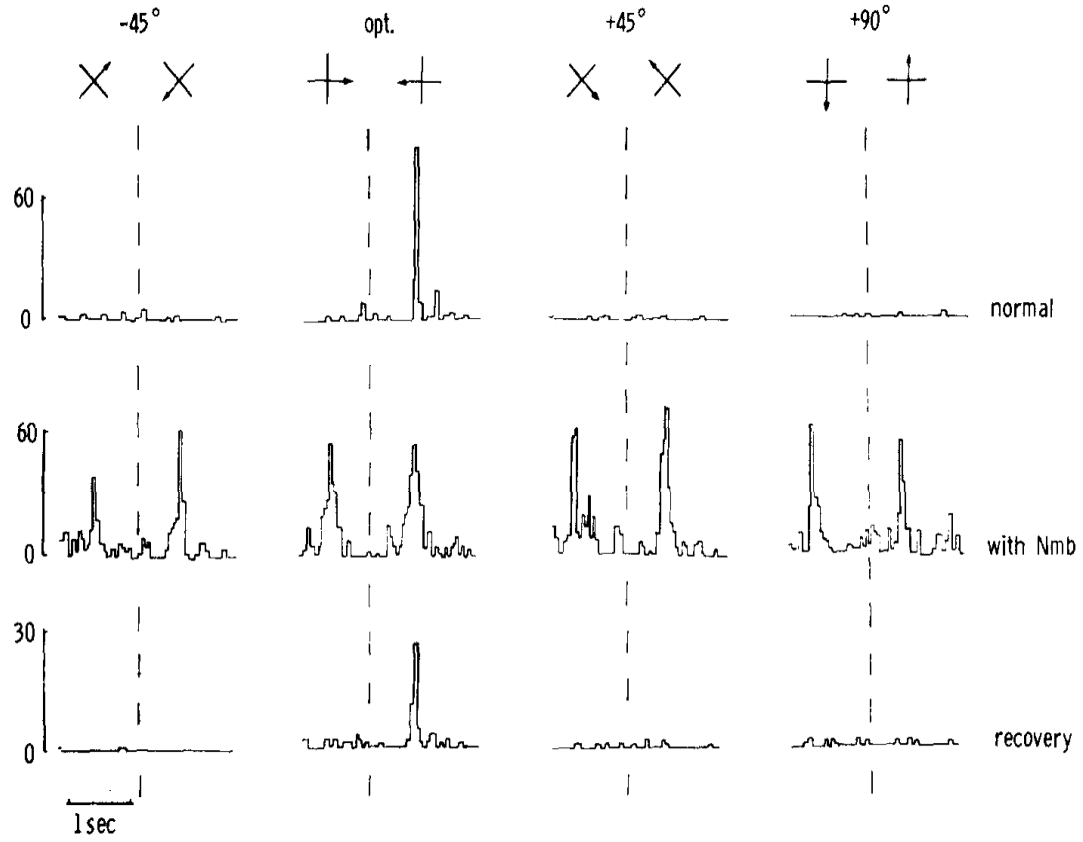
Evidence for Cortical columns from Hubel & Wiesel (1962)





How can we test the Hubel & Wiesel Model for Orientation Selectivity?

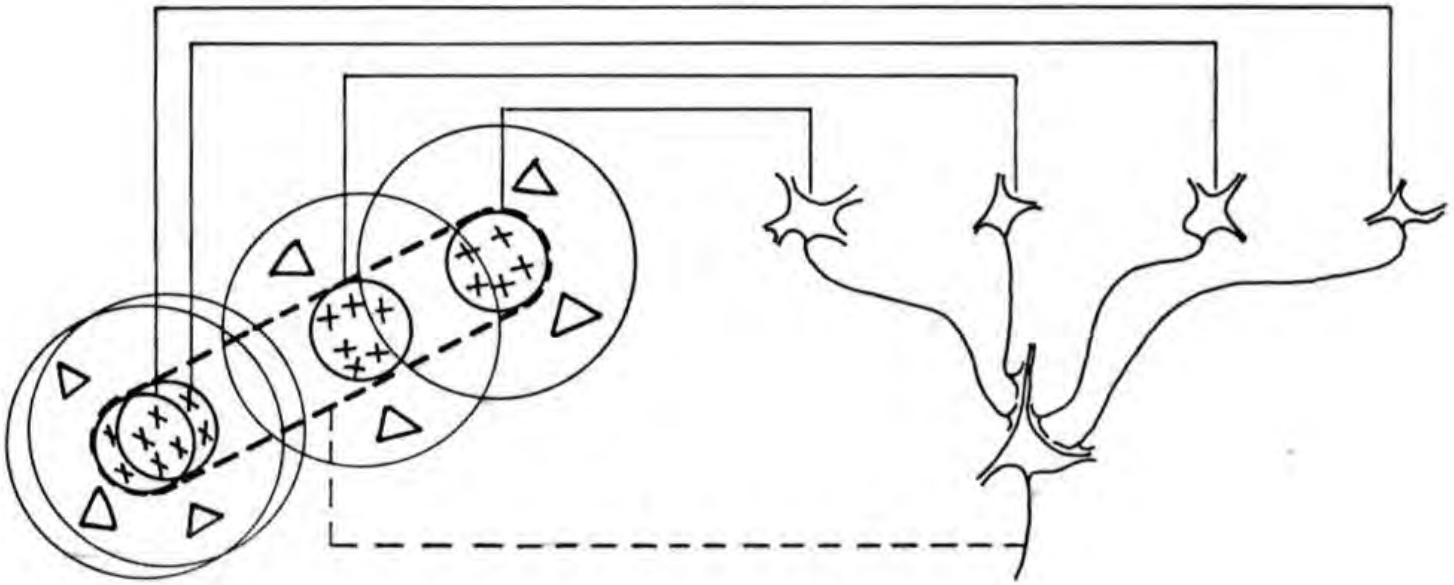
Sillito showed that blocking GABA-mediated inhibition with N-methyl- bicuculline made cortical cells respond to all orientations.



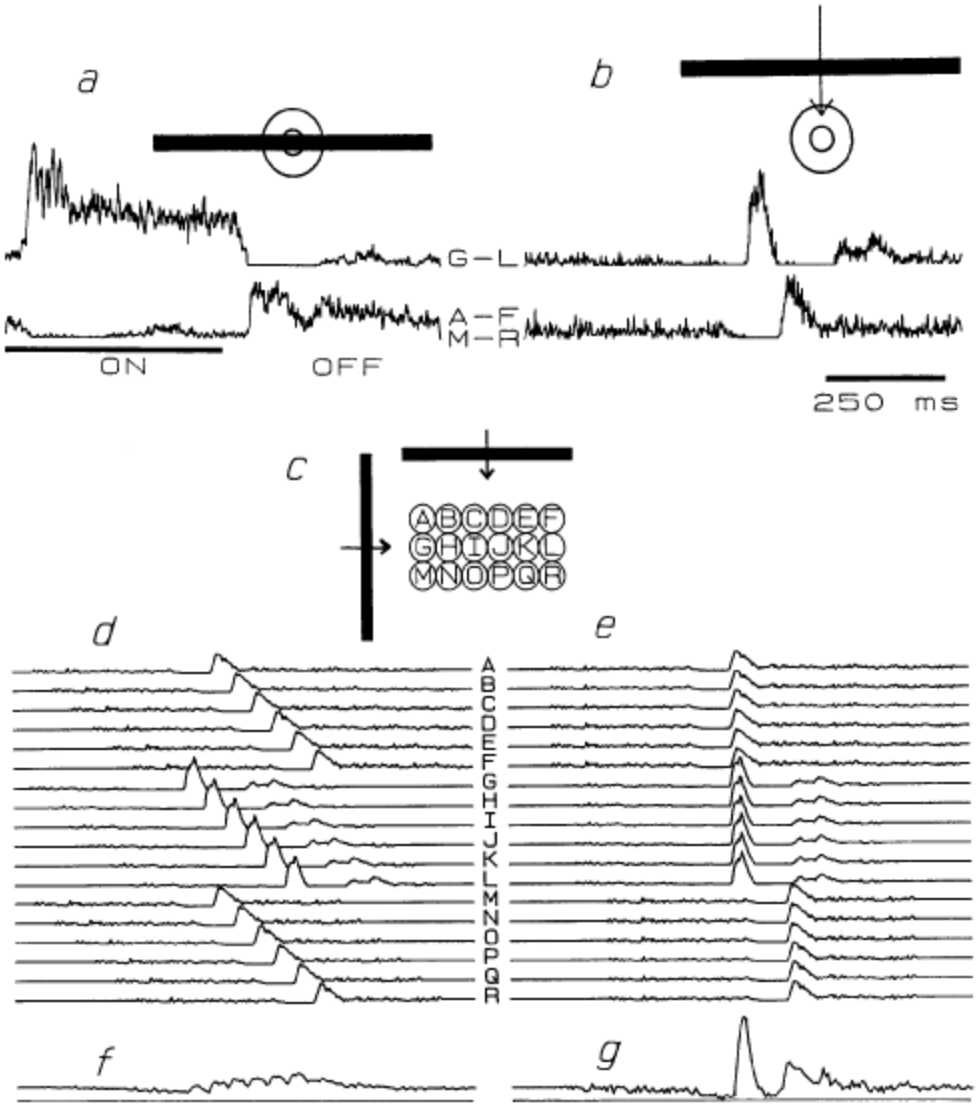
Sillito et al (1980) Brain Res

How can we test the Hubel & Wiesel Model for Orientation Selectivity?

First, why doesn't the H&W Model Simple Cell fire spikes to all orientations, as it did for Sillito?



Ferster (1986, 1987) showed that the relative timing of PSPs combined with a threshold can account for thalamocortical orientation selectivity.



Effect of RF length on orientation Tuning in Ferster (1987) Model

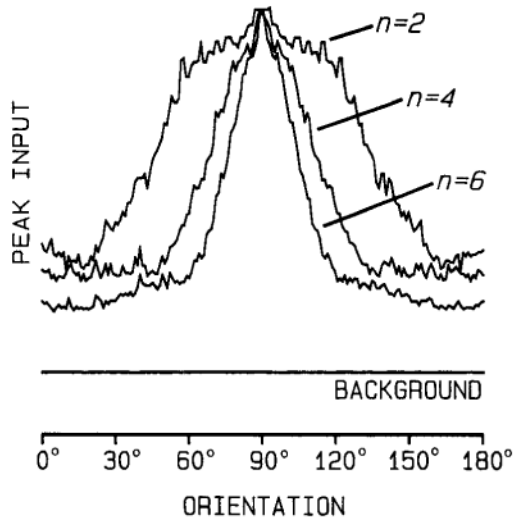
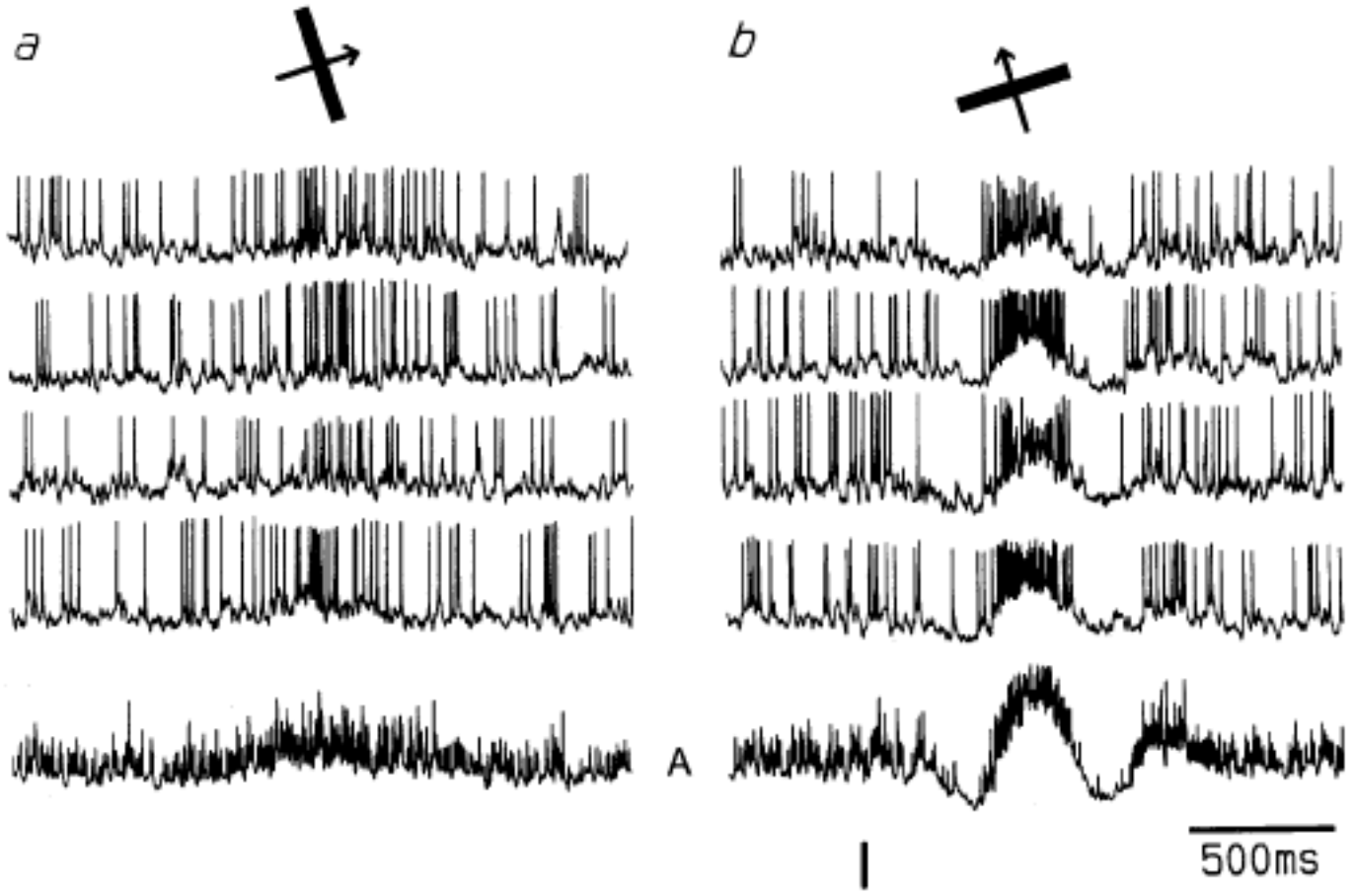


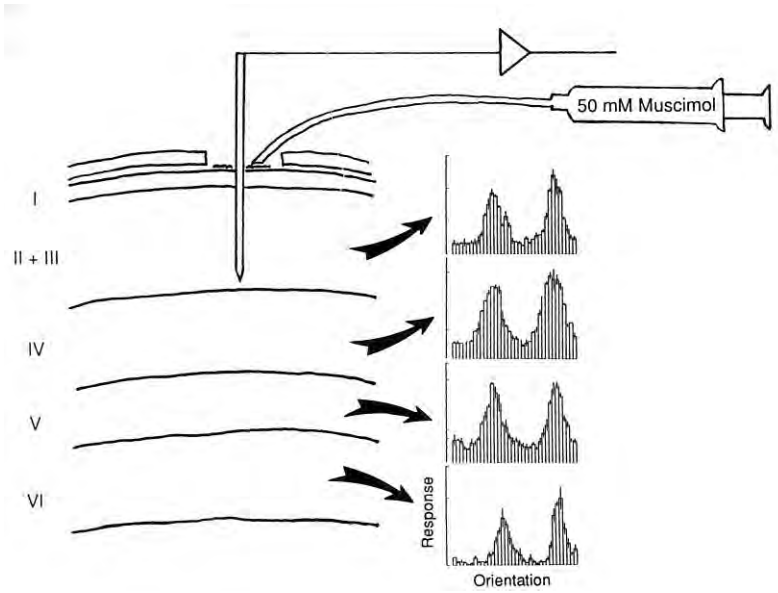
Figure 3. Orientation-tuning curves predicted by the model for the simple cell of Figure 4, with different numbers (n) of presynaptic geniculate neurons making up the 3 subfields. For $n = 6$, the curve is

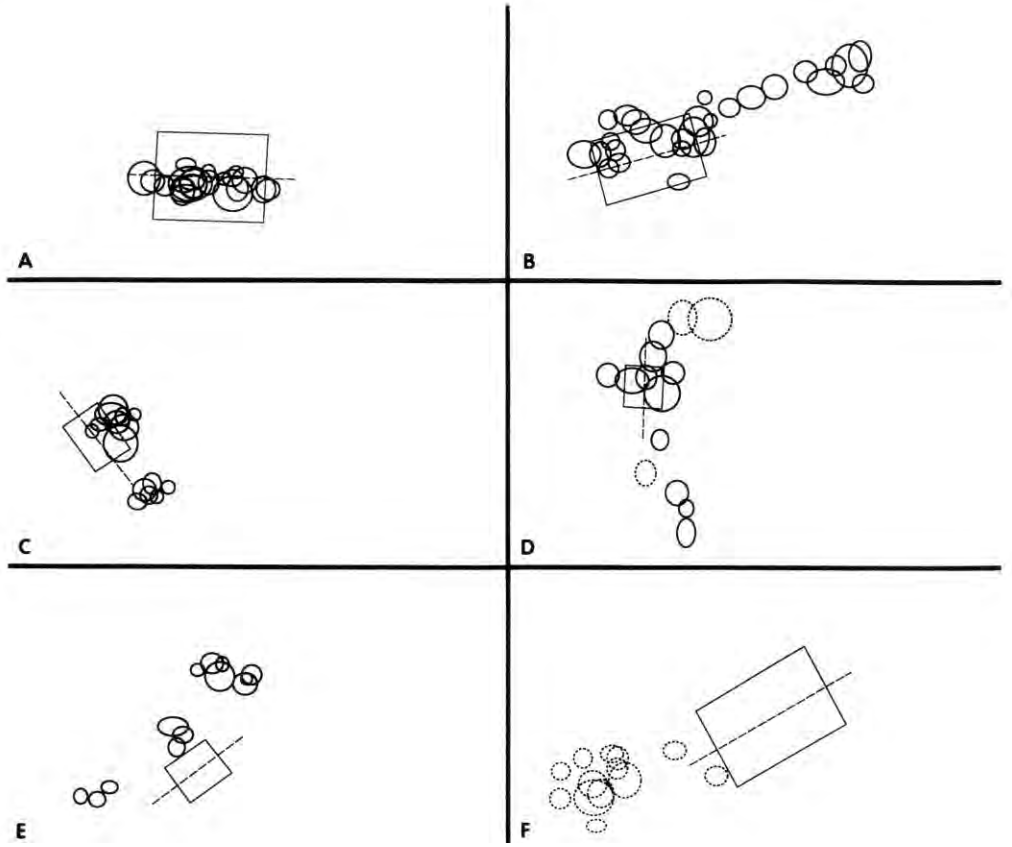
Second, Ferster (1987) showed that intracellularly recorded EPSPs in Simple Cells matched the H&W Model.

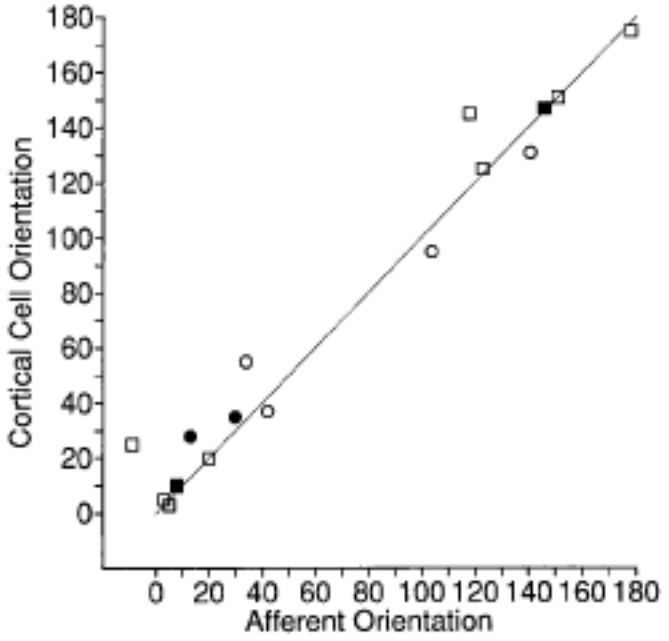


But how do we know that those PSPs are from the LGN?

Chapman et al (1991) silenced cortex pharmacologically to allow measurement of LGN afferent RFs that arborize within a single cortical column and found them dispersed over elongated region parallel to preferred orientation of column.





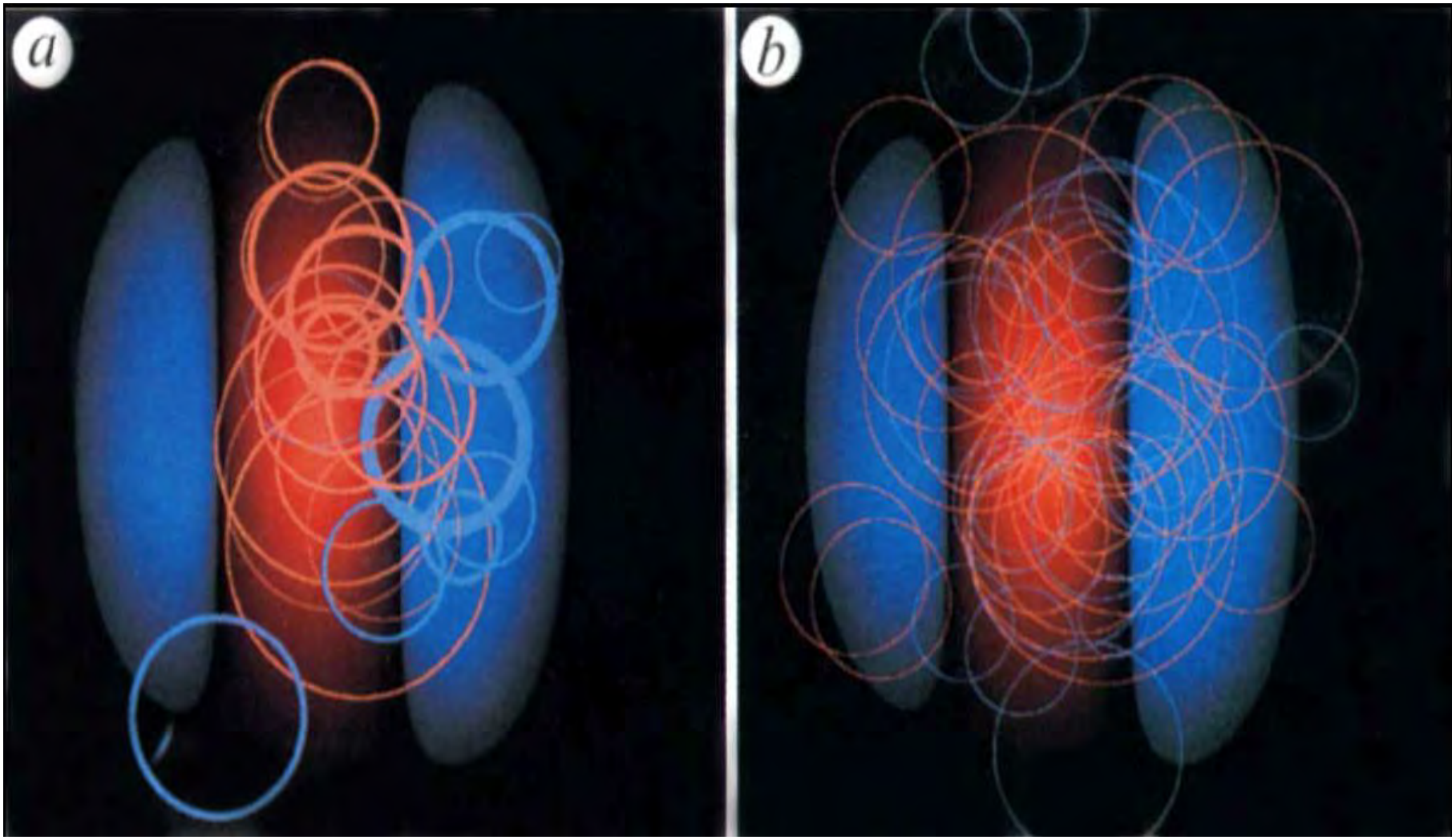


From Chapman et al (1991)

Monosynaptic Geniculate Inputs to Simple Cells in Cat V1 from Cross Correlation

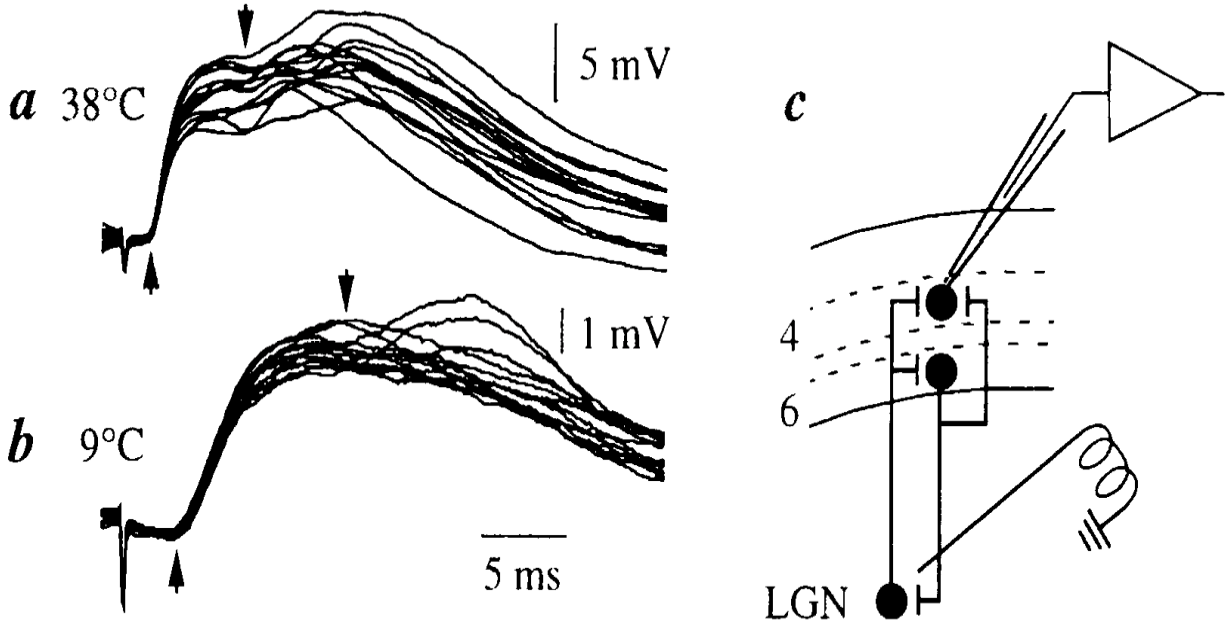
Connected Pairs

Unconnected pairs



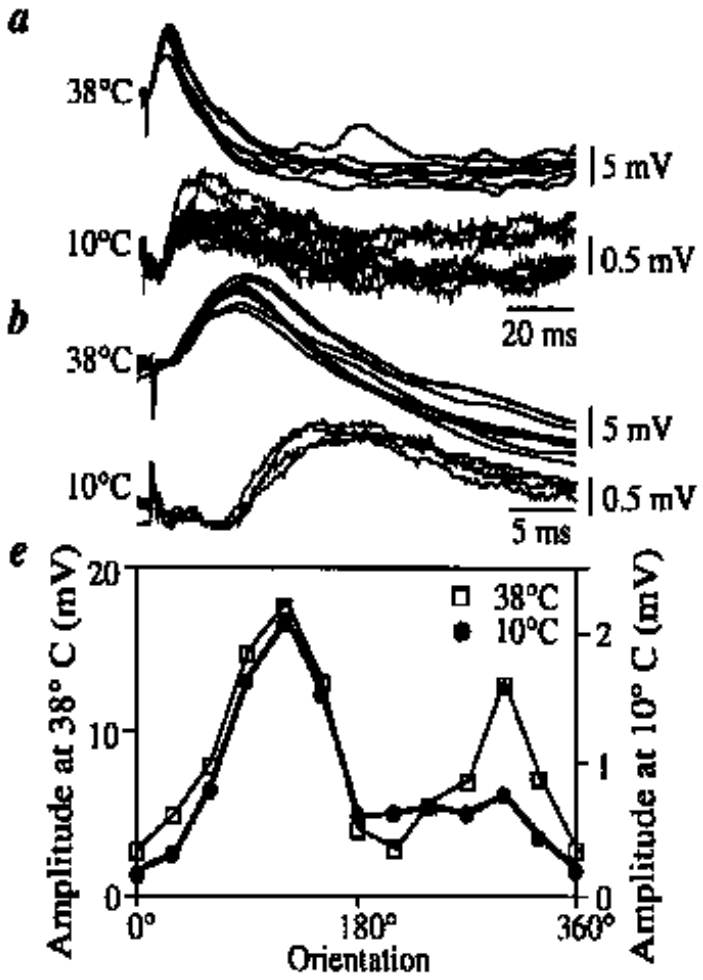
Alonso & Reid (1995) Nature

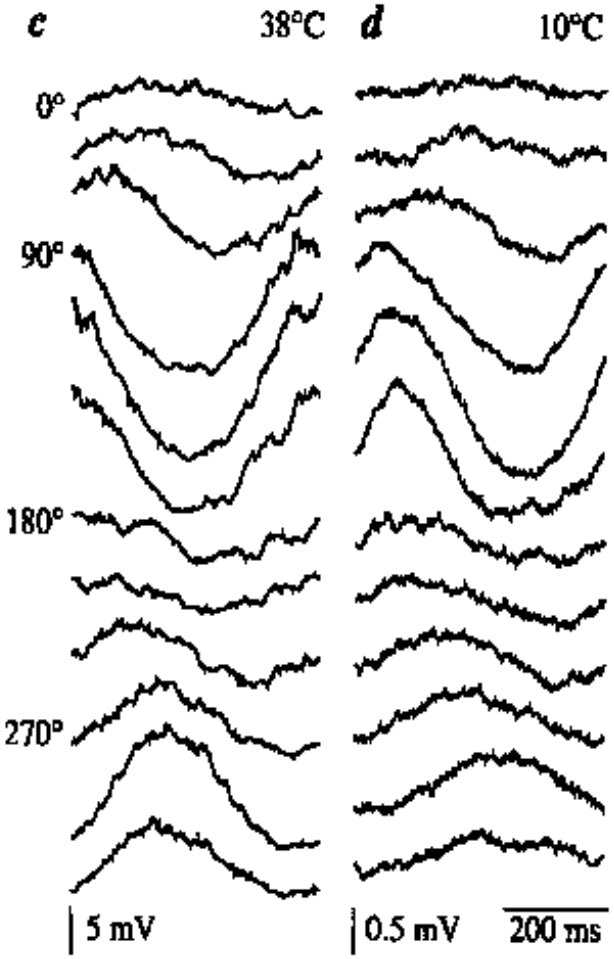
Ferster Cooled Cortex To Eliminate All Input to Simple Cells From Within the Cortex, Leaving Only Monosynaptic LGN Input

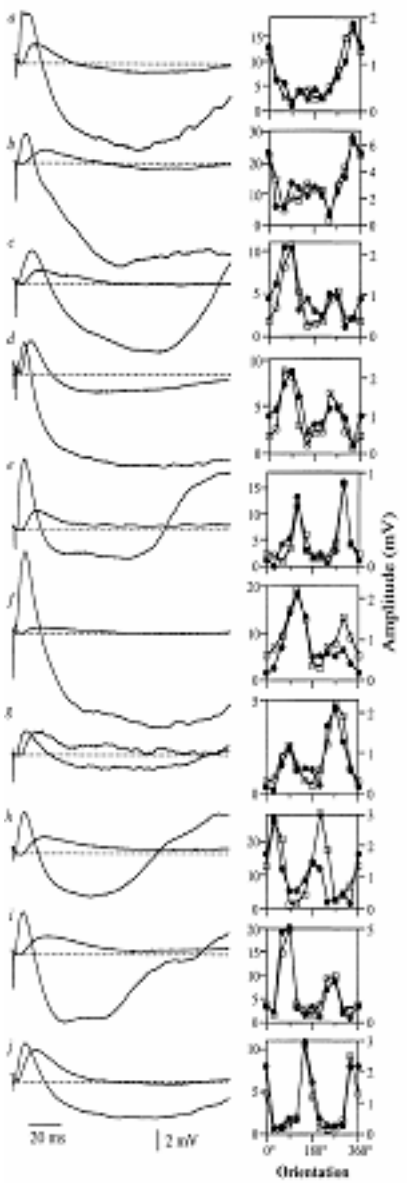


Ferster et al (1996) Nature

Cooling Cortex Does Not Change Orientation Tuning of Excitatory Input to Simple Cells







Ferster et al (1996) Nature

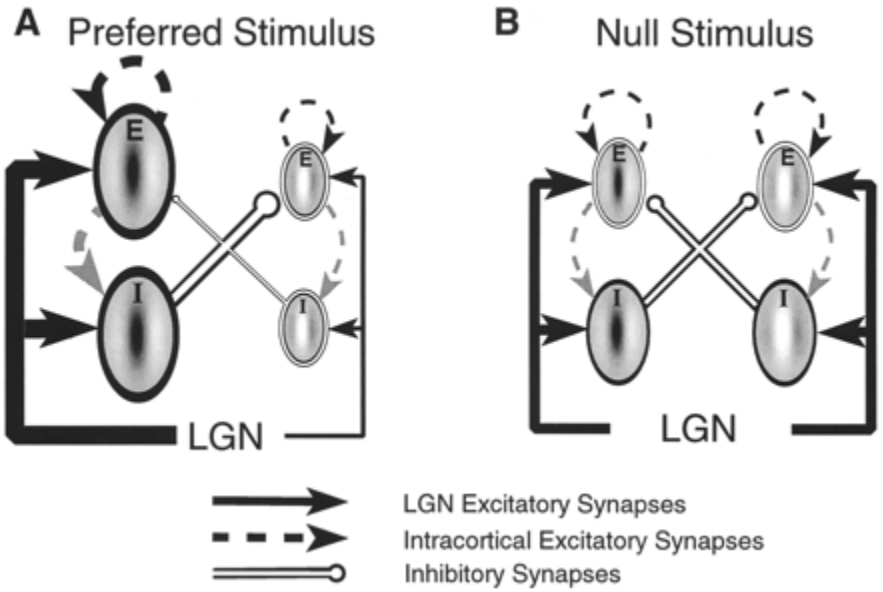
Both Reid's and Ferster's labs used repetitive electrical stimulation to profoundly inhibit nearly all cortical cells and thereby get rid of intracortical inputs to Simple Cells.

They both saw with intracellular (Ferster) or white noise stimuli and cross-correlation (Reid) that the remaining input to Simple Cells was elongated and oriented.

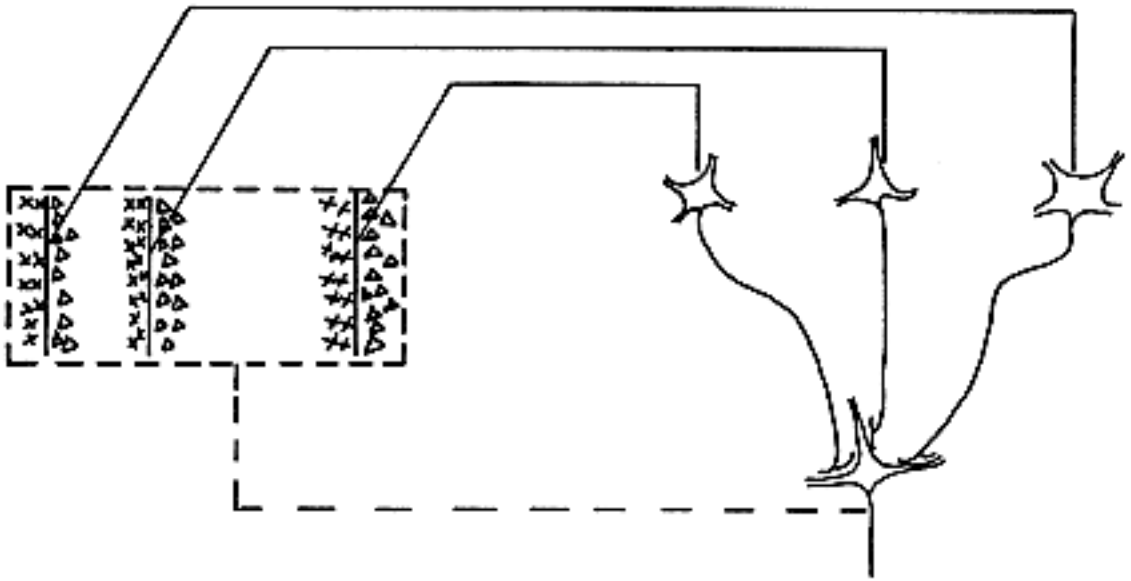
These findings confirm the H&W Simple Cell model. How then do we explain Sillito's bicuculline finding?

Much of current work addresses the problem of the contrast invariance of orientation selectivity.

One proposal from Ken Miller's lab involves orientation-specific connections to and from inhibitory cells. Troyer et al (1998)



A second major issue is the hierarchy. Are Complex Cells made up of a composite of inputs from Simple Cells of the same orientation but different RF positions?



Intracortical cross-correlations have provided little evidence either way, and some on both sides. In addition, monosynaptic LGN input is stronger in Simple Cells but is not absent in Complex Cells.

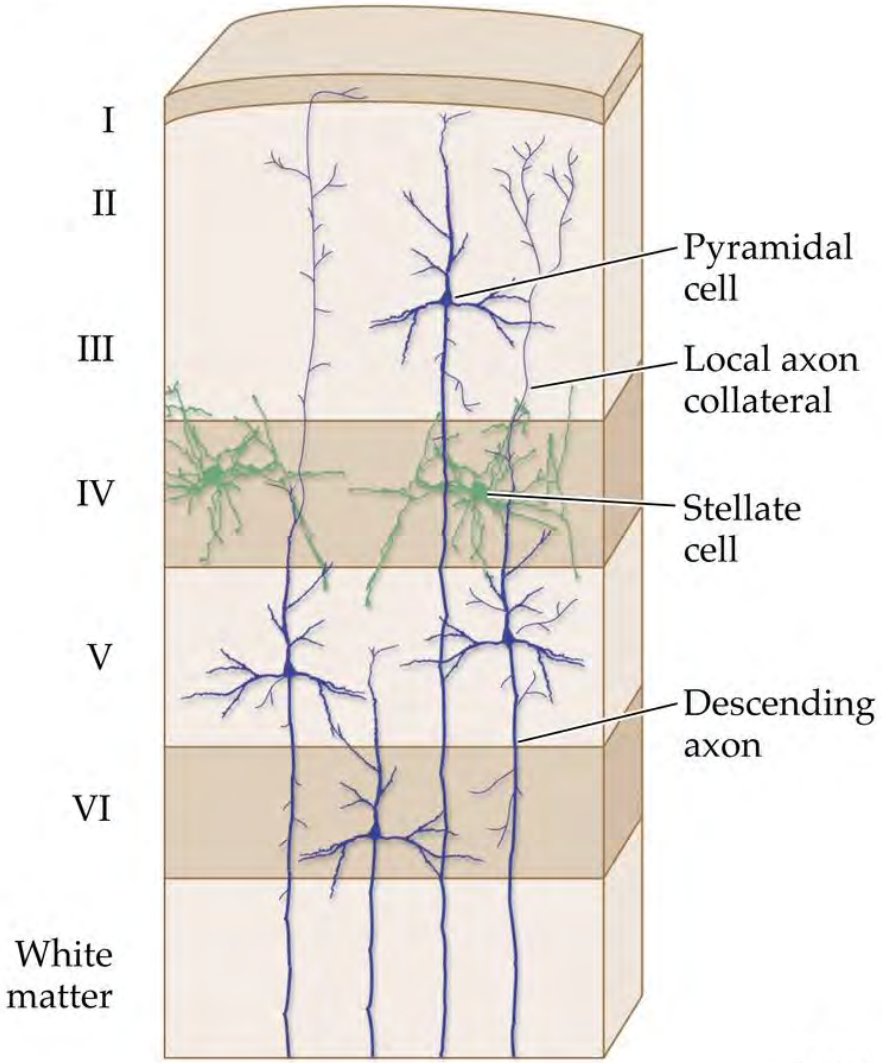
These questions about circuitry come down to something like

Which are the cells that provide input to a given cell under study, and what are their properties?

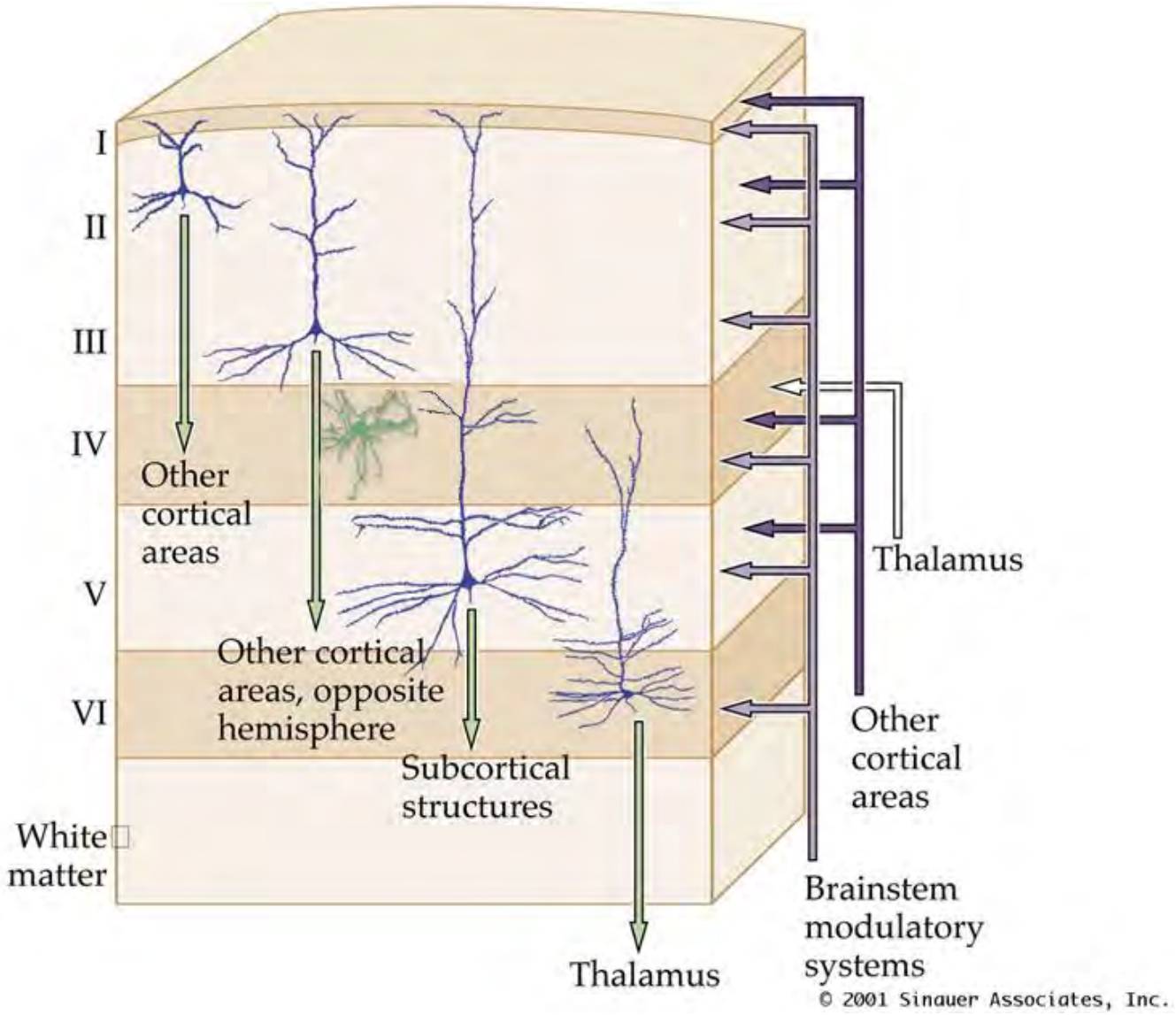
Until the past 5 years, there was in general no way for systems neuroscience to answer this question definitively. We tried to answer it by inference, from a combination of physiology, anatomy, and modeling approaches.

There is still not an easy general way to answer this question. But at the end of the lecture we shall talk about advances that will make it possible in your careers.

Anatomists recognized several different cell types in cortex



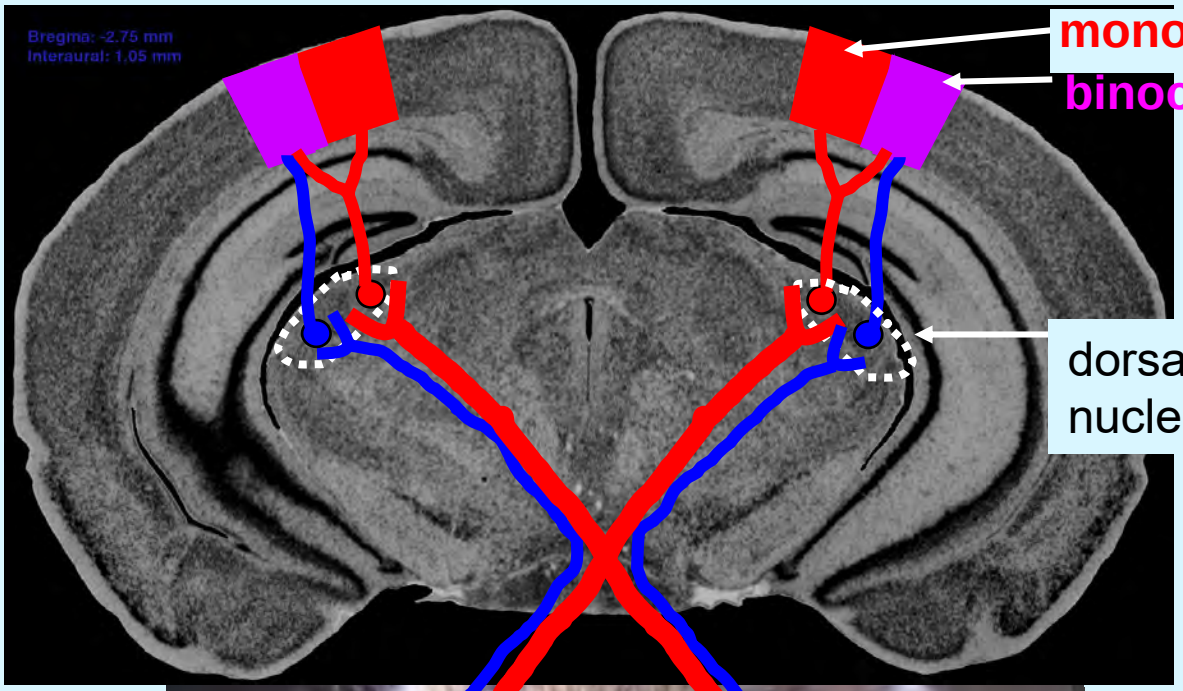
Traditional anatomical techniques were sufficient to work out some of the basic circuitry



Old time genetics in mice, exploiting random mutations collected at Jackson Laboratories or mutations appearing in mice irradiated at the nuclear weapon research facilities in Harwell, UK, and Oak Ridge,TN, had led to some important findings, particularly in the *reeler* line, where two groups showed that neurons in an inside out cortex could make proper connections and receptive fields. In retrospect, these studies are important because they showed that the mouse cortex could be studied physiologically.

The advent of genetic manipulation in mice made some of us initially and, increasingly over the years, most of the field turn to mice.

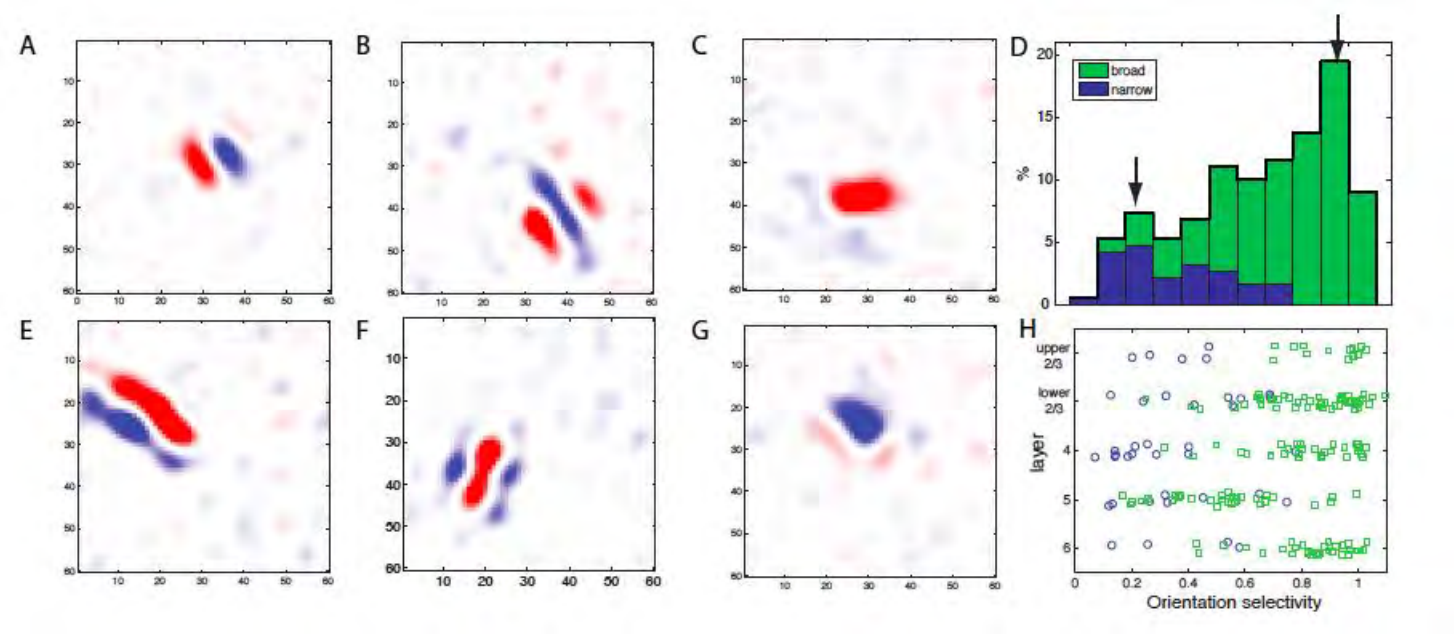
Retino-geniculo-cortical pathway in the mouse



monocular
binocular
} primary visual cortex (V1)

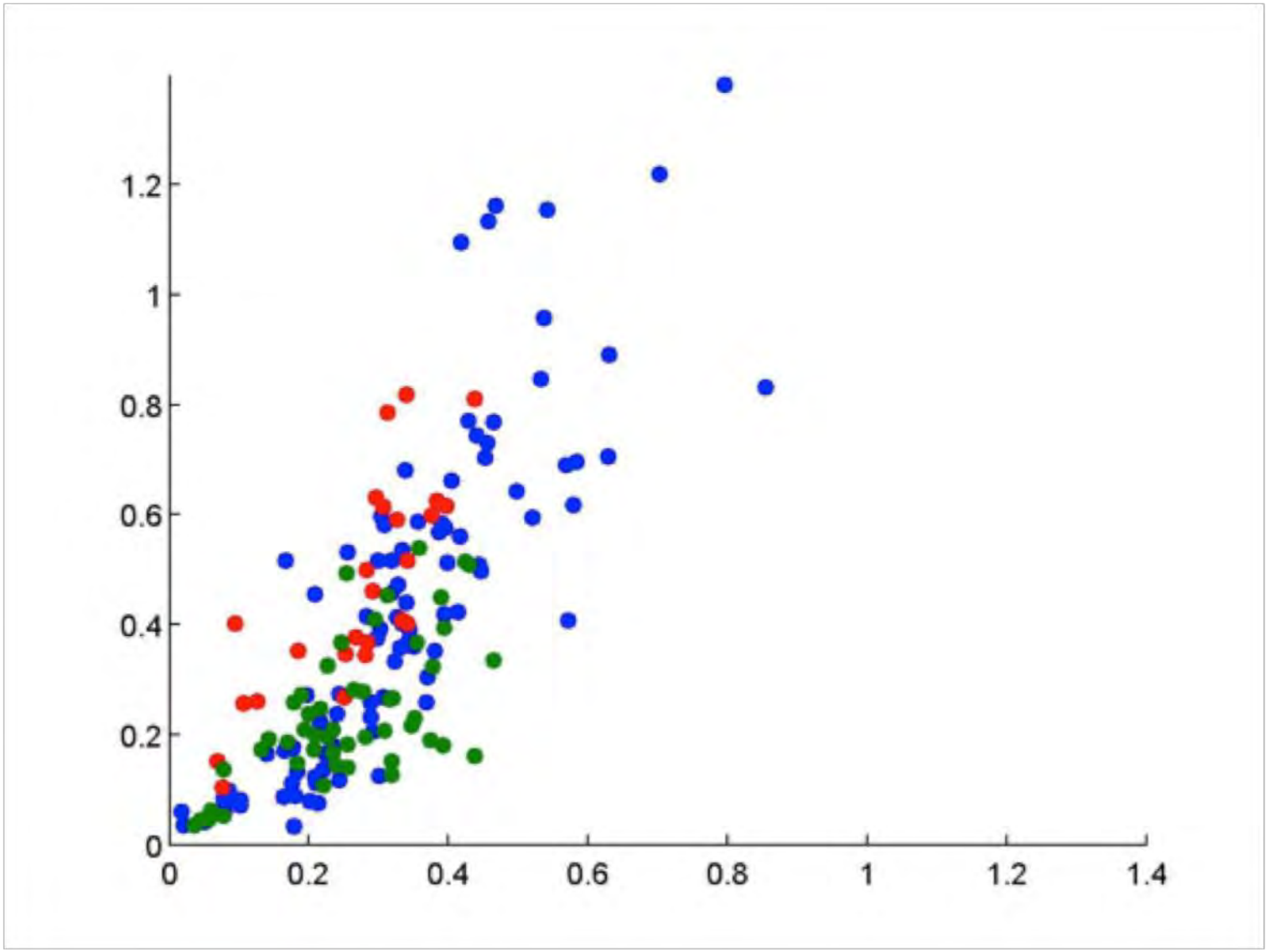
dorsal lateral geniculate nucleus (dLGN)

Receptive fields of neurons in mouse V1 are similar to those in cats primates.

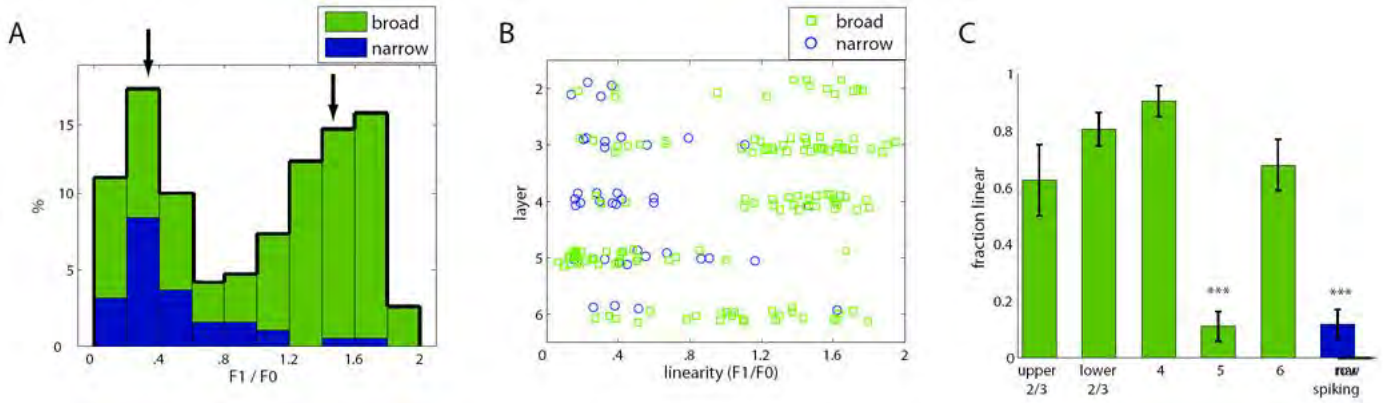


Niell 2008

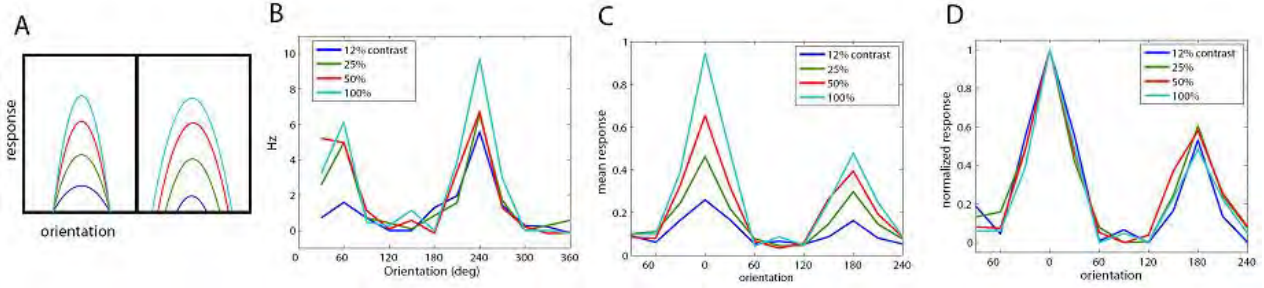
Gabor parameters of Monkey, Cat and Mouse RFs



Most excitatory neurons are highly linear …

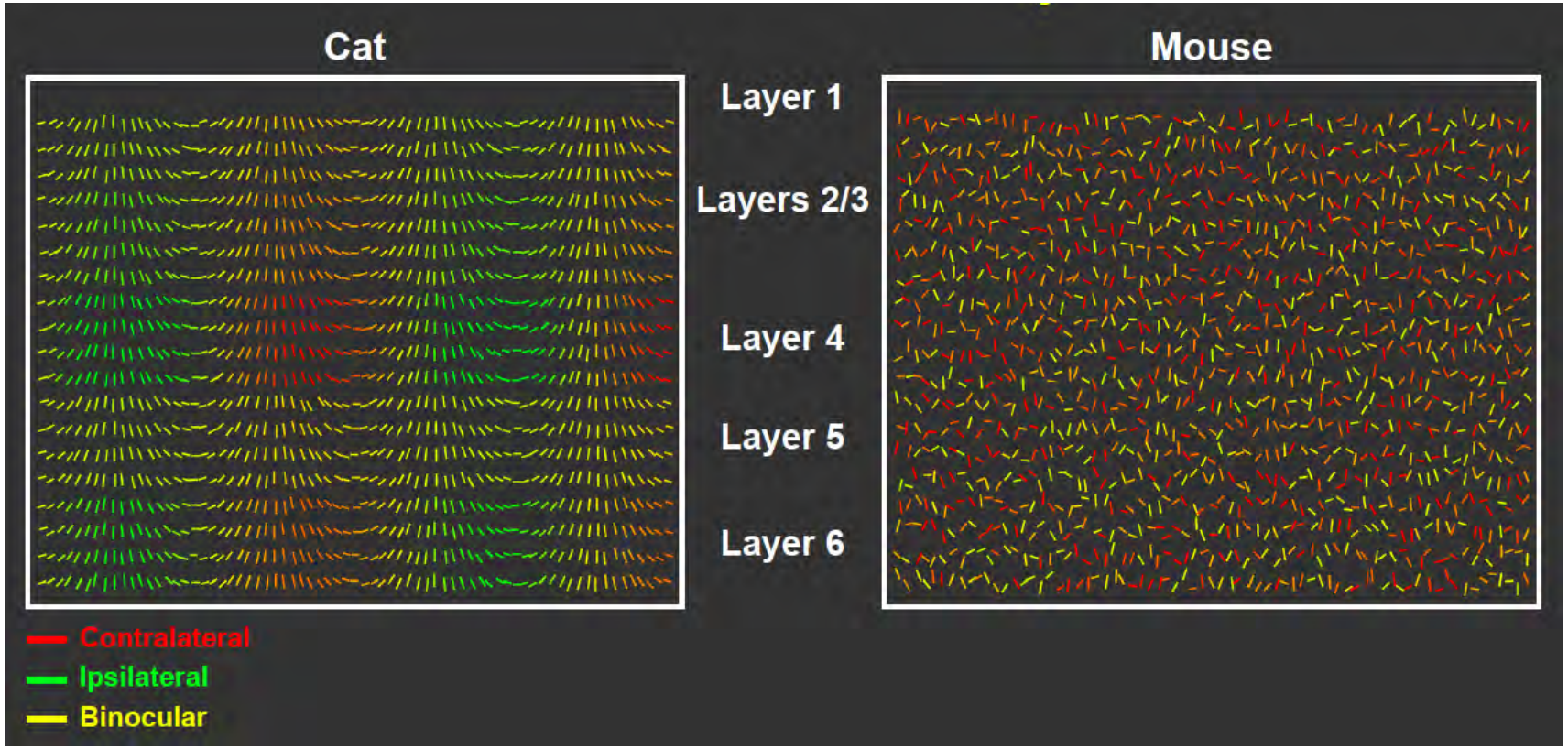


and show highly sophisticated features, such as contrast-invariant orientation tuning



Niell & Stryker, 2008

Mouse visual cortex lacks orientation and ocular dominance columns



A few prominent differences between mouse and human (or other higher primate and carnivore) visual cortex.

Mouse and other rodents lack orientation and ocular dominance columns.

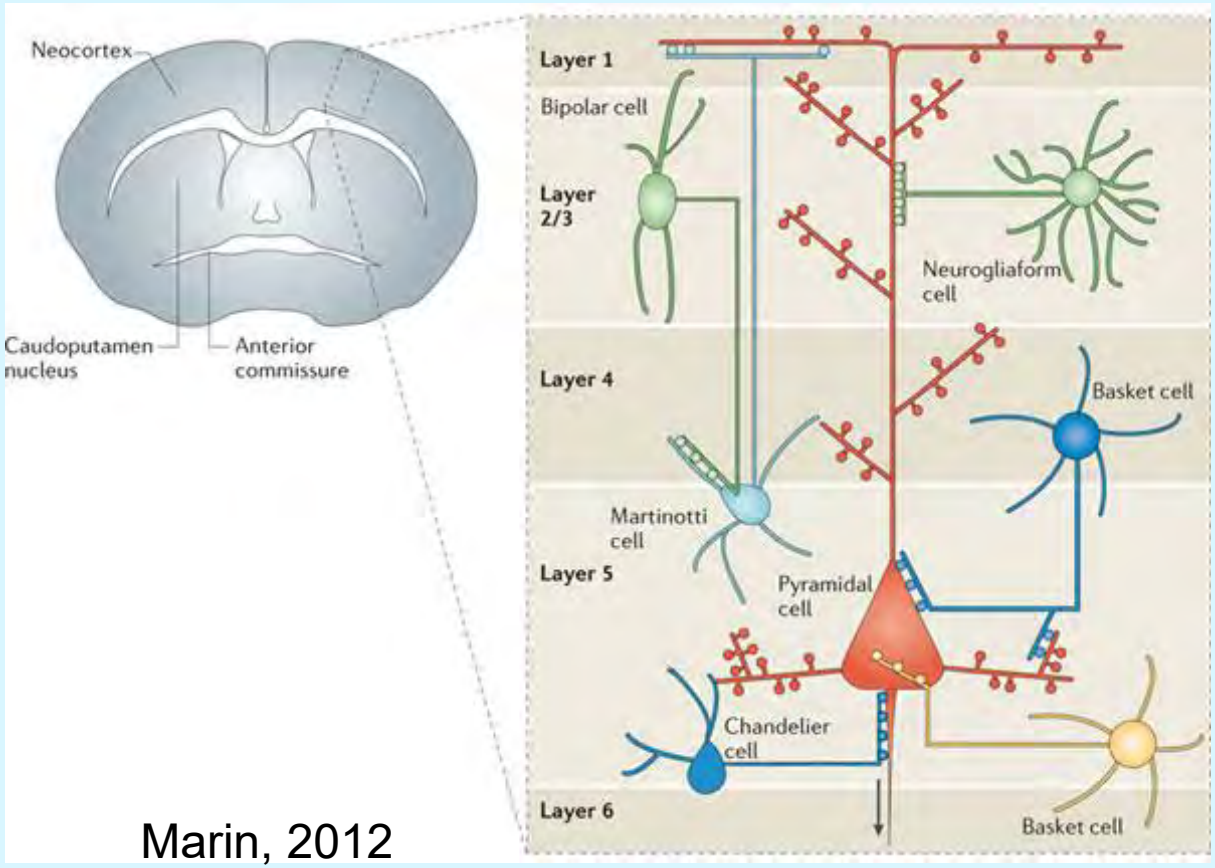
Many compelling similarities.

Same basic structure and cell types as defined by morphology, inputs and outputs, and in may cases gene expression.
Similar sequence of developmental events.
Both have multiple visual areas, dorsal and ventral streams.
Similar response properties and receptive fields of single neurons.
Similar activity-dependent plasticity.

Mouse retina and cortical visual system are a good model for study of human peripheral vision, not foveal vision.

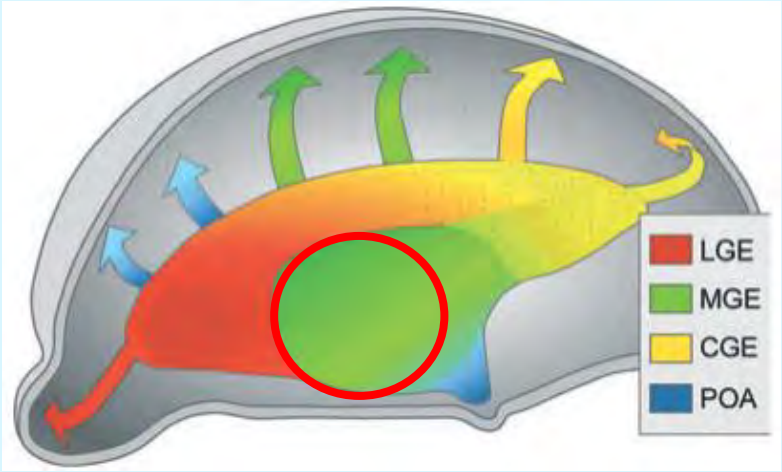
Cortical GABAergic Interneurons

- Provide GABAergic inhibition to post-synaptic neurons
- Integral part of cortical neural circuits
- Defects implicated in neurological diseases: epilepsy, schizophrenia, Alzheimer's disease, etc.

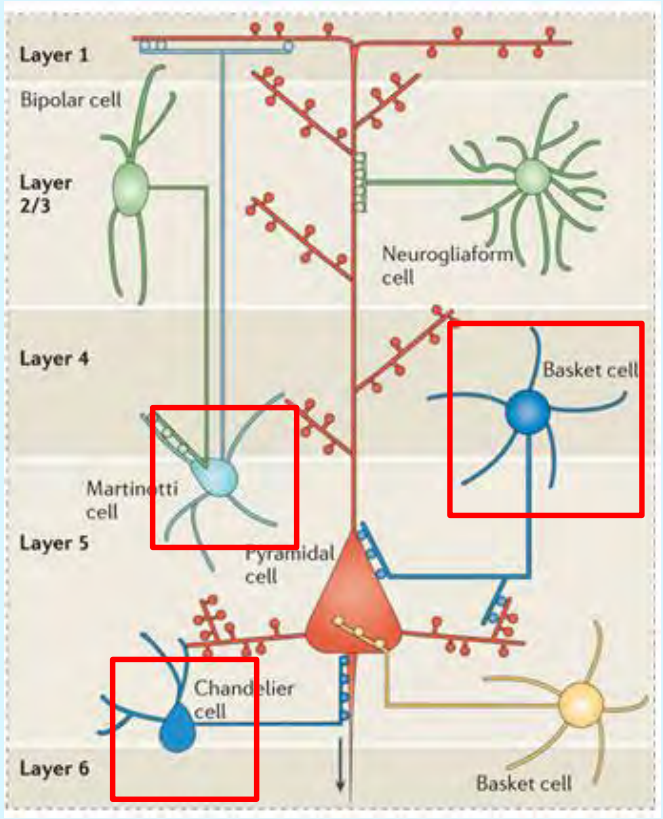


Marin, 2012

The Origin of Cortical Interneurons



Gelman and Marín, 2012



Marín, 2012

How do the interneurons participate in the cortical circuit?

Inhibition of inhibition in visual cortex: the logic of connections between molecularly distinct interneurons

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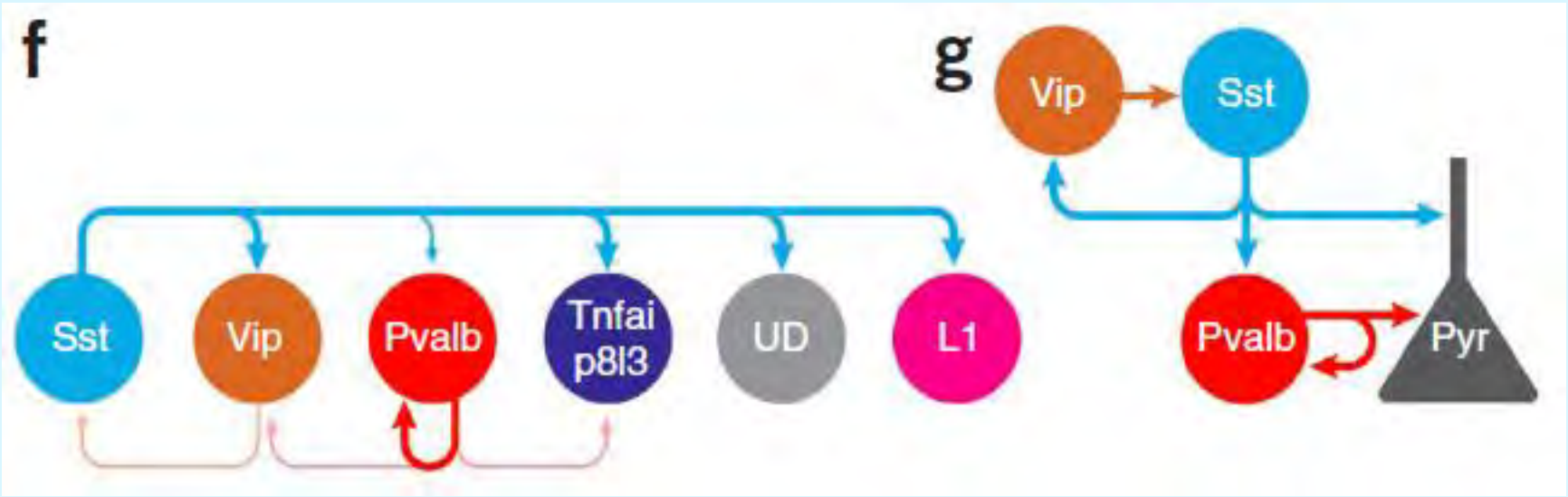
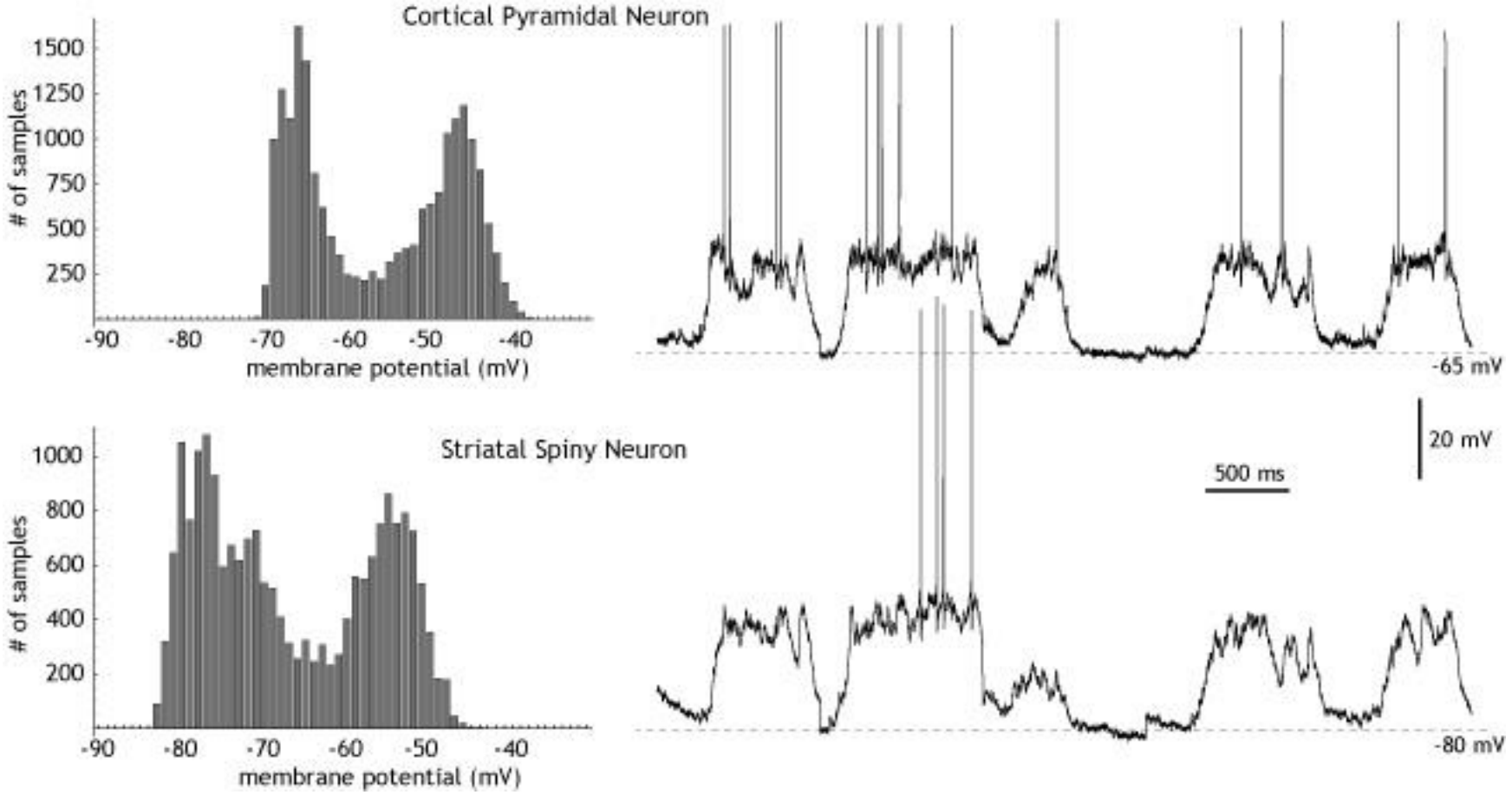
NATURE NEUROSCIENCE ADVANCE ONLINE PUBLICATION



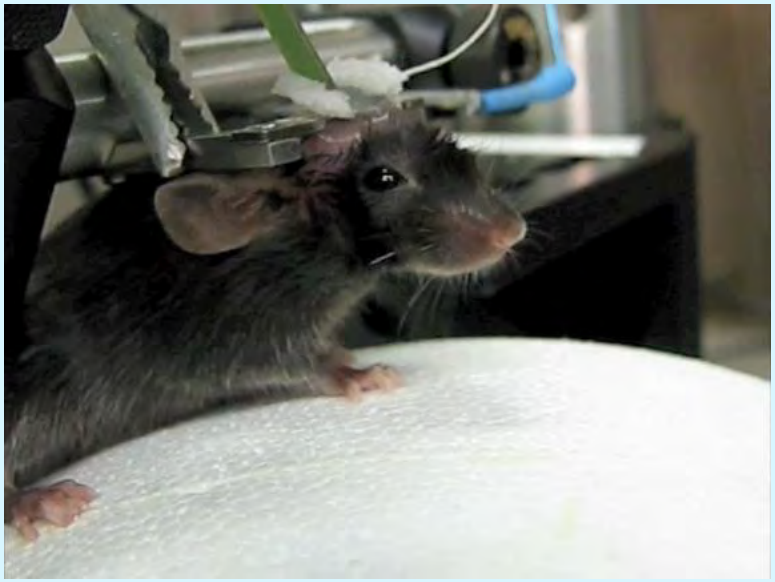
Pfeffer et al., *Nature*, 2013

Cortical States: Studies of mostly of anesthetized animals had shown that the cortex could exhibit several different "states".

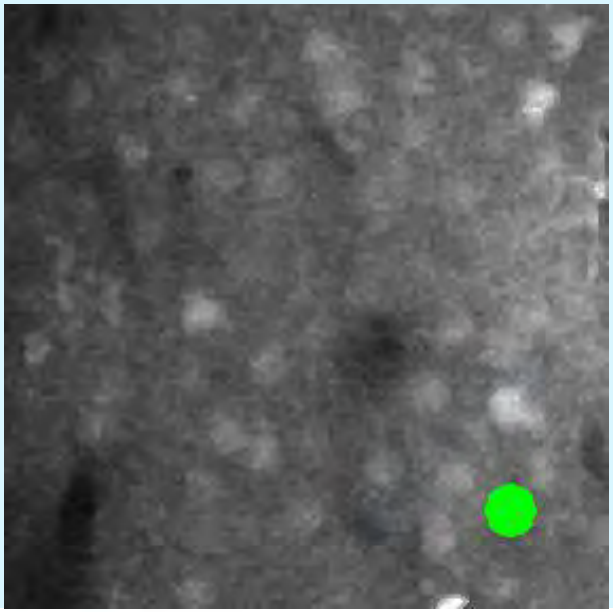
Most prominently there were UP states, in which neurons were more depolarized and more active, and DOWN states

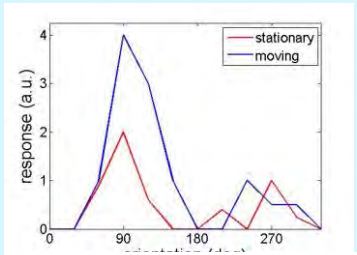


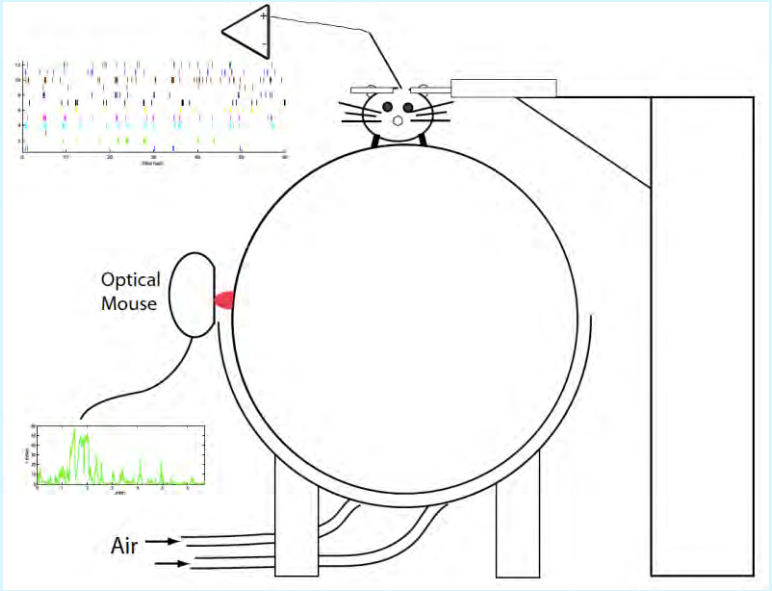
Locomotion enhances cortical responses
Alert animals are mostly or always in the UP state

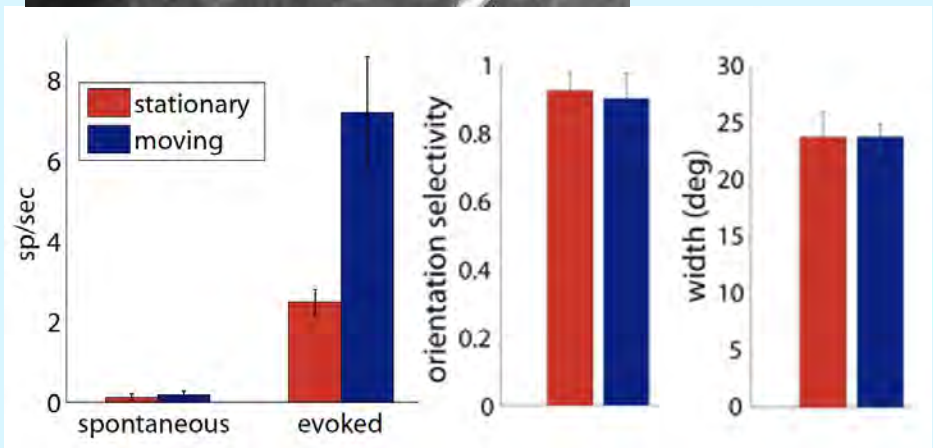


Locomotion specifically increases visual responses in cortex, with no change in LGN or in tuning or spontaneous rate.









Niell & Stryker *Neuron* 2010

There are many highly specific neuromodulatory inputs. For example, the different populations of inhibitory neurons receive distinct inputs from basal forebrain cholinergic nuclei.

In addition there are noradrenergic (from locus coeruleus), serotonergic (from the midline raphé nucleus), and dopaminergic inputs, along with inputs from distant cortical areas.

So it is a tremendous oversimplification to talk about only 2 cortical states. There is much more to learn.

However, the field has now moved away from studying anesthetized preparations, and most modern work favors alert animals, or even animals engaged in a task.

Techniques for studying the cortex

Electrophysiology recording:

Extracellular single-unit recordings and multiple extracellular unit recordings: *measure spike responses and infer connectivity from cross-correlations. Numbers of recording sites are expanding dramatically, now hundreds, soon thousands.*

Intracellular (sharp) recordings: *measure postsynaptic potentials as voltage changes. Low yield in vivo.*

Intracellular (whole-cell or patch) recordings: *measure transmembrane currents in voltage clamp. Low yield in vivo.*

Electro- and Magneto- encephalography (EEG/MEG) and evoked potential recordings: *Slow potential changes related to large scale organized patterns of activity, with good timing resolution (~msec) but poor spatial resolution, and with aliasing difficulties. Frequency tagging and source localization is possible, though rare.*

Ecog to measure surface or local field potentials that sometimes are lawfully related to more or less local underlying neuronal activity. Relationship sometimes related to activity in different frequency bands (α β δ θ γ) *The advantage here is that this can be done in humans*.

Techniques for studying the cortex

Imaging of neural activity was historically useful only for response properties that are locally clustered:

•2-deoxyglucose or activity-dependent gene expression: *past 5-30 minutes of glucose utilization or unknown activity, non-survival, only 1 or 2 stimuli, resolution 50 μm to single cell.*

•Intrinsic signal imaging: *> 4 sec of metabolic activity, averaged for 1-min to a few hours, resolution better than 50 μm, survival.*

•Voltage sensitive dye imaging: < 1 msec response time to changes in local postsynaptic voltage in a population of neurons, usually averaged for *several min, resolution better than 50 μm, survival.*

•Functional magnetic resonance imaging (fMRI): *resolution no better than 4-8 mm^3 at present, averaged to stimulus or response, survival.*

Techniques for studying the cortex

Now imaging can be highly local using intracellular markers such as calcium fluorescence. Genetically encoded calcium sensors can target calcium recordings to particular cell types.

Wide-field calcium imaging can reveal the average activity of many cells in a large area, similar to intrinsic signal imaging but with more rapid signal onset.

More important, 2-photon calcium imaging can show the spiking activity of many single cells simultaneously. Furhtermore, the same cells can be studied over days to wekks to months, permitting studies of plasticity at the single cell level.

Limitations are that only part of the dynamic range of responses can be captured and that in general increases can be measured.

Not only calcium but also neurotransmitters (eg, GluSnFR) and other signaling molecules can be measured with optical techniques when fluorescent sensors are created. These can be cell-type specific.

Techniques for studying the cortex

Anatomy:

Single cell reconstruction, possibly in combination with physiology. Both cells and large afferent terminals can be recorded from and filled. *Both filling and reconstruction are very difficult for connections longer than 1-2 mm.*

Anterograde connection tracing from extracellular deposits of labeled amino acid, sugar, lectin, or other molecule, detected by fluorescence, by histochemistry or by immunohistochemistry. *Generally lacks single cell resolution.*

Retrograde connection tracing from extracellular deposits of labeled lectin or other molecule, detected by fluorescence, by histochemistry or by immunohistochemistry. *Generally lacks single cell resolution.*

Multi-photon imaging can show dendrites, axons, spines and presynaptic boutons, and changes over time can be observed.

Electron microscope reconstructions can show actual connections in detail.

Molecular resolution can be achieved with super-resolution microscopy.

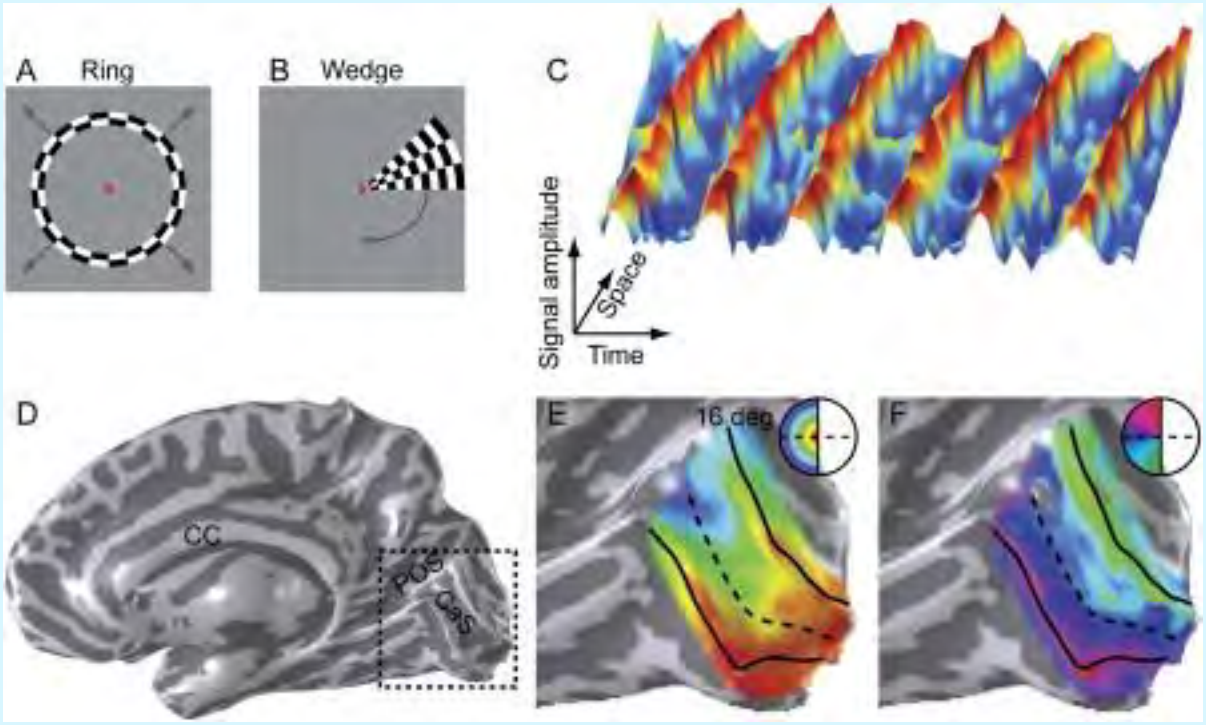
Techniques for studying the cortex

Optogenetics (channelrhodopsin, halorhodopsin, etc) and chemo-genetics (eg DREADs, ivermectin, etc) has allowed cell-type specific perturbation of activity. These techniques are now routine in mice and becoming available in other species.

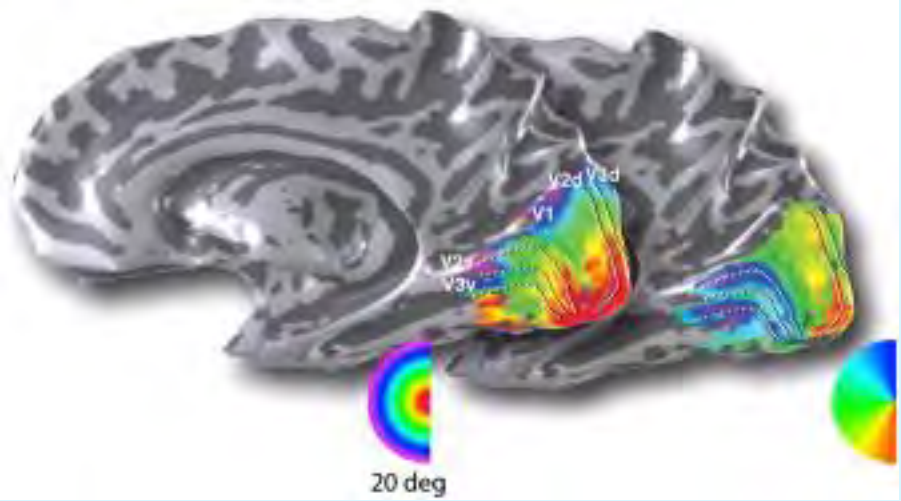
Advances in optics allow these perturbations to be made in real time with single cell resolution.

fMRI in humans

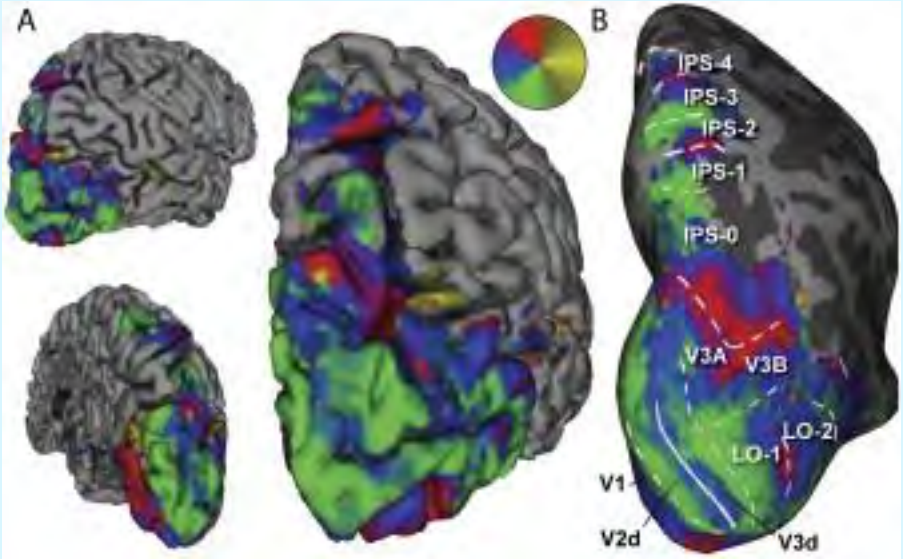
Wandell (2007) Visual field maps in human cortex. Neuron 56 : 366-383



V1, V2 & V3



V3A, V3B, V6,
and IPS-X

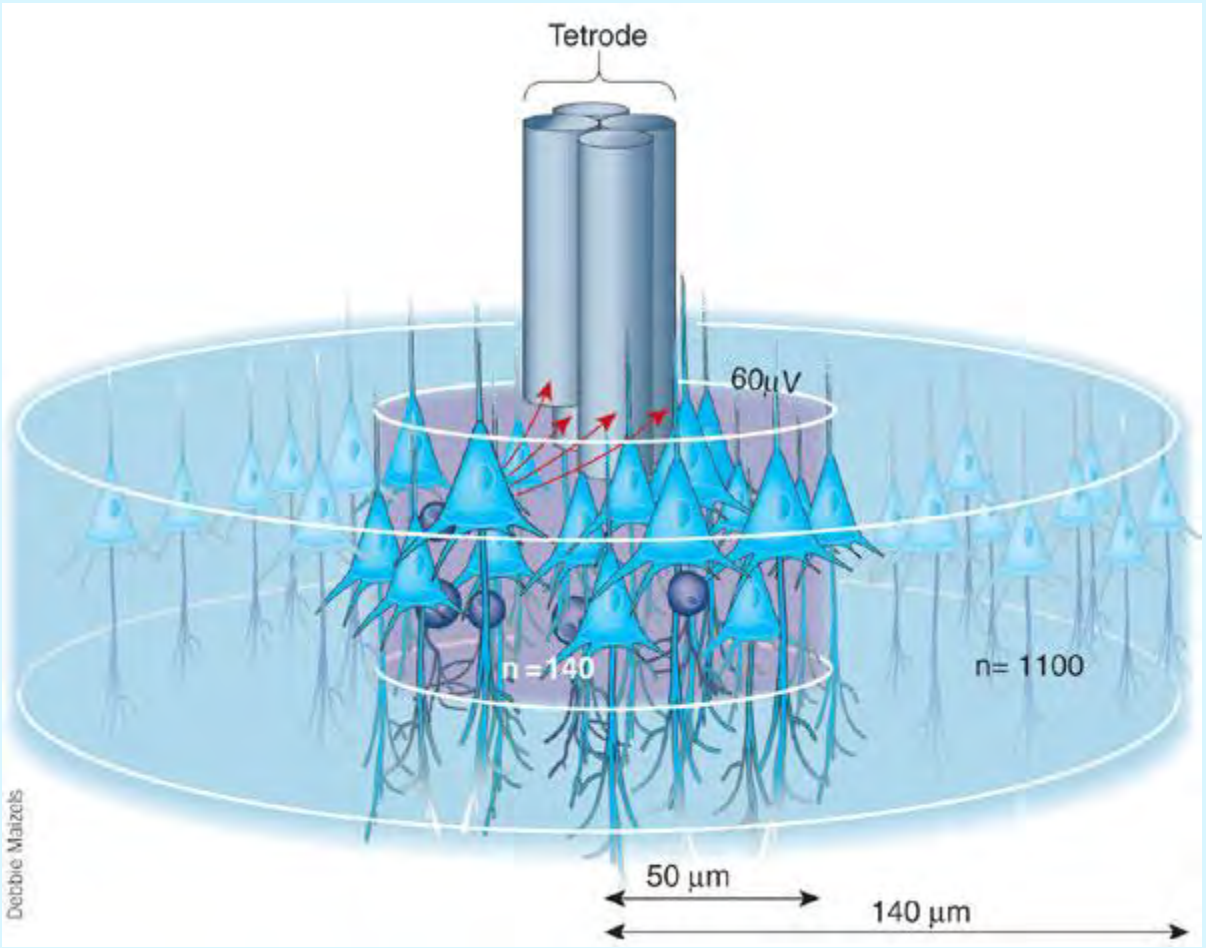


New techniques: Studying the visual responses of many neurons simultaneously with multielectrode extracellular recording.

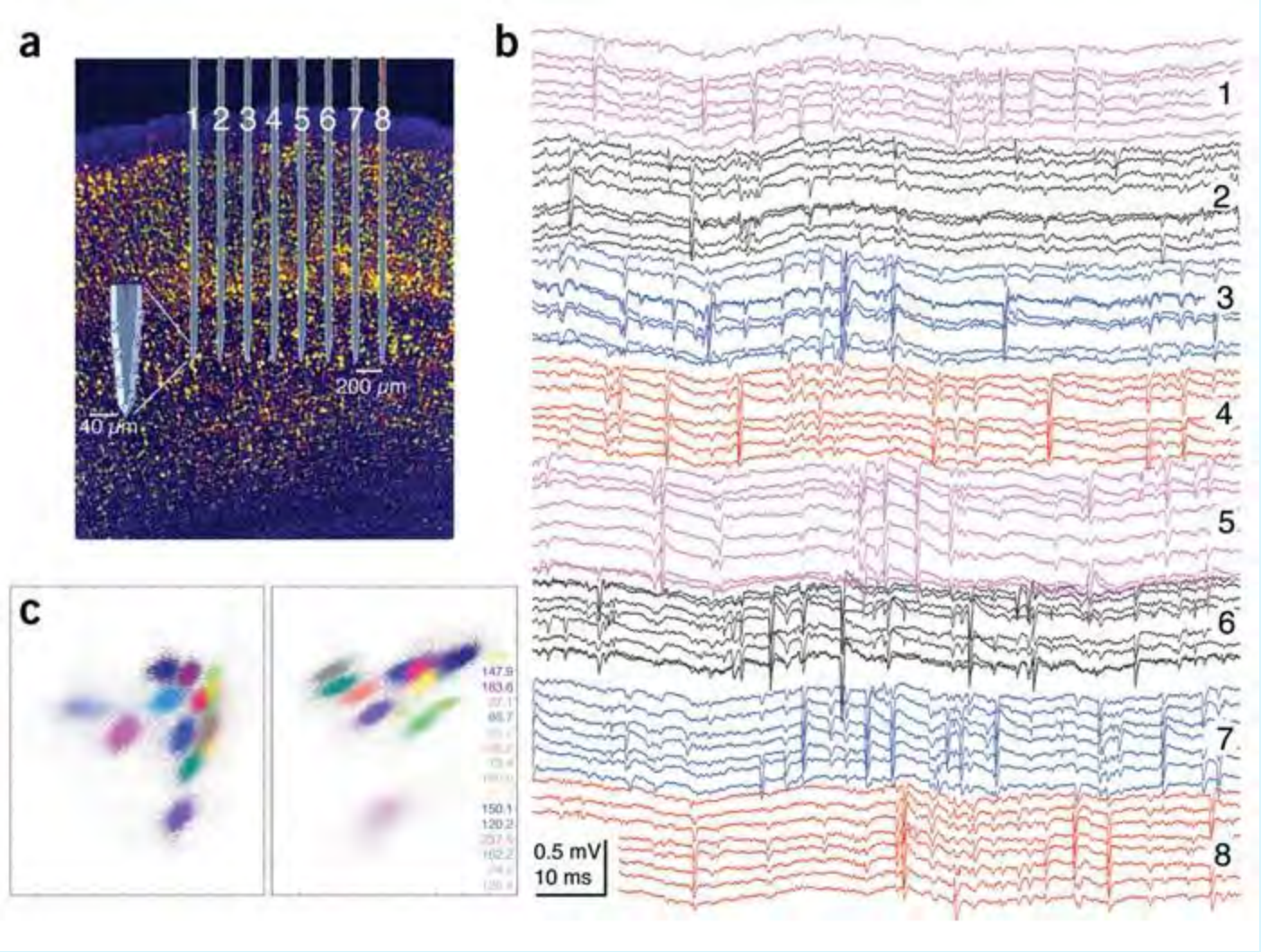
G. Buzsáki (2004) Large-scale recording of neuronal ensembles *Nature Neuroscience* 7: 446 – 451.

Unit isolation quality varies as a function of distance from the electrode.

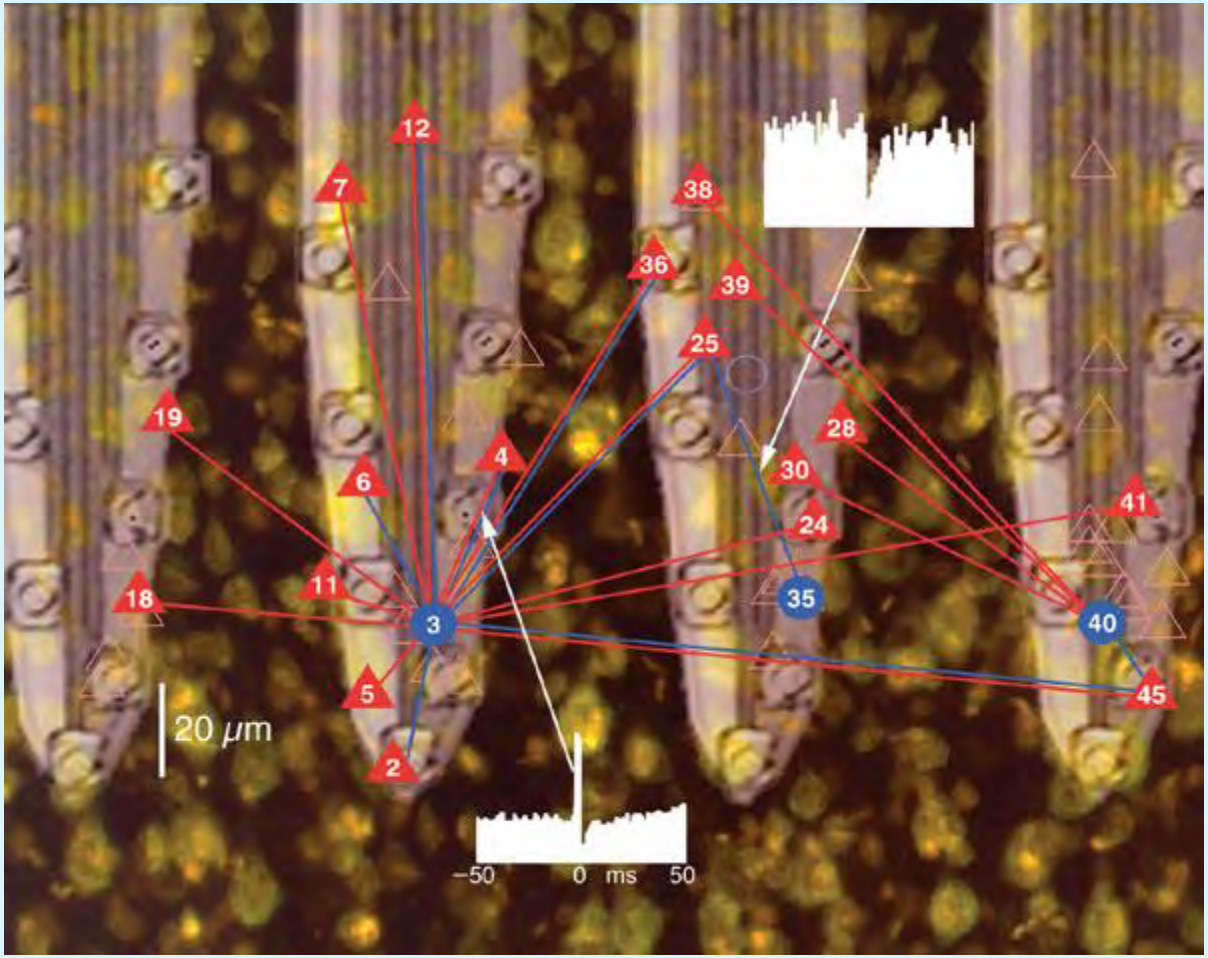
Multisite electrodes (a wire tetrode, for example) can estimate the position of the recorded neurons by triangulation. Distance of the visible electrode tips from a single pyramidal cell (triangles) is indicated by arrows. The spike amplitude of neurons (>60 muV) within the gray cylinder (50 mum radius), containing approx 100 neurons, is large enough for separation by currently available clustering methods. Although the extracellularly recorded spike amplitude decreases rapidly with distance, neurons within a radius of 140 mum, containing approx1,000 neurons in the rat cortex can be detected.



High-density recording of unit activity in the somatosensory cortex of the rat. An eight-shank silicon probe in layer 5. Buzsáki (2004)

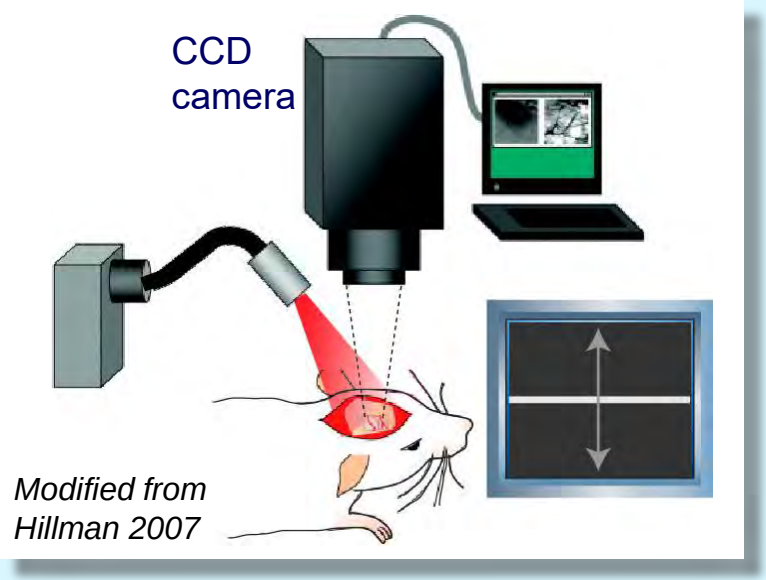


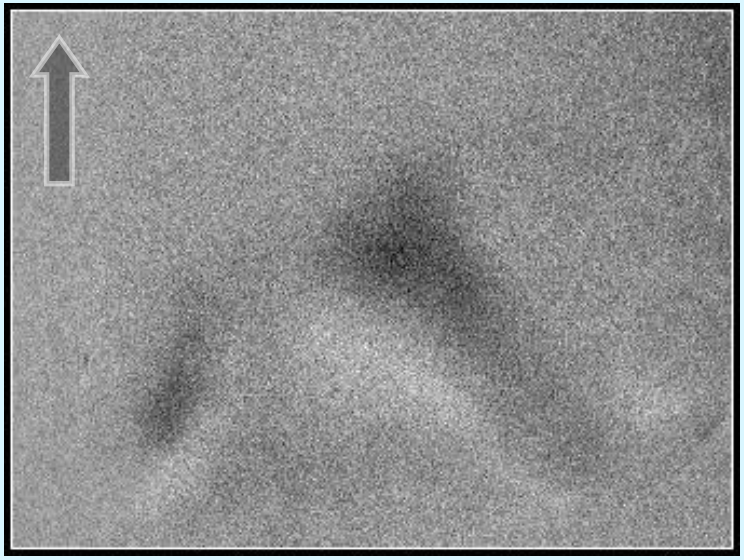
Functional topography and connectivity inferred from cross-correlation in the rat somatosensory cortex. Filled symbols show participating pyramidal cells (red triangles) and interneurons (blue circles). Empty symbols show neurons that are not connected functionally. Buzsáki (2004)



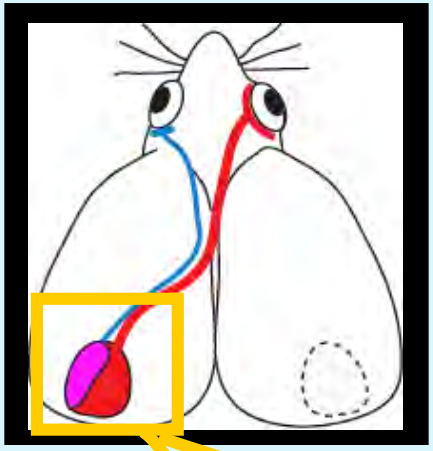
Optical Imaging of Intrinsic Signals

- Intrinsic signals = changes in light reflectance in brain tissue due to hemodynamic responses evoked by neural activity
- Most important factor is greater absorption of red light by deoxyhemoglobin than oxyhemoglobin *(Bonhoeffer and Grinvald, 1996*).





Measuring visual responses using intrinsic signal imaging



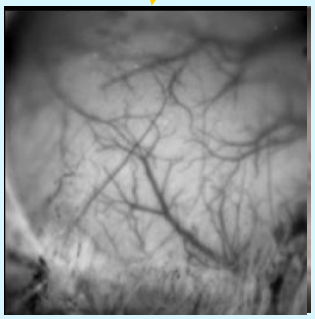
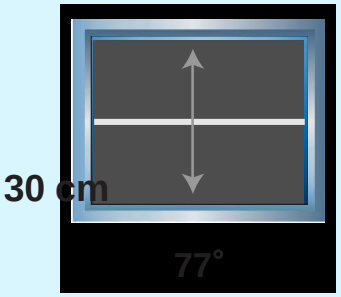
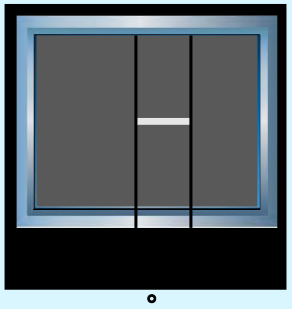


image through intact skull

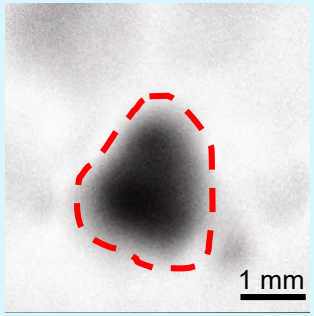
binocular + monocular areas



binocular area

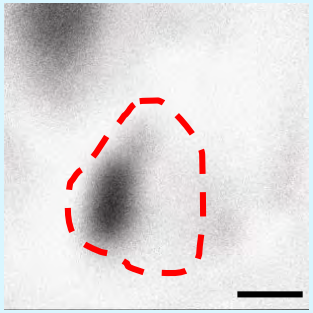


Contra (right) eye stimulation

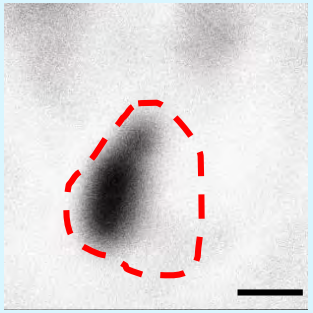


ΔR/R x10⁴

Ipsi eye stim.



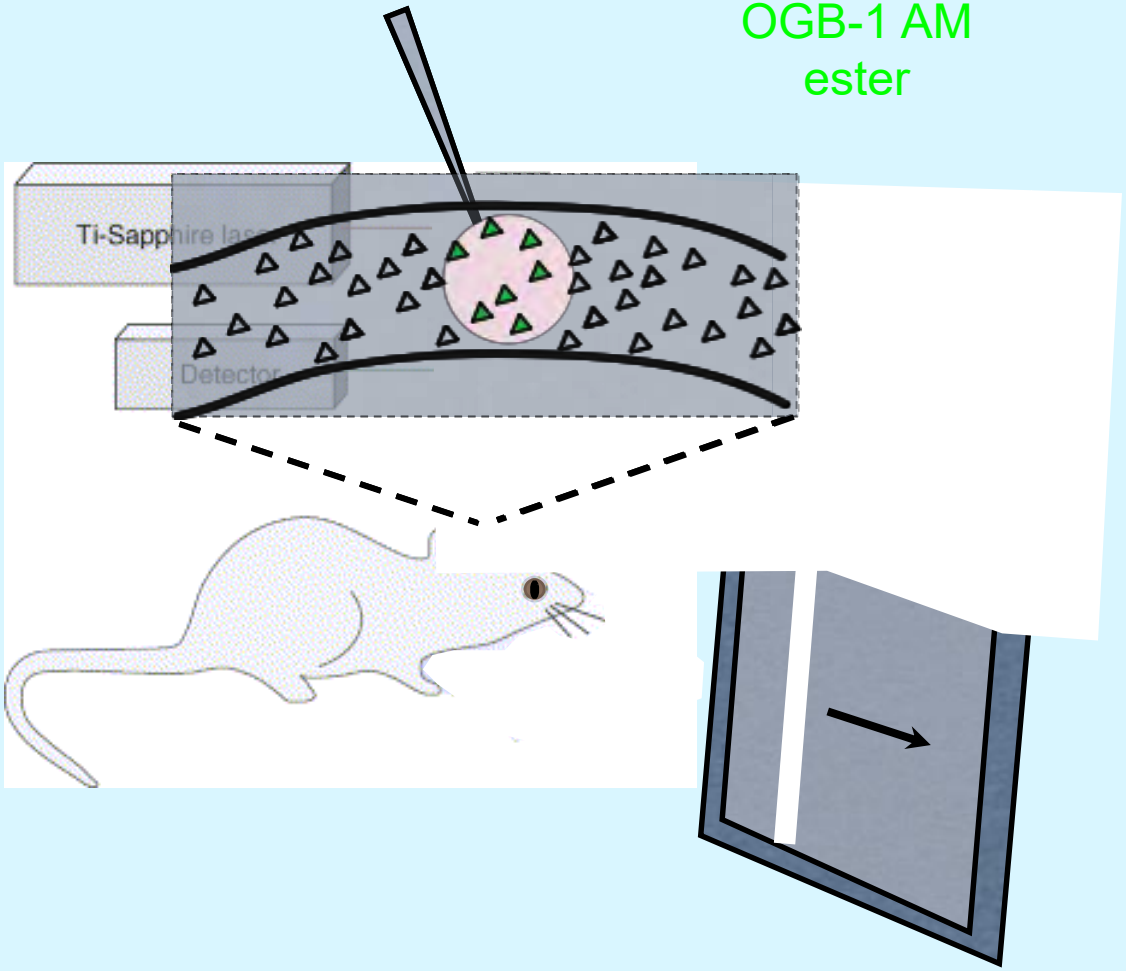
Contra eye stim.



Ocular dominance index (ODI) = (C-I) / (C+I)

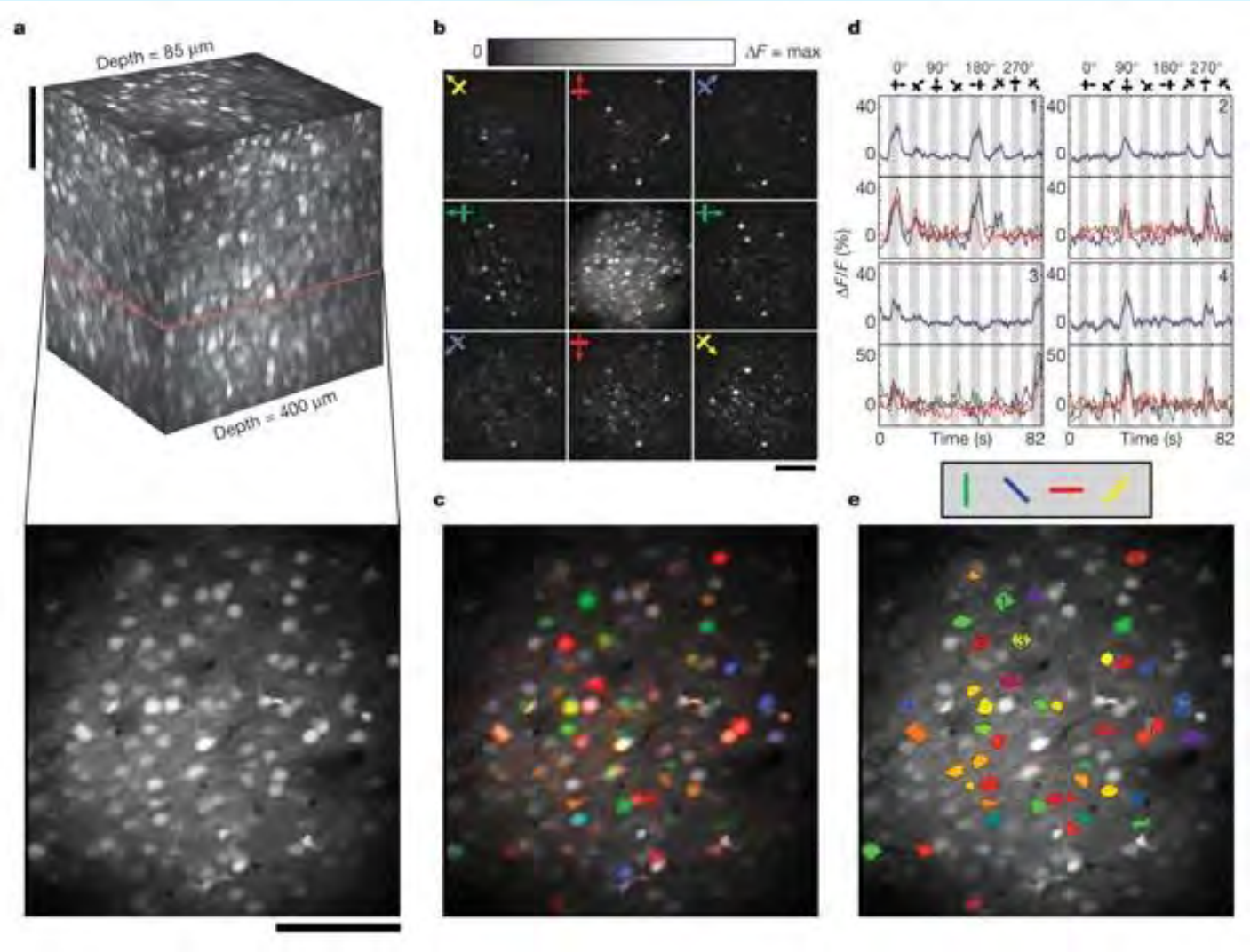
New techniques: Studying the visual responses of many neurons simultaneously with 2-photon optical recording of calcium transients, pioneered by Arthur Konnerth lab (Stosiek et al, PNAS, 2003)

Bulk loading of calcium dye into neurons *in vivo*

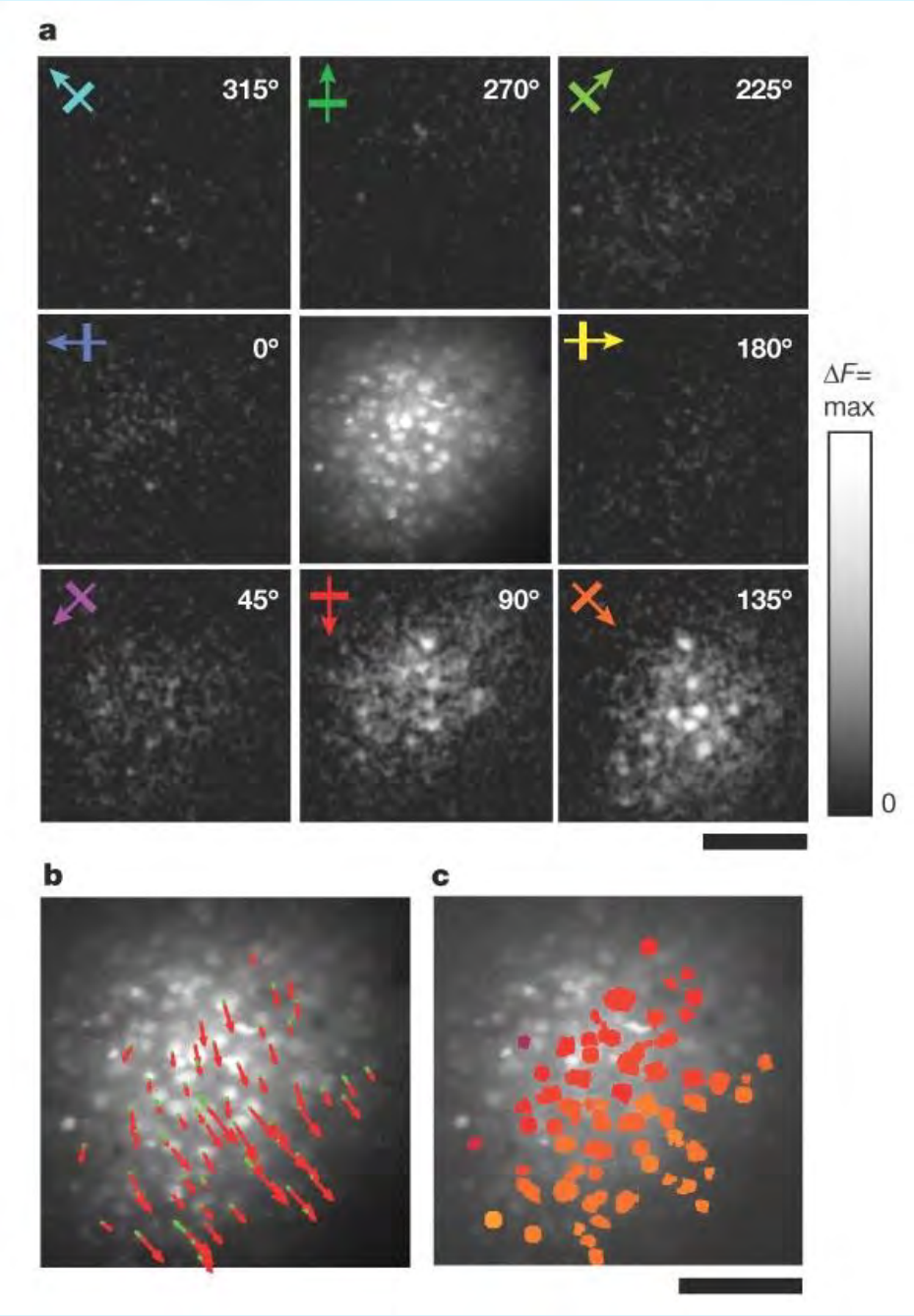


First use in visual system by Clay Reid lab. Ohki K, Chung S, Ch'ng YH, Kara P, Reid RC. (2005) Functional imaging with cellular resolution reveals precise micro-architecture in visual cortex. *Nature*. 433:597-603.

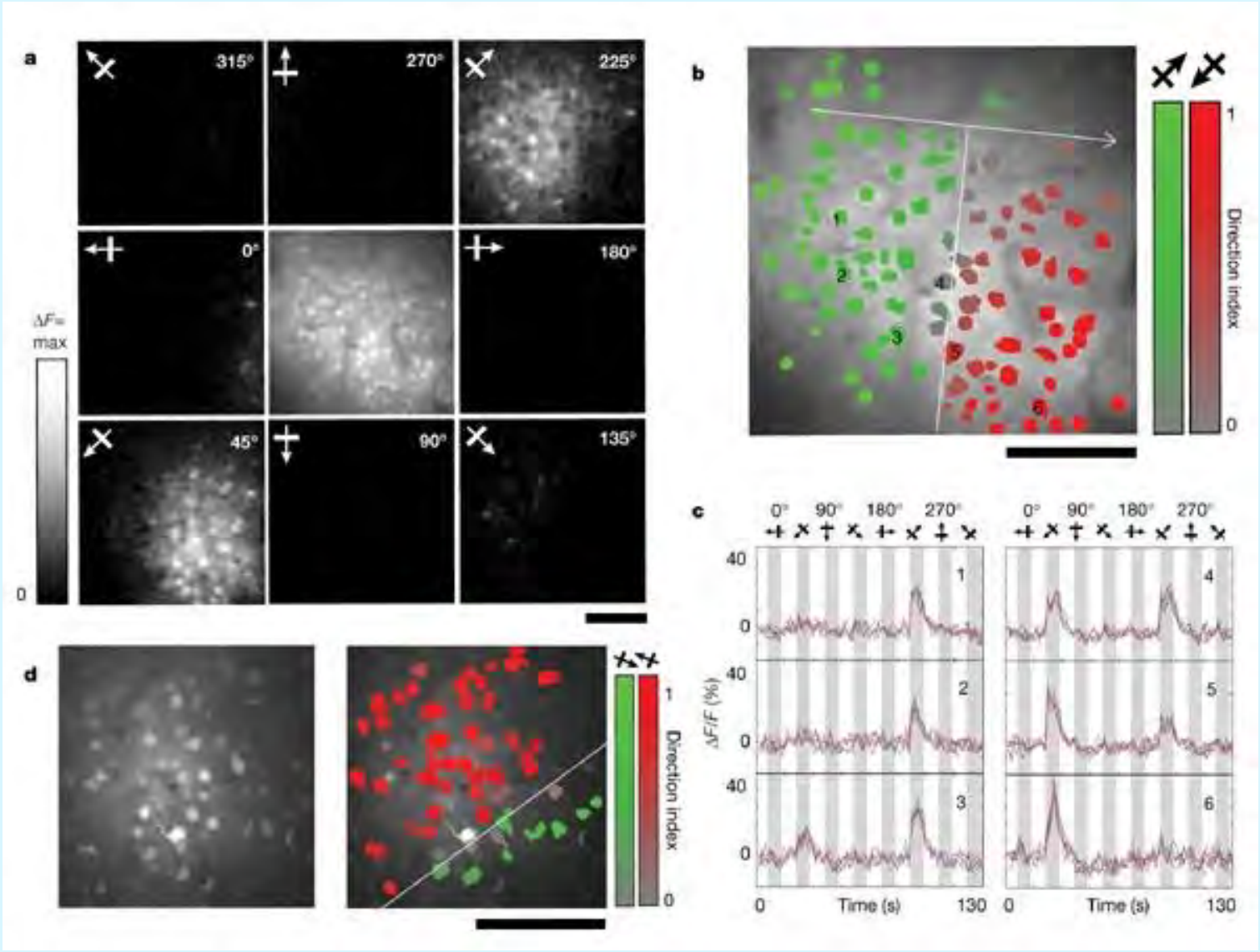
Functional maps of selective responses in rat visual cortex with single-cell resolution.



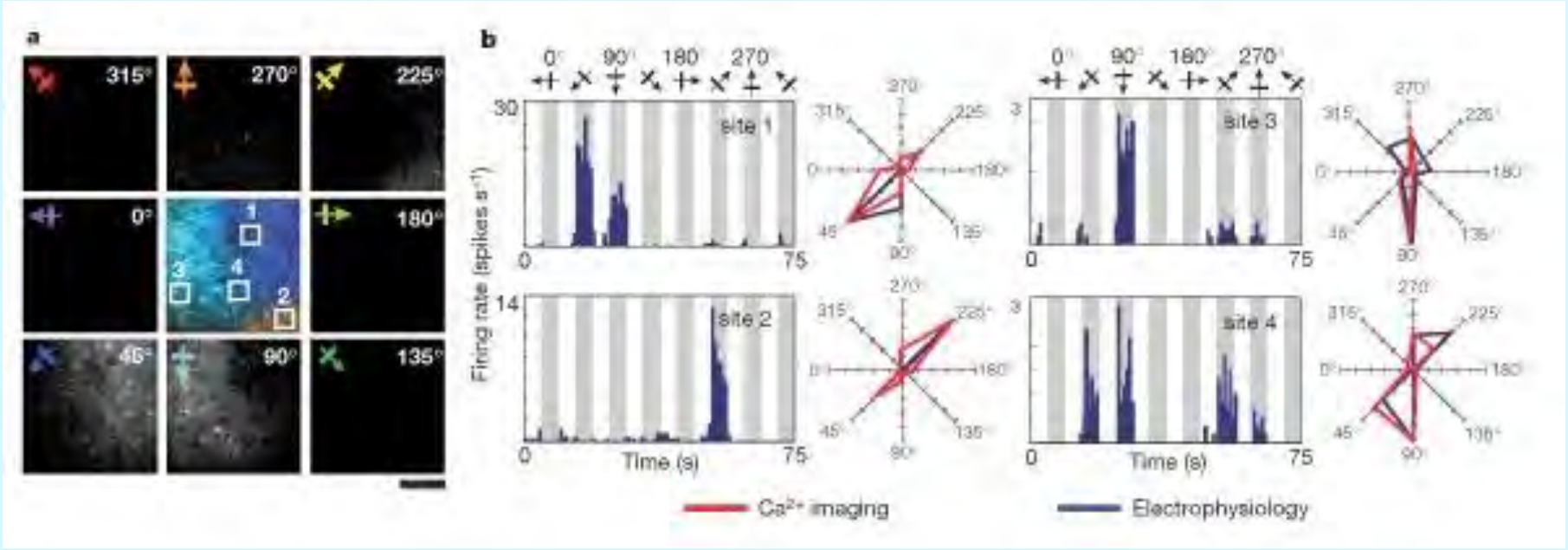
Smoothly changing direction map in cat visual cortex. (Ohki et al, 2005)



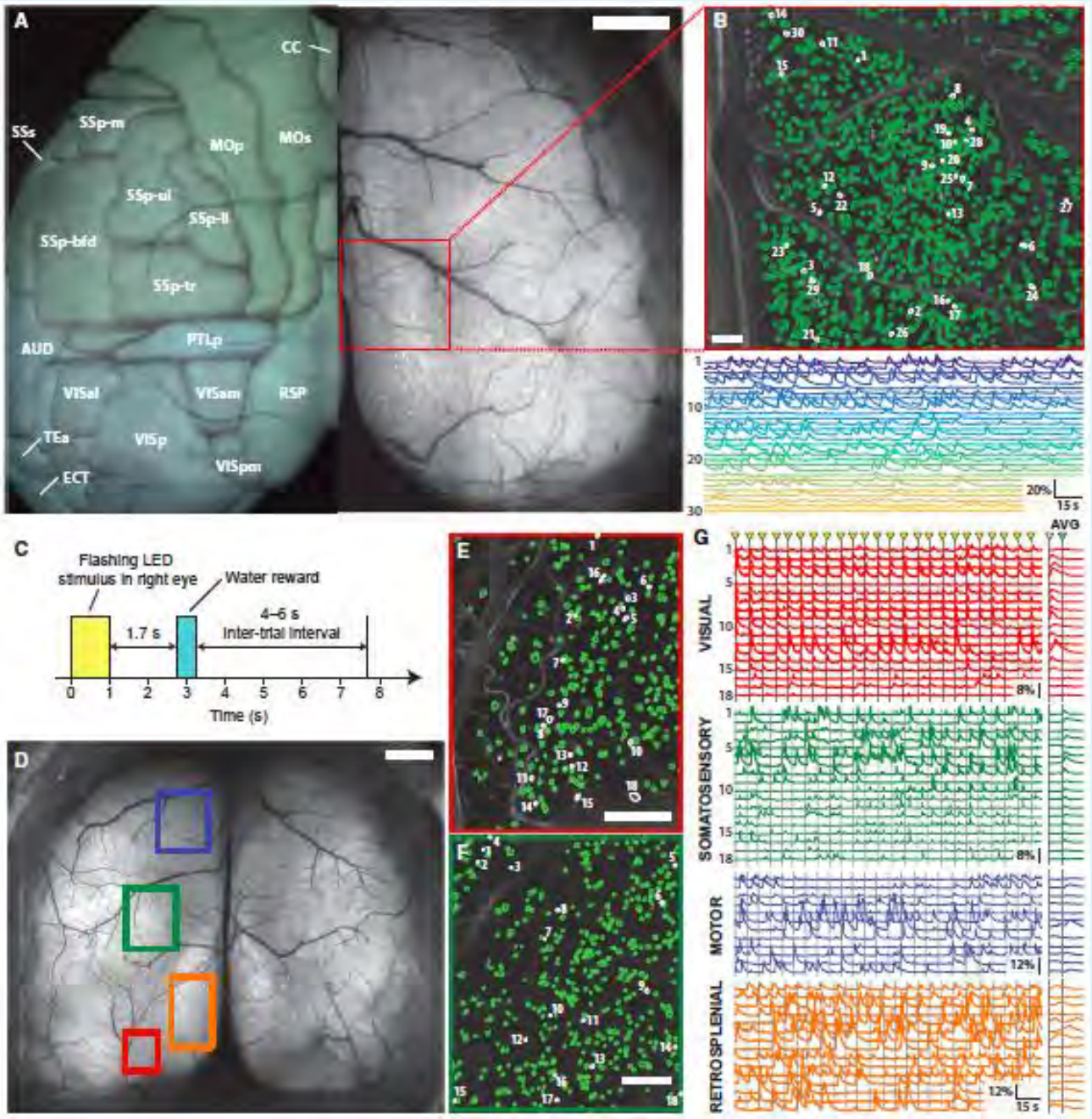
Direction discontinuity in cat visual cortex. (Ohki et al, 2005)



Correspondence of direction tuning obtained by calcium imaging and single-unit electrophysiology in cat visual cortex. (Ohki et al, 2005)

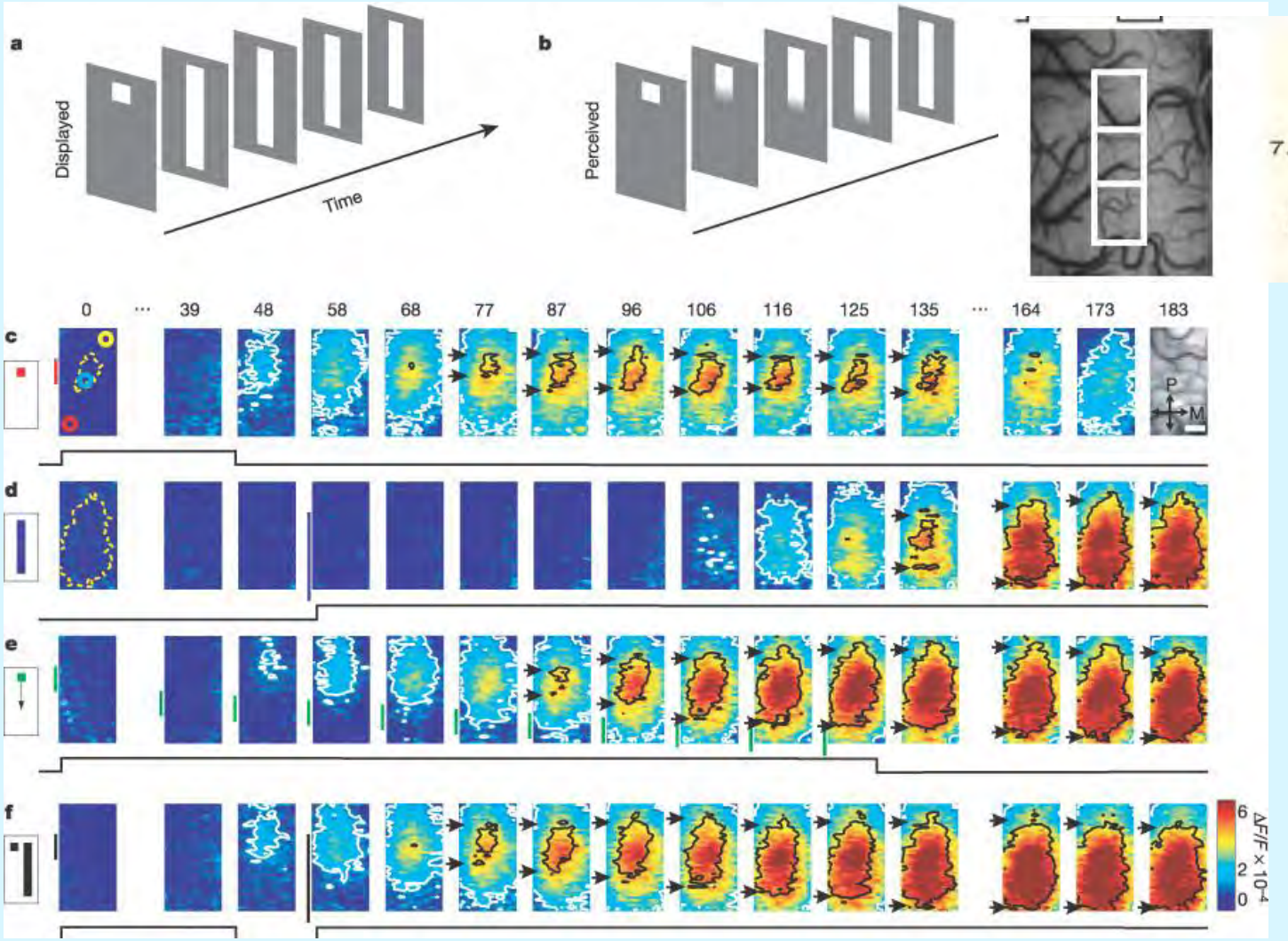


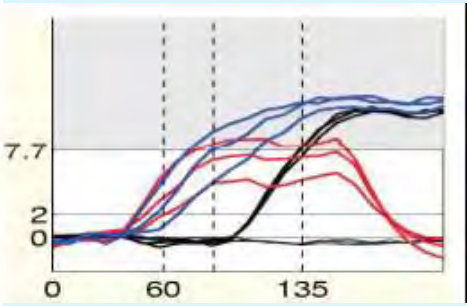
Both wide and single-cell activity with genetically encoded calcium sensors



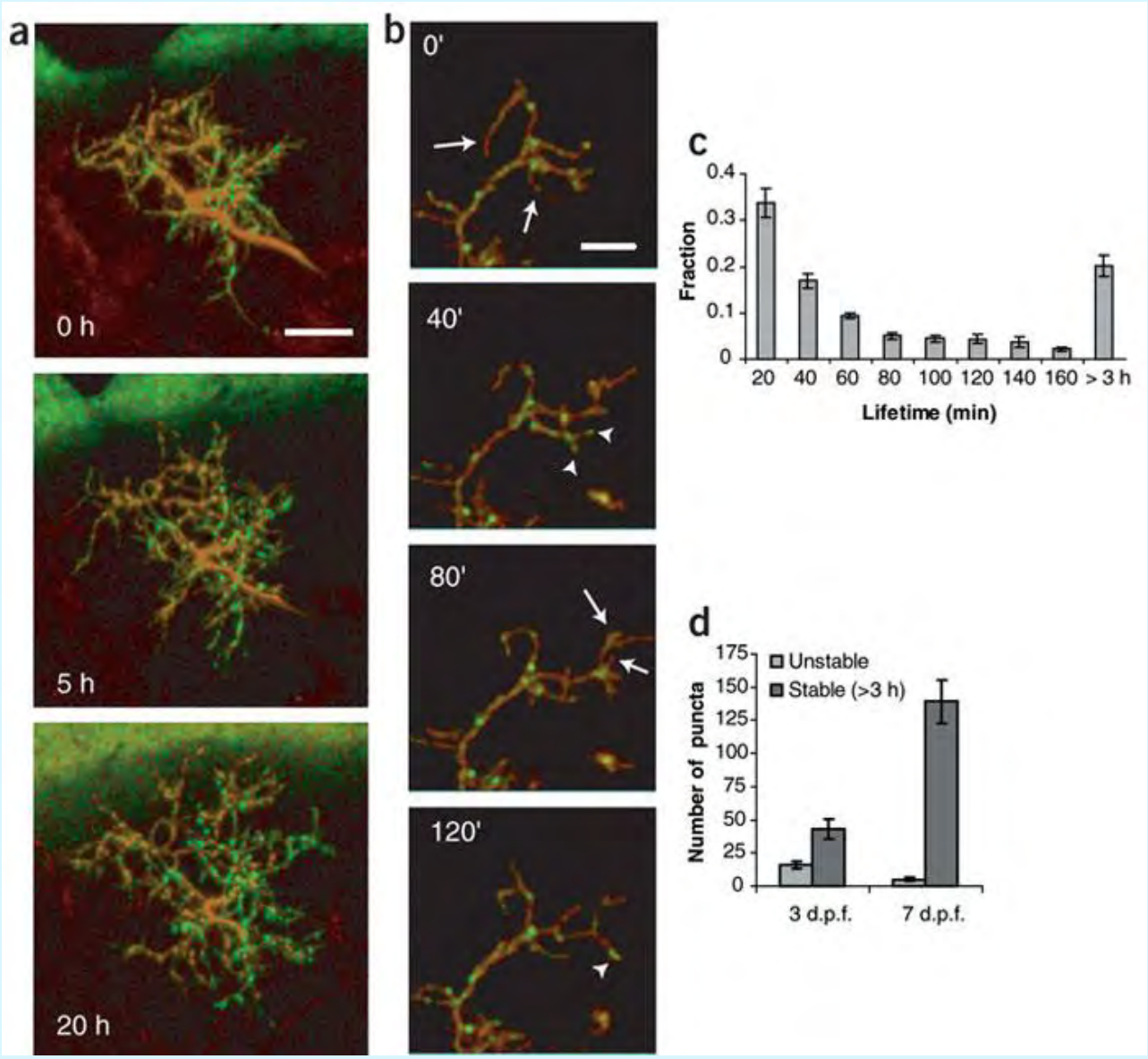
Kim et al., 2016, Cell Reports 17, 3385–3394

Voltage sensitive dye imaging of a motion illusion in cat visual cortex. Jancke, D, Chavane, F, Naaman, A & Grinvald, A (2004) Imaging cortical correlates of illusion in early visual cortex *Nature* 428: 423-426

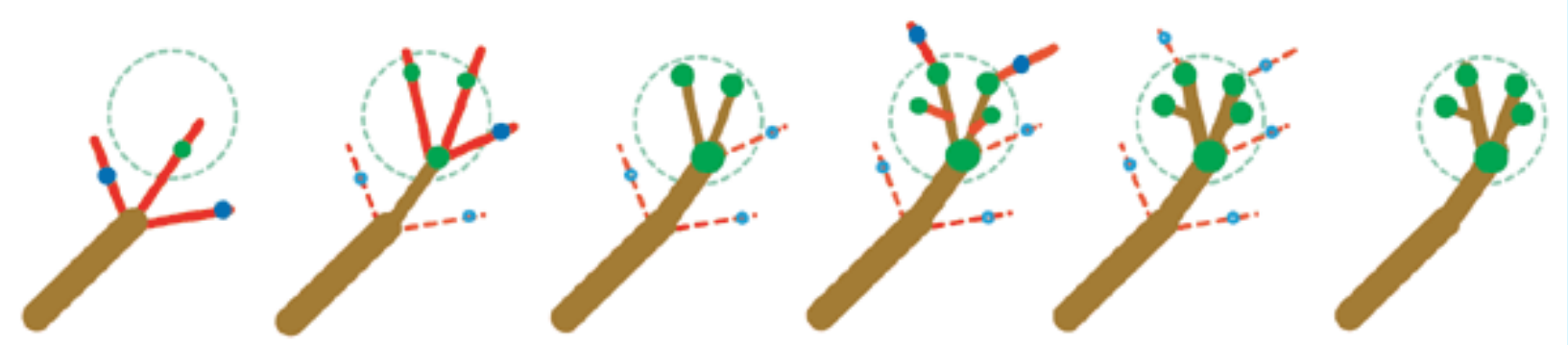




New Techniques: Anatomy can be dynamic. Niell, CM, Meyer, MP & Smith, SJ (2004) In vivo imaging of synapse formation on a growing dendritic arbor *Nature Neuroscience* 7: 254 – 260. Zebrafish dendritic filopodia and boutons (arrowheads)



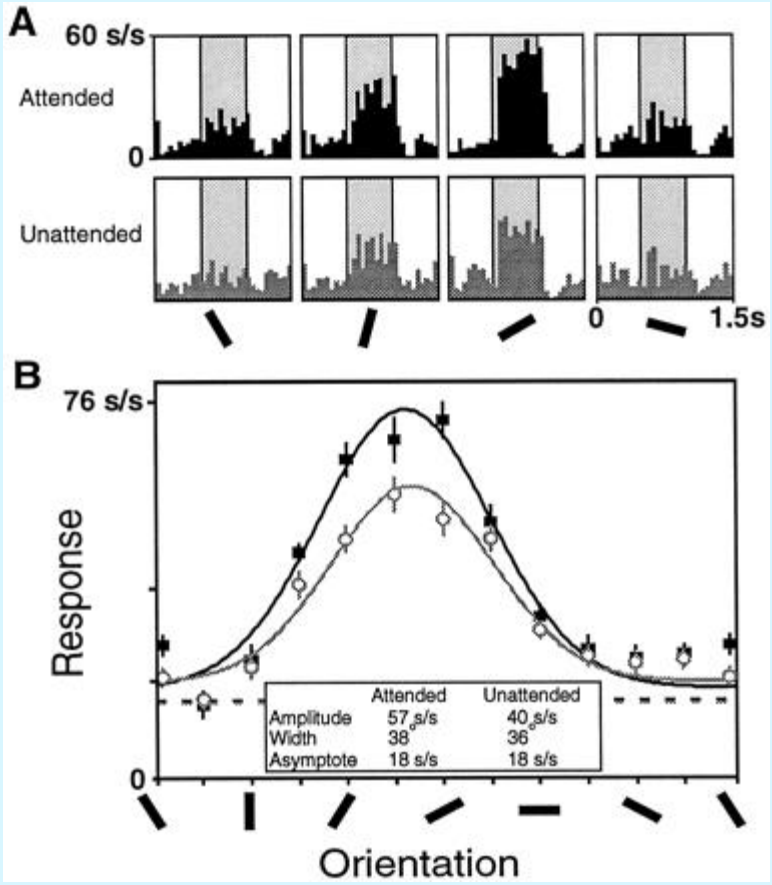
New Techniques: Models for development or function can be inferred from dynamic anatomy. (Niell at al, 2004)



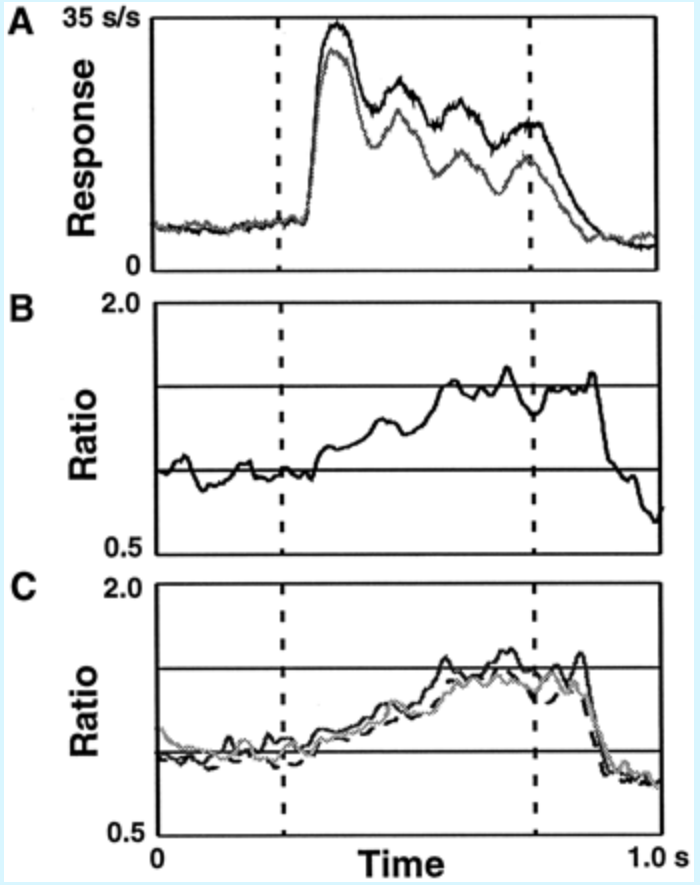
Model of synaptotropic guidance of dendrite growth.

A number of filopodia (solid red) extend from a dendritic branch. Those that encounter correct partners and form synaptic contacts (green dots) are stabilized as new branches (brown), whereas those that establish inappropriate contacts (blue dots) are retracted (dashed red). Successive rounds of selective stabilization result in arborization within a field of appropriate synaptic connections (dashed green region)

New Techniques: Alert animals can allow one to investigate attention, perceptual learning, and vision in a behavioral context. McAdams CJ, Maunsell JH. (1999) Effects of attention on orientation-tuning functions of single neurons in macaque cortical area V4. *J Neurosci*. 19:431-41.

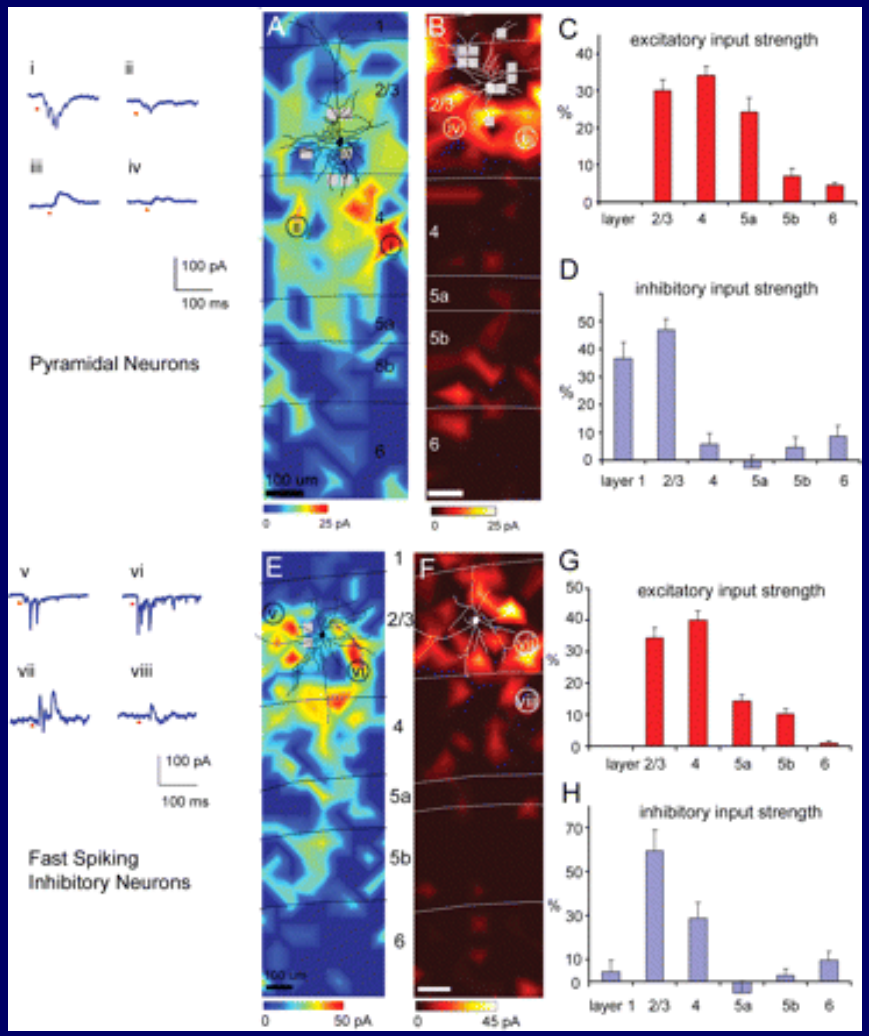


Data from one V4 cell showing enhanced responses in the attended mode (*black*) relative to the unattended mode (*gray*)



The time course of the attentional effect. attended (*black*) and unattended (*gray*) modes

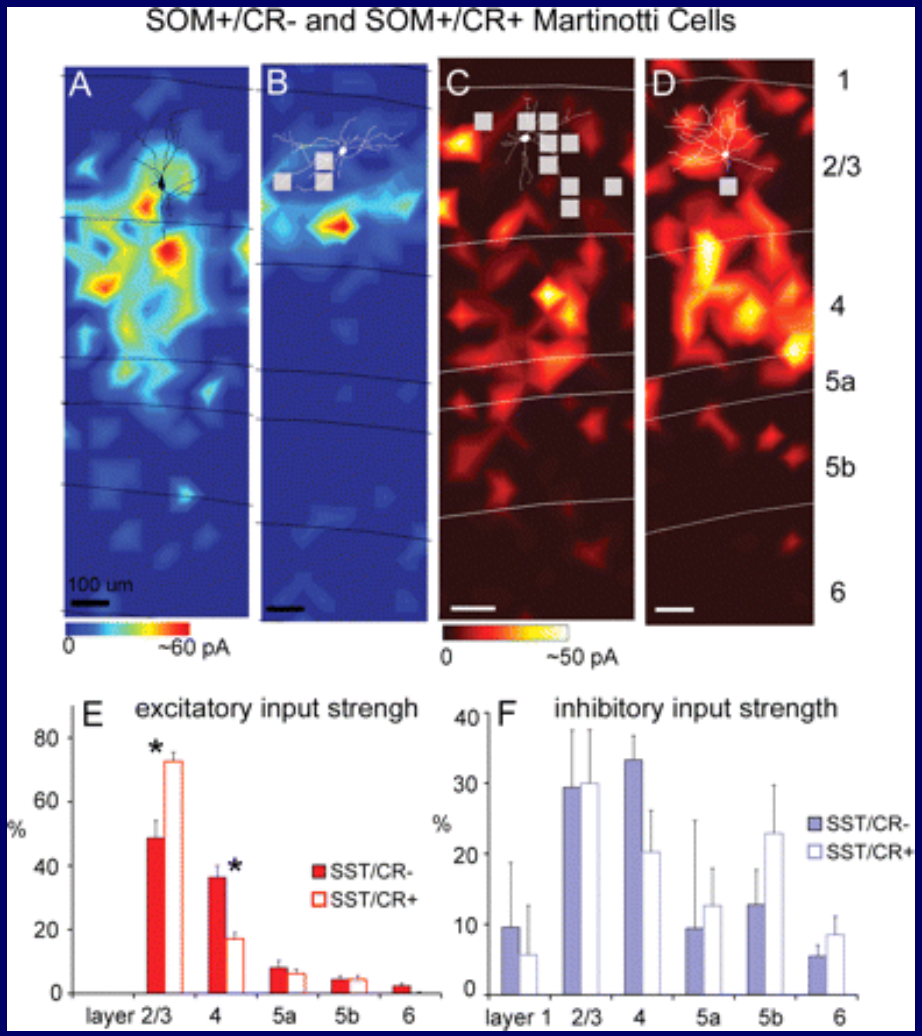
Laser scanning photo-stimulation reveals Laminar patterns of excitatory and inhibitory input to pyramidal cells and fast-spiking cells



Xu, X. et al. J. Neurosci. 2009;29:70-85

SOCIETY FOR NEUROSCIENCE The Journal of Neuroscience

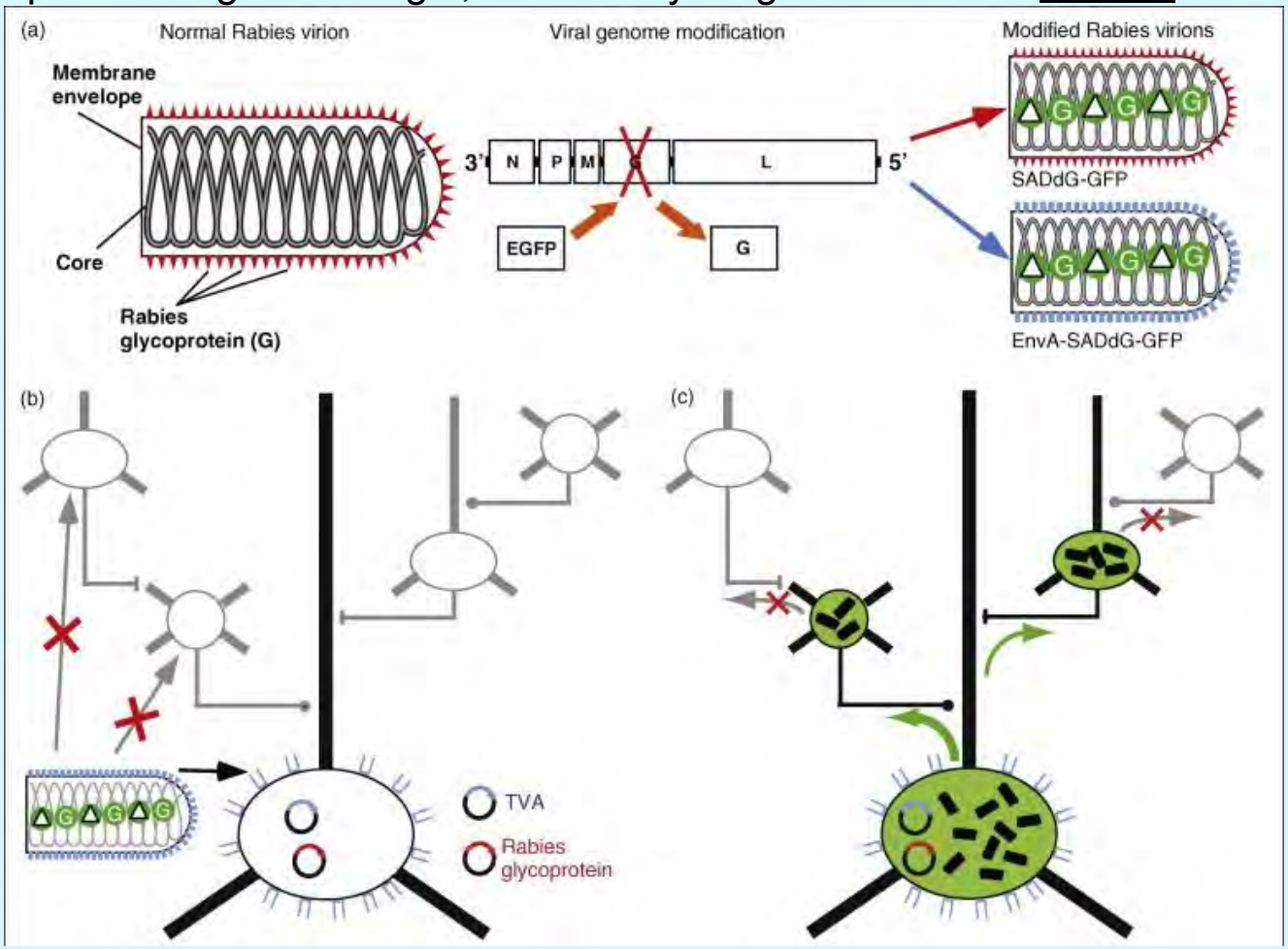
A second example: Laminar patterns of excitatory and inhibitory input to two subtypes of Martinotti cells



Xu, X. et al. J. Neurosci. 2009;29:70-85

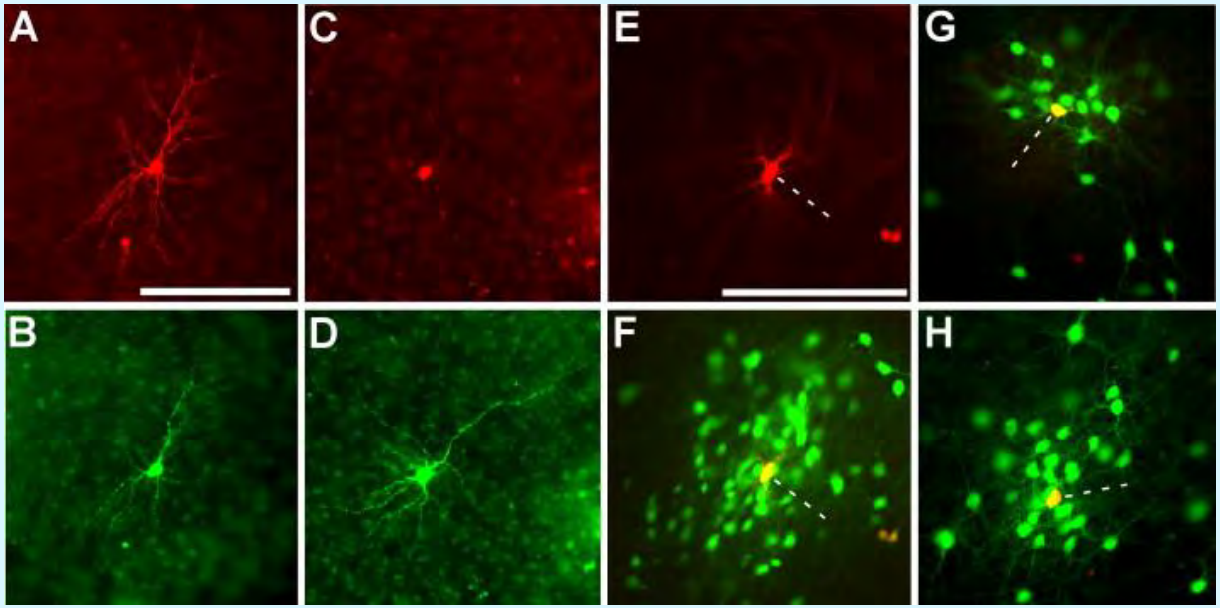
SOCIETY FOR NEUROSCIENCE The Journal of Neuroscience

The Solution from the Callaway lab: Wickersham et al (2007) Monosynaptic Restriction of Transsynaptic Tracing from Single, Genetically Targeted Neurons Neuron 53: 639-647



Pseudotyped rabies virus for tracing connections of targeted neurons. (a) Normal and modified rabies virions. The normal rabies virion (left) includes an RNA core within the viral envelope. The envelope is coated with the rabies glycoprotein (RG). It is possible to produce modified rabies virions in which the RG gene has been deleted from the genome and replaced with coding sequence for EGFP (middle). When these modified virions are grown in culture they can be coated with RG, conferring the normal infectious properties of rabies virus (right, top), or pseudotyped with an envelope protein from another virus (e.g. EnvA), conferring the infectious properties of that virus (right, bottom). Although these modified virions are capable of infecting cells and replicating to produce large quantities of EGFP, they are not able to spread out of those cells without the help of another DNA expression vector that provides RG. This is because RG is absolutely essential for viral spread. (b) Selective infection. EnvA Pseudotyped rabies virus can be used to selectively infect neurons that have been targeted for expression of the EnvA receptor, TVA. Because there are not endogenous receptors for EnvA in the mammalian brain, other cells are not infected. Because, the RG gene has been deleted from the rabies genome, complementation is required to allow spread of the virus from infected cells. This can be accomplished by targeted expression of RG in the same cells that express TVA. (c) Monosynaptically restricted spread. Following infection and RG complementation in the initially infected neurons, the rabies virus is able to spread retrogradely to directly presynaptic neurons. However, because these presynaptic neurons lack RG expression, the virus cannot spread beyond these cells..

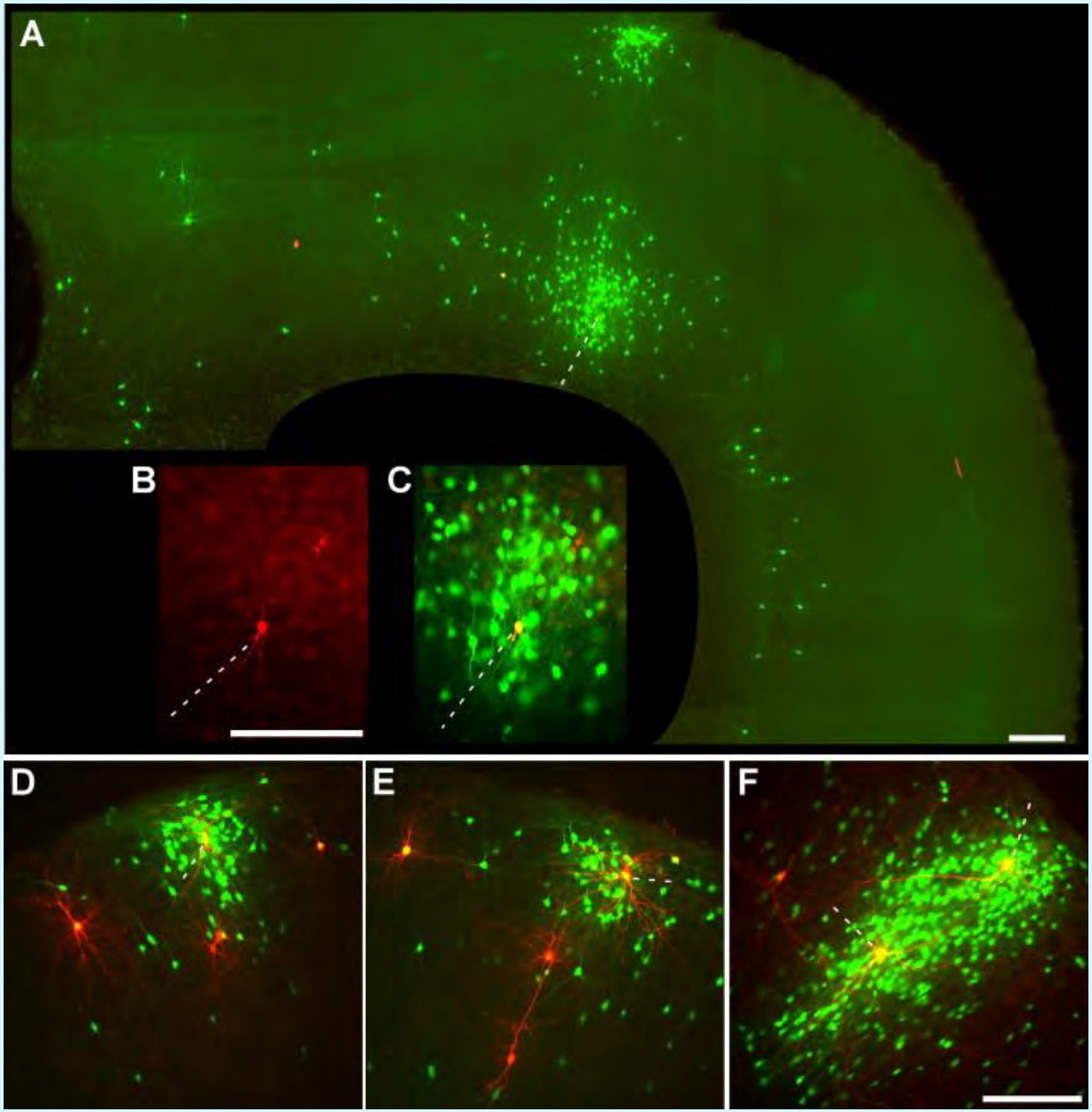
Wickersham et al: Selective Infection and In Situ Complementation in Slice Culture



(A–D) Initial infection is restricted to cells expressing the ASLV-A receptor, TVA. In these control experiments to test infection selectivity, isolated neurons in cultured brain slices were transfected using the gene gun with two genes, one encoding TVA, and the other, DsRed2. ASLV-A-pseudotyped rabies virus [SADΔG-EGFP(EnvA)] was applied the next day and images were taken 6 days following. (A and C) DsRed2 expression marking transfection with TVA and (B and D) EGFP expression indicating subsequent selective infection of the same TVA-expressing cells with pseudotyped virus.

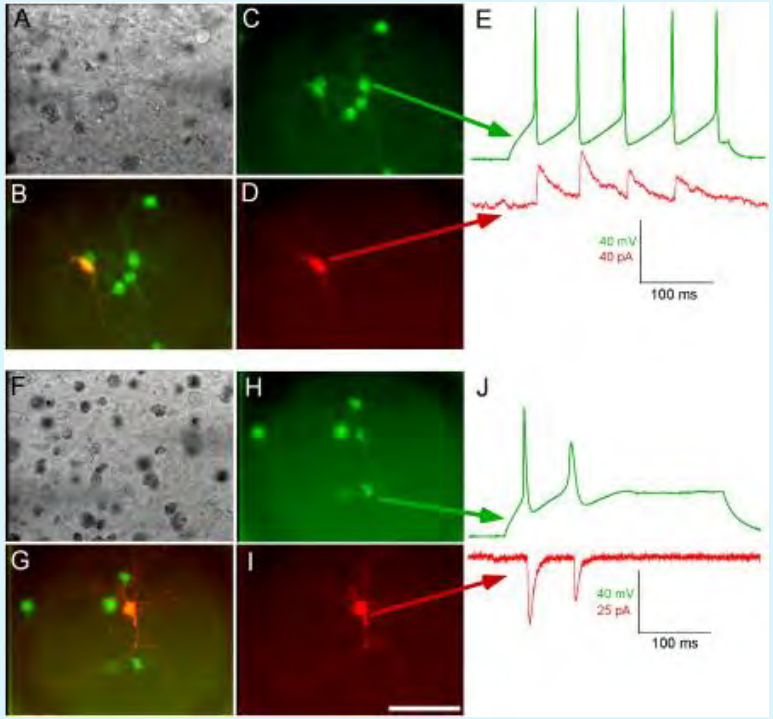
(E–H) In situ complementation permits transsynaptic spread from a single initially infected cell to a cluster of monosynaptically connected cells. Isolated neurons were transfected with three genes encoding TVA to permit viral infection, DsRed2 to mark transfected cells, and the rabies virus glycoprotein gene to complement the deletion in the viral genome. (E) DsRed2 fluorescence indicating a transfected cell, marked with a dotted line, at the center of the cluster shown in (F). (G and H) Two more examples of clusters surrounding a single transfected cell. The initially infected cells expressing both EGFP and DsRed2 appear yellow. Scale bars: 200 μm.

Wickersham et al: Another example in slice culture



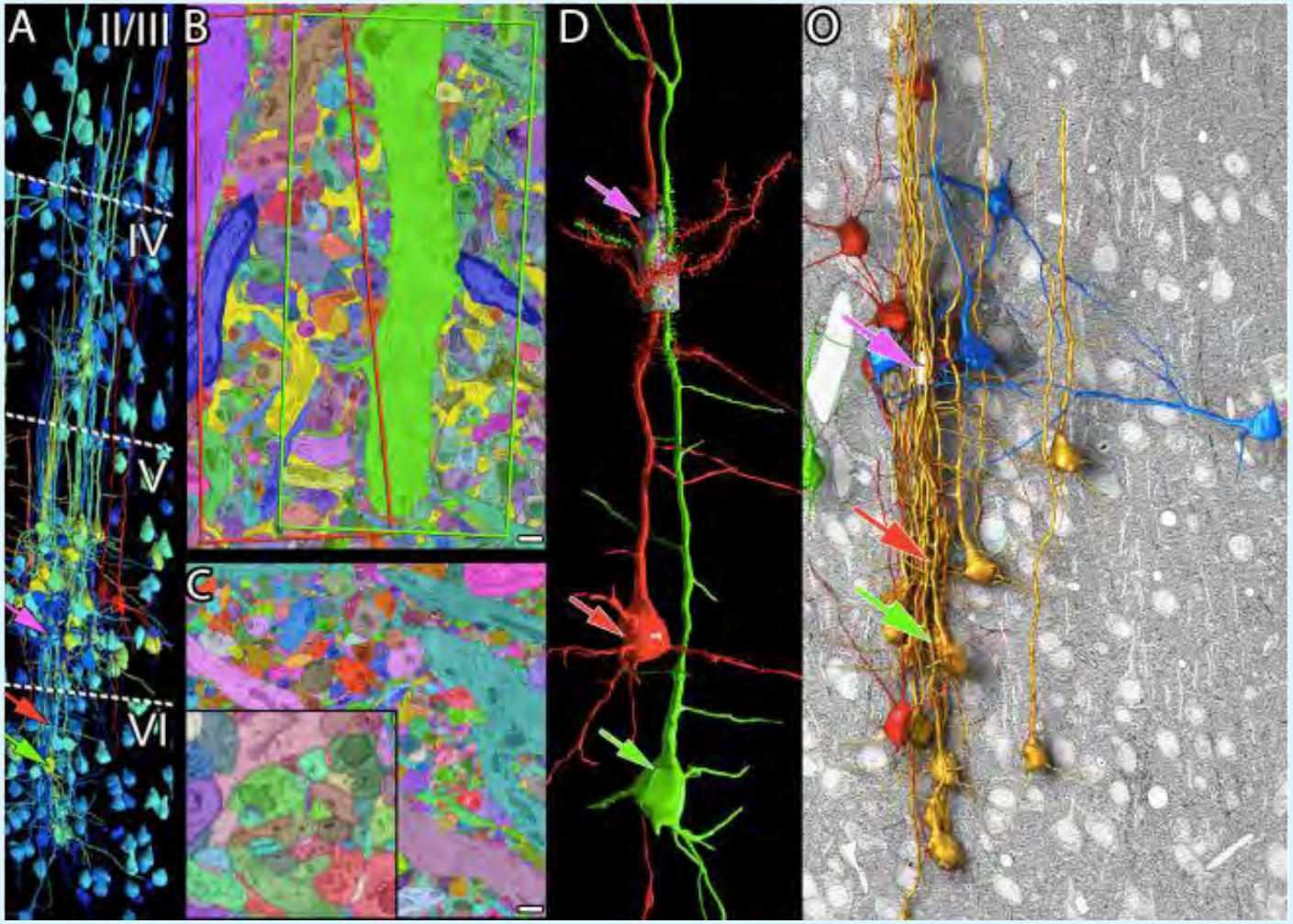
(A–C) Long-range viral spread from a single initially infected cell. (A) A huge cluster of green cells surrounds a single red/green deep-layer cortical neuron (dotted line) at 8 days postinfection. Another dense cluster of cells is also infected in the superficial cortical layers immediately above it, consistent with known projections of superficial layers to deeper ones; distant deep-layer pyramidal cells are also infected, again consistent with known patterns of long-range intralaminar connectivity. To the left of the putatively initially infected cell is a second yellow (double-labeled) cell, apparently secondarily—and recently—infected because of the lack of green cells surrounding it. (B–C) Closeup of central cluster from (A). (D–F) More examples of in situ complementation: clusters of infected cells surrounding isolated putatively postsynaptic ones. Scale bars: 200 μm.

Wickersham et al: Viral Spread Is Specific to Cells Presynaptic to the Initially Infected Cell

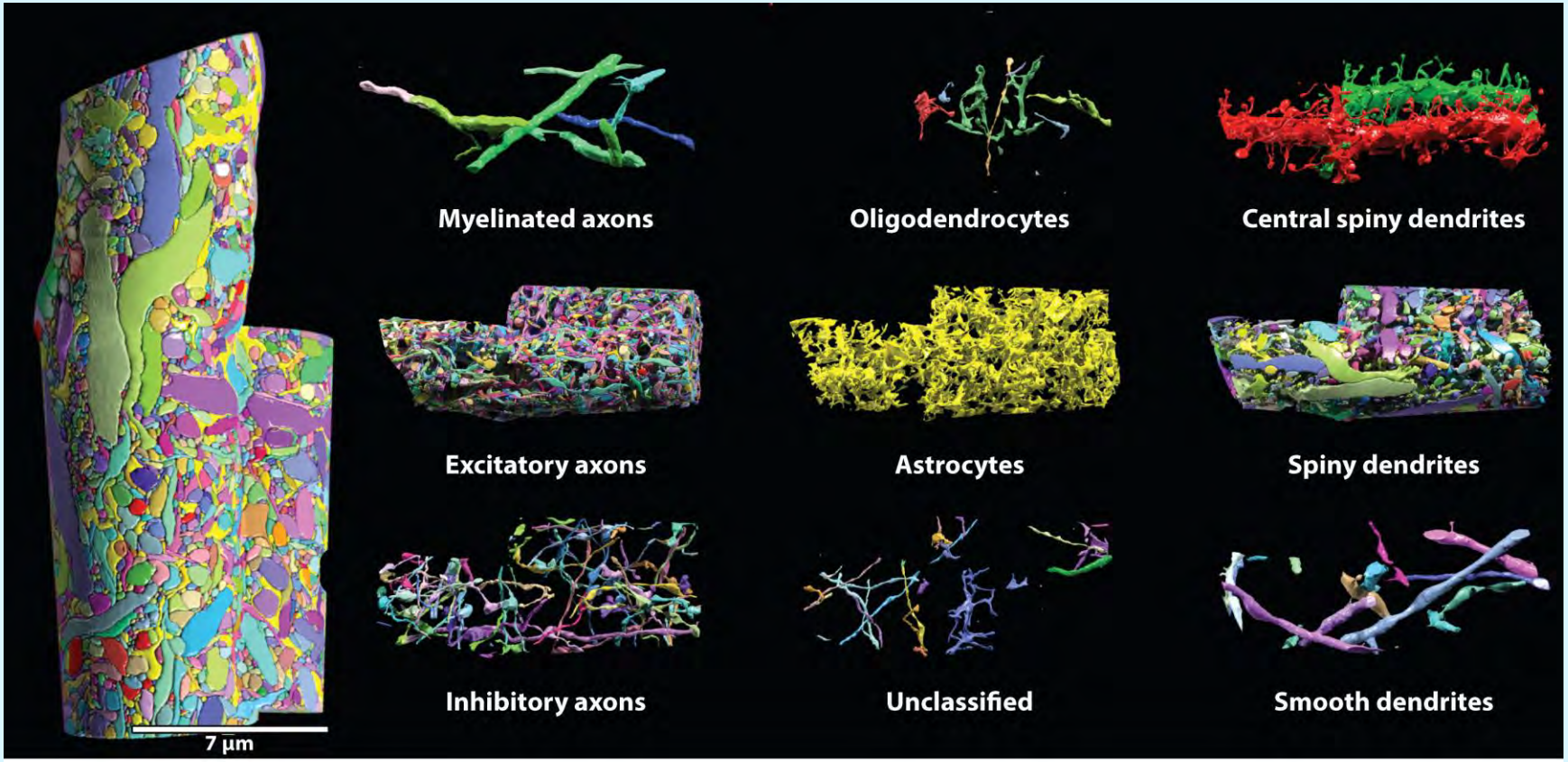


(A) DIC image of slice and recording pipettes targeting putatively pre- and postsynaptic neurons, (B) combined fluorescent image, and (C–D) single-channel fluorescence images. (E) Inhibitory postsynaptic currents in the putatively postsynaptic cell are coincident with action potentials in a nearby infected one, demonstrating a monosynaptic connection. (F–J) Similar demonstration of spread to an excitatory presynaptic cell. Scale bar: 100 μm, applies to all panels.

The real circuits are more complicated, as we are just beginning to learn from Electron Microscopic reconstructions



The different types of neurons and glia can now be identified



Swanson LW, Lichtman JW. 2016.
Annu. Rev. Neurosci. 39:197–216

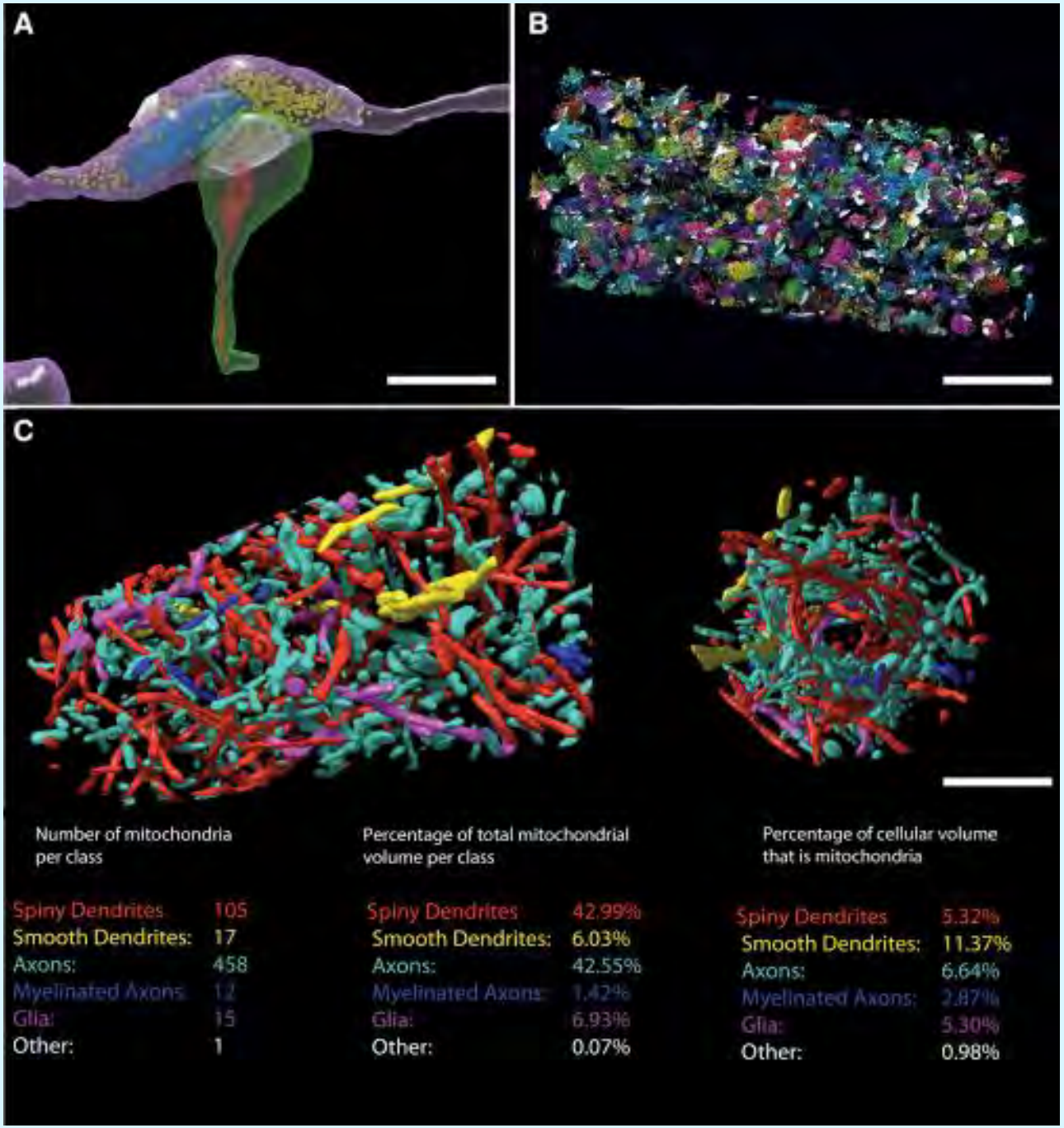
And we can zoom in at great detail

Reconstruction of Subcellular Organelles

(A) A reconstruction of a single synapse showing the innervating excitatory axon and its en passant varicosity (purple), postsynaptic dendritic spine (green), synaptic vesicles (yellow), a presynaptic mitochondrion (blue), the postsynaptic density (white), and spine apparatus (red).

(B) All of the synaptic vesicles in cylinder 1 (n = 162,259) and their corresponding postsynaptic densities (white) are shown.

(C) All of the mitochondria (n = 635) contained in cylinder 1 from side view of the cylinder (left) and end-on view (right).



One of the papers for the Wednesday discussion, if you want to read it on the weekend (there will be others):

Packer AM, Russell LE, Dalgleish HW, Häusser M. (2105) Simultaneous all-optical manipulation and recording of neural circuit activity with cellular resolution in vivo. Nat Methods. 12:140-6. PMID 25532138, PMC4933203
http://www.nature.com/nmeth/journal/v12/n2/full/nmeth.3217.html