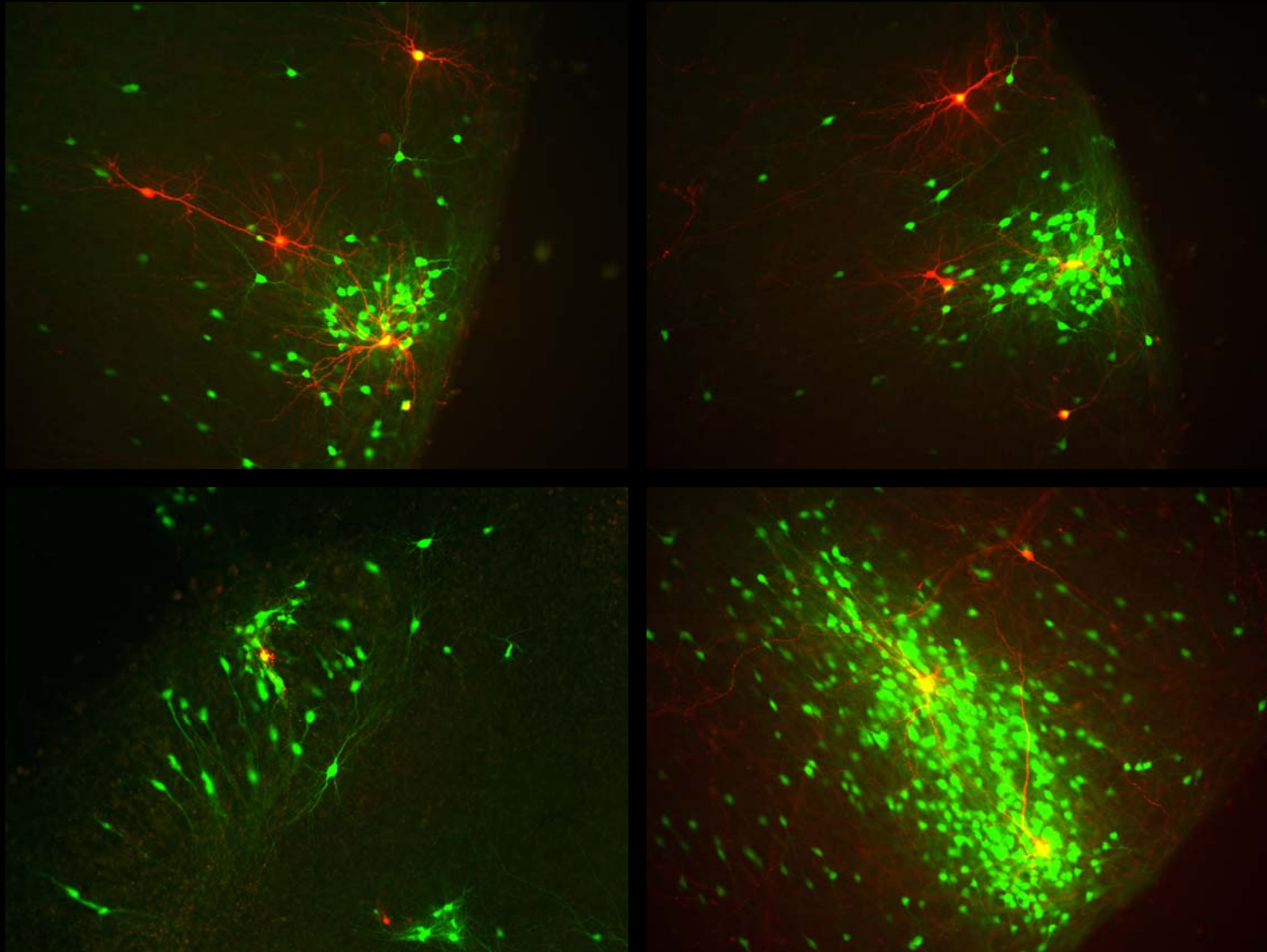


New Methods for Revealing and Manipulating Highly Specific Neural Circuits

Edward Callaway
Salk Institute

KITP Brain08 Apr 3, 2008

New Methods for Revealing and Manipulating Highly Specific Neural Circuits



How do neural circuits generate perception and behavior?

What we need to know or do:

1. What is the circuit?

- Standard anatomical approaches reveal possible connections.
- Newer methods reveal that only a subset of possible connections are manifested. Fine-scale and cell-type specificity.

2. How does circuitry relate to function?

- Record from identified cell types.
- Directly correlate connectivity and function of single neurons.
- Correlate neural activity to behavior.

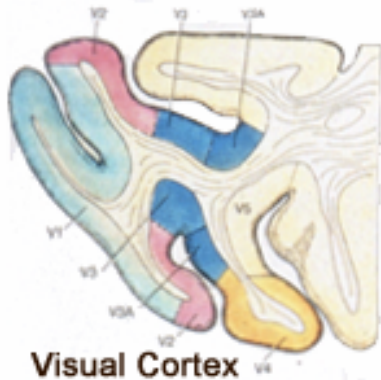
3. Because neural circuits and behaviors are complex and not intuitively understood, we must generate testable models.

4. Test hypotheses by perturbing circuits.

- Use molecular and genetic methods to selectively manipulate specific components of neural circuits (e.g specific cell types).

Reductionist View of Cortical Circuits

Areas



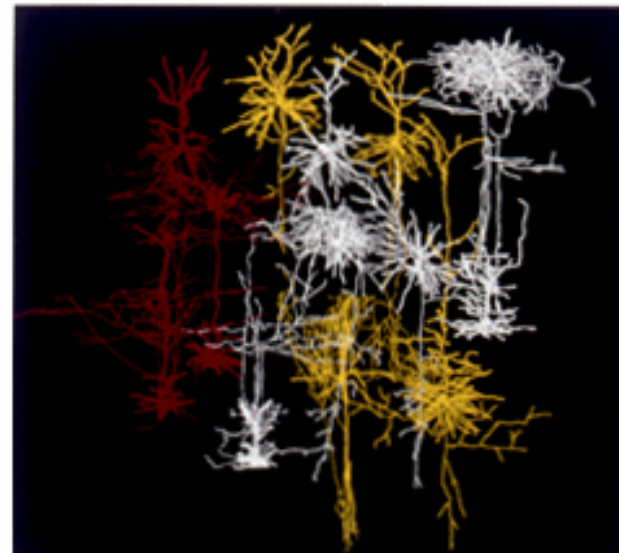
Neurons



Modules



Microcircuits



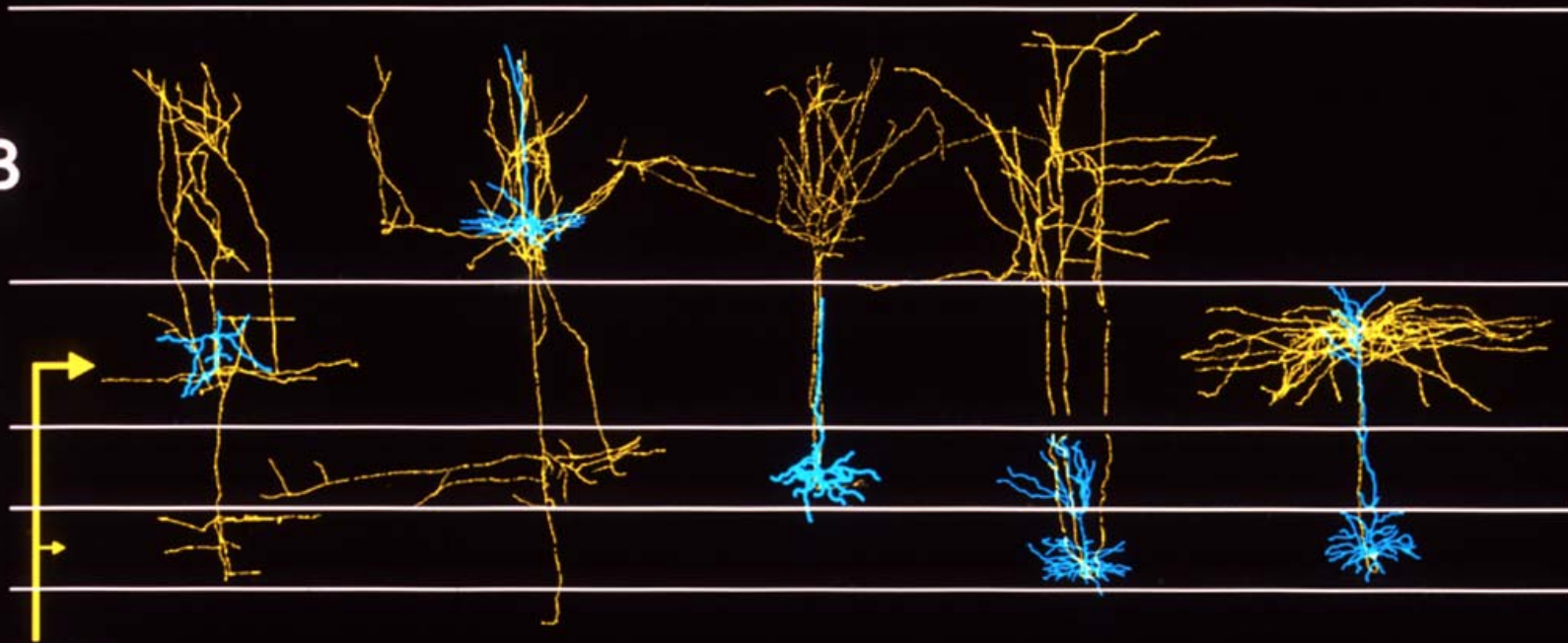
2-4B

4C

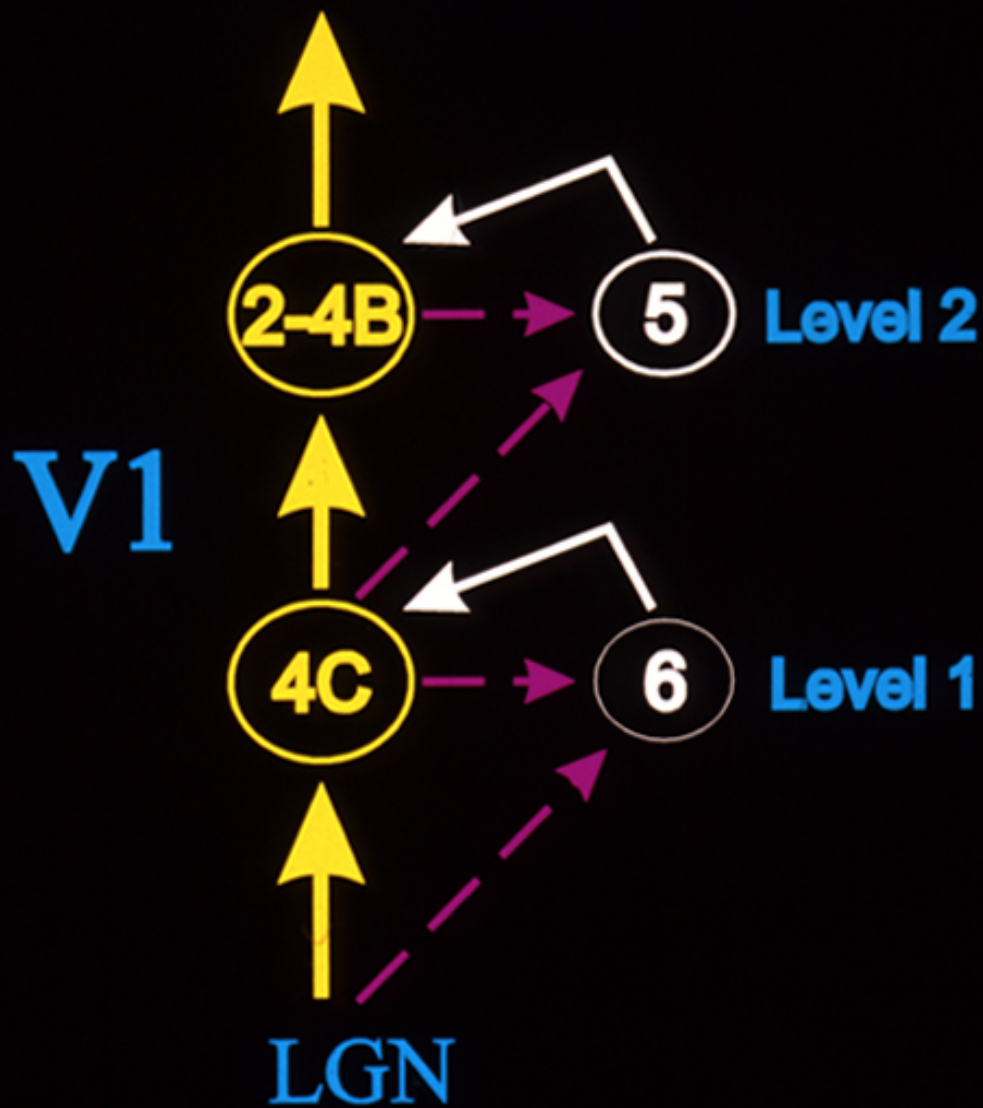
5

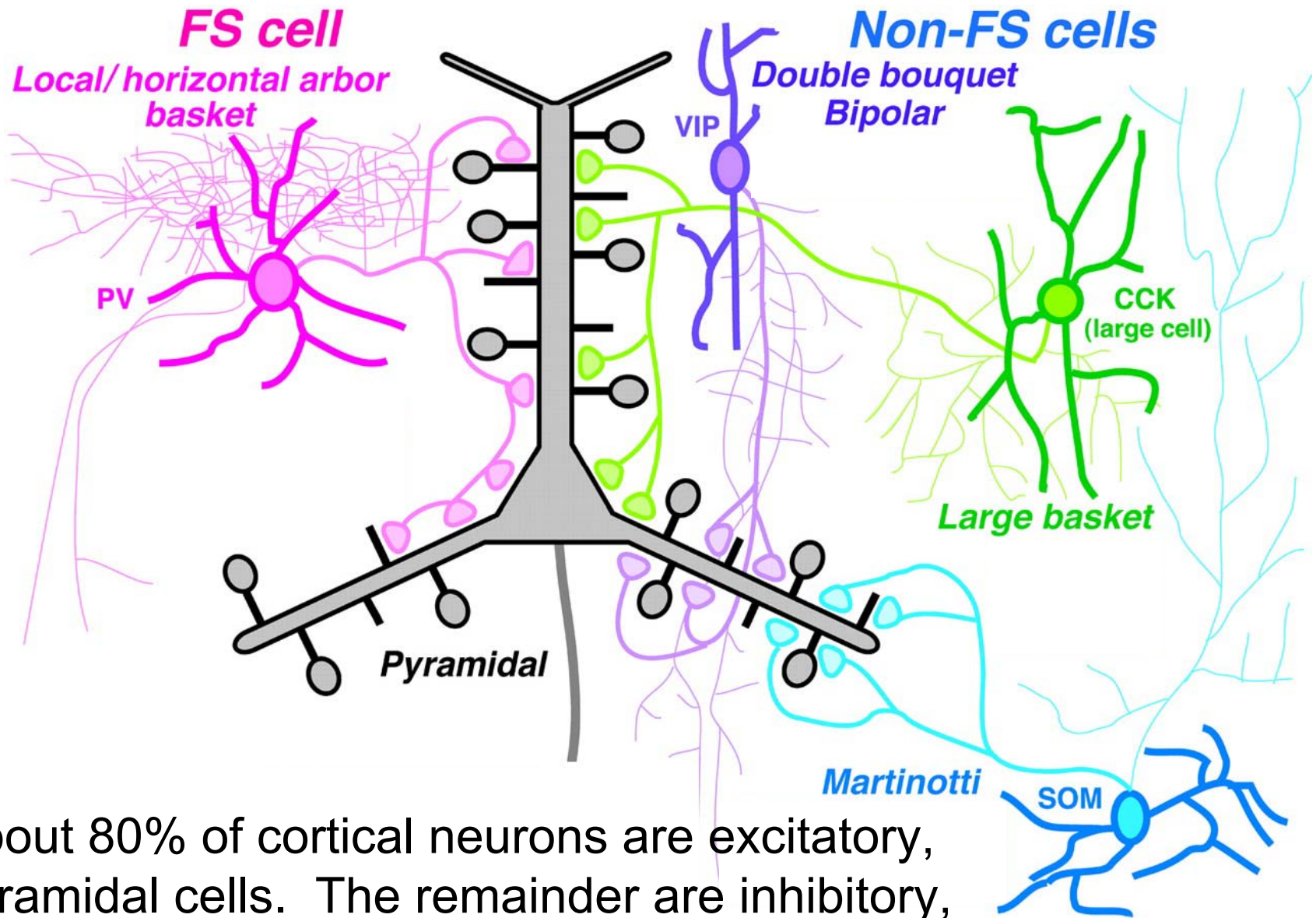
6

LGN



Extrastriate Cortex





About 80% of cortical neurons are excitatory, pyramidal cells. The remainder are inhibitory, of which there are at least a dozen types.

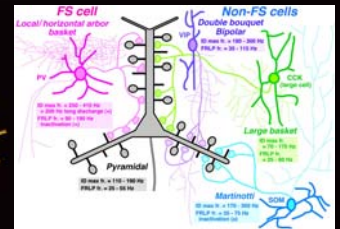
2-4B

4C

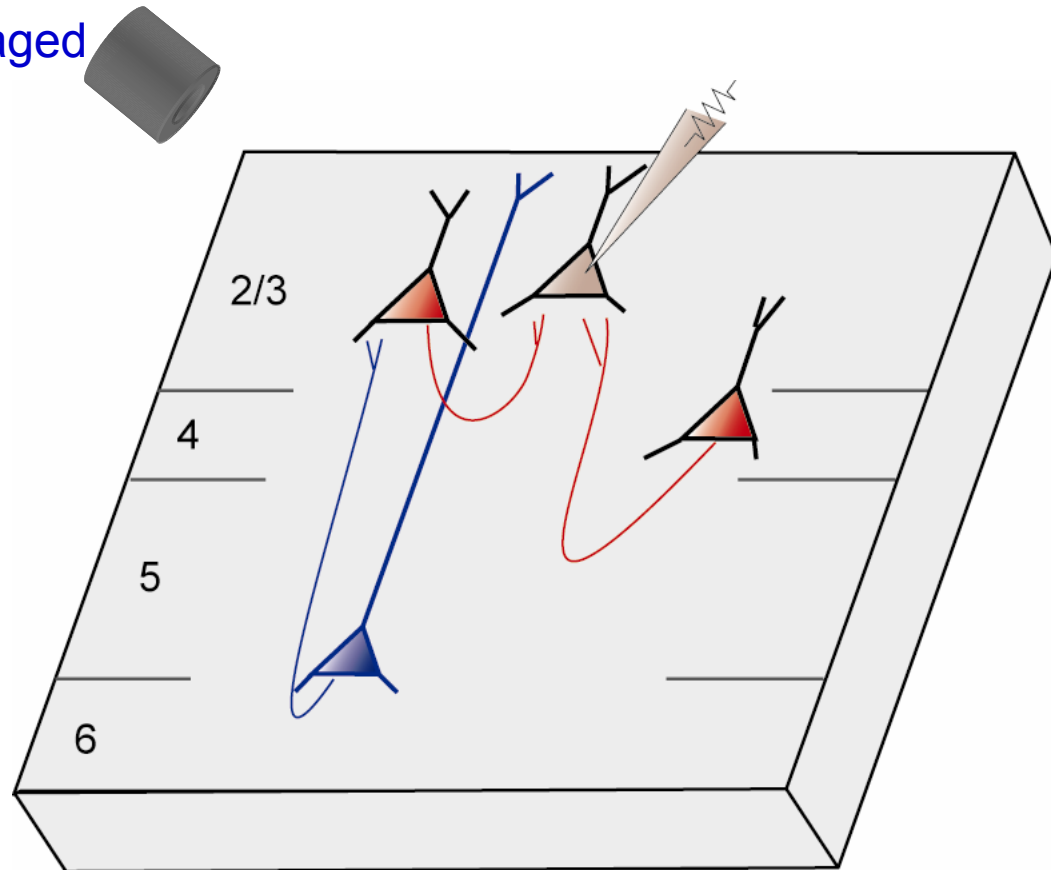
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6

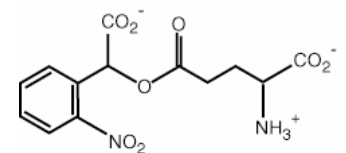
LGN



1. Brain slice bathed in caged glutamate

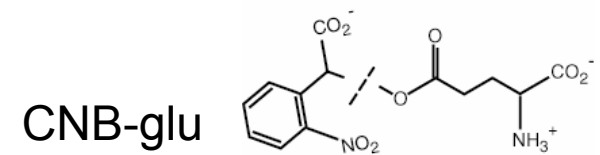
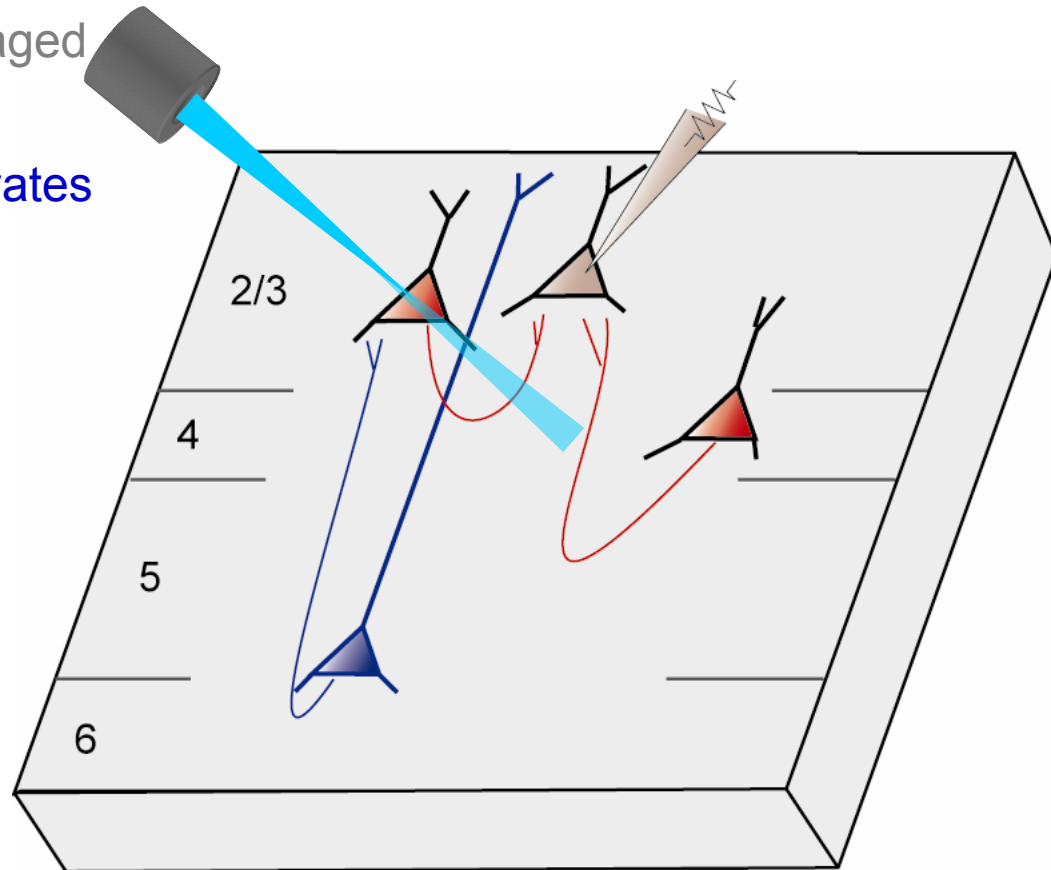


CNB-glu



1. Brain slice bathed in caged glutamate

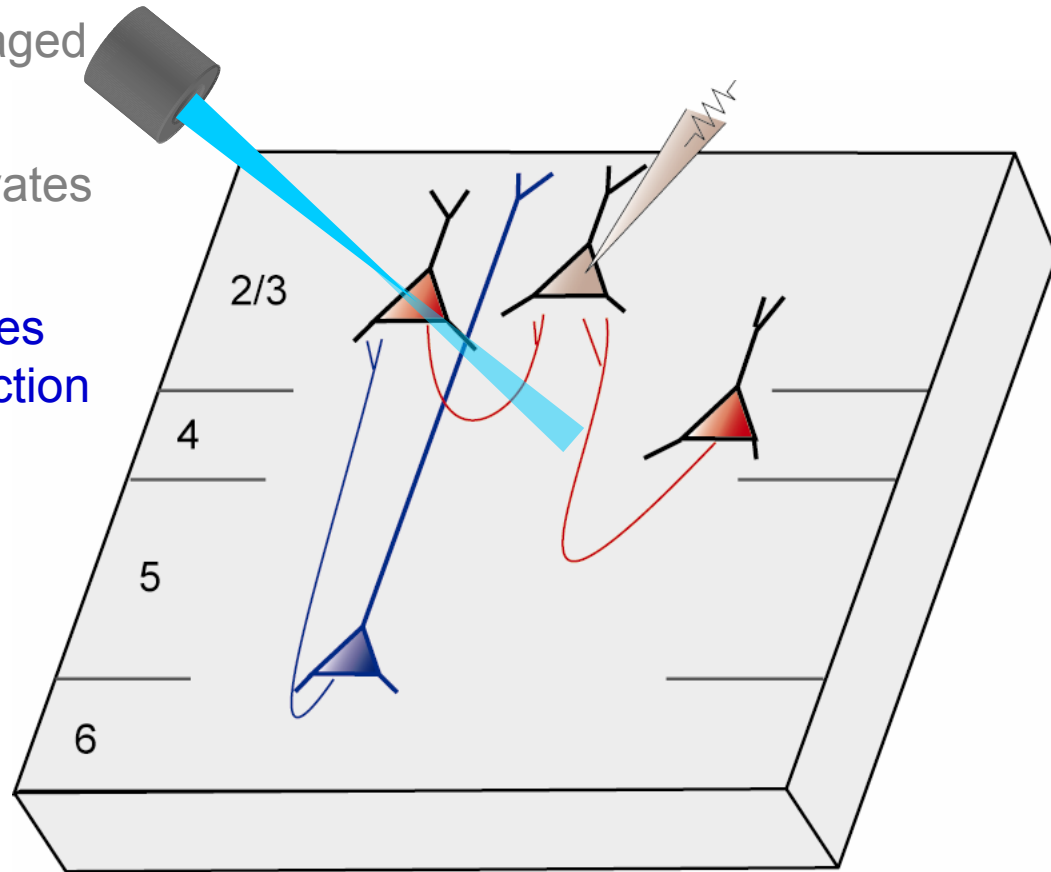
2. Focused UV light activates glutamate



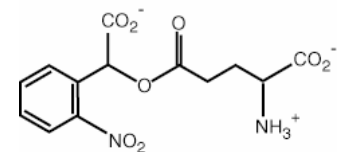
1. Brain slice bathed in caged glutamate

2. Focused UV light activates glutamate

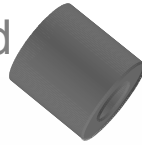
3. Neurons with cell bodies close to focal point fire action potentials



CNB-glu



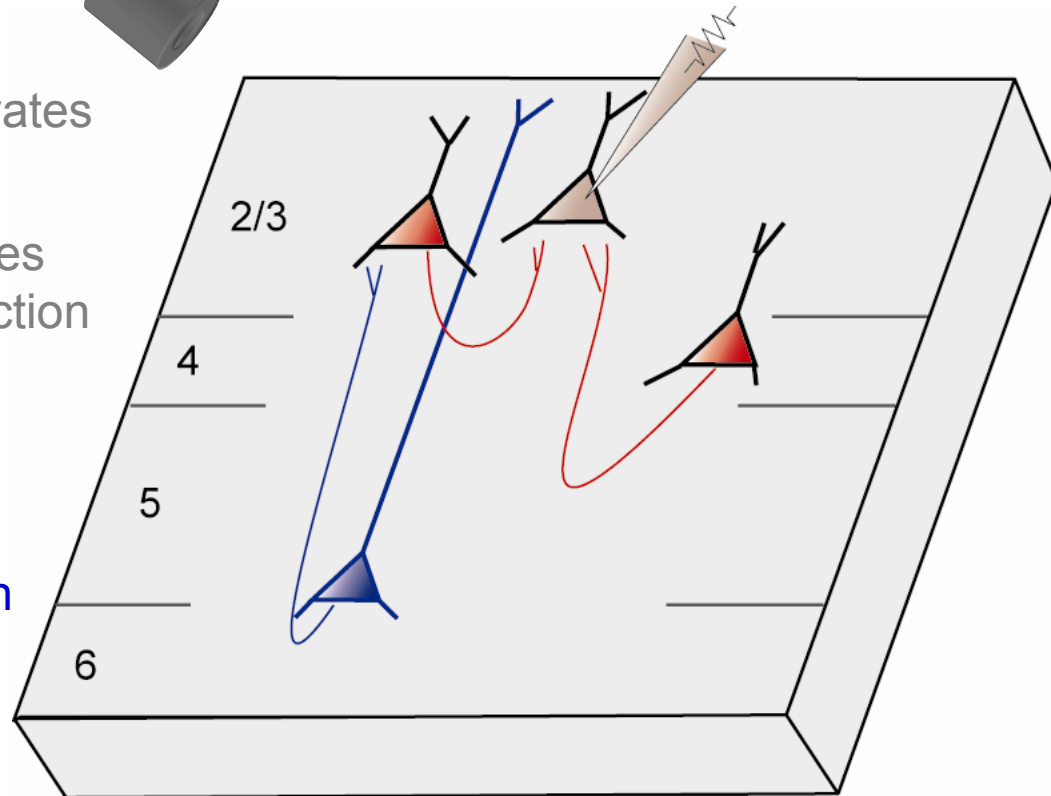
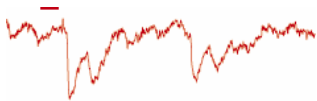
1. Brain slice bathed in caged glutamate



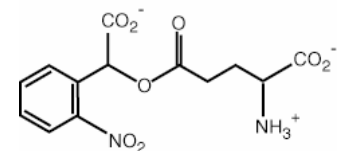
2. Focused UV light activates glutamate

3. Neurons with cell bodies close to focal point fire action potentials

4. Record responses in postsynaptic neuron
monosynaptic connection



CNB-glu

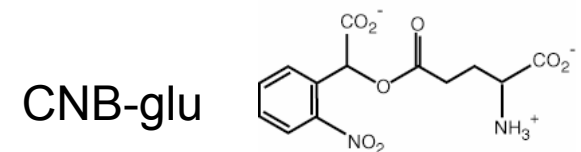
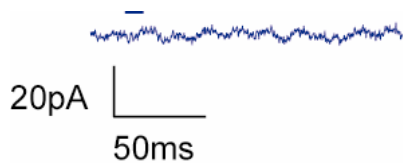
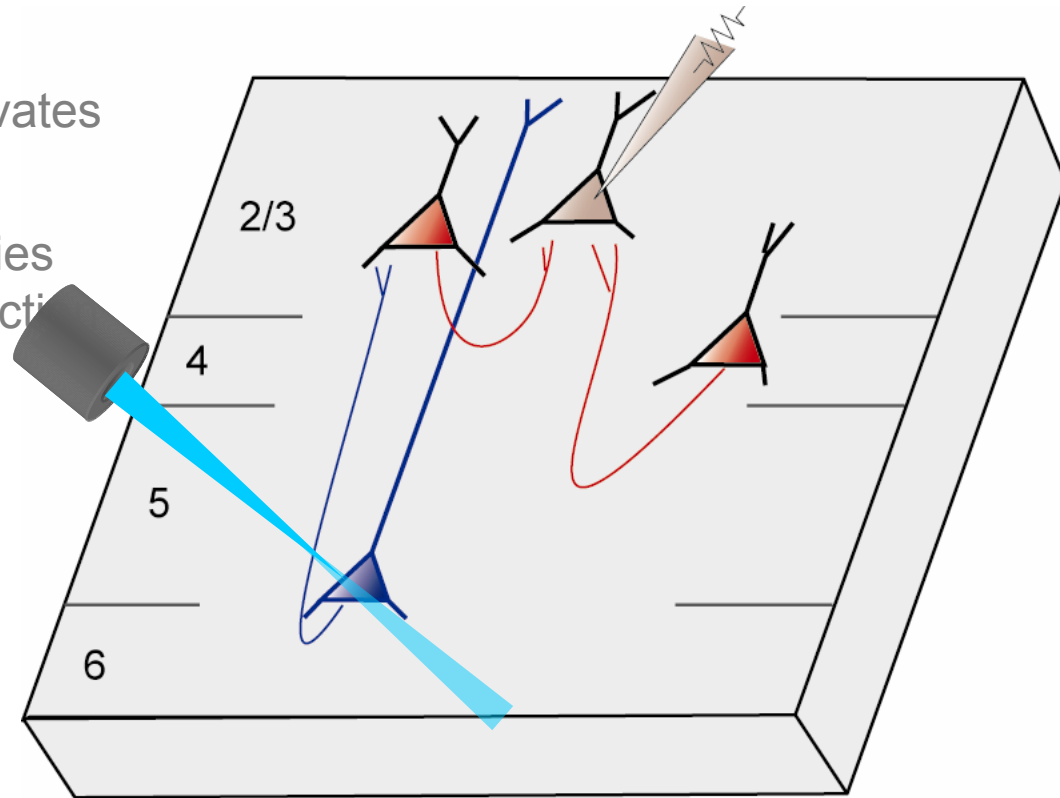


1. Brain slice bathed in caged glutamate

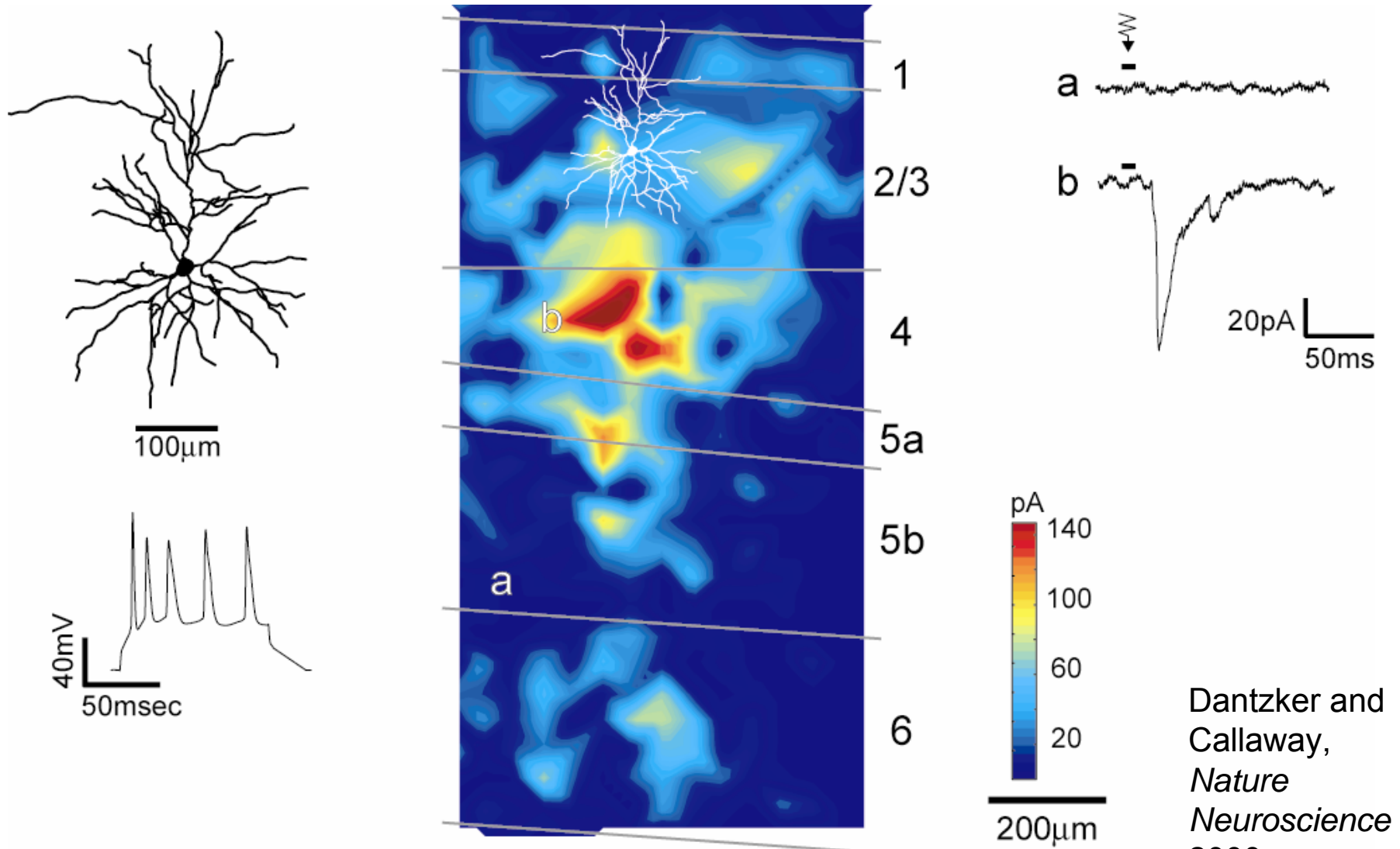
2. Focused UV light activates glutamate

3. Neurons with cell bodies close to focal point fire action potentials

4. Record responses in postsynaptic neuron
NO monosynaptic connection

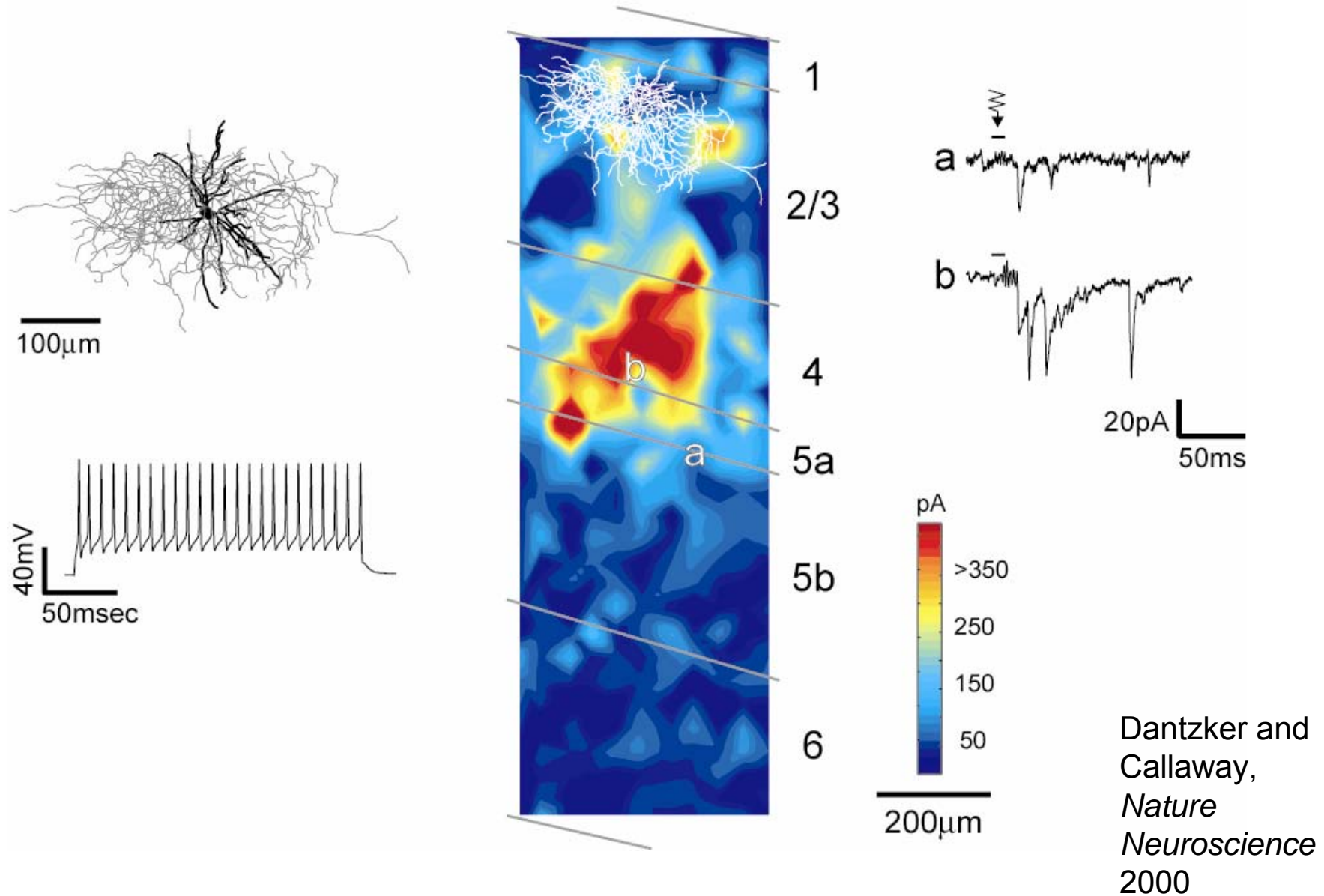


Pyramidal neuron receives most synaptic input from middle cortical layers

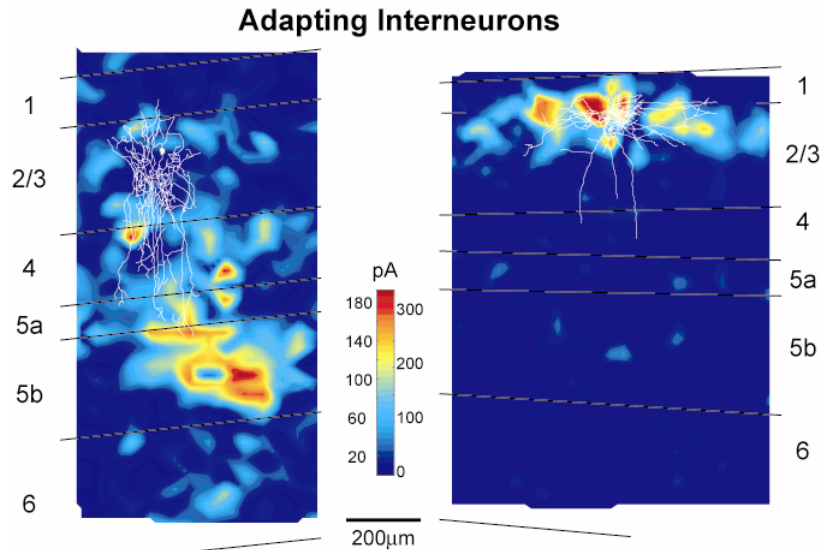
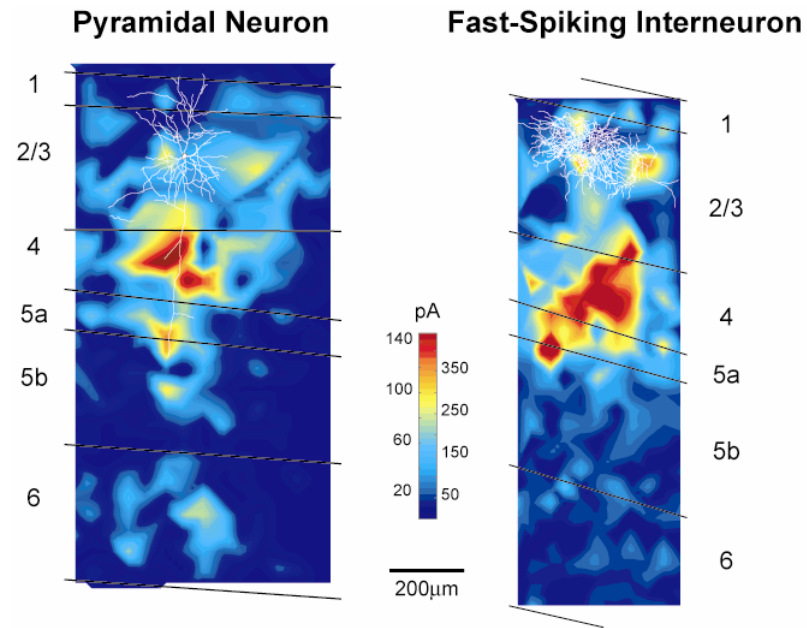


Dantzker and
Callaway,
*Nature
Neuroscience*
2000

Fast spiking interneuron receives most synaptic input from middle cortical layers



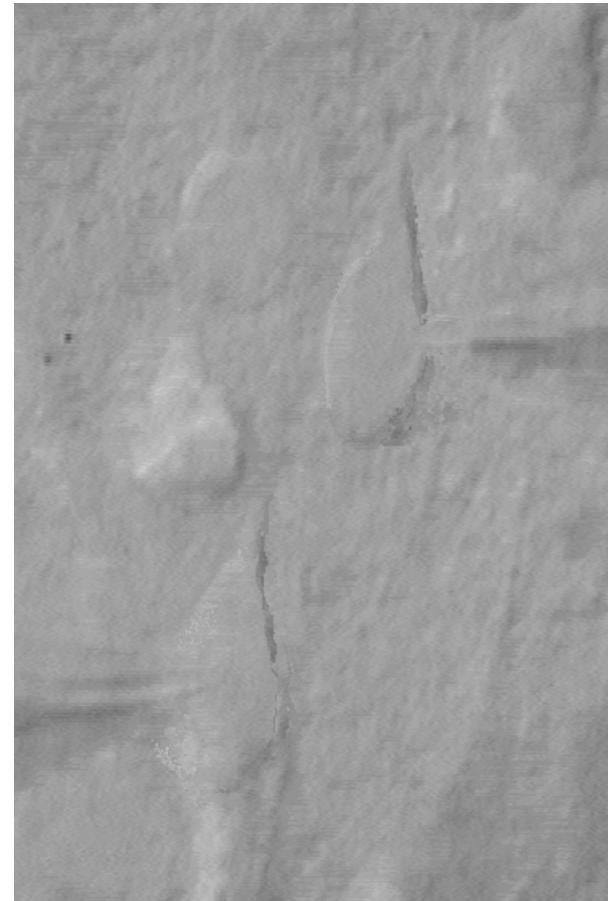
There is Cell-Type Specificity of Functional Cortical Connections



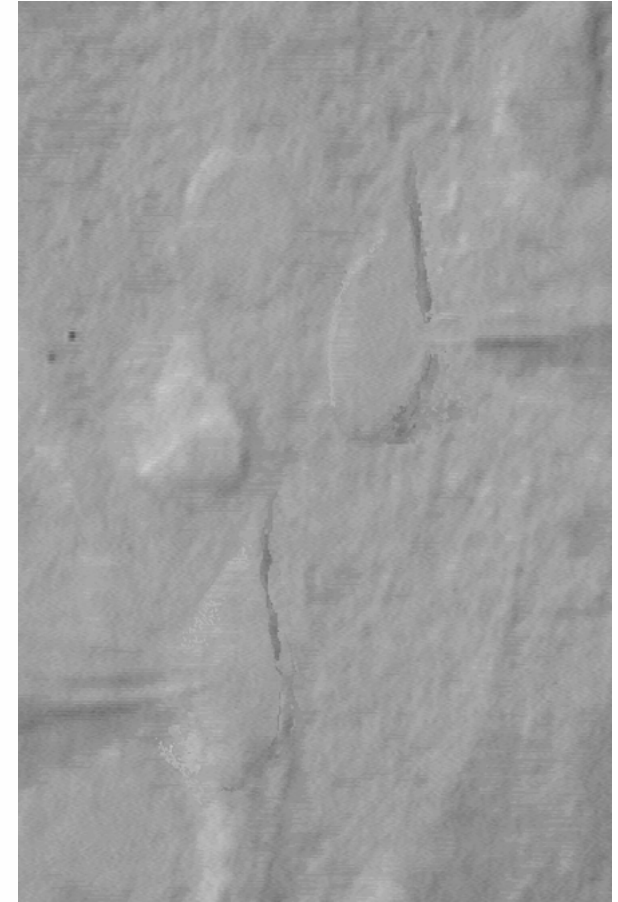
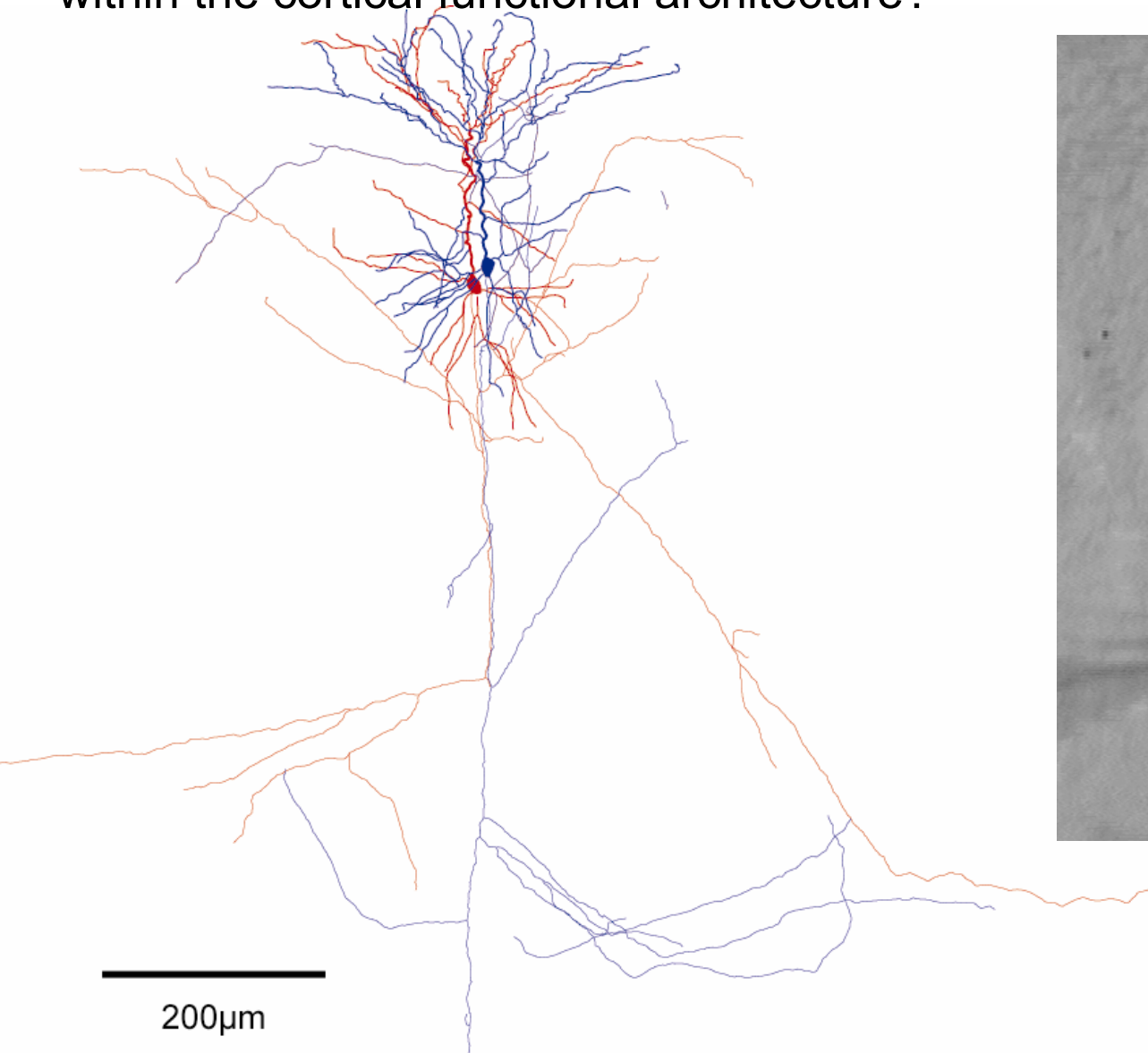
Dantzker and
Callaway,
*Nature
Neuroscience*
2000

Can connections be specific amongst individual neighboring cells of the same type?

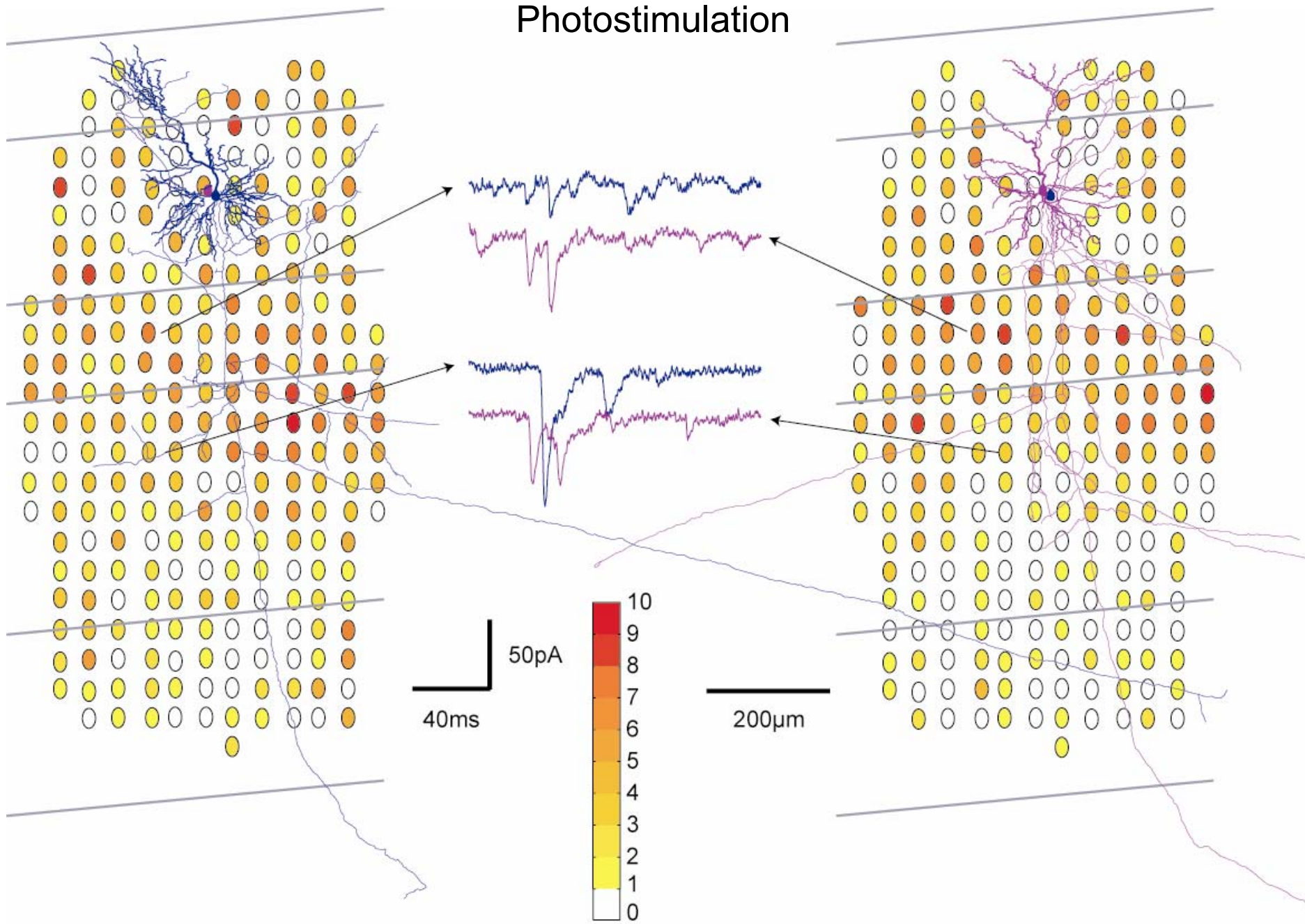
1. We can combine photostimulation with cross-correlation analyses to test fine-scale specificity of connections to neighboring cells.
2. There is fine-scale selectivity of excitatory cortical connections.
3. There is also fine-scale specificity of inhibitory cortical connections, but it depends on the type of inhibitory cell and its direct connections with excitatory cells.



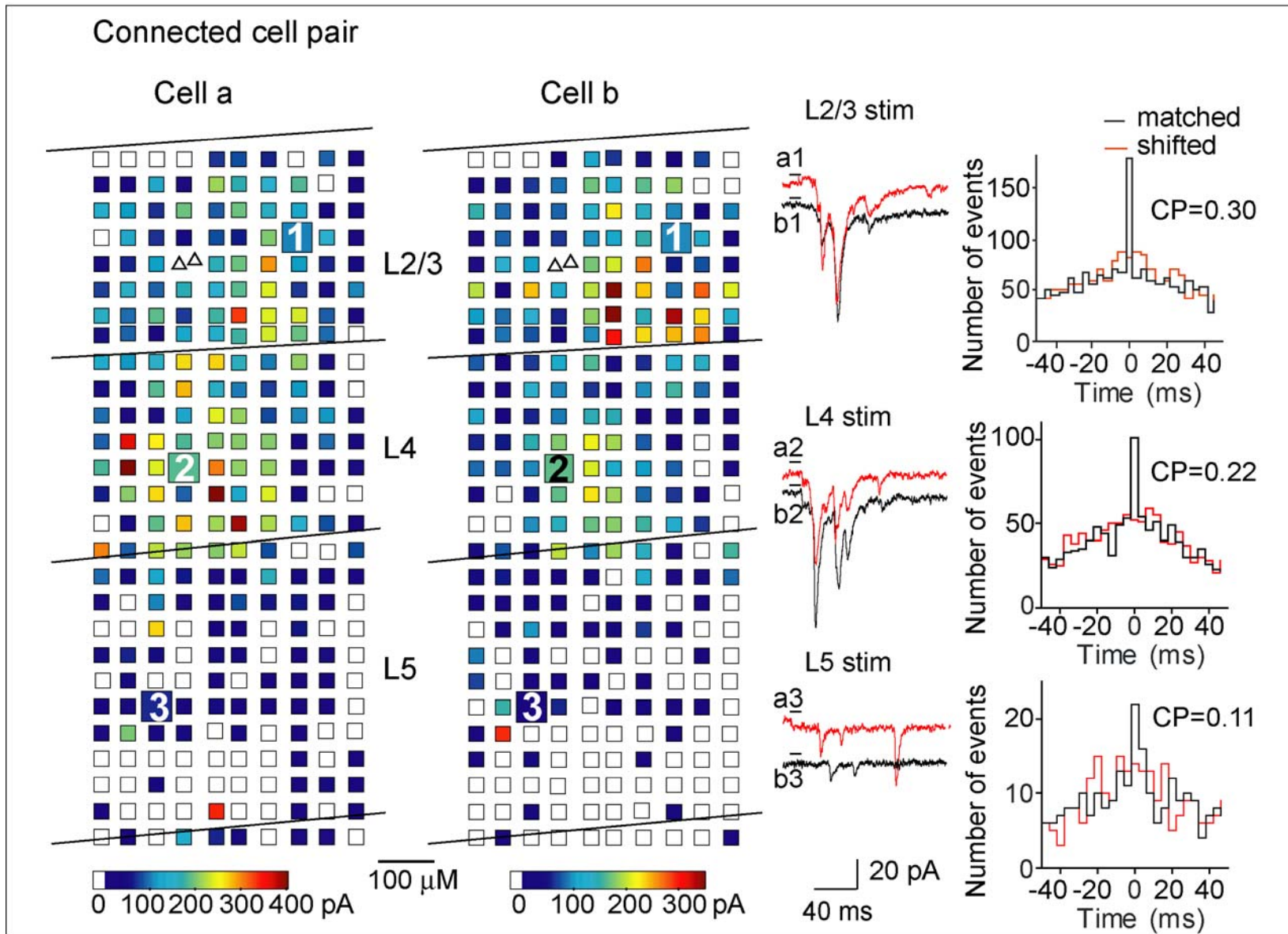
Only one out of five neighboring excitatory neurons are directly connected. Does this reflect a randomly distributed network? Or is there a finer scale organization that creates relatively independent sub-networks embedded within the cortical functional architecture?



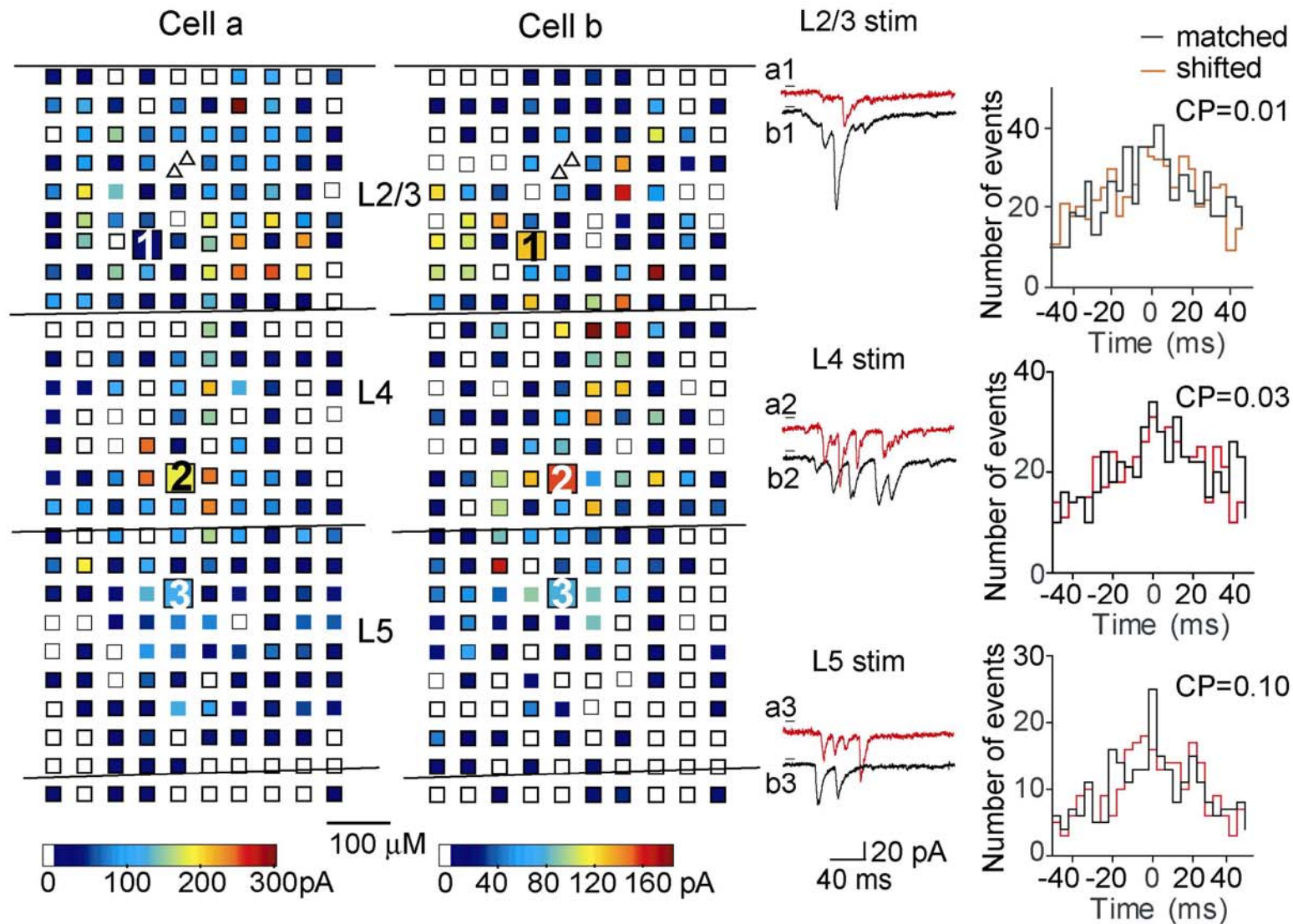
Simultaneous EPSC Recordings Following Photostimulation



Input Maps and EPSC Correlograms for a Pair of *Connected* Layer 2/3 Pyramids

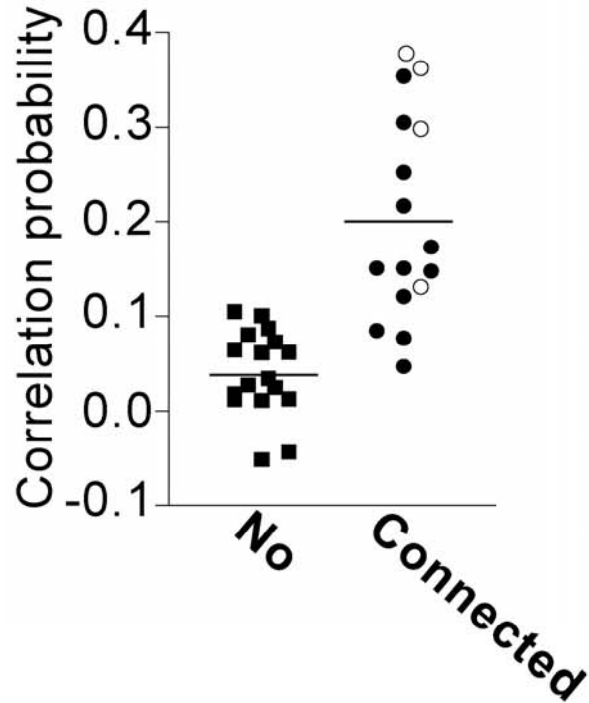


Input Maps and EPSC Correlograms for a "*Typical*" Pair of layer 2/3 Pyramids

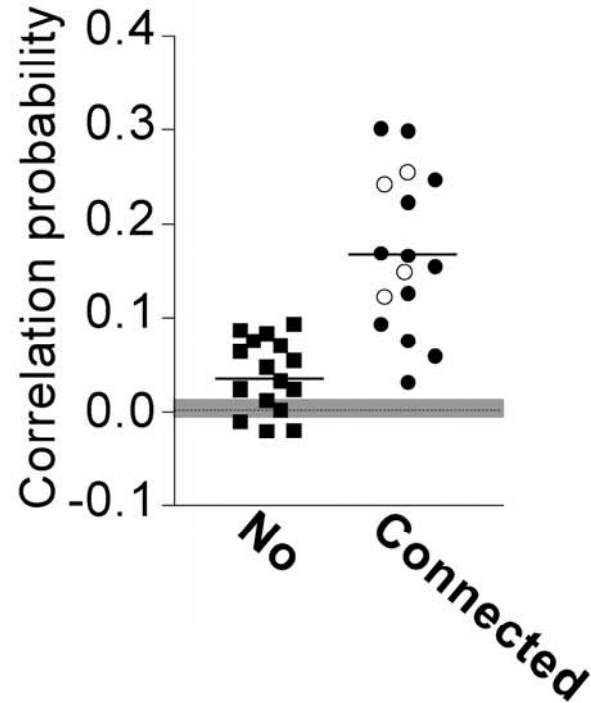


Excitatory inputs in layer 2/3 pyramidal cell pairs

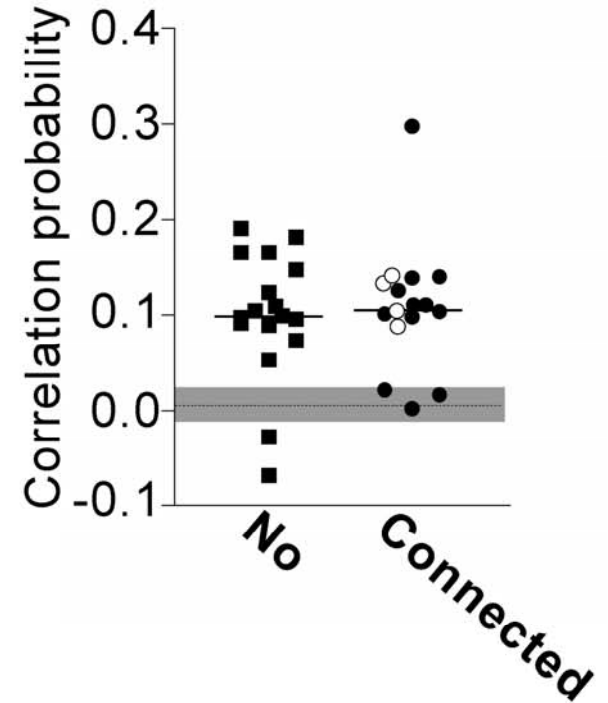
i. L2/3 stim



ii. L4 stim

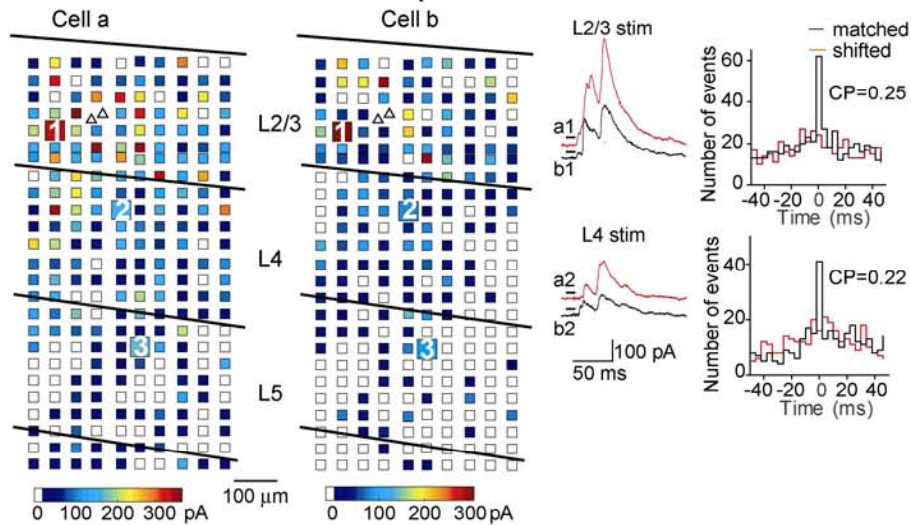


iii. L5 stim

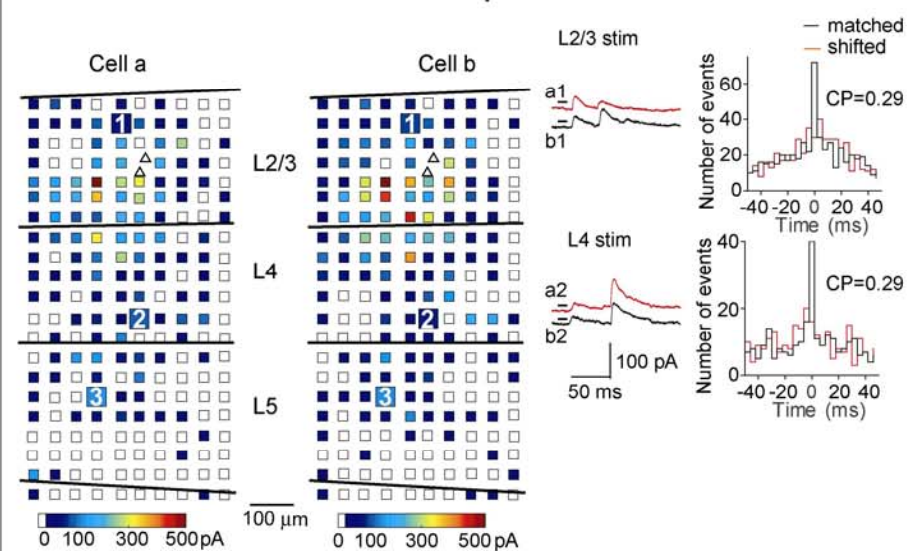


Input Maps and IPSC Correlograms for an Unconnected and a Connected Pair of Pyramidal Neurons

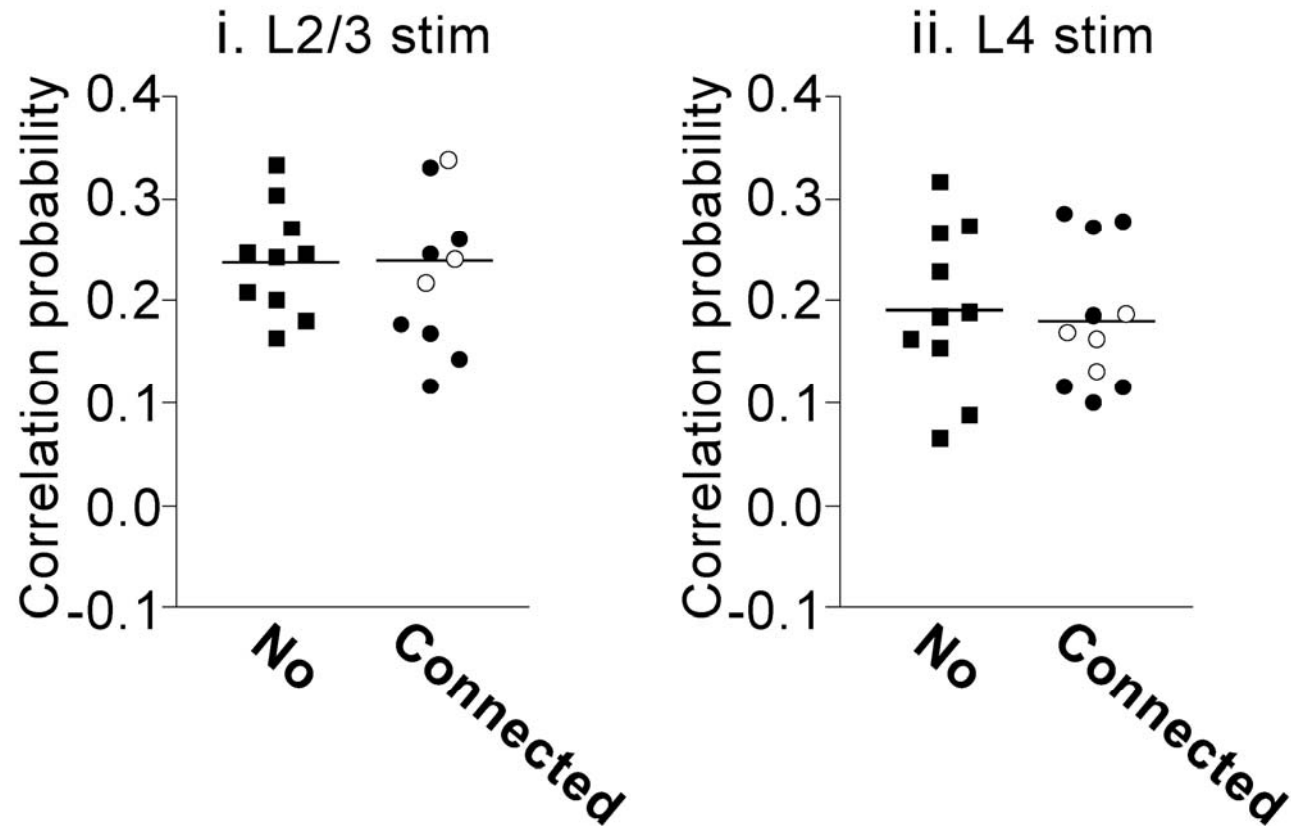
Connected cell pair



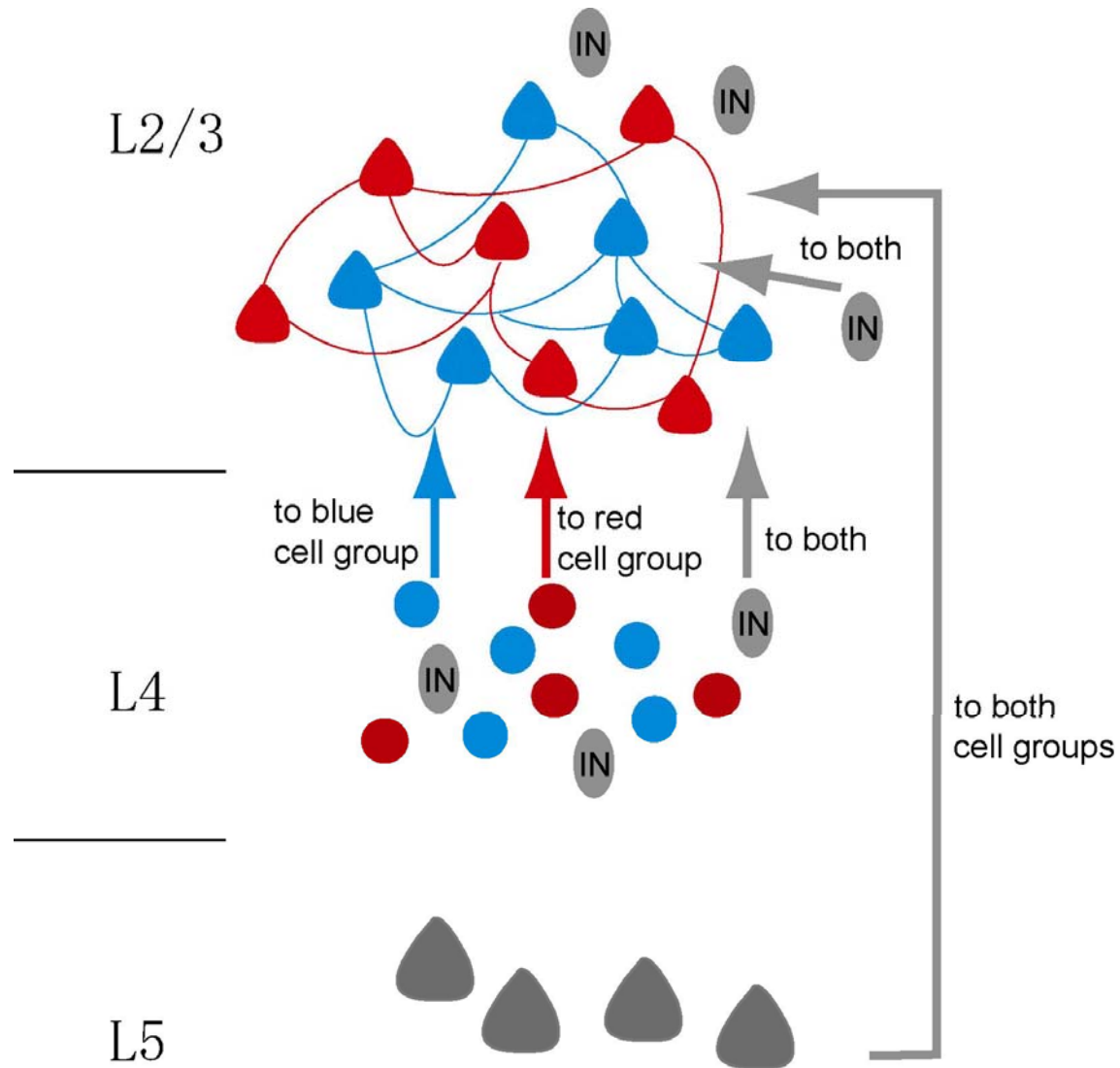
Unconnected cell pair



Inhibitory inputs in layer 2/3 pyramidal cell pairs



Fine-Scale Specificity of Excitatory Cortical Circuits

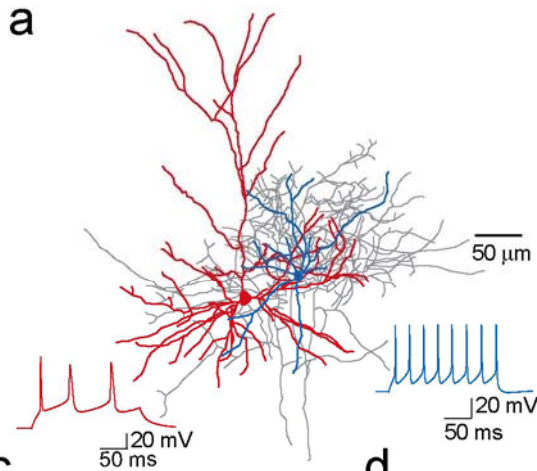


More Questions

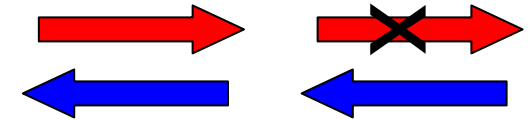
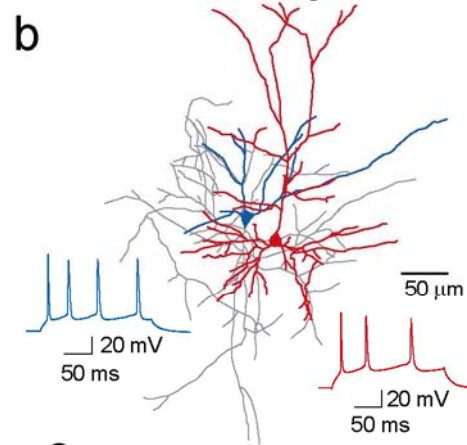
1. Can inhibitory connections be specific on a fine scale?
2. Is there fine-scale specificity of excitatory connections to inhibitory neurons and does it depend on the inhibitory cell type?
3. Does the direct connectivity between neighboring inhibitory and excitatory neurons depend on whether they are part of the same fine-scale excitatory subnetwork?

Fast Spiking Interneurons Preferentially Connect to the Same Pyramids that Excite Them

Fast-Spiking



Adapting



88%
(7/8)
172pA

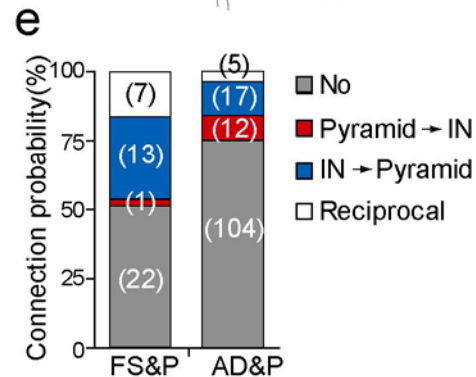
37%
(13/35)
54pA

43 FS/Pyramid Pairs

Not Connected, 22

Pyramid to IN, 8 (7 reciprocal)

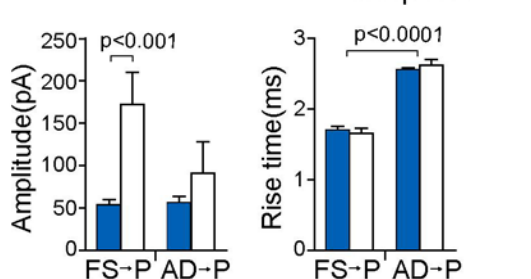
IN to Pyramid 20 (7 reciprocal)



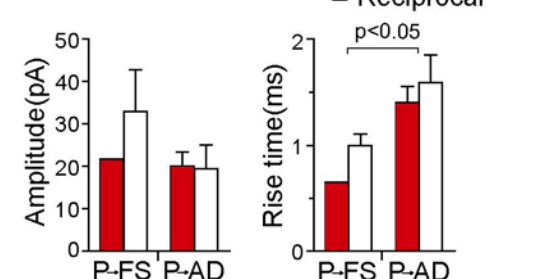
FS interneurons preferentially connect to the same pyramids that excite them.

(88% versus 37%, $p=0.017$)

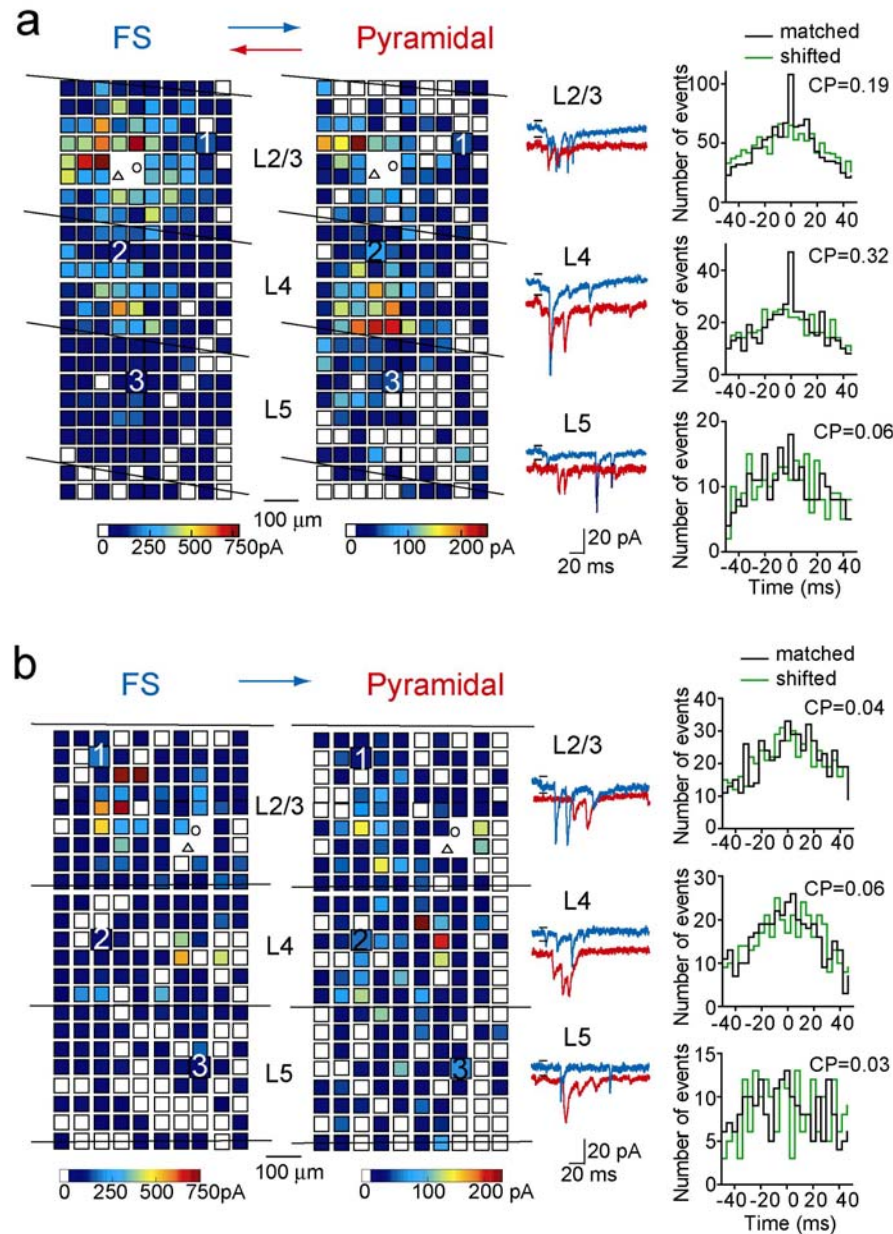
f Spike-evoked IPSCs



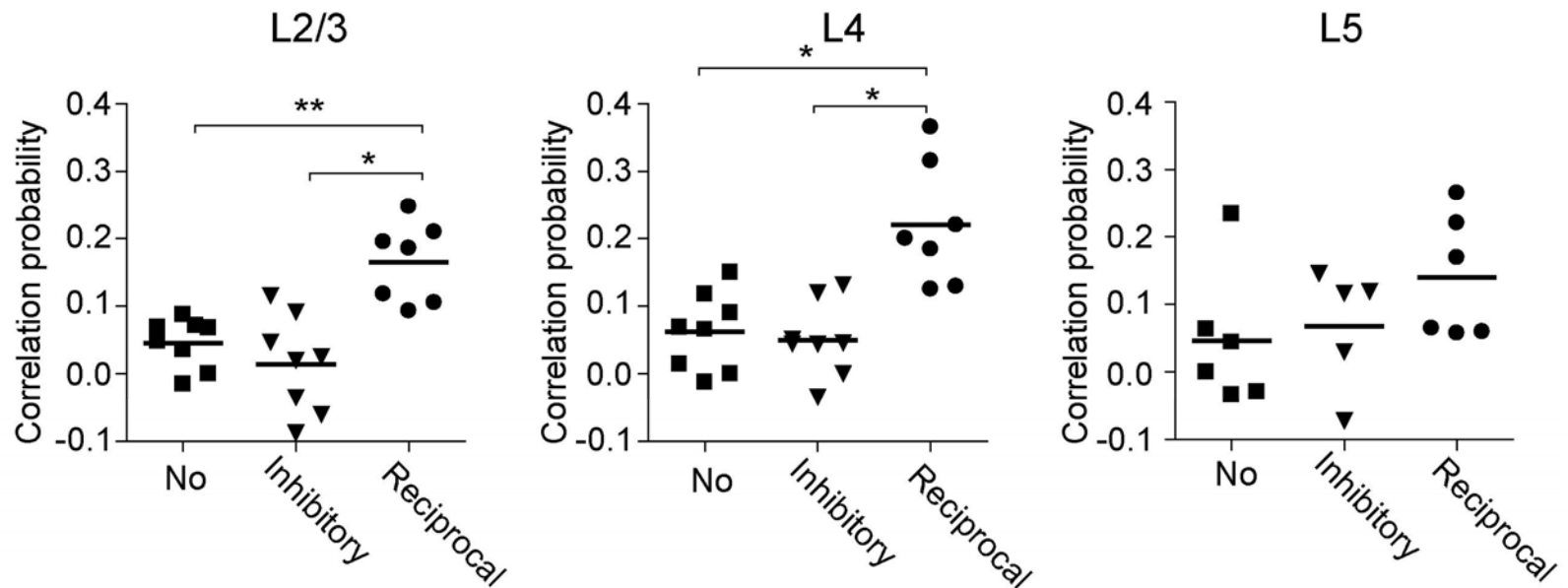
g Spike-evoked EPSCs



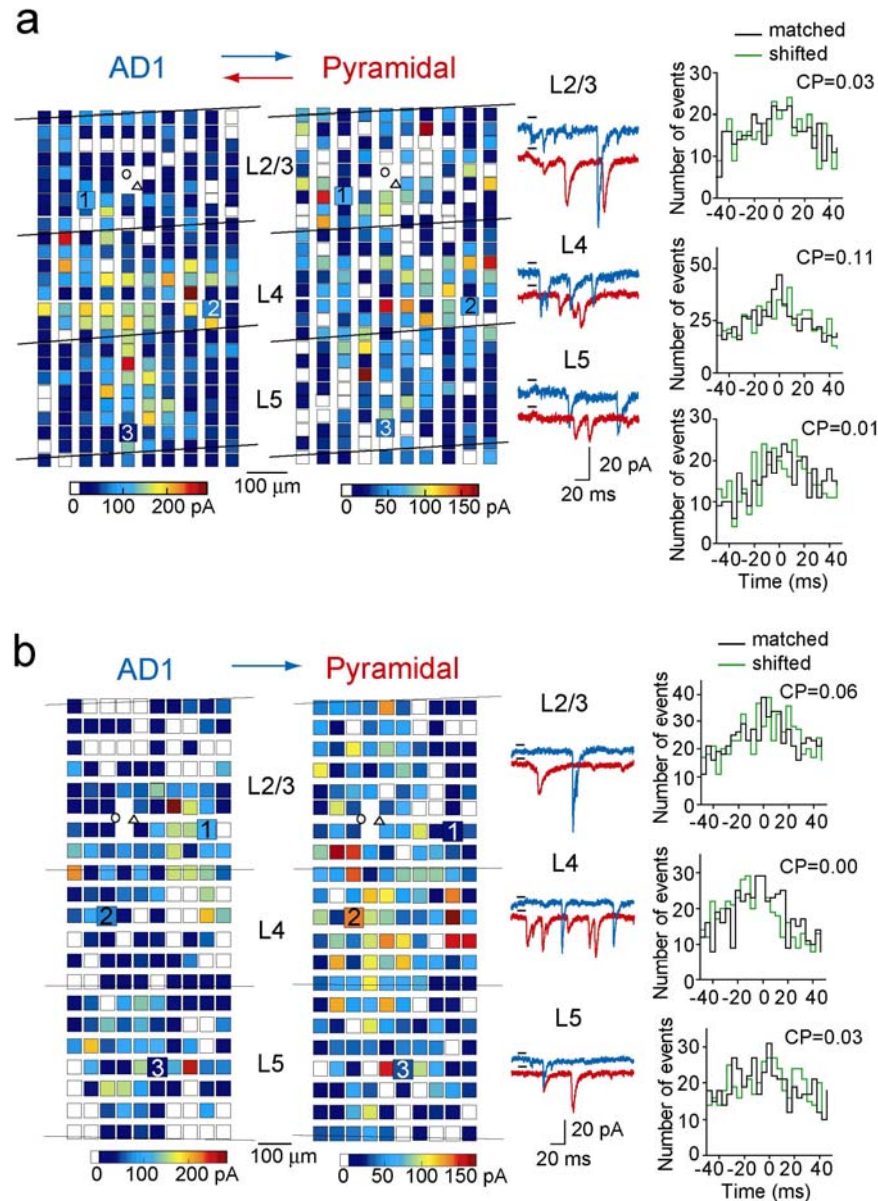
Input Maps and EPSC Correlograms for Fast-Spiking Interneuron – Pyramidal Neuron Pairs



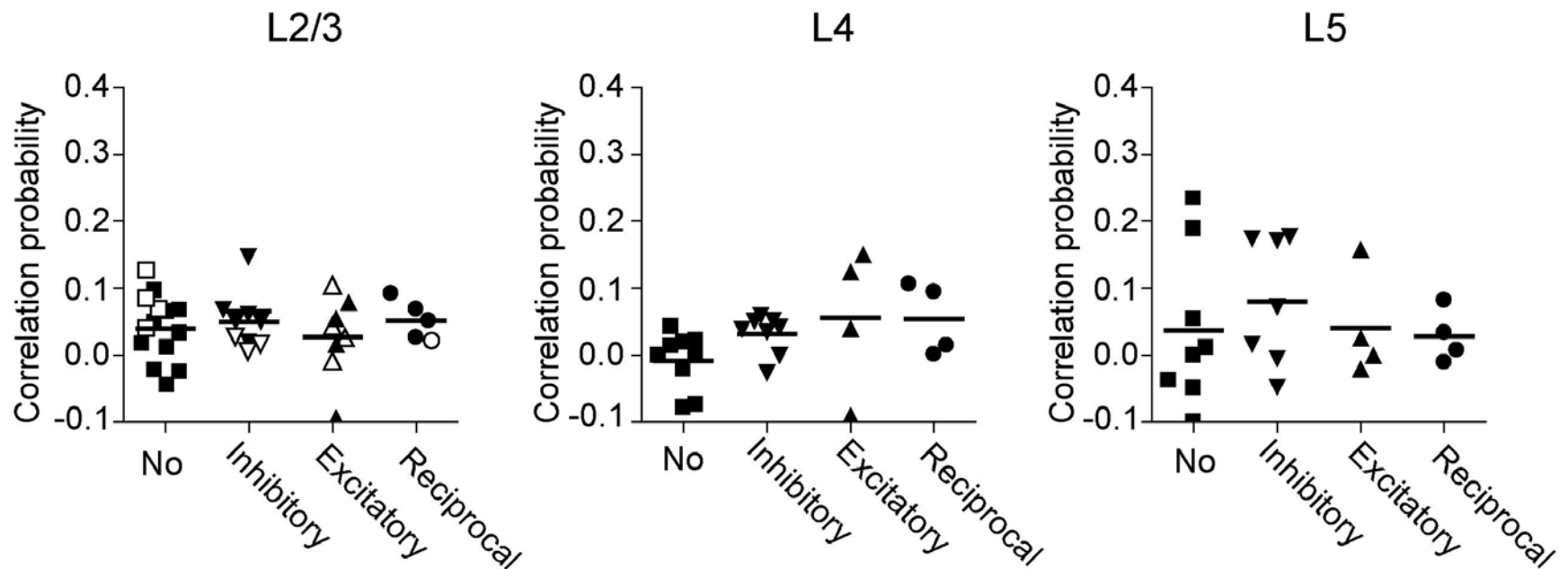
Correlation Probabilities for Fast-Spiking Interneuron – Pyramidal Neuron Cell Pairs (Excitatory Inputs)



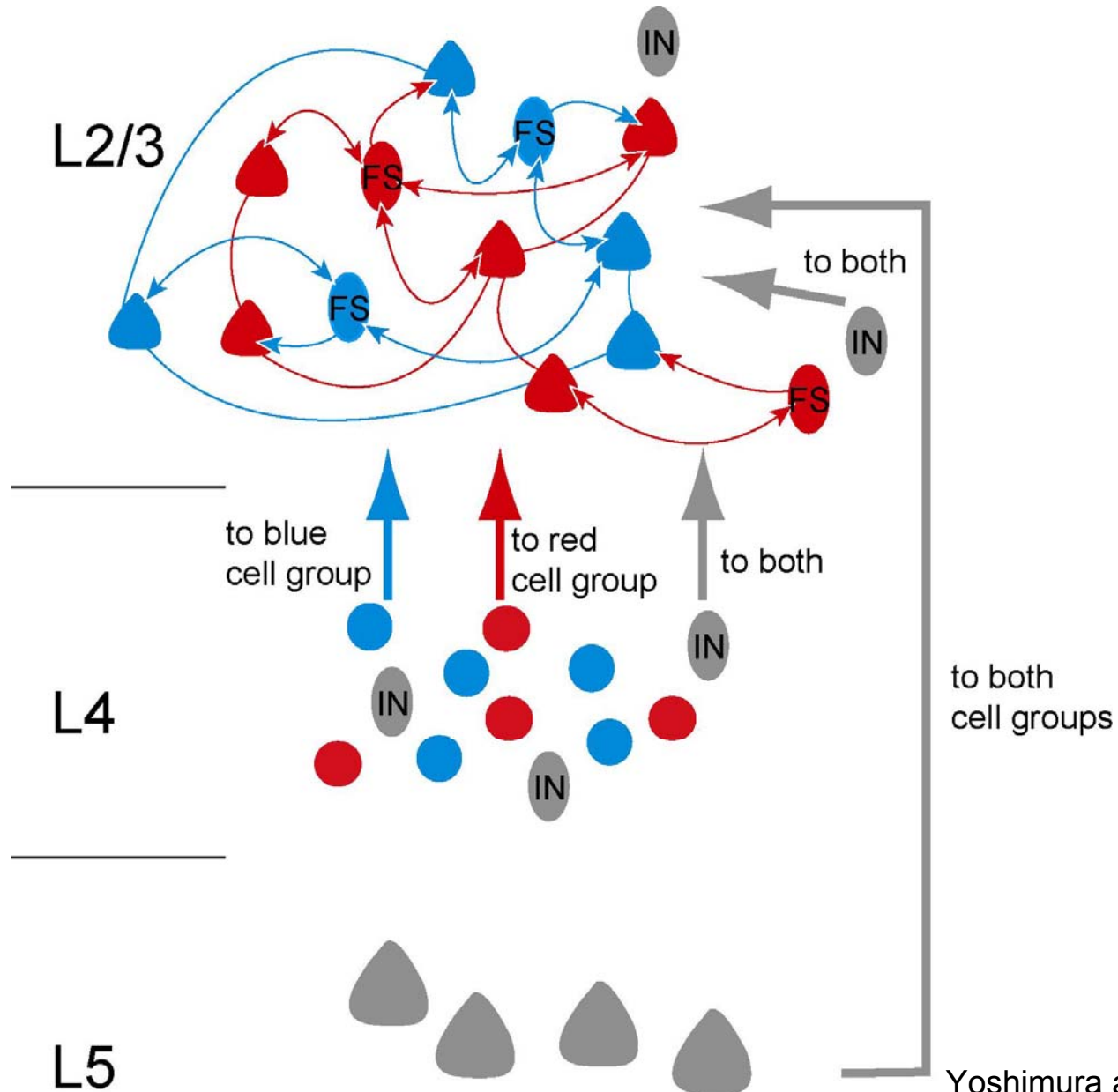
Input Maps and EPSC Correlograms for Adapting Interneuron – Pyramidal Neuron Pairs



Correlation Probabilities for Adapting Interneuron – Pyramidal Neuron Cell Pairs (Excitatory Inputs)

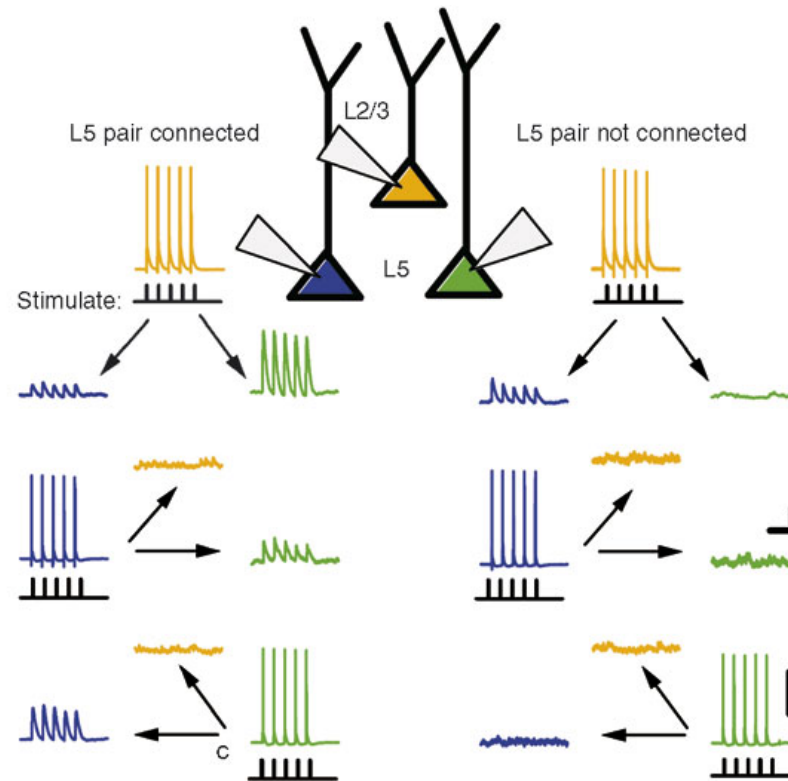


Fine-Scale Specificity of Excitatory and Inhibitory Cortical Circuits

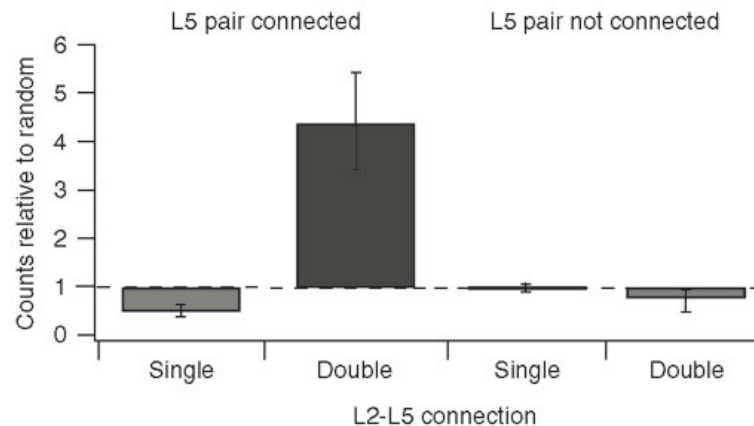


Connected Layer 5 Pyramids Share Common Layer 2/3 Input

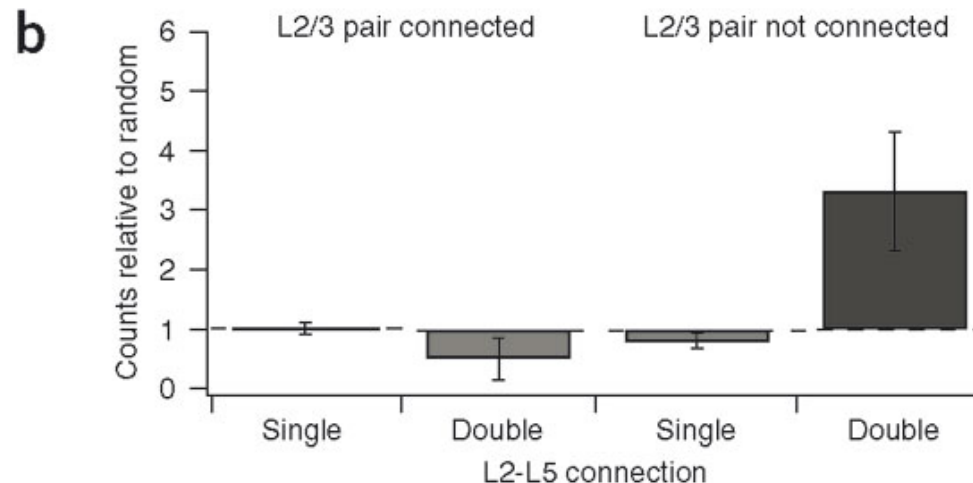
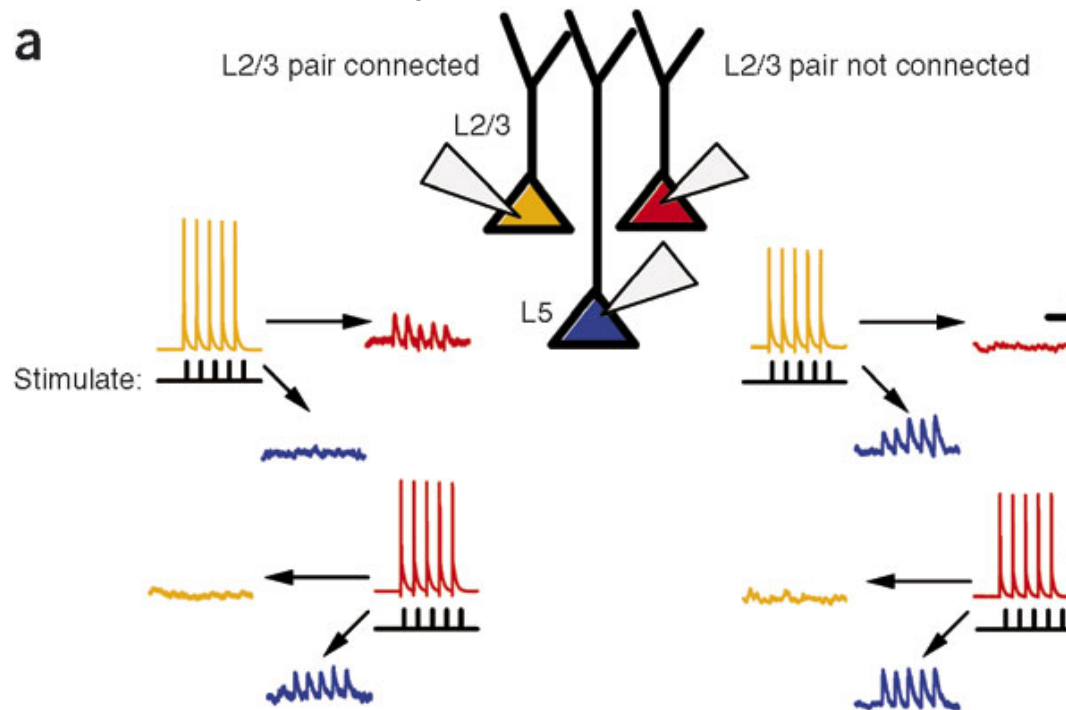
a



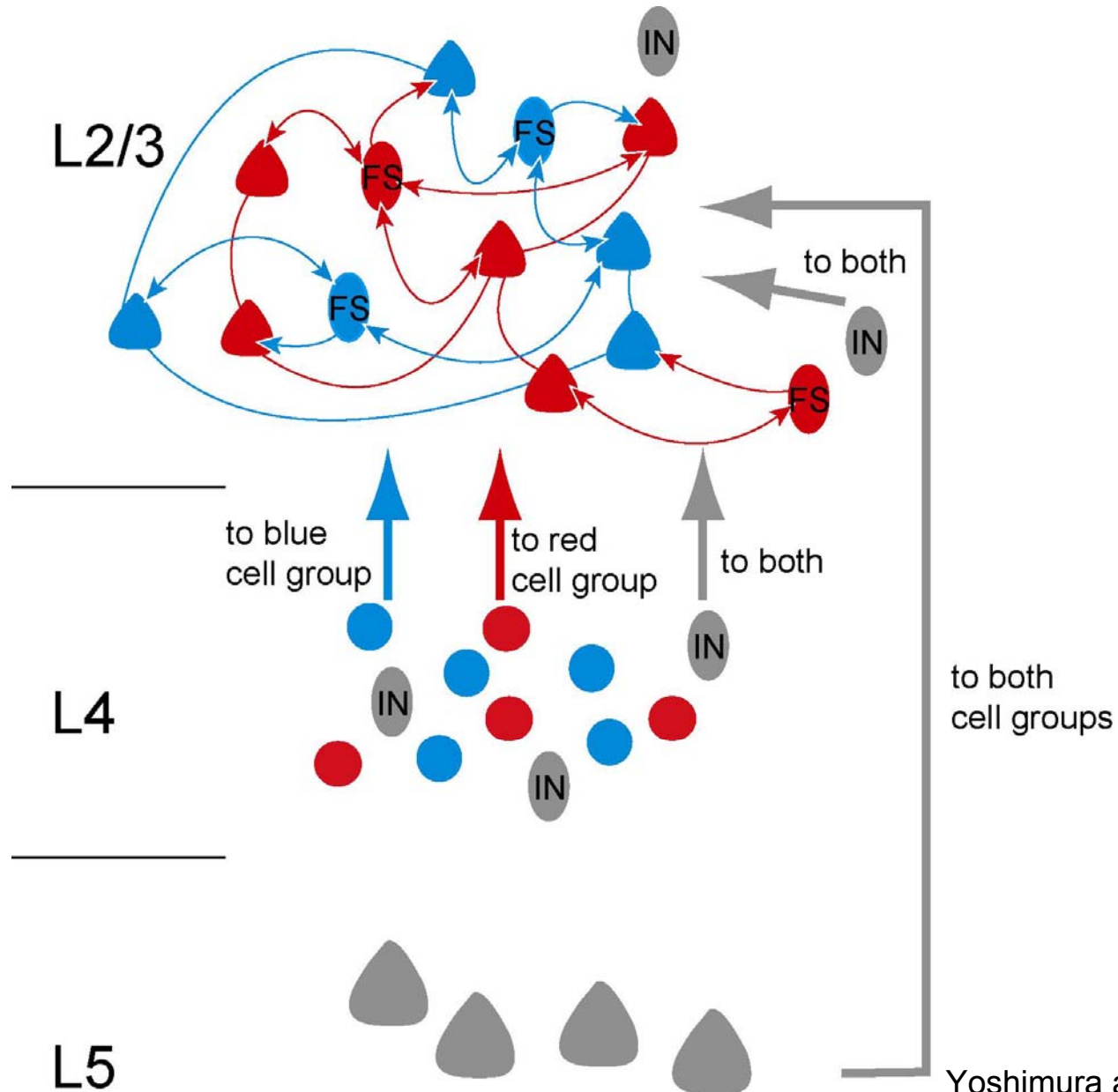
b



Layer 2/3 Pyramids Converge on a Common Layer 5 Cell When They are **Not** Connected



Fine-Scale Specificity of Excitatory and Inhibitory Cortical Circuits



Conclusions

1. Fast spiking interneurons preferentially connect to neighboring pyramids that provide them with reciprocal excitation.
2. Fast spiking interneurons share common fine-scale excitatory input with neighboring pyramidal neurons only when the two cells are reciprocally connected, and not when there is no connection or a one-way, inhibitory to excitatory connection.
3. Adapting inhibitory neurons share little or no common input with neighboring pyramids, regardless of their direct connectivity.

Successes in Systems Neuroscience Now and in The Future

- Systems Neuroscience has been successful in reducing to cell types and microcircuits by studying reduced systems.
- And we have been successful in bringing studies to the behavioral level in awake monkeys. But not at the level of cell types and microcircuits.
- How will we bridge this gap? Molecular and Genetic methods in vivo.

Genetic Methods for Studying Microcircuits with Cell Type Specificity

How to Get Cell Type Specific Expression?

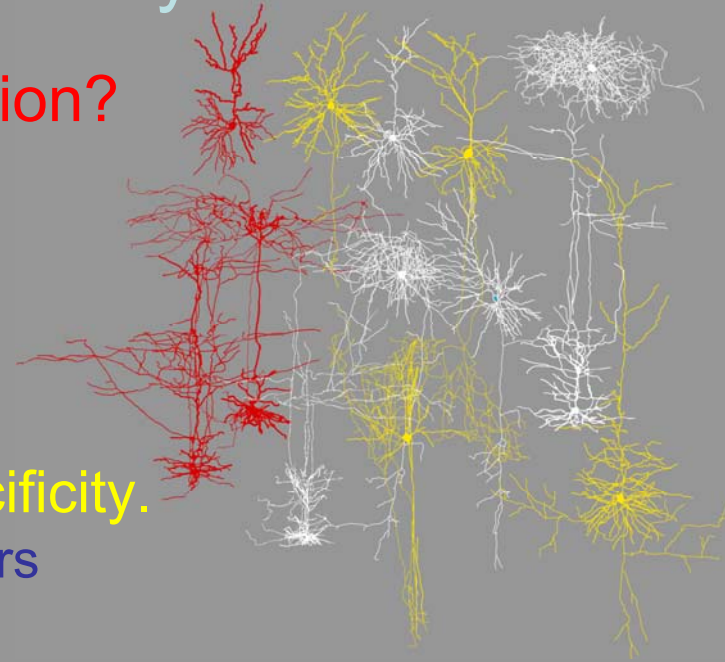
1. Transgenic Mice. This works.

Driver lines that express with cell type specificity.

- E.g. Cre-recombinase, Tet-transactivators

Cross with lines for conditional expression of effector genes.

- Genetically-Expressed Sensors of Activity
- Genes for activation or inactivation
- Genes for mapping connections



Genetic Methods for Studying Microcircuits with Cell Type Specificity

2. Targeting Gene Expression to Cell Types in Species Where Transgenics are not Practical

Viral Infection to Introduce Transgenes - Easy

- Allows Genetic Manipulation in Primates

- Allows Targeting to Brain Area of Interest

- Adeno-Associated Virus (AAV),
Lentivirus, HSV Amplicons and more

Cell Type Specific Promoters - Hard

- Allows Targeting to Cell Types of Interest

- Fugu promoters, Use BACs in HSV amplicons

Retrograde Infection of Specific Types of Projection Neurons – Works but ...

- Allows Targeting to Specific Types of Projection Neurons

- HSV vectors

- Rabies Pseudotyped Lentivirus

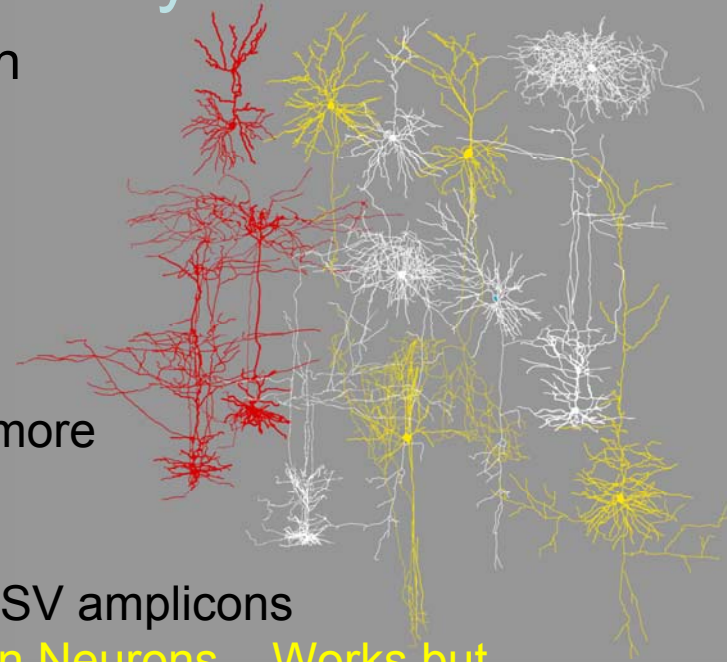
- Adenovirus

- G-deleted Rabies

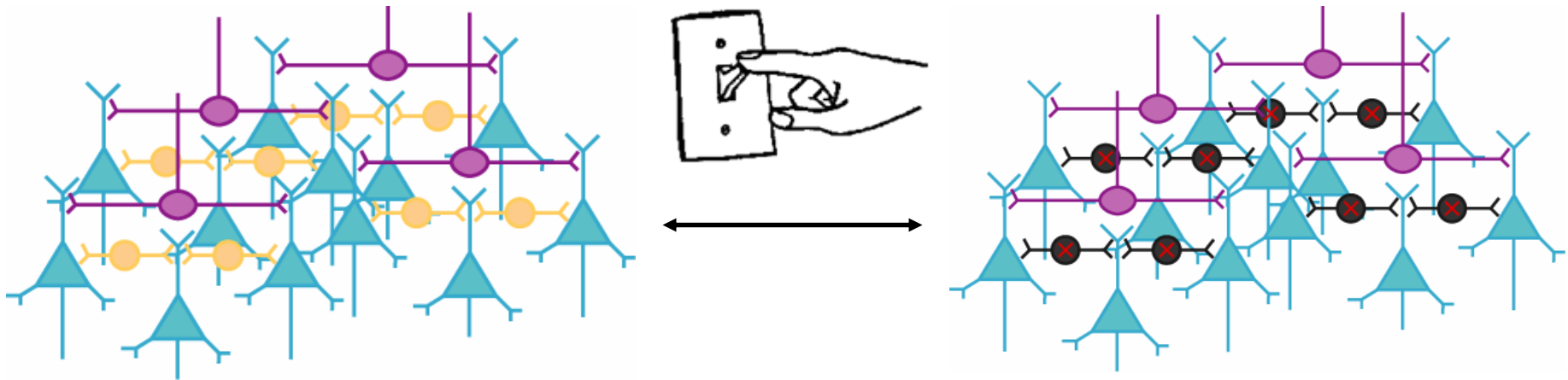
Engineered Viral Tropism – Recent In Vivo Attempts Look Promising

- Targeting based on viral uptake via cell surface receptors

- TVA/Ligand Bridge Proteins, Antibody Bridges

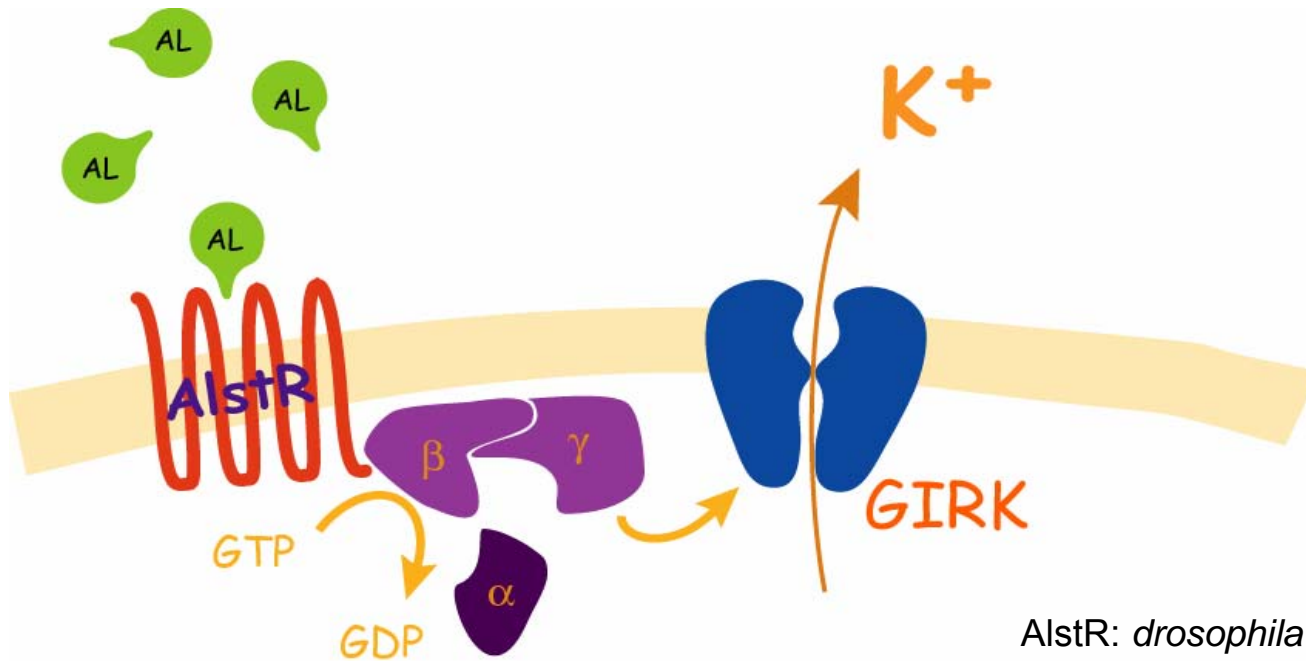


How does circuitry underlie function?



- Directly compare circuit properties in presence/absence of targeted cell type

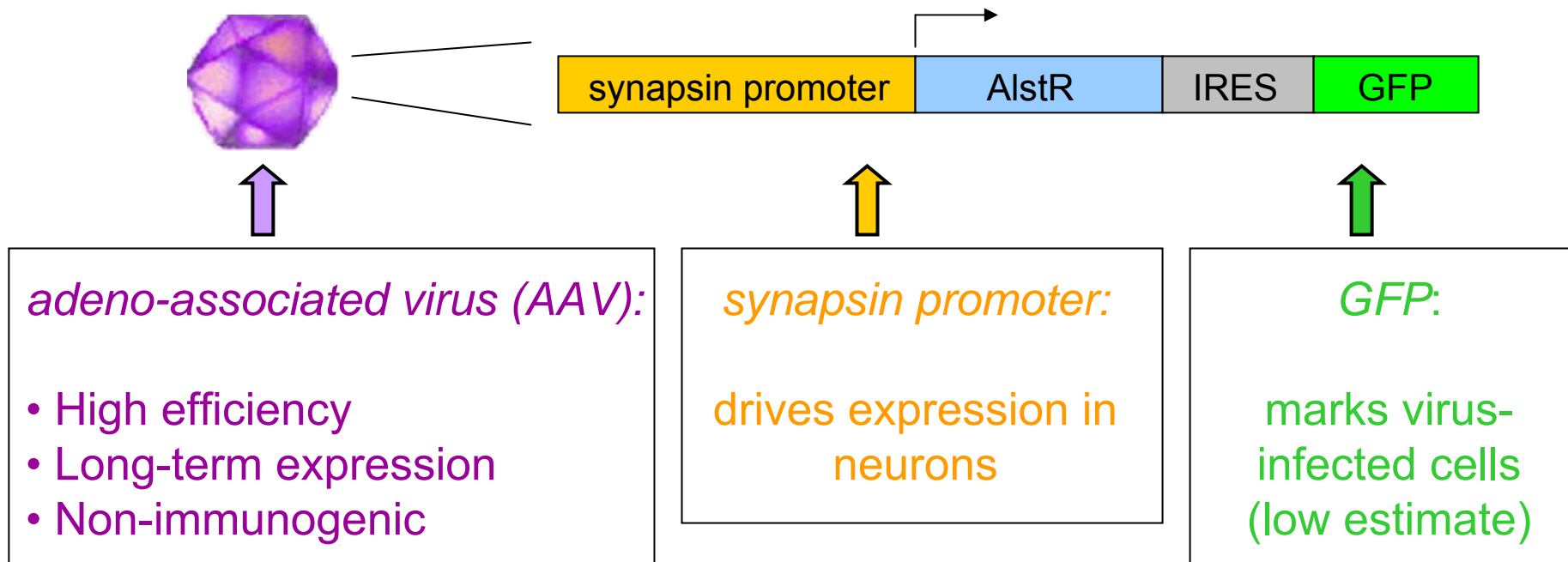
The molecular switch: *Allatostatin receptor (AlstR) system*



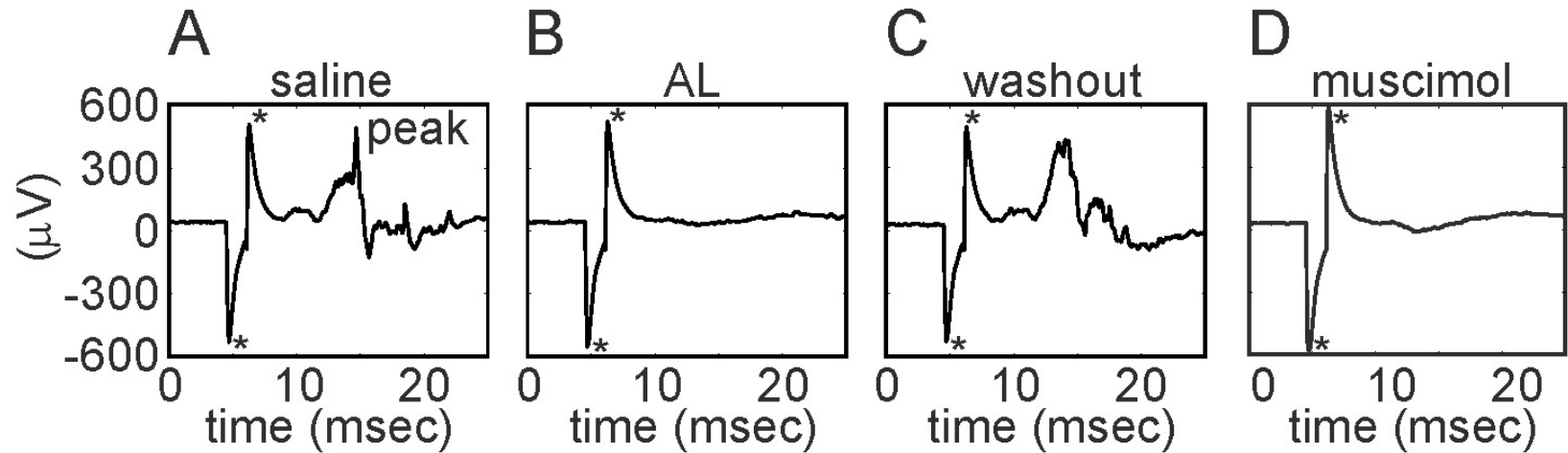
AlstR: *drosophila* allatostatin receptor type 1
AL : allatostatin peptide

- K⁺ channels open when AL applied → excitability decreases
- normal excitability when AL not present

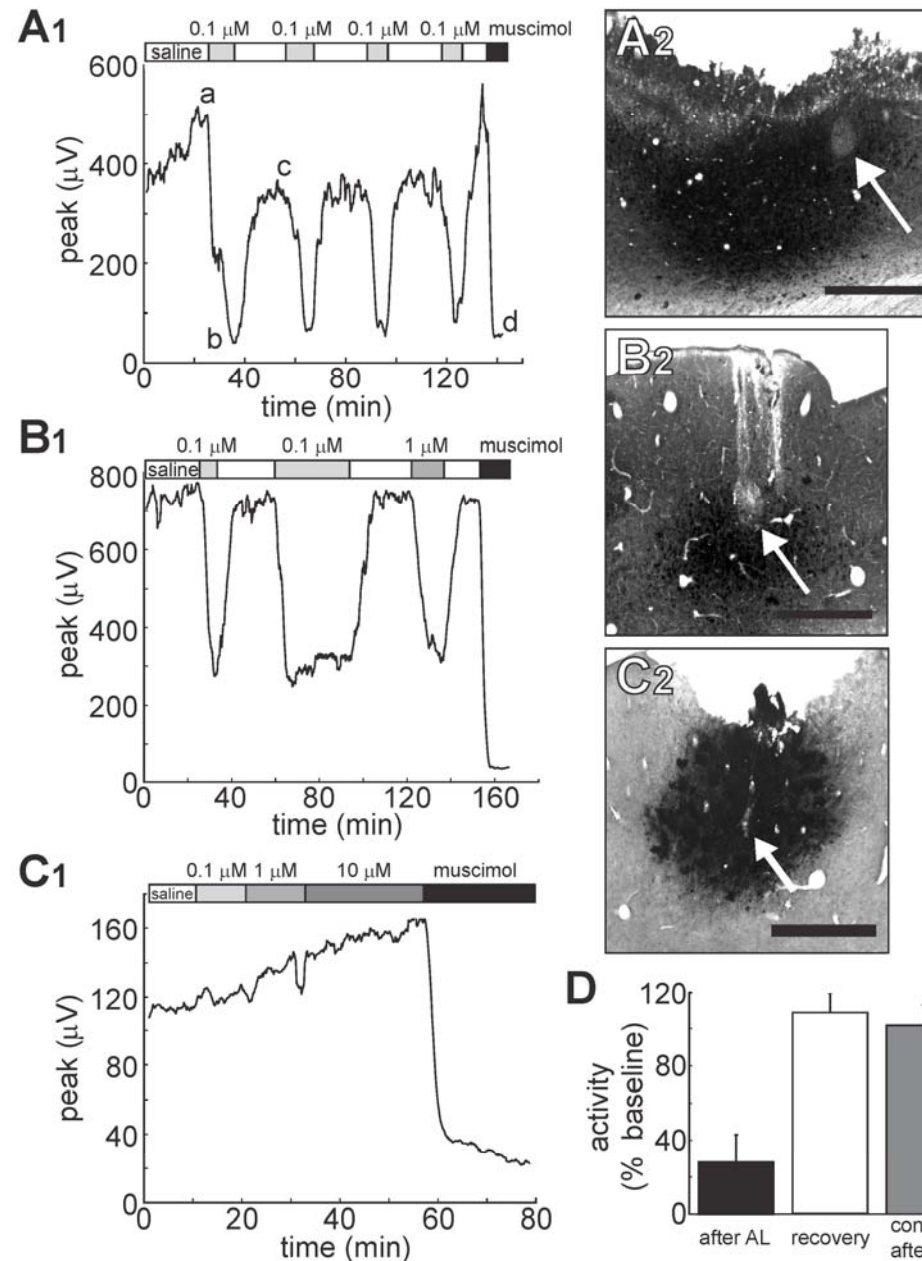
Virus-mediated delivery of AlstR



AlstR Inactivation of LFPs Recorded in Rat Barrel Cortex



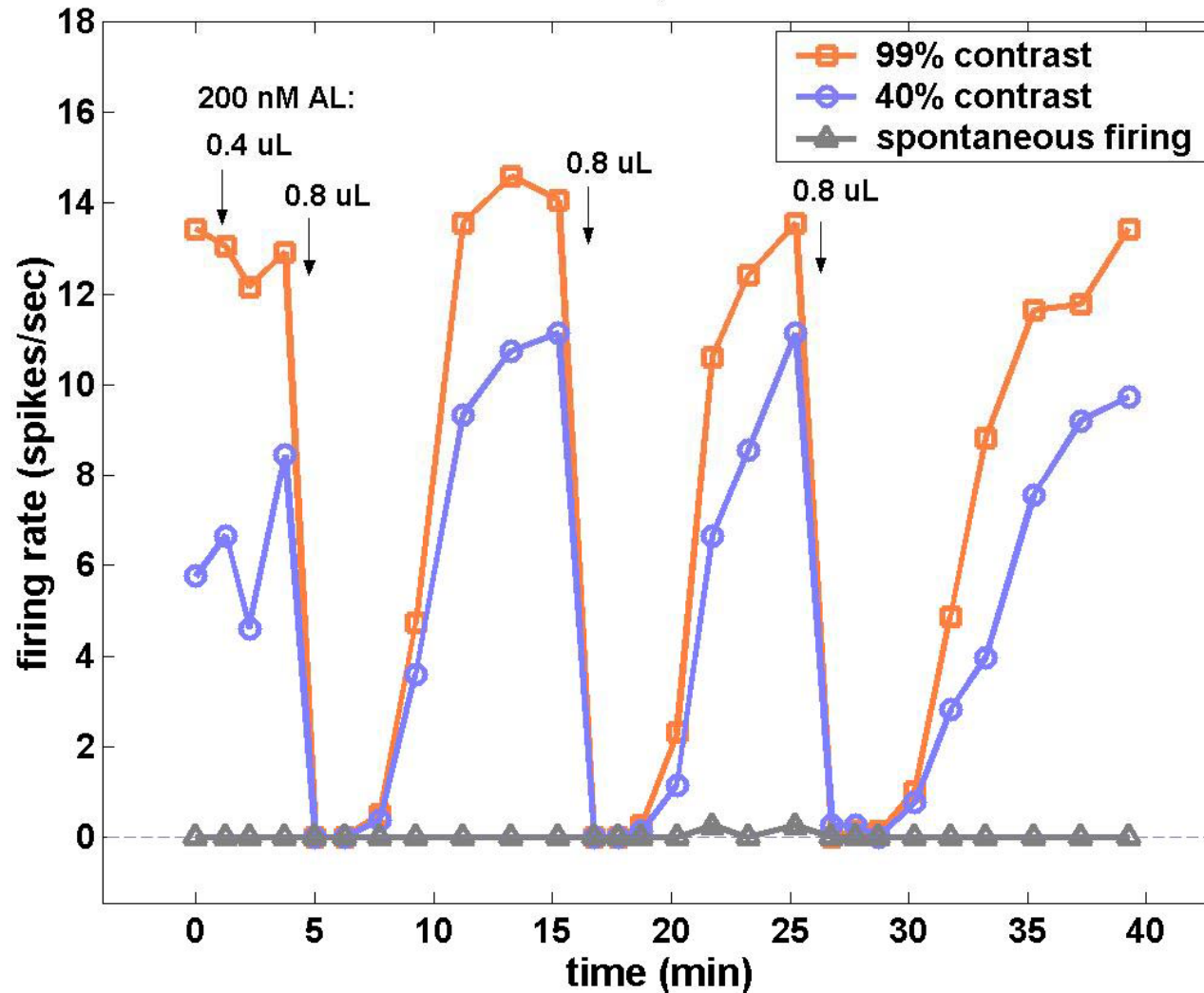
AlstR Inactivation of LFPs Recorded in Rat Barrel Cortex



Monkey LGN

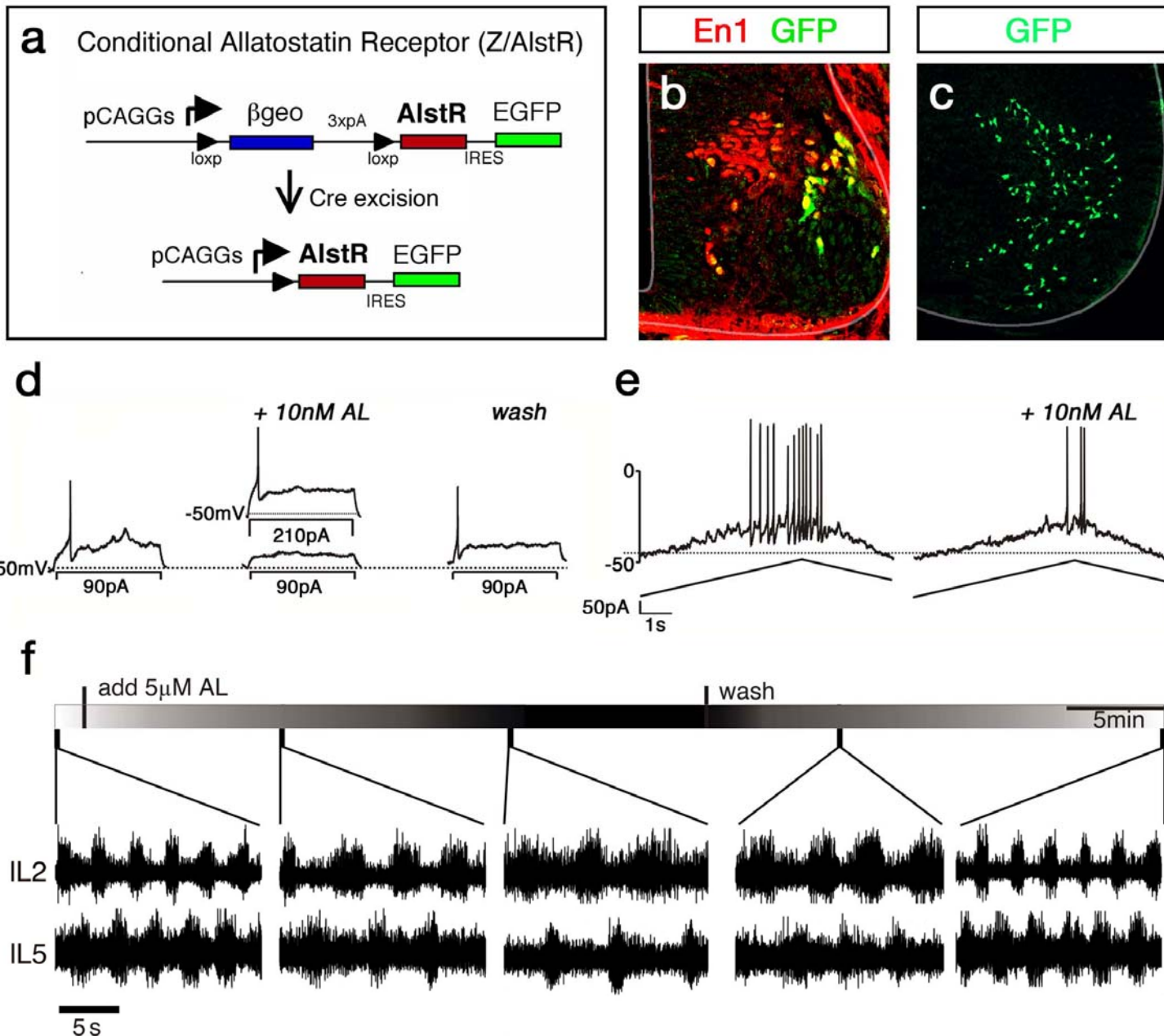
Single-Unit Recording

fred2, u007



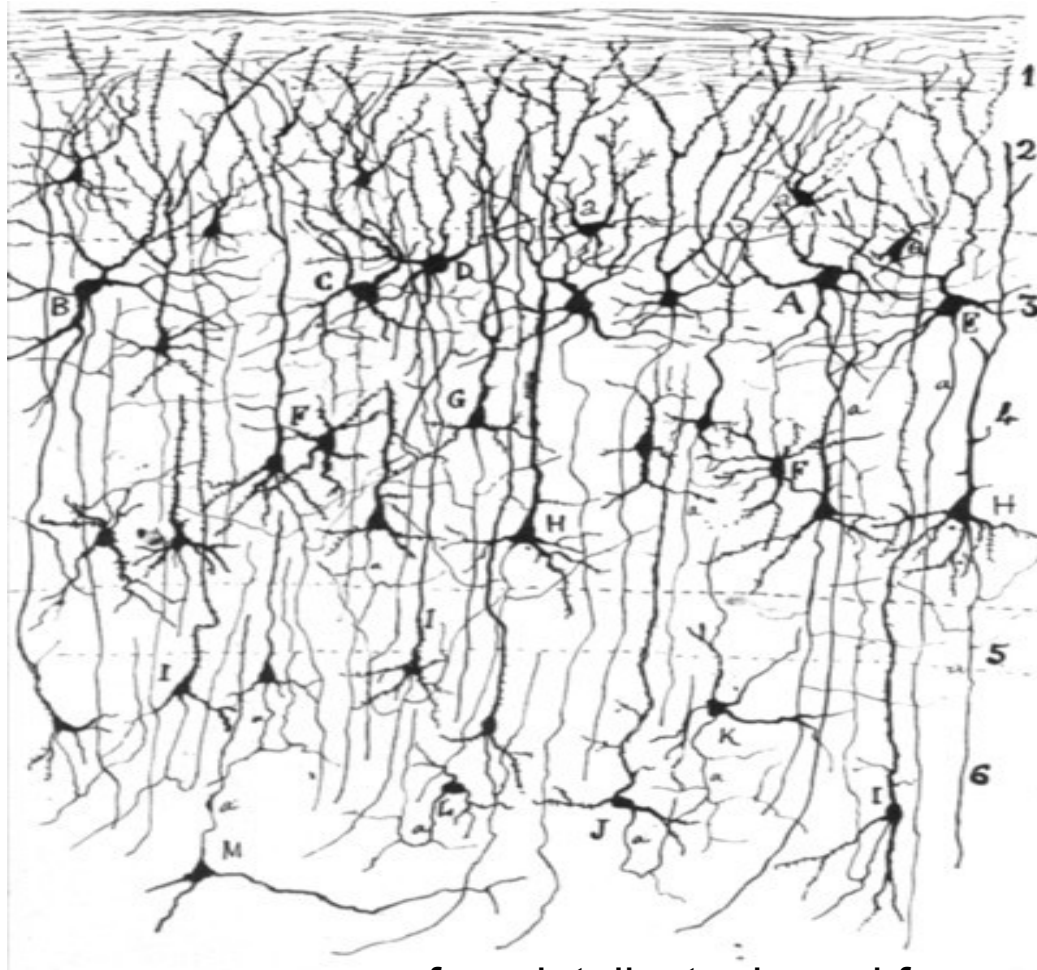
- first injection probably reflects reflux of fluid up pipette
- started to pick up another unit at around 33 minutes

Selective and Reversible Inactivation of V1 Interneurons Prolongs Step Cycle



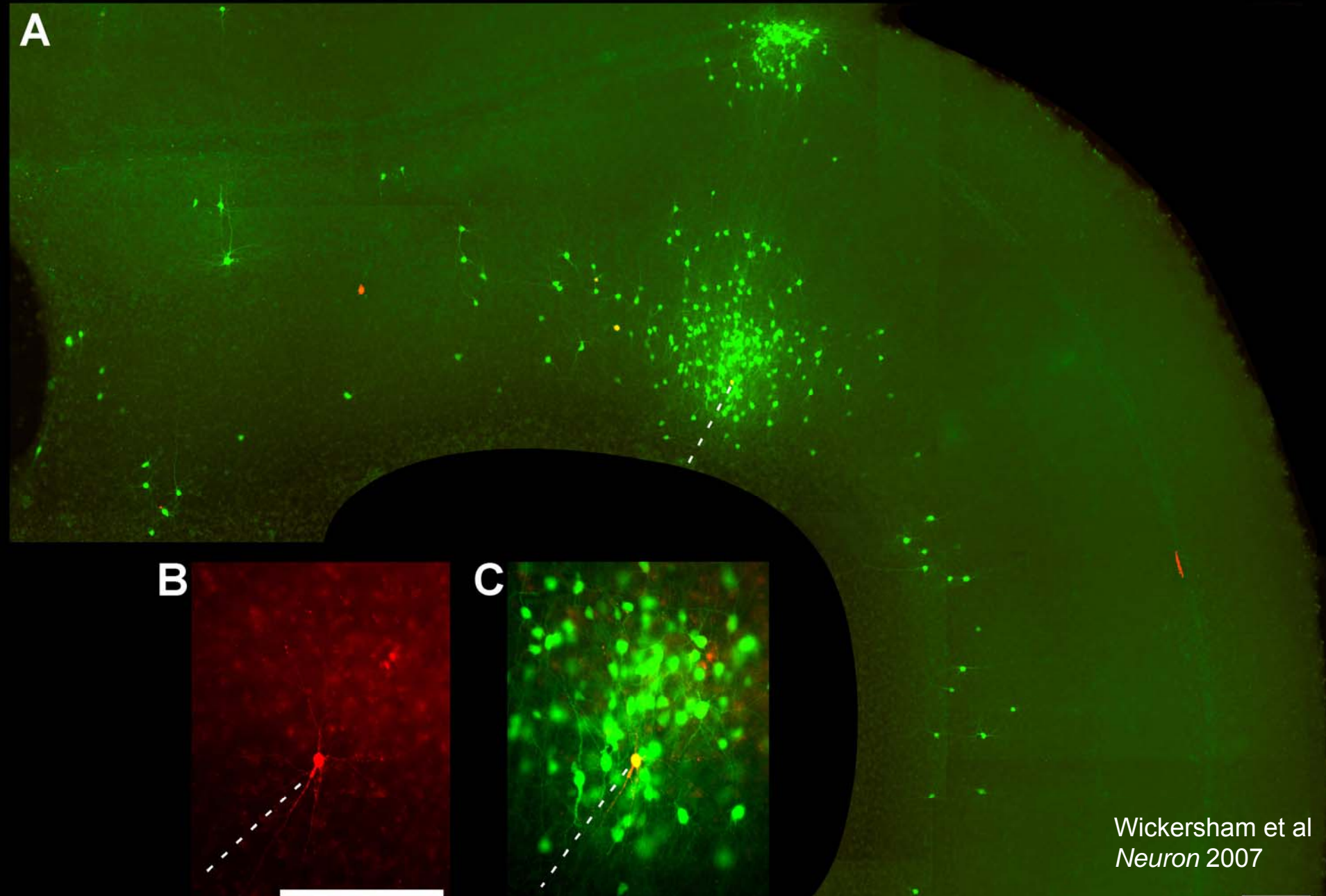
Brain Structures are Characterized by a Jungle of Neurons with their Axons and Dendrites Intimately Entangled

Short of 3-D EM reconstruction of the whole brain, how do we untangle this mess?



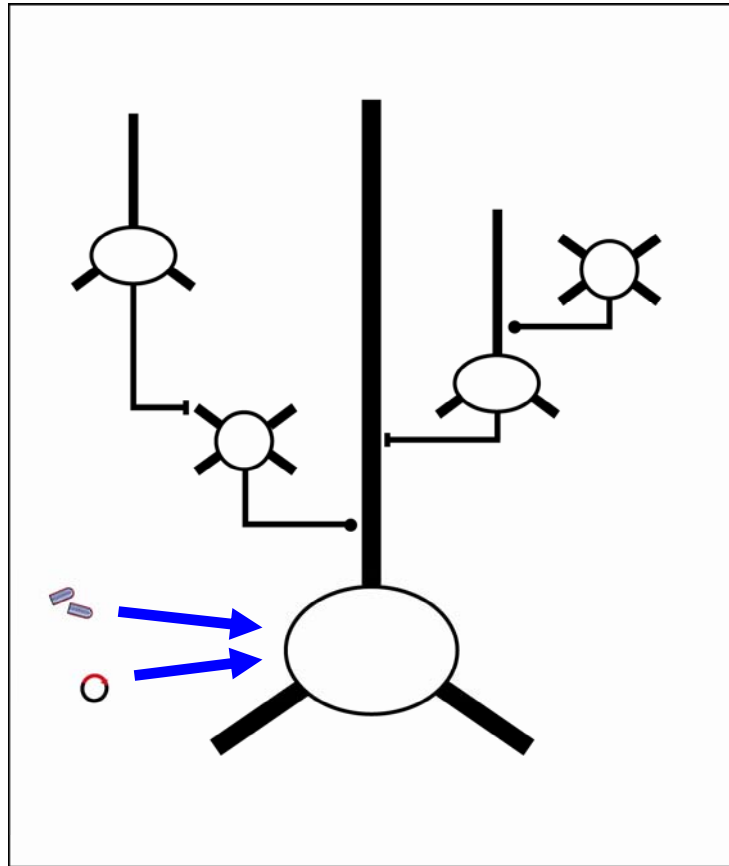
Unfortunately, nature seems unaware of our intellectual need for convenience and unity, and very often takes delight in complication and diversity. *Santiago Ramon y Cajal*

Identifying Neurons that are Directly Presynaptic to a Single Neuron or to a Genetically Targeted Neuronal Population



TRANSCOMPLEMENTED TRANSSYNAPTIC TRACING

CORE CONCEPT

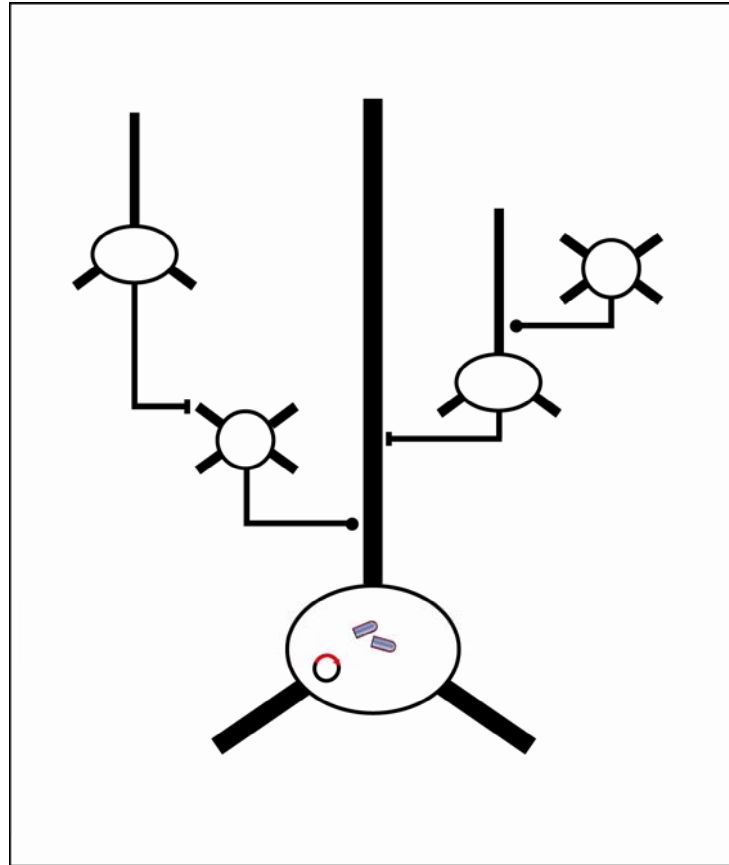


INTRODUCE INTO A CELL BOTH

- 1) A DELETION-MUTANT TRACING VIRUS AND
- 2) THE MISSING VIRAL GENE

TRANSCOMPLEMENTED TRANSSYNAPTIC TRACING

CORE CONCEPT

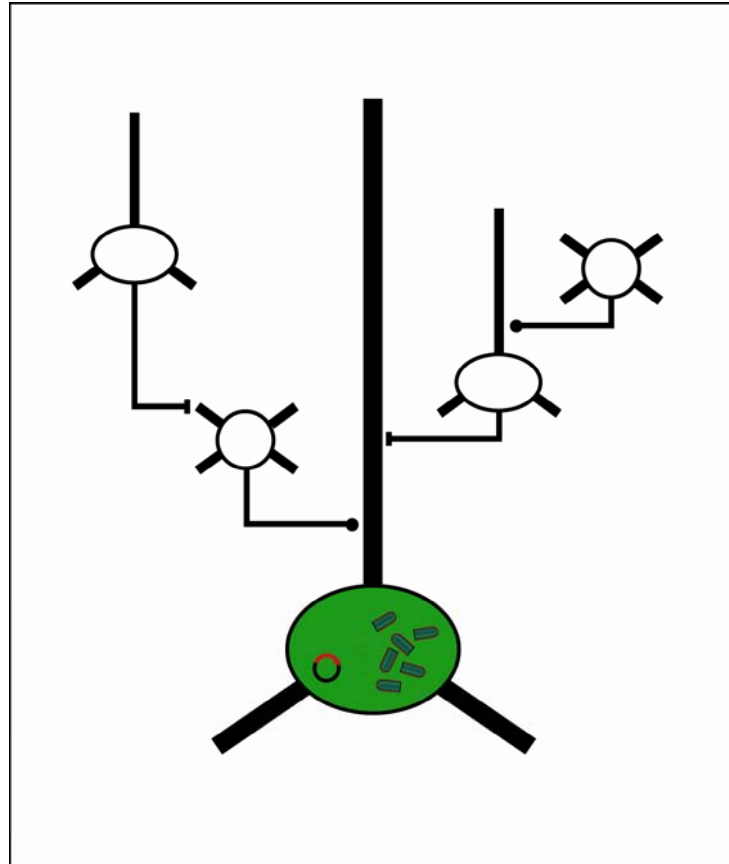


INTRODUCE INTO A CELL BOTH

- 1) A DELETION-MUTANT TRACING VIRUS AND
- 2) THE MISSING VIRAL GENE

TRANSCOMPLEMENTED TRANSSYNAPTIC TRACING

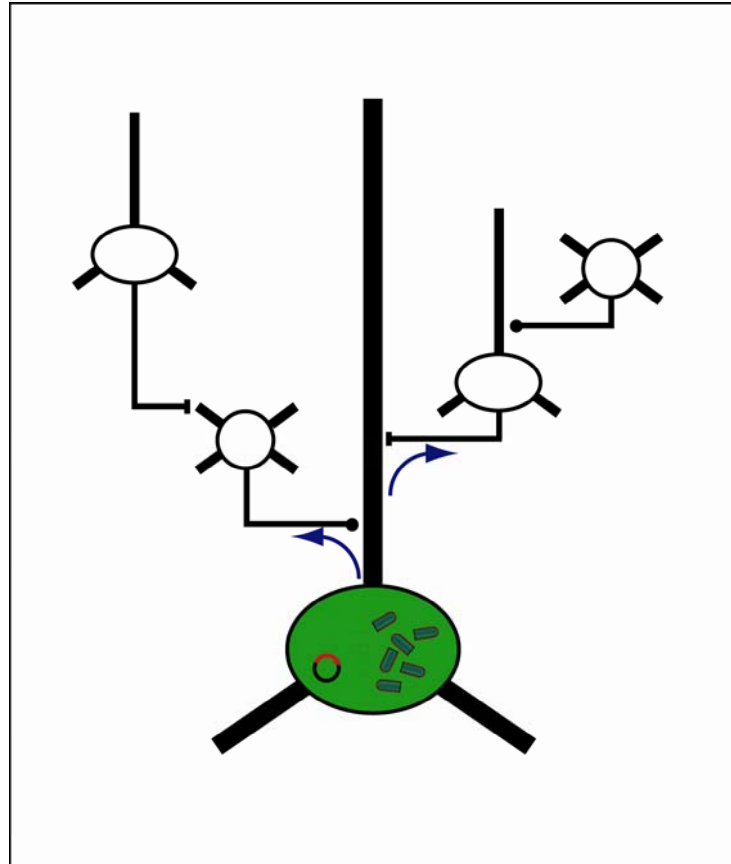
CORE CONCEPT



INITIALLY INFECTED CELL FILLS WITH GFP

TRANSCOMPLEMENTED TRANSSYNAPTIC TRACING

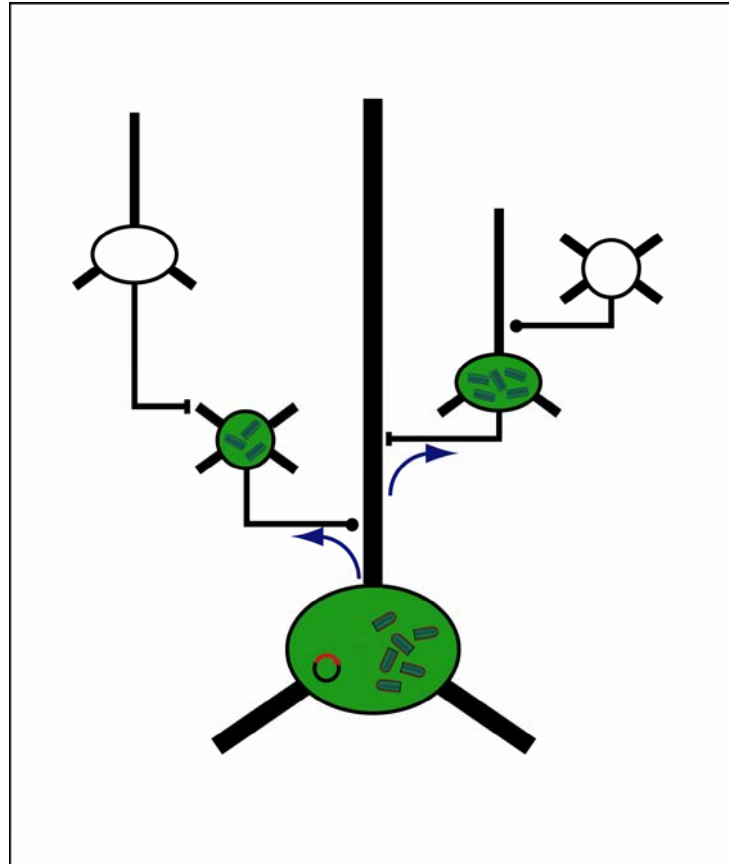
CORE CONCEPT



TRANSCOMPLEMENTED IN THIS CELL,
THE VIRUS REPLICATES AND SPREADS

TRANSCOMPLEMENTED TRANSSYNAPTIC TRACING

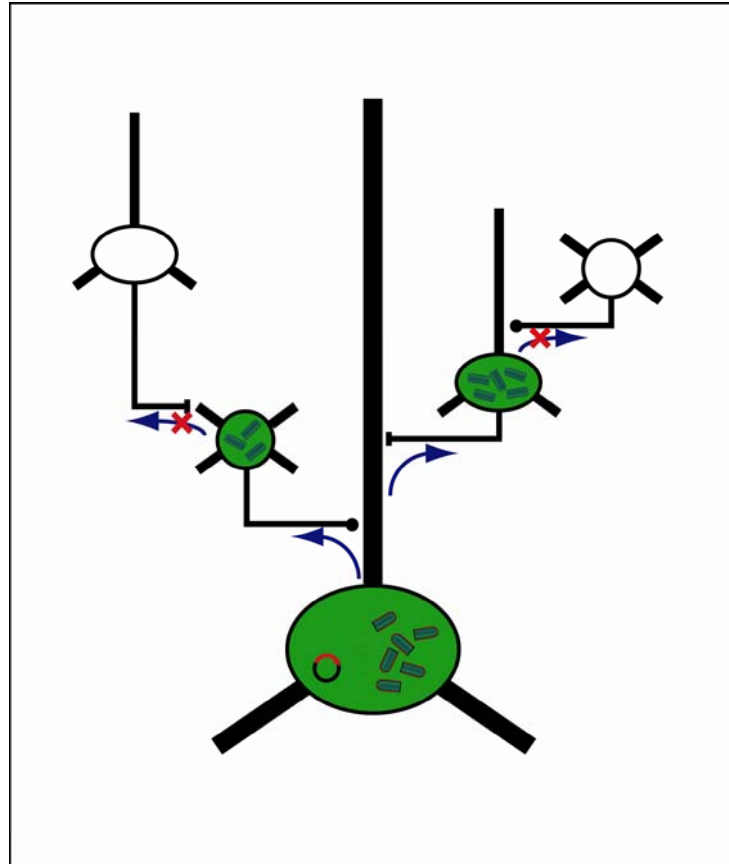
CORE CONCEPT



NEWLY INFECTED NEURONS FILL WITH GFP

TRANSCOMPLEMENTED TRANSSYNAPTIC TRACING

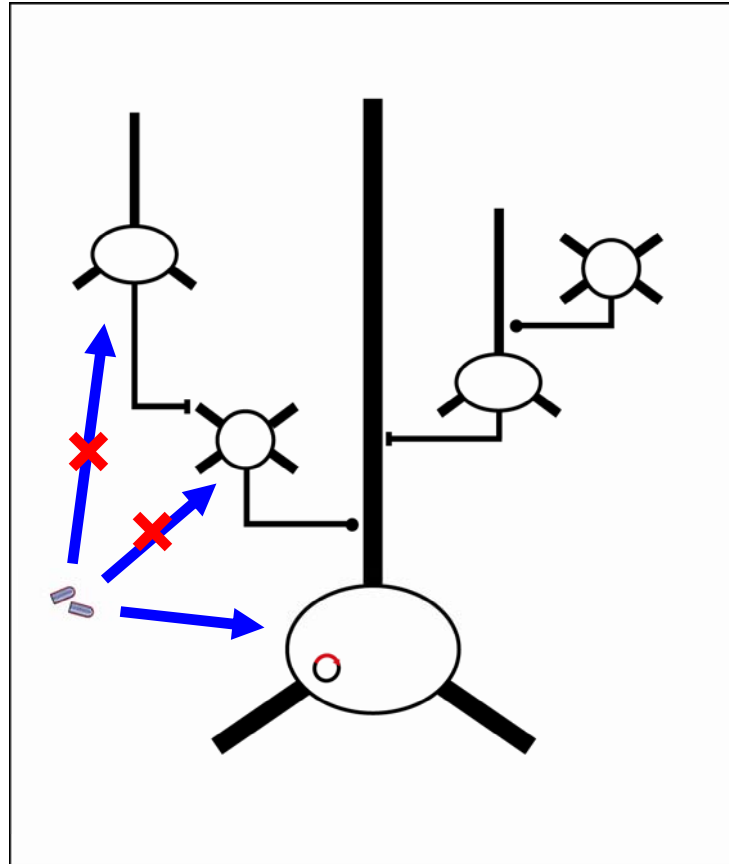
CORE CONCEPT



SINCE MISSING GENE NOT PRESENT IN THE NEWLY
INFECTED CELLS, VIRUS STOPS THERE

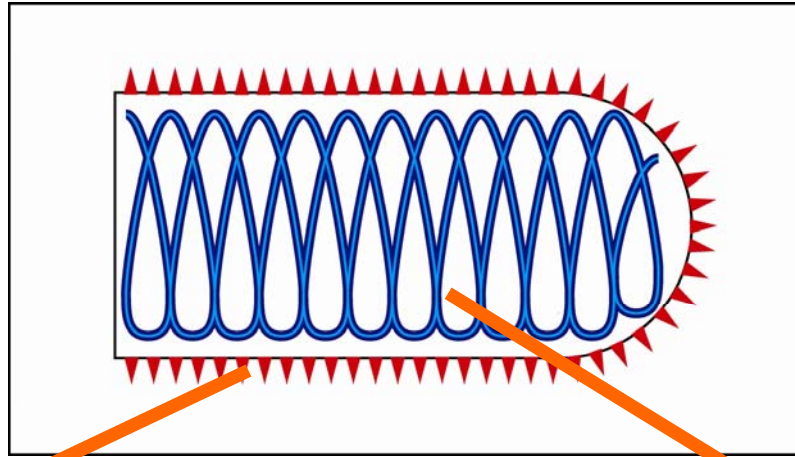
TRANSCOMPLEMENTED TRANSSYNAPTIC TRACING

& HOW TO SELECTIVELY INFECT?



INITIAL INFECTION & GENE MUST BE
RESTRICTED TO CELL(S) OF INTEREST

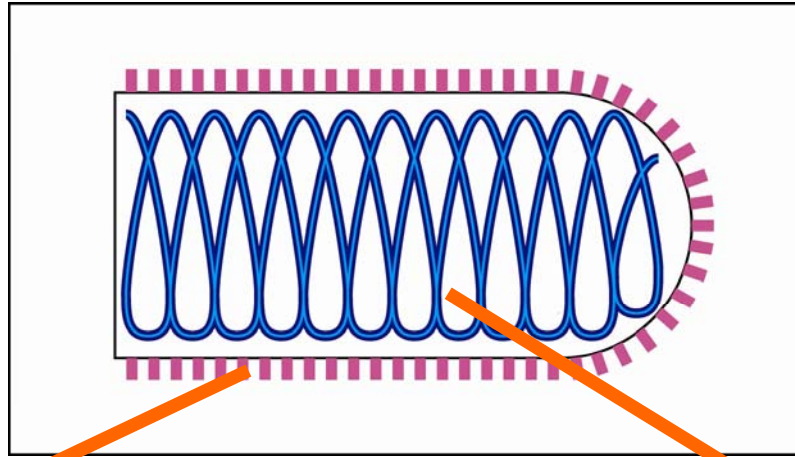
TRANSCOMPLEMENTED TRANSSYNAPTIC TRACING TARGETING RV INFECTION



**GLYCOPROTEIN
IN MEMBRANE**

**GLYCOPROTEIN GENE
DELETED FROM GENOME**

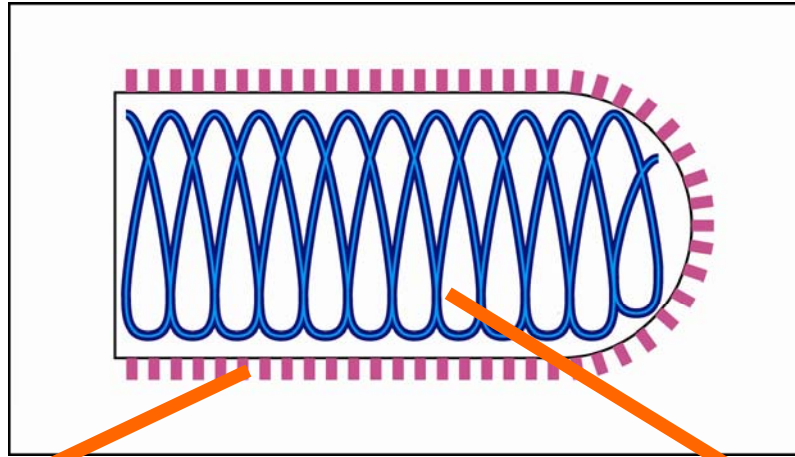
TRANSCOMPLEMENTED TRANSSYNAPTIC TRACING TARGETING RV INFECTION



**ALIEN
GLYCOPROTEIN
IN MEMBRANE**

**GLYCOPROTEIN GENE
DELETED FROM GENOME**

TRANSCOMPLEMENTED TRANSSYNAPTIC TRACING TARGETING RV INFECTION



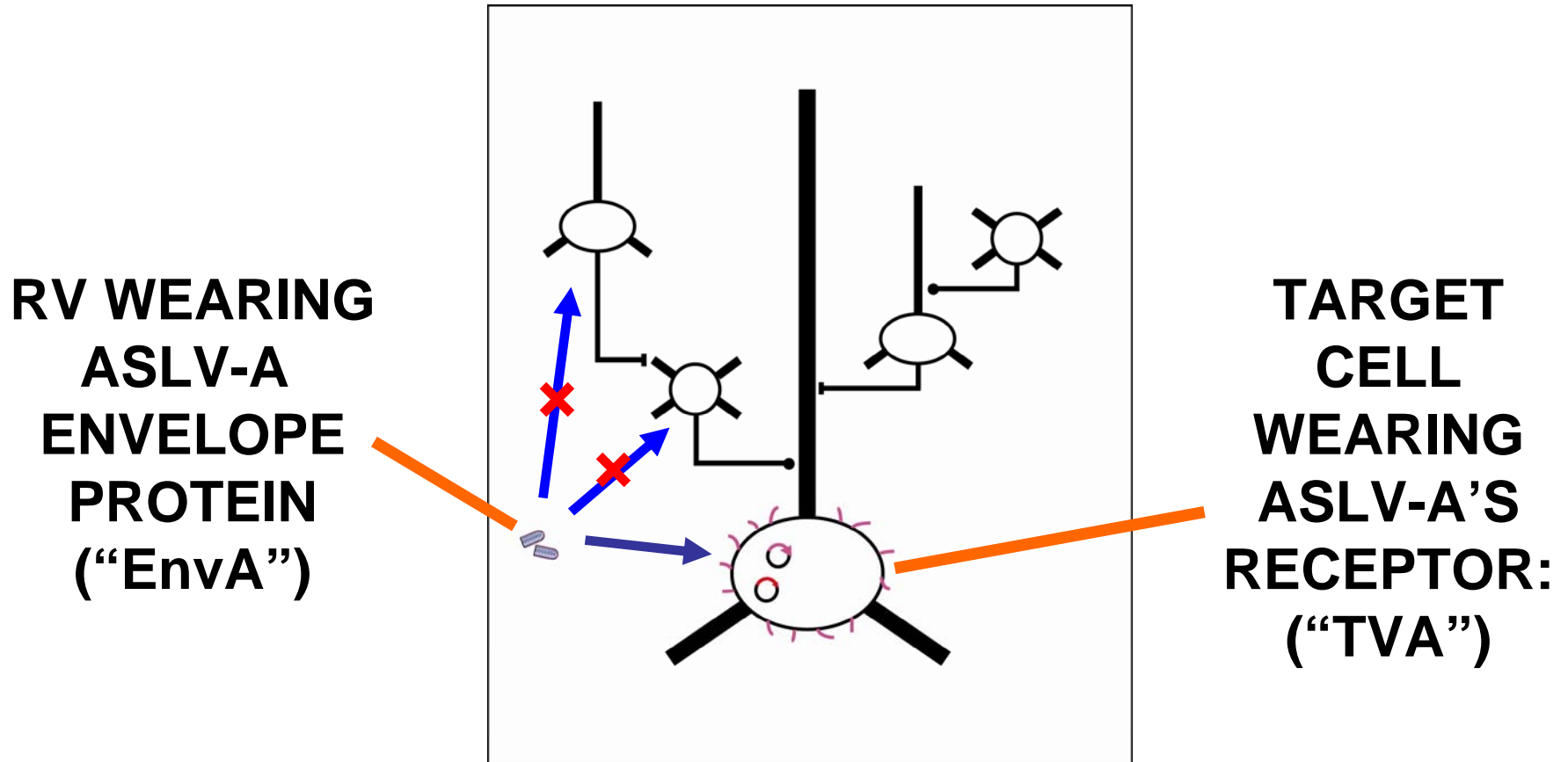
ALIEN
GLYCOPROTEIN
IN MEMBRANE

GLYCOPROTEIN GENE
DELETED FROM GENOME

GLYCOPROTEIN IN ENVELOPE REPLACED
WITH ONE FROM A DIFFERENT VIRUS
("PSEUDOTYPING")

TRANSCOMPLEMENTED TRANSSYNAPTIC TRACING

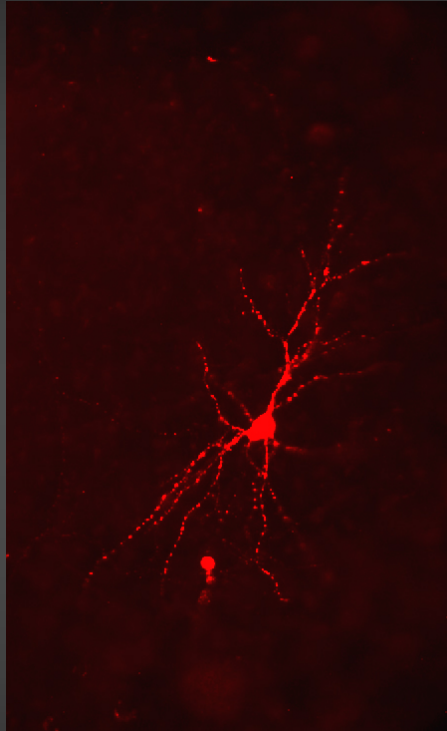
RV PSEUDOTYPED WITH ASLV-A



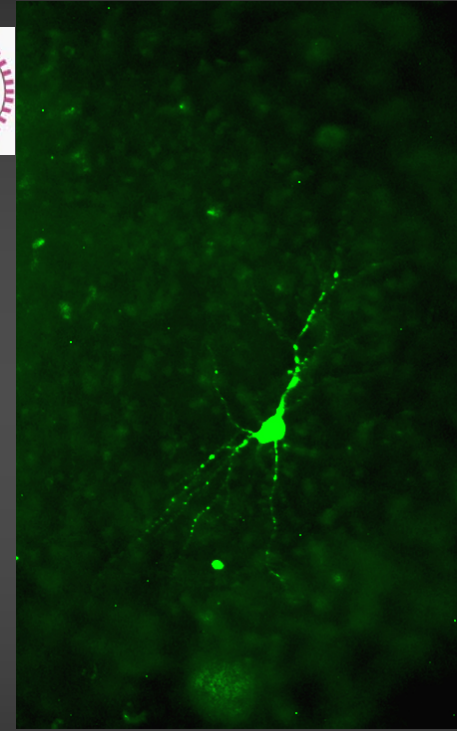
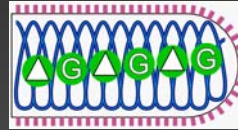
IN COLLABORATION WITH RICHARD BARNARD
AND JOHN YOUNG

Control: Shoot Plasmids for TVA and DSRED

Infect with EnvA- Δ G, GFP Rabies



DSRED2

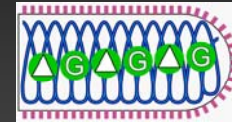
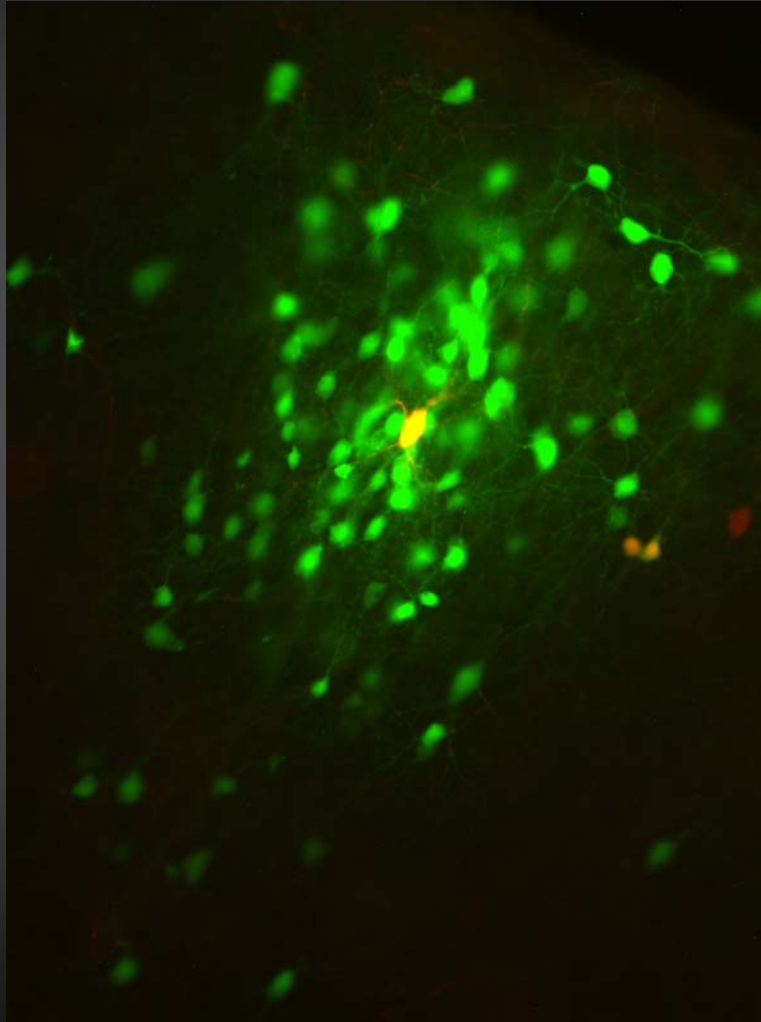


GFP

EnvA Pseudotyped Virus Infects
TVA-expressing Cells

Wickersham et al
Neuron 2007

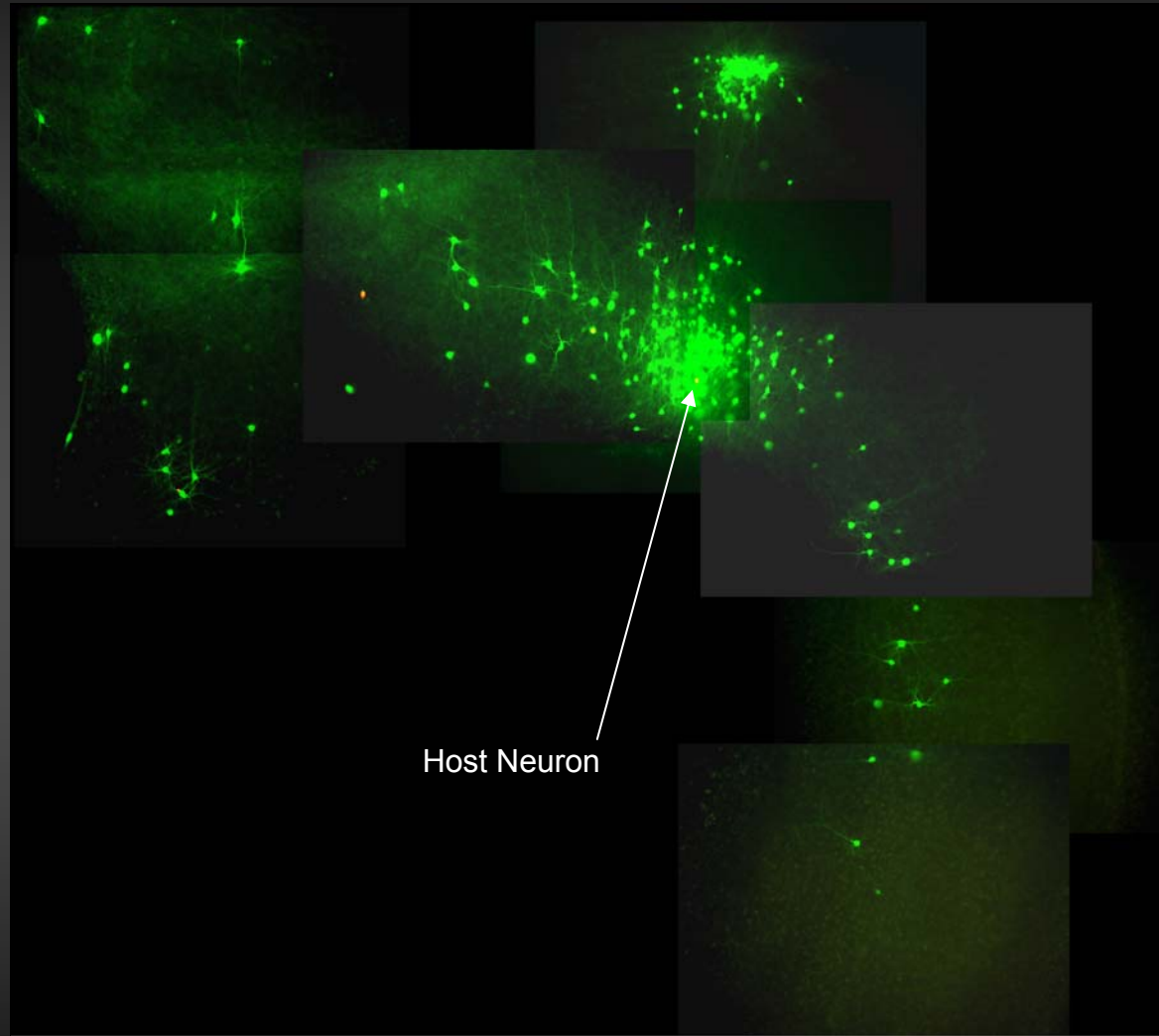
Shoot All 3 Plasmids – TVA, DSRED and Rabies Glycoprotein

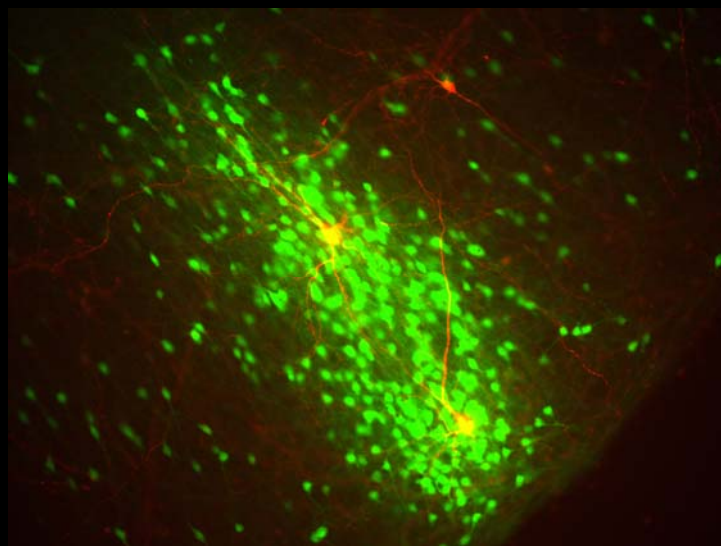
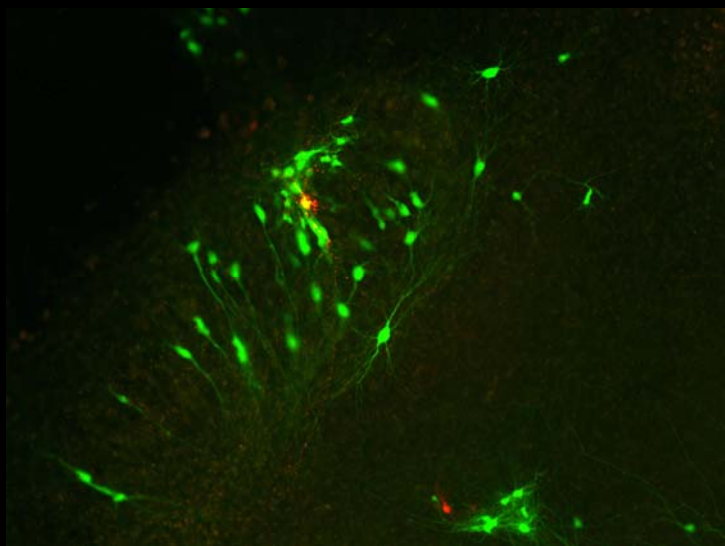
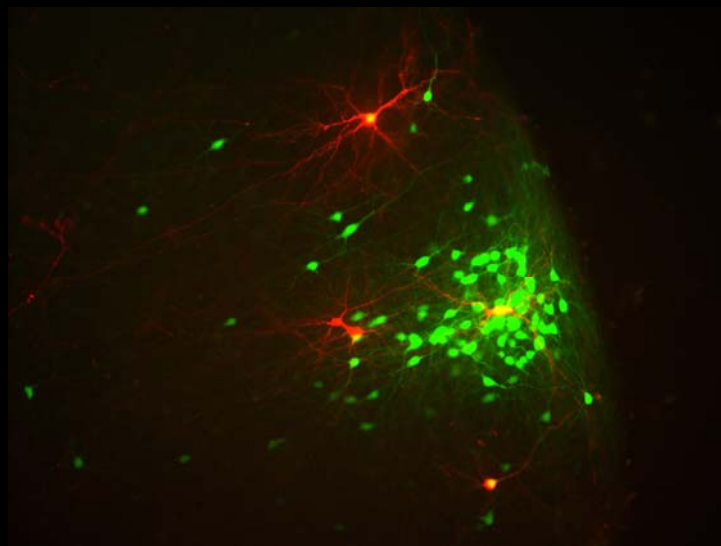
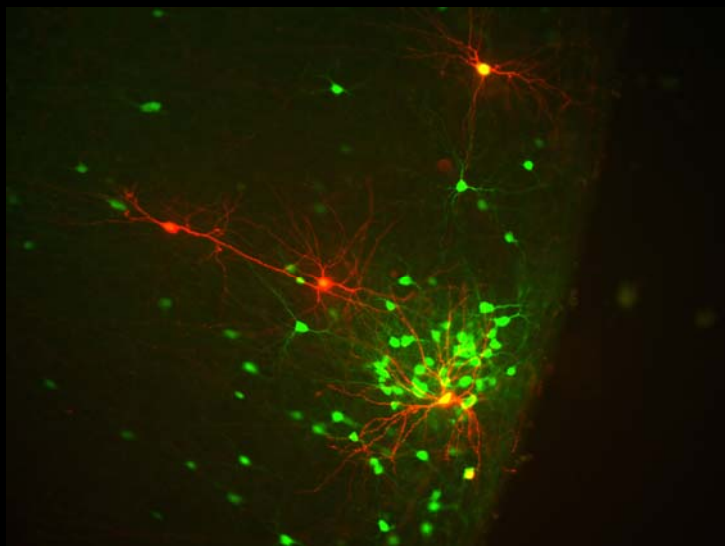


Infect with
EnvA-▲G, GFP Rabies

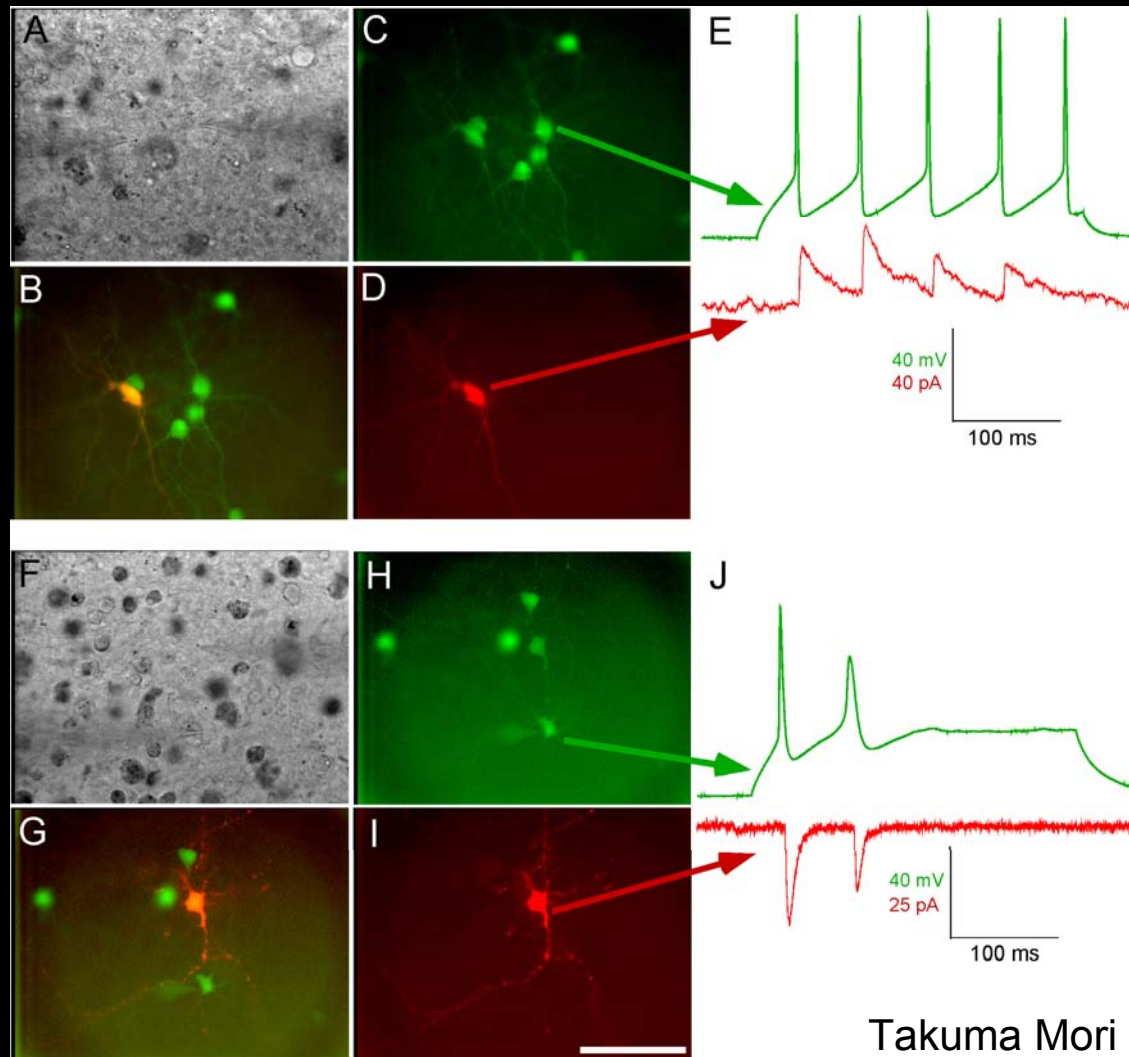
Wickersham et al
Neuron 2007

Shoot All 3 Plasmids – TVA, DSRED and Rabies Glycoprotein





Confirming Specificity of Spread – Paired Recordings



Postsynaptic Cell – Host neuron.

Presynaptic Cells – monosynaptically connected to host.

Wickersham et al
Neuron 2007

Three Ways to Use this Method for Real Questions About Brain Circuitry

1. Label direct input to a population of cells of a single, genetically targeted type.

Cre-dependent expression of TVA and RG, followed by infection with EnvA-▲RG, GFP rabies.

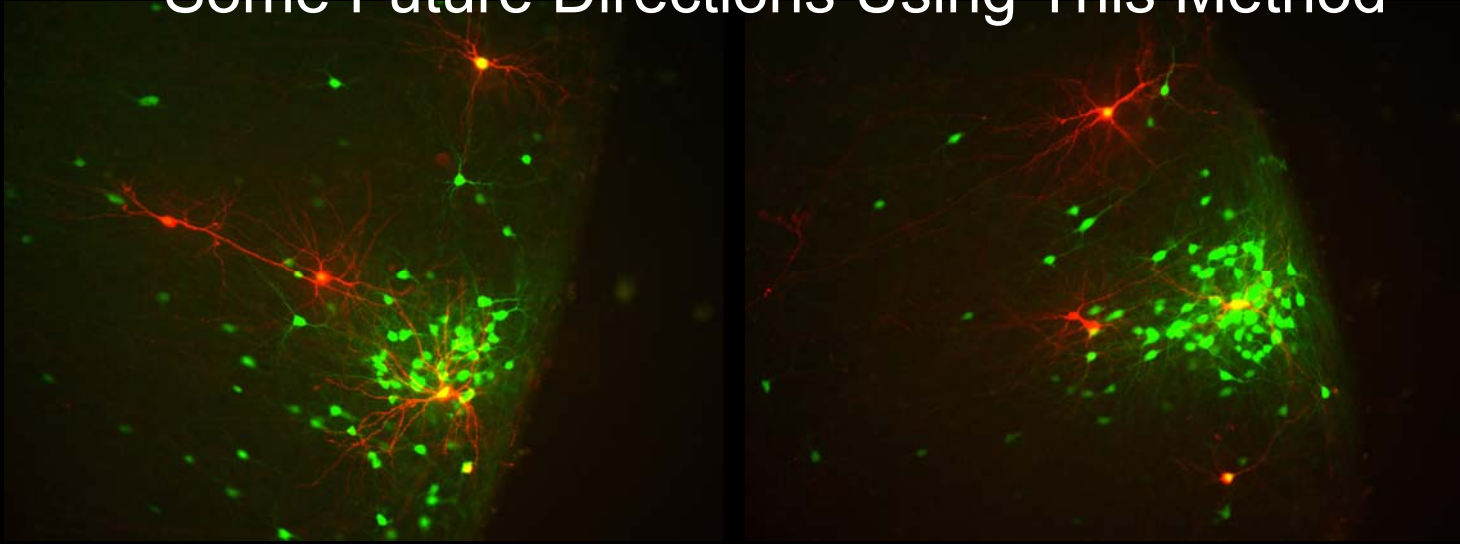
2. Label direct input to a population of cells of a single type of projection neuron.

Co-injection of ▲RG, GFP rabies (not EnvA pseudotyped) and a retrogradely infecting helper virus (e.g. HSV amplicon) that expresses RG.

3. Label inputs to a single neuron in vivo.

Single cell electroporation to express TVA and RG, followed by infection with EnvA-▲RG, GFP rabies.

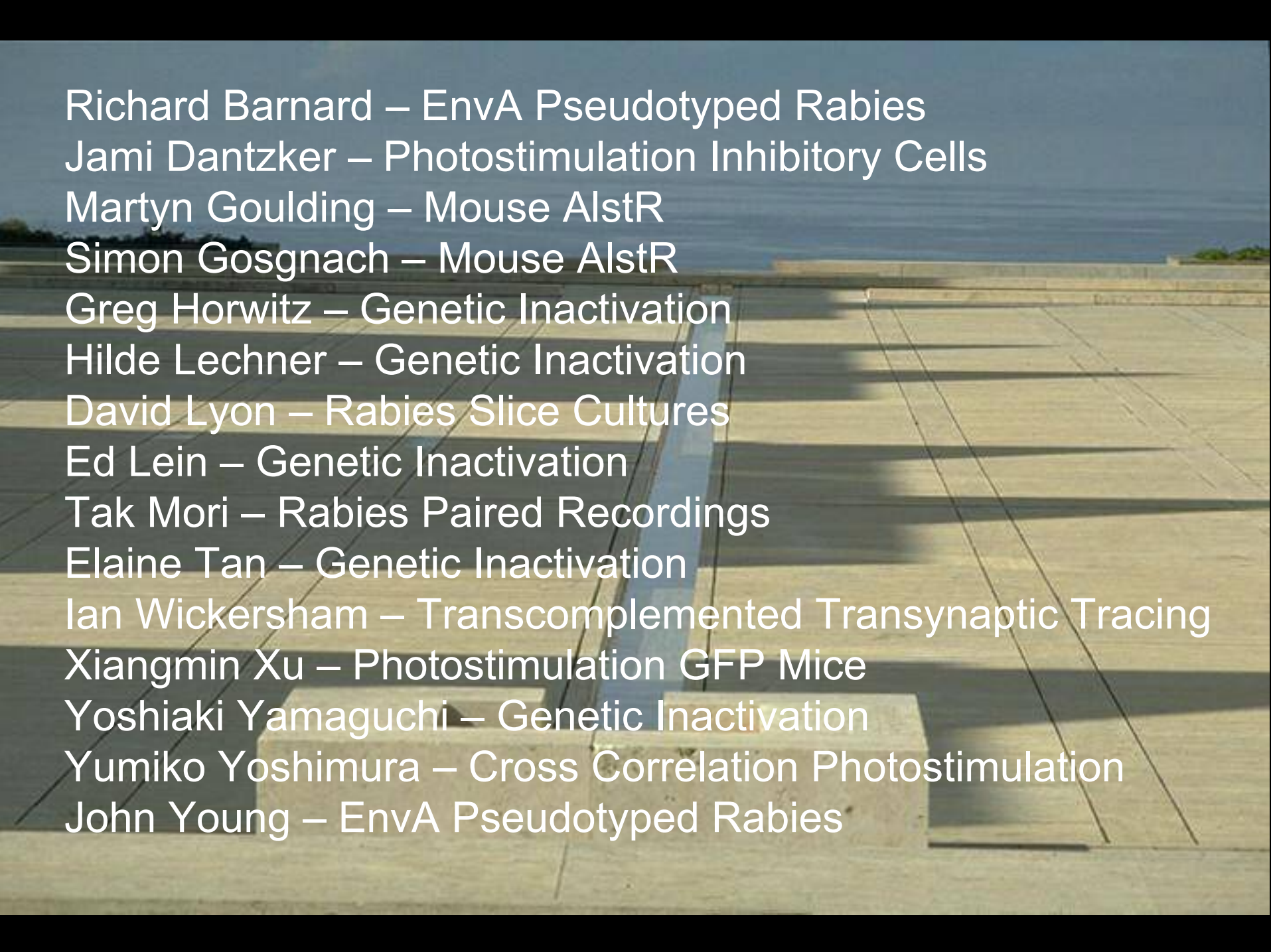
Some Future Directions Using This Method



Deliver Genes *in vivo* Through Single Cell Electroporation.

Then:

- 1) Whole brain quantitative description of rates of **connections to single neurons** of all mouse cortical cell types.
- 2) Record functional properties of a single neuron prior to electroporation and subsequently label all of the monosynaptic inputs to that cell. This would directly **relate functional properties to connectivity of the same neuron**.
- 3) Use in vivo 2-photon imaging to measure the functional properties of neurons that are presynaptic to a single cell – calcium dye imaging or expression of genetically expressed sensor. This would allow **simultaneous measurements of the activity of connected neuronal populations**.



Richard Barnard – EnvA Pseudotyped Rabies
Jami Dantzker – Photostimulation Inhibitory Cells
Martyn Goulding – Mouse AlstR
Simon Gosgnach – Mouse AlstR
Greg Horwitz – Genetic Inactivation
Hilde Lechner – Genetic Inactivation
David Lyon – Rabies Slice Cultures
Ed Lein – Genetic Inactivation
Tak Mori – Rabies Paired Recordings
Elaine Tan – Genetic Inactivation
Ian Wickersham – Transcomplemented Transynaptic Tracing
Xiangmin Xu – Photostimulation GFP Mice
Yoshiaki Yamaguchi – Genetic Inactivation
Yumiko Yoshimura – Cross Correlation Photostimulation
John Young – EnvA Pseudotyped Rabies