9.17 Systems Neuroscience Laboratory

Neurohistology

Why do we do neurohistology ? (systems perspective)

Basic methods of neurohistology

The neocortex

Immuno**cyto**chemistry v. Immuno**histo**chemistry

Immunocytochemistry

- used to assess the presence of a specific protein or antigen in cells (cultured cells, cell suspensions) by use of a specific antibody, thereby allowing visualization and examination under a microscope.
- Samples include blood smears, aspirates, swabs, cultured cells, and cell suspensions.
- surrounding extracellular matrix removed

Immunohistochemistry

- sections of biological tissue, where each cell is surrounded by tissue architecture and other cells normally found in the intact tissue
- Samples include organs, muscle, brain, etc.

Often used incorrectly/interchangeably!

What is the right tool for the job?

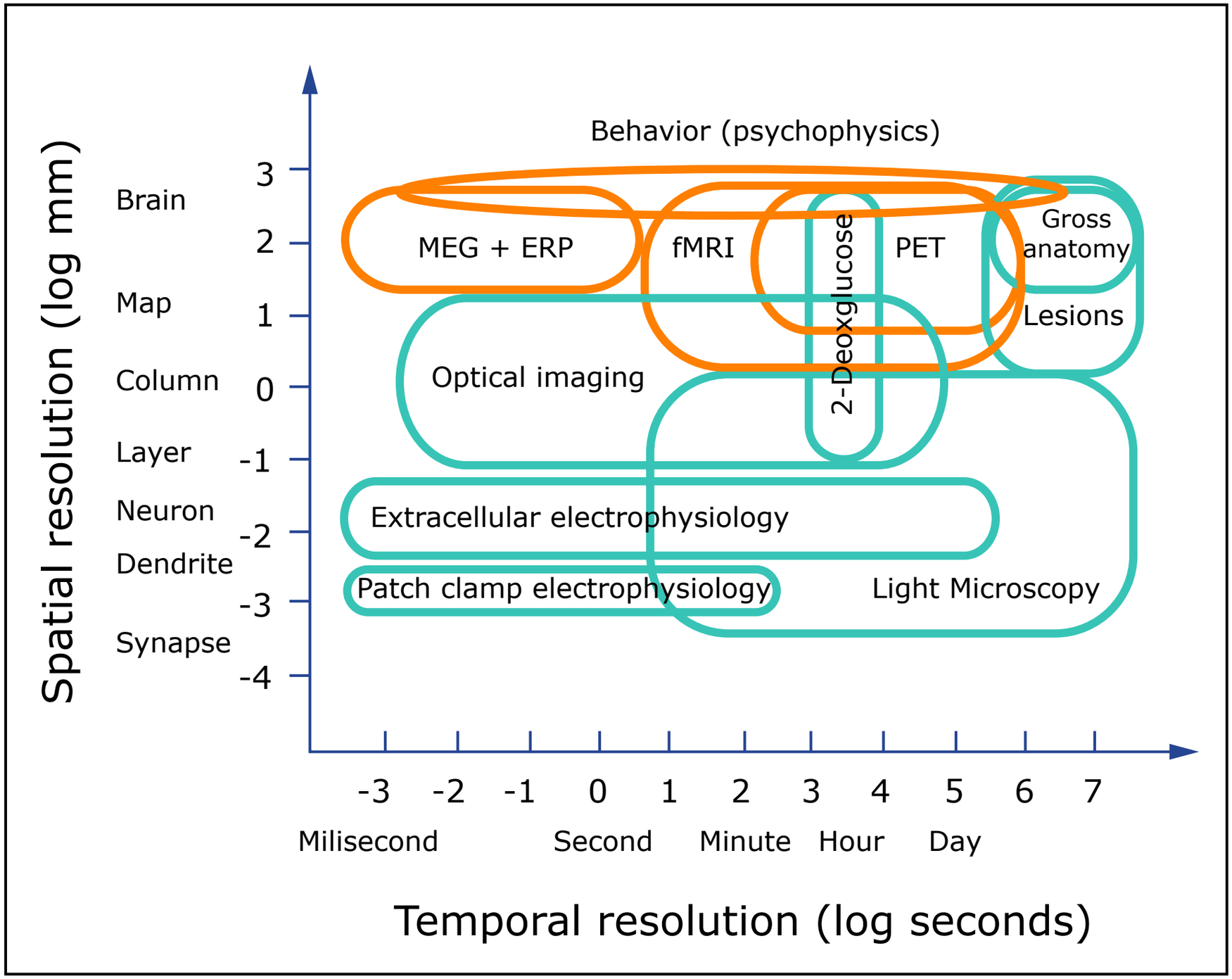
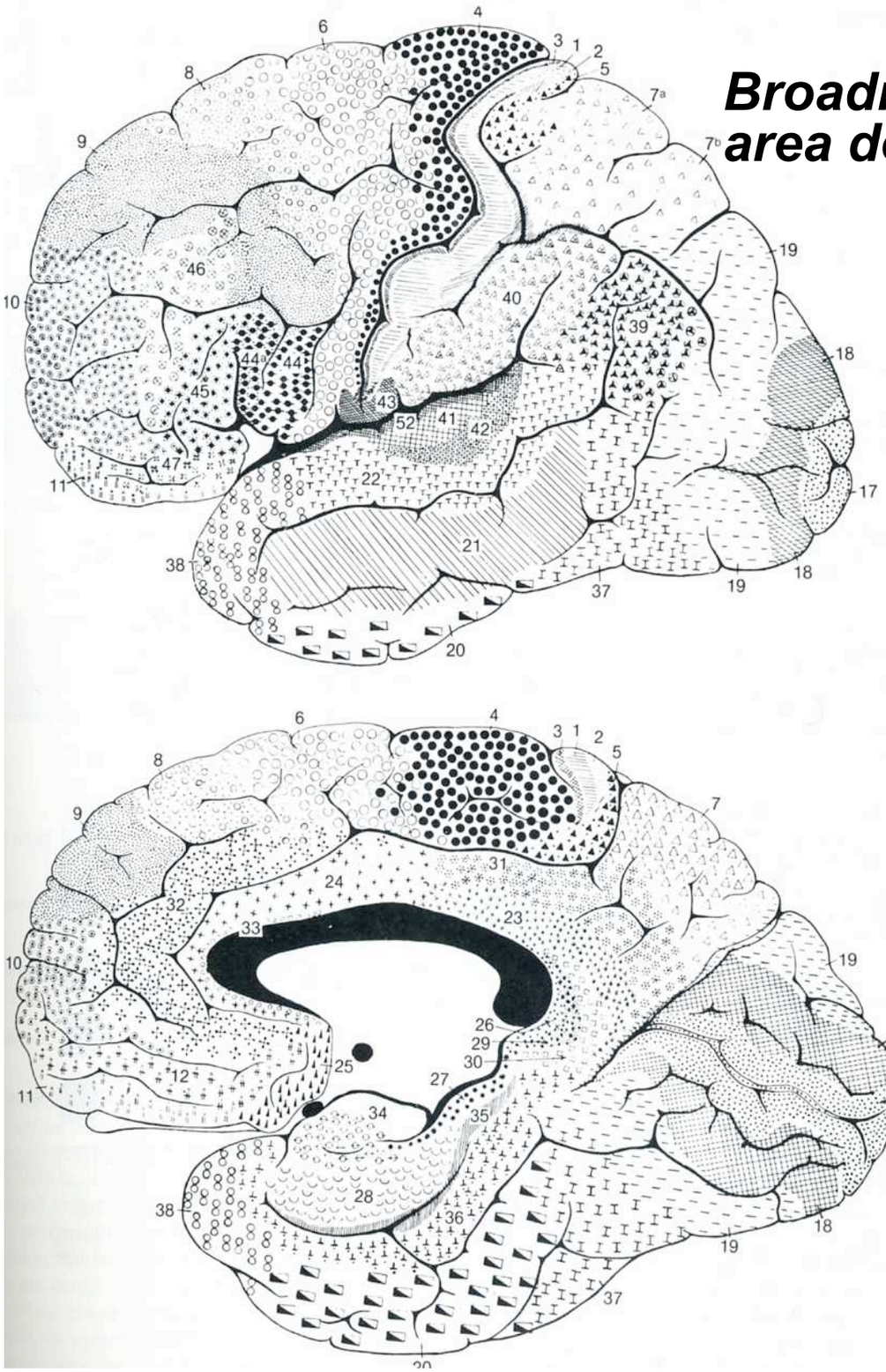


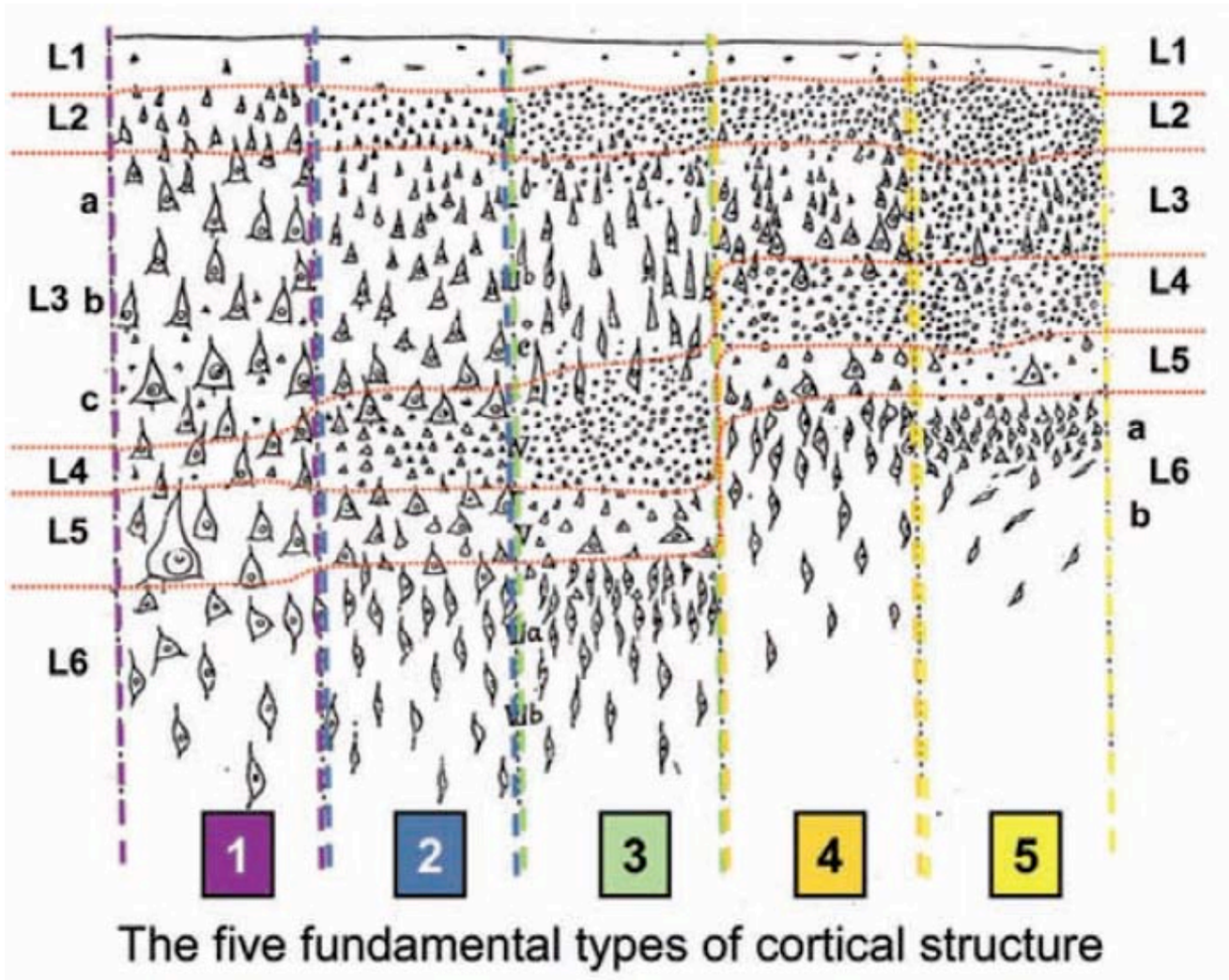
Image by MIT OpenCourseWare.

The contribution of neurohistology: Example 1 (cortical areas)

Broadman cortical area designations



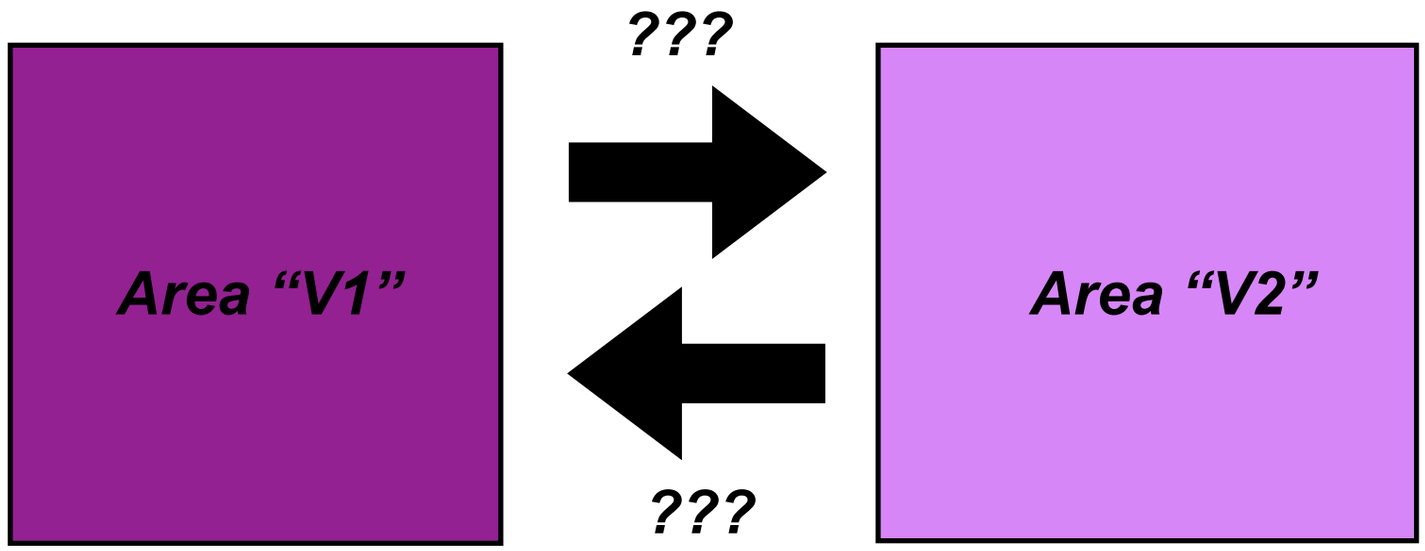
Public Domain. Brodmann, Korbinian. "The Cortical Areas of the Lateral and Medial Surfaces of the Human Cerebral Hemispheres." *Localisation in the Cerebral Cortex.*



Courtesy of Soren Van Hout Solari and Rich Stoner. Used with permission. CC BY-NC. "Cognitive Consilience: Primate Non-primary Neuroanatomical Circuits Underlying Cognition." *Frontiers in Neuroanatomy* 5, no. 65 (2011). doi: 10.3389/fnana.2011.00065.

The contribution of neurohistology: Example 2 (cortical hierarchy)

Image removed due to copyright restrictions. Fig. 2. Map of cortical areas in the macaque. Felleman, D.J. and D.C. Van Essen." Distributed Hierarchical Processing in Primate Visual Cortex." *Cerebral Cortex* 1 (1991): 1-47.



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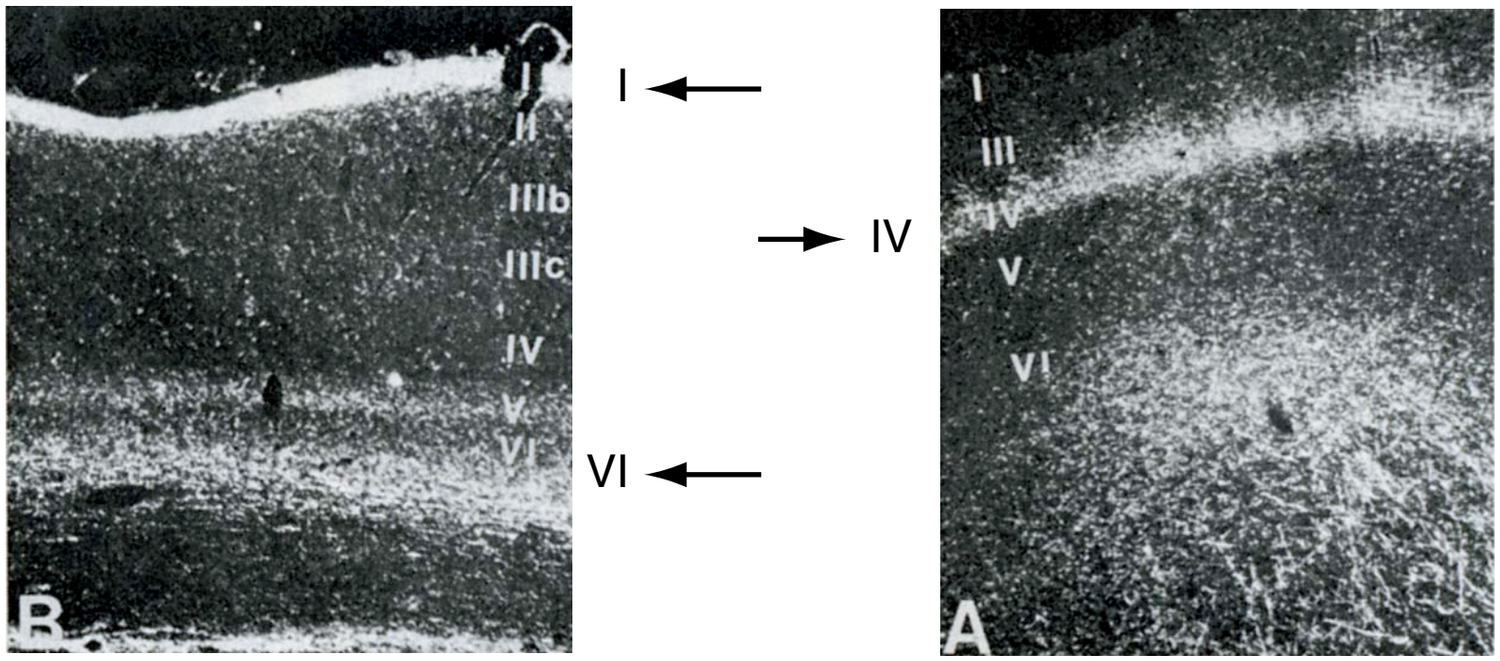

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The contribution of neurohistology: Example 2 (cortical hierarchy)

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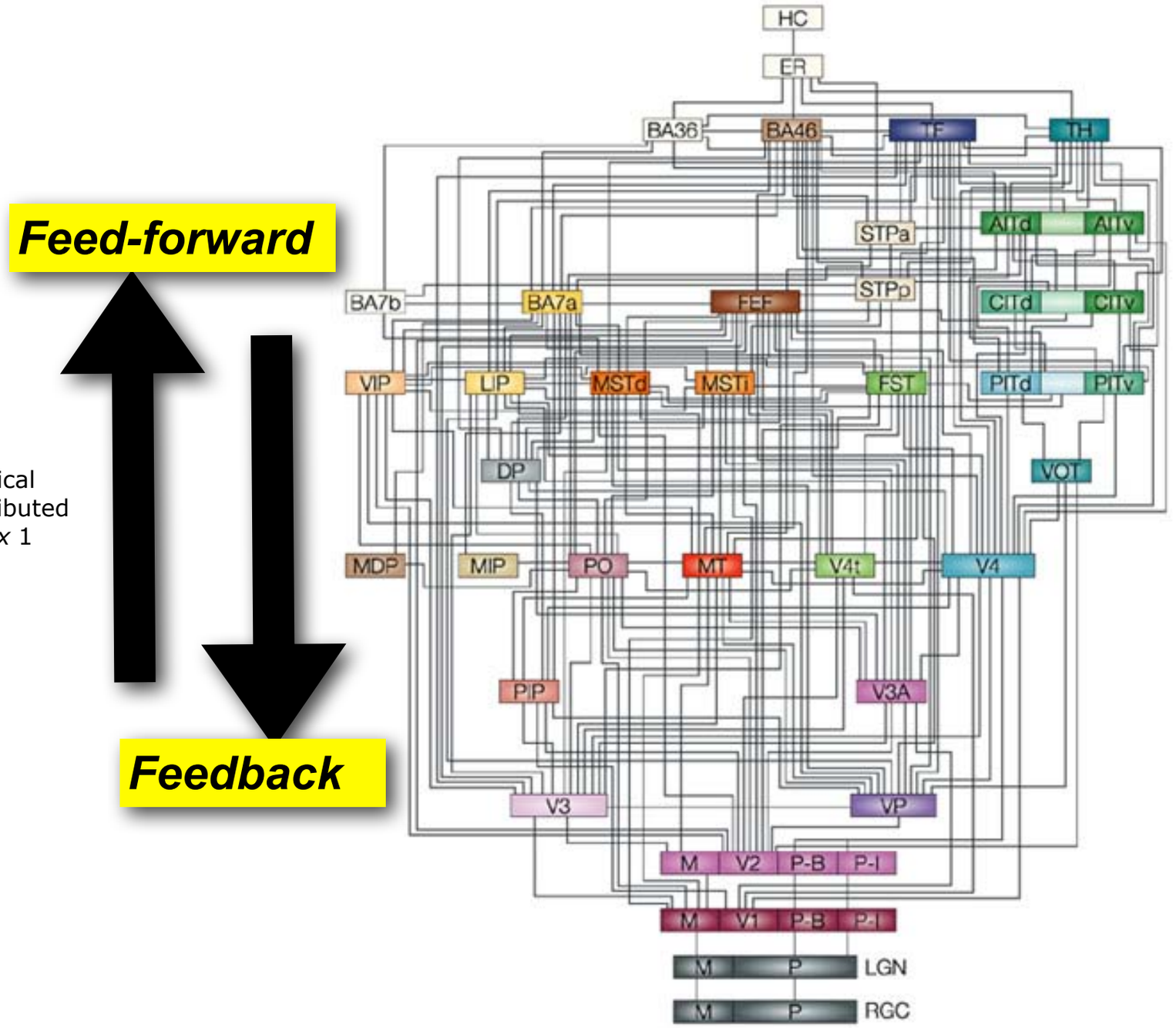
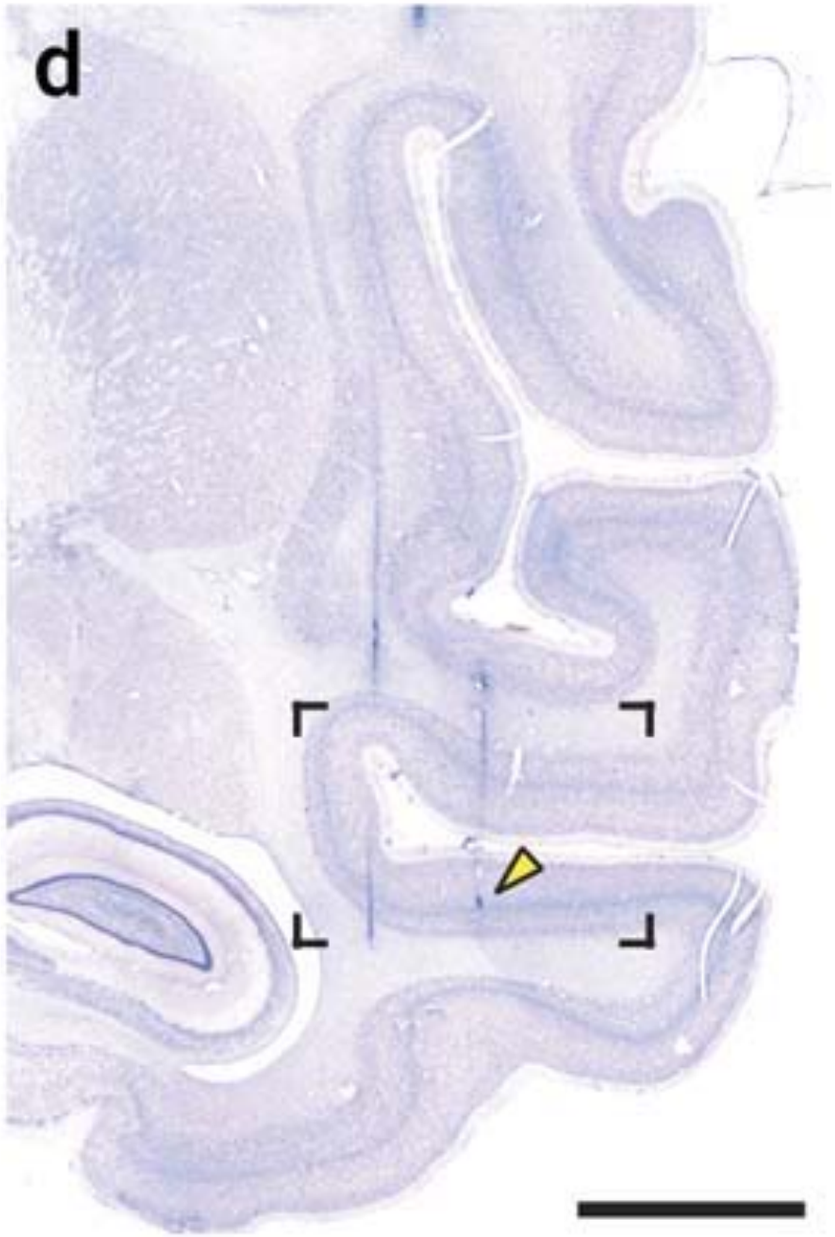


Monkey Cerebral Cortex
Felleman and Van Essen 1991

Reprinted by permission from Macmillan Publishers Ltd: *Nature Reviews Neuroscience*. Source: Figure 3A. Rees, Geraint, Gabriel Kreiman, et al. "Neural Correlates of Consciousness in Humans." *Nature Reviews Neuroscience* 3 (2002): 261-70. © 2002.

The contribution of neurohistology: Example 3 (functional mapping)

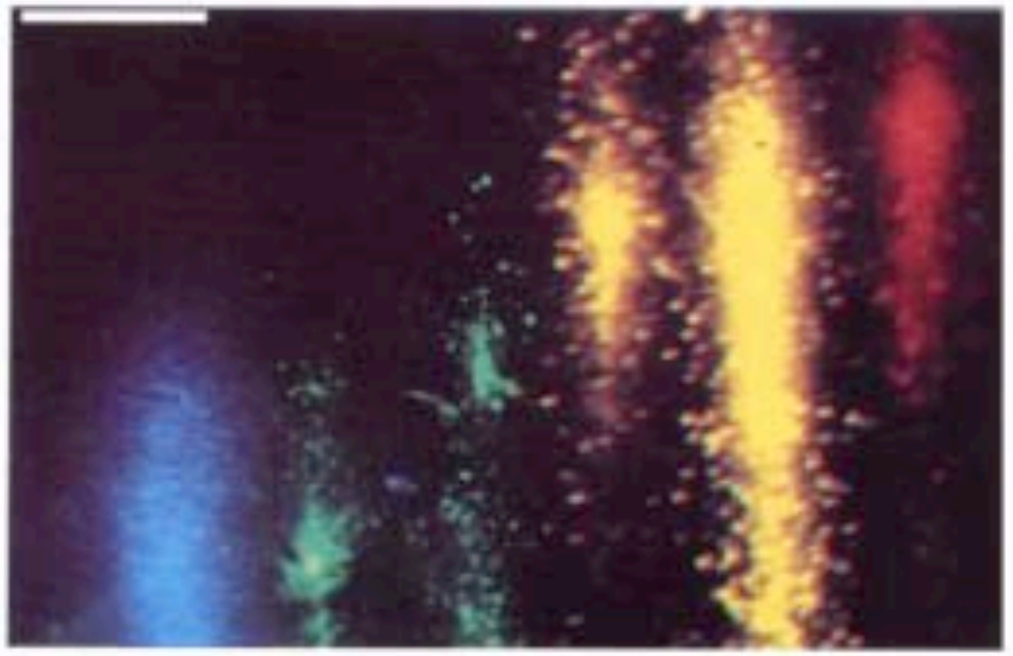
I recorded from a neuron. Where is it located in the brain?



microlesions at recording sites

Reprinted by permission from Macmillan Publishers Ltd: *Nature Methods*. Source: Matsui, Teppei, Kenji W. Koyano, et al. "MRI-based Localization of Electrophysiological Recording Sites within the Cerebral Cortex at Single-voxel Accuracy." *Nature Methods* 4 (2006): 161-8. © 2006.

fluorescent dye on electrodes



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DiCarlo et al, J Neurosci Methods (1996).

The contribution of neurohistology: Example 3 (functional mapping)

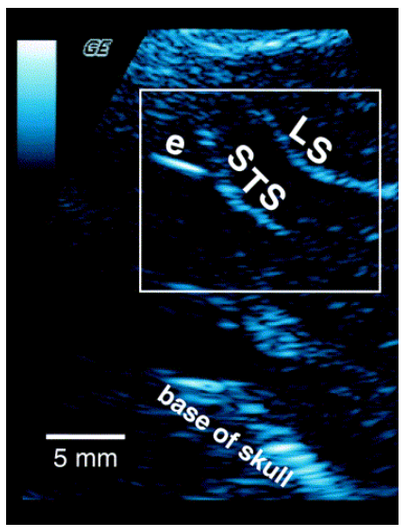
Fig. 1. A stereo microfocal X-ray 3-dimensional (3D) imaging system removed due to copyright restrictions. See Cox, DD, AM Papanastassiou, et al. "High-resolution Three-dimensional Microelectrode Brain Mapping using Stereo Microfocal X-ray Imaging." *Journal of Neurophysiology* 100 (2008): 2966–2976.doi:10.1152/jn.90672.2008.

X-ray localization

Cox, Papanastassiou, Oreper, Andken, and DiCarlo J Neurophys. Innovative Methodology (2008)

Ultrasound localization

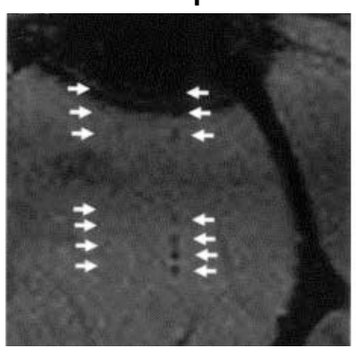
Ultrasound



Glimcher et al. 2001; Courtesy of Elsevier, Inc., http://www.sciencedirect.com. Used with permission.

MRI based localization

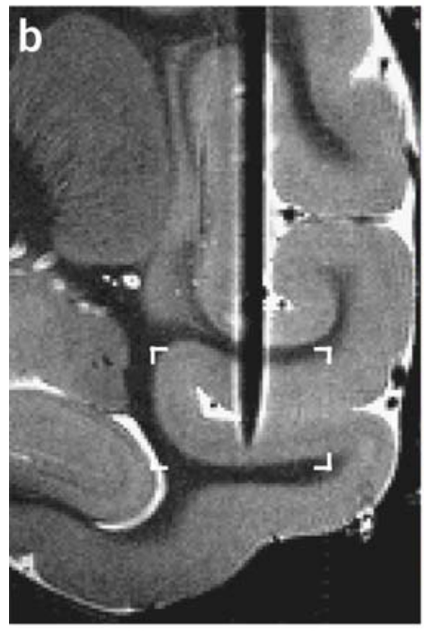
Iron deposits



Fung et al, 1998.

Image removed due to copyright restrictions. Fig 1 A. Tsao, DY, Freiwald, WA, Tootell, RBH and Livingstone, MS (2006) "A cortical region consisting entirely of face-sensitive cells. *Science* 311 (2006): 670-674.

Tsao et al., 2006



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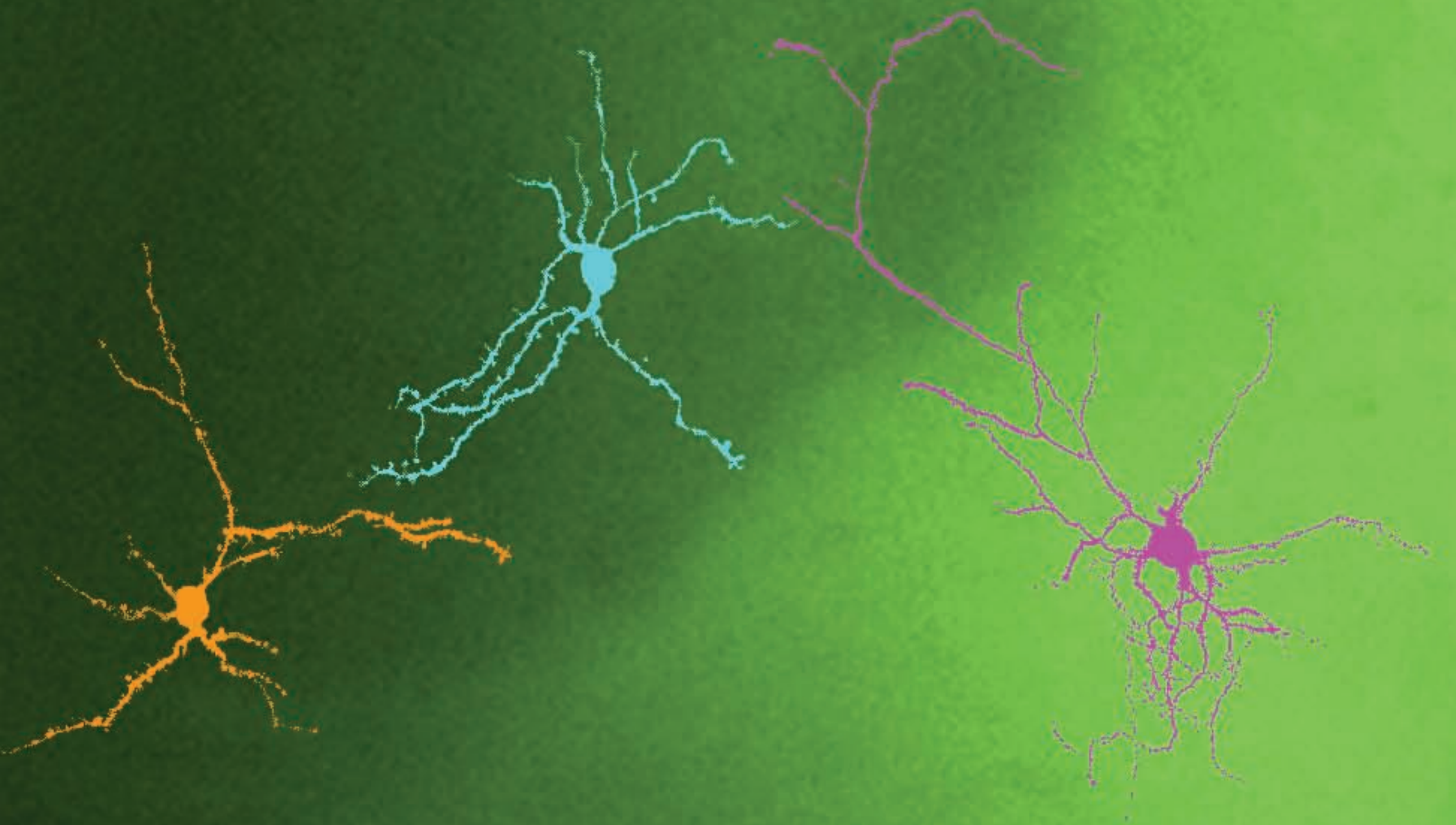
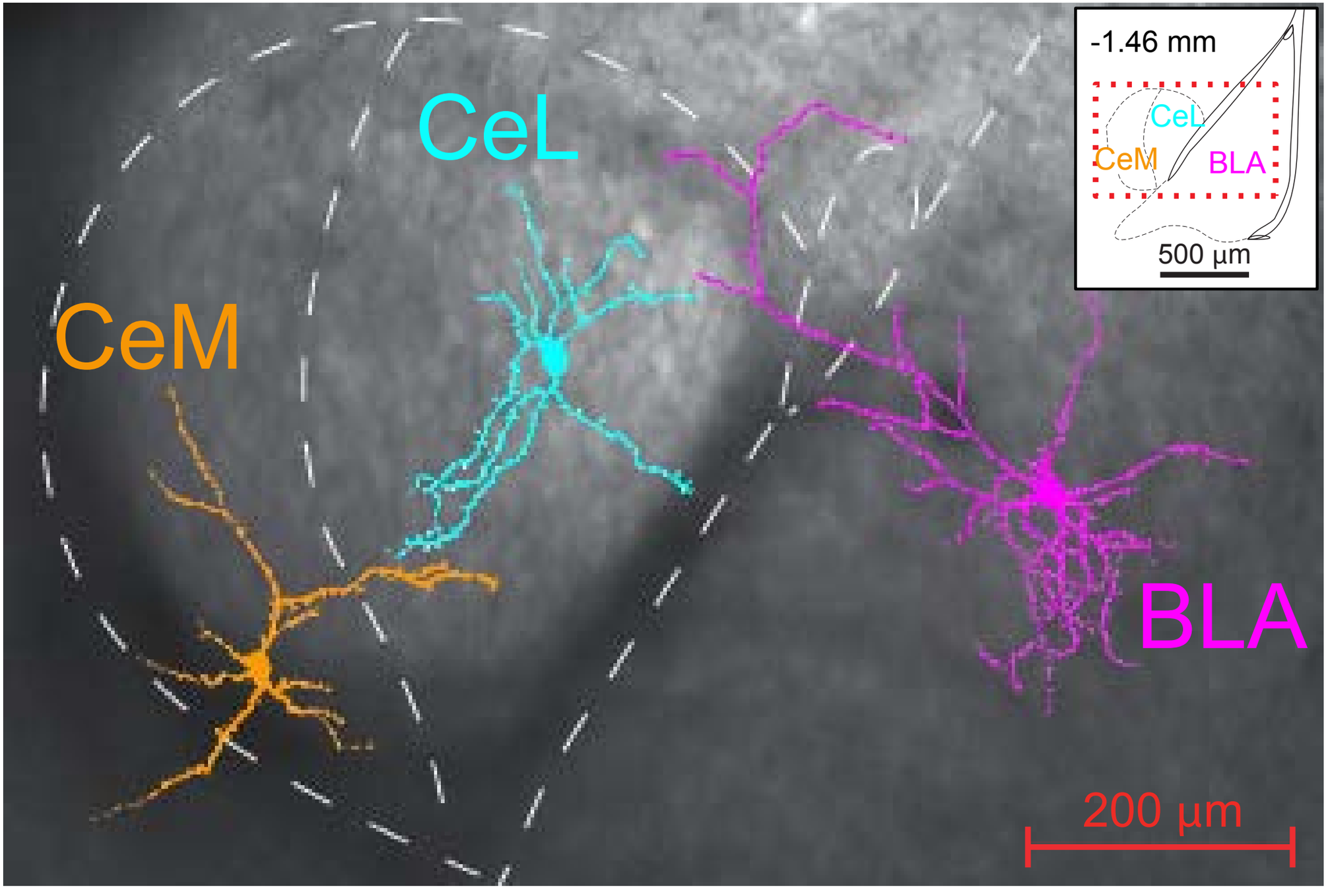
The contribution of neurohistology: Example 3 (functional mapping)
Dissecting circuits by linking anatomy to function



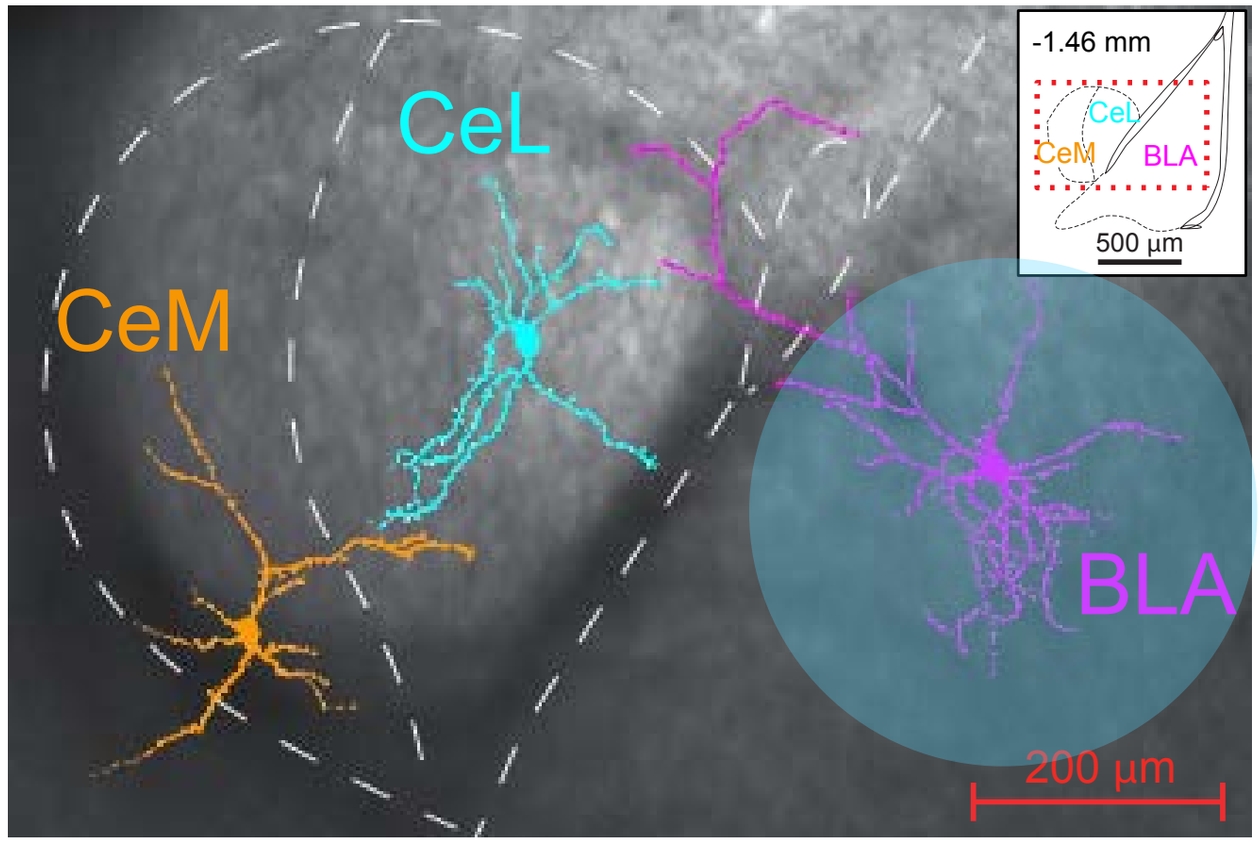
Tye et al., 2011

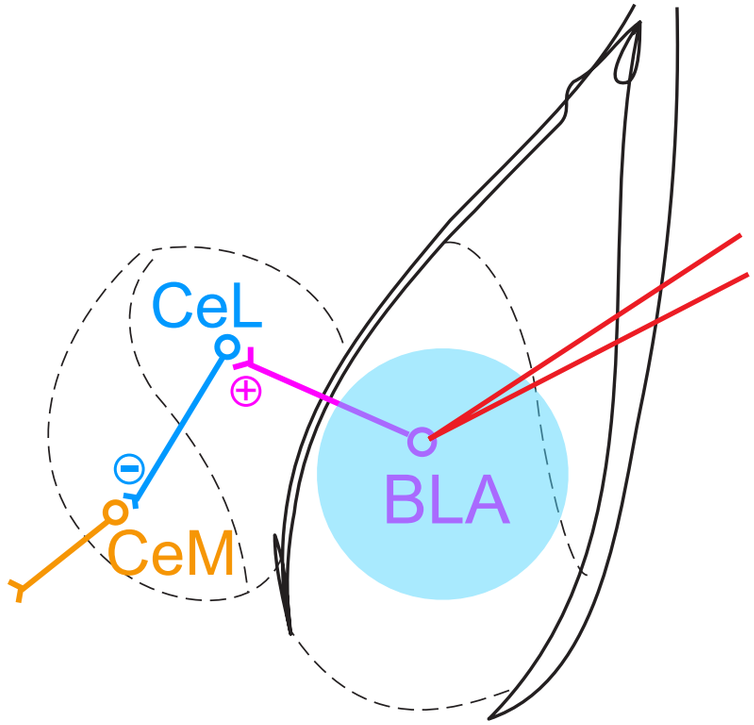
Visual and functional dissection of the BLA-CeL-CeM microcircuit

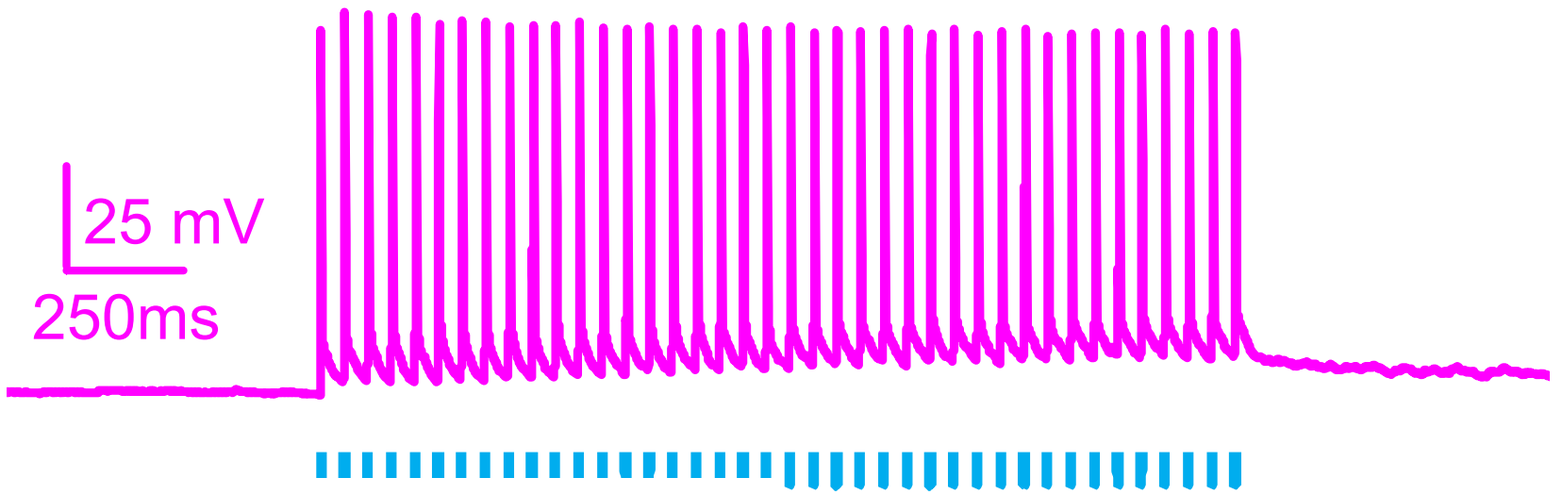


Visual and functional dissection of BLA-CeL-CeM circuit

Upon direct illumination of BLA neurons expressing ChR2, we observe high-fidelity spiking



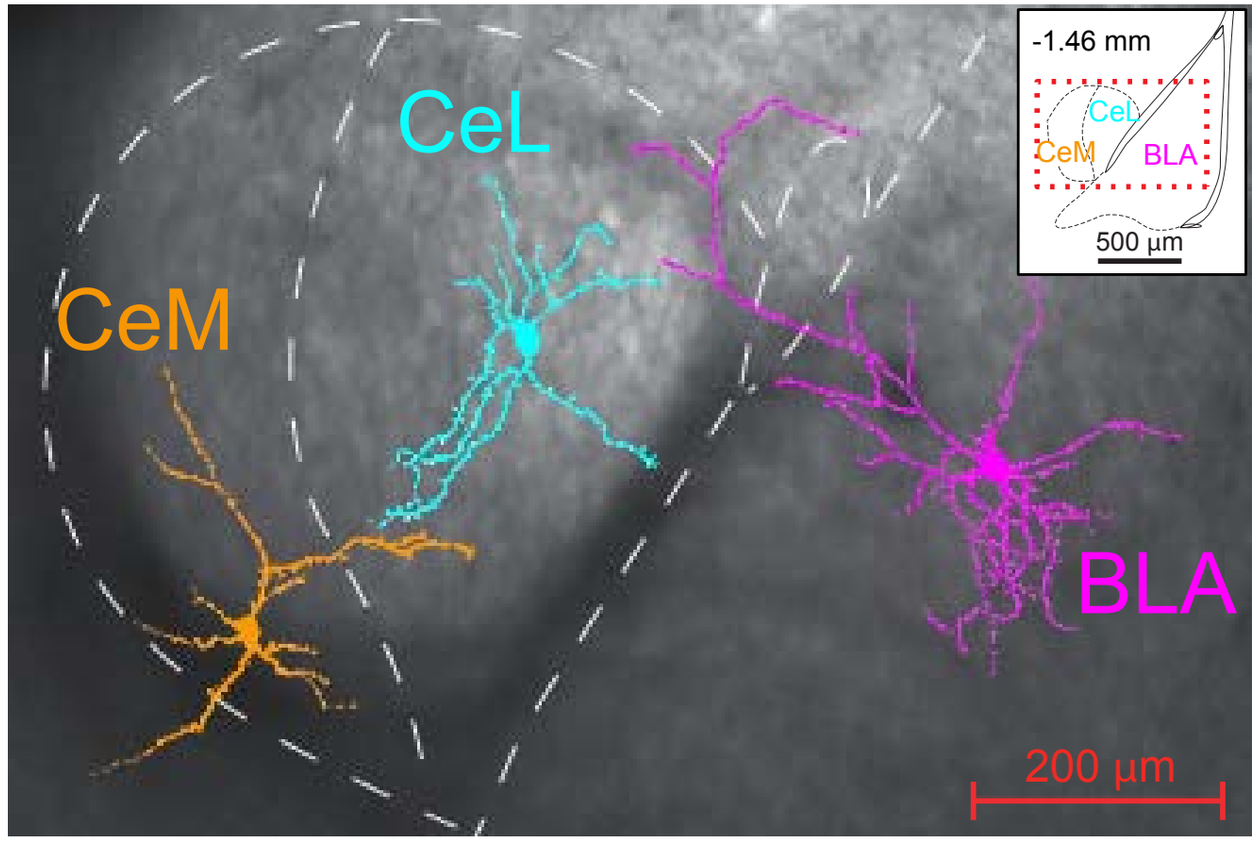


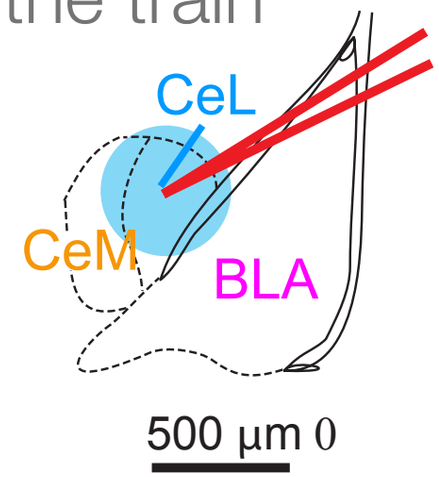


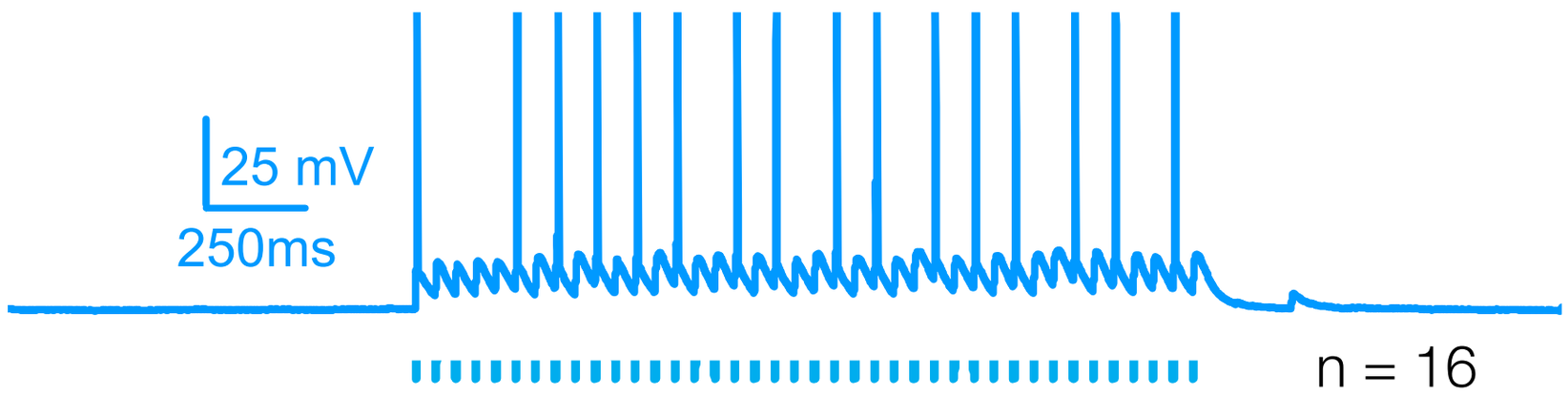
Tye et al., *Nature* (2011)

Selective illumination of BLA terminals induces vesicle release onto CeL cells

Illumination of BLA-CeL synapses induces both sub- and supra-threshold excitatory responses in the postsynaptic CeL cell that are stable across the train







Tye et al., *Nature* (2011)

9.17 Systems Neuroscience Laboratory

Neurohistology

Why do we do neurohistology ?

Basic methods of neurohistology

The neocortex

Basic neurohistology method sequence

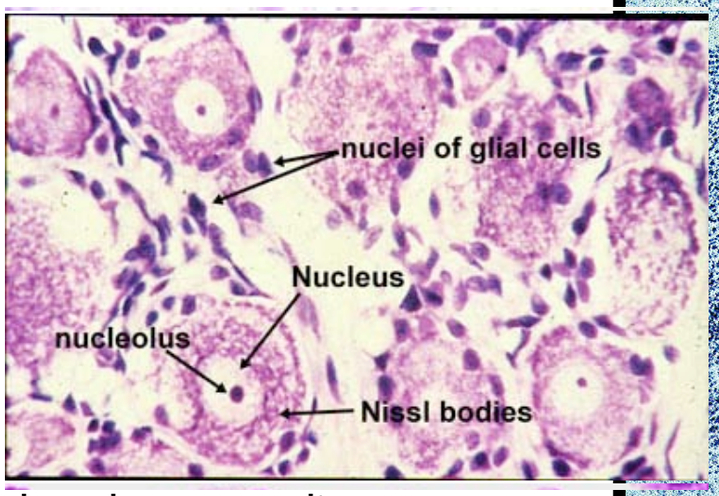
0. Experimental manipulation e.g. rat whisker removal (sensory deprivation)
   e.g. electrode marking

1. Euthanasia / perfusion / fixation
2. Brain extraction
3. Photograph / cut blocks (large brains, optional)
4. Cut sections
5. Staining (if needed)
6. Mount sections (for some stains, we mount BEFORE staining)
7. Coverslip (if needed)
8. Microscopy / documentation

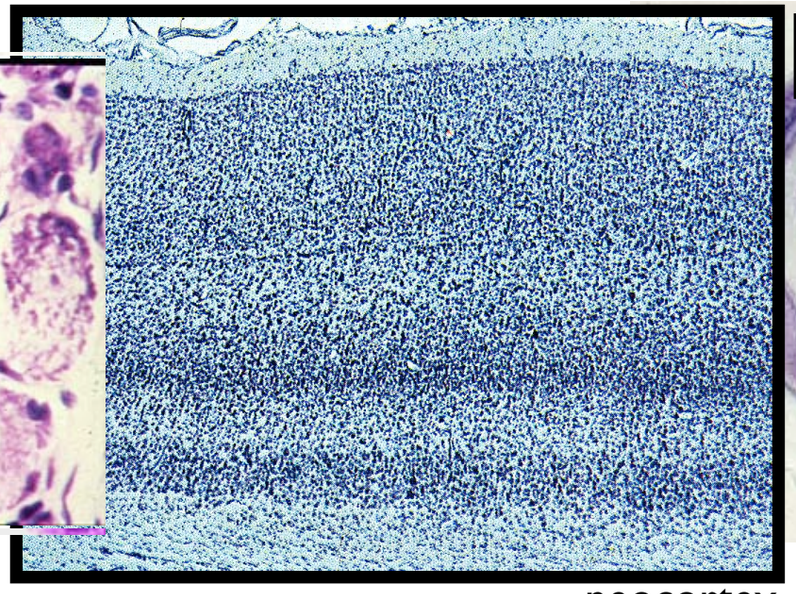
Staining: a wide array of existing stains

Nissl stain

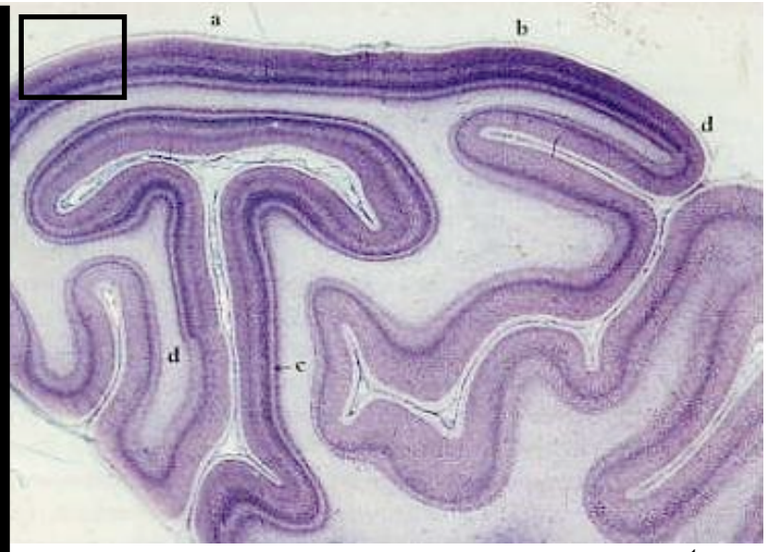
(RNA, neurons and glia)



dorsal root ganglion



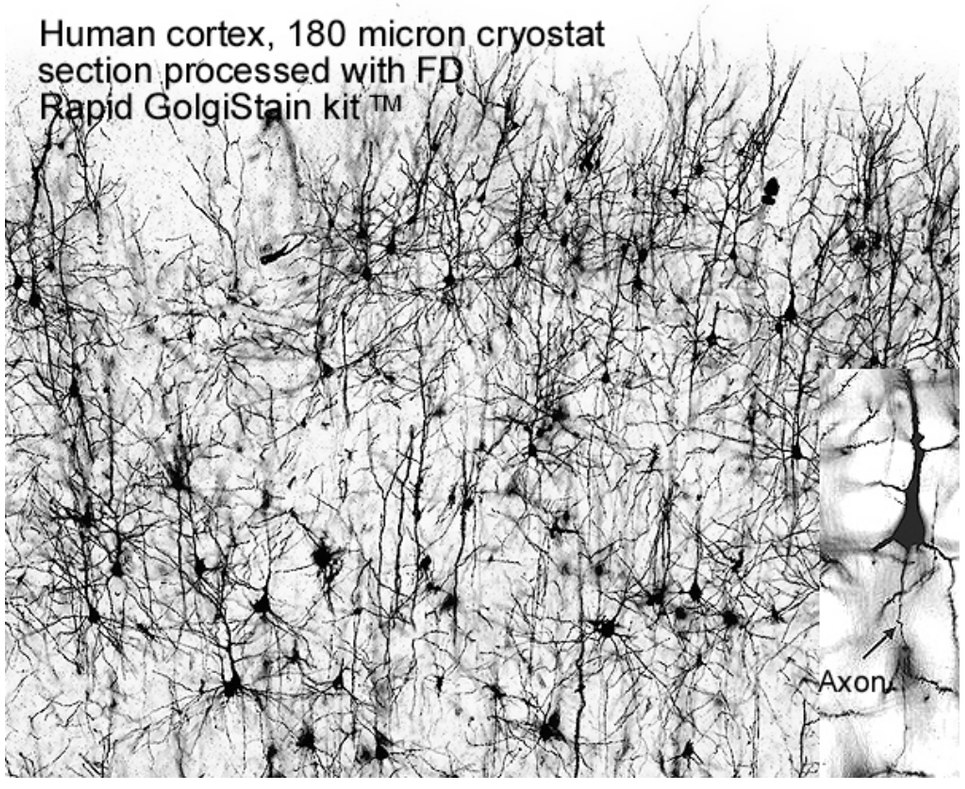
neocortex



neocortex

Golgi stain

(~random, small number of neurons, fills axons and dendrites)



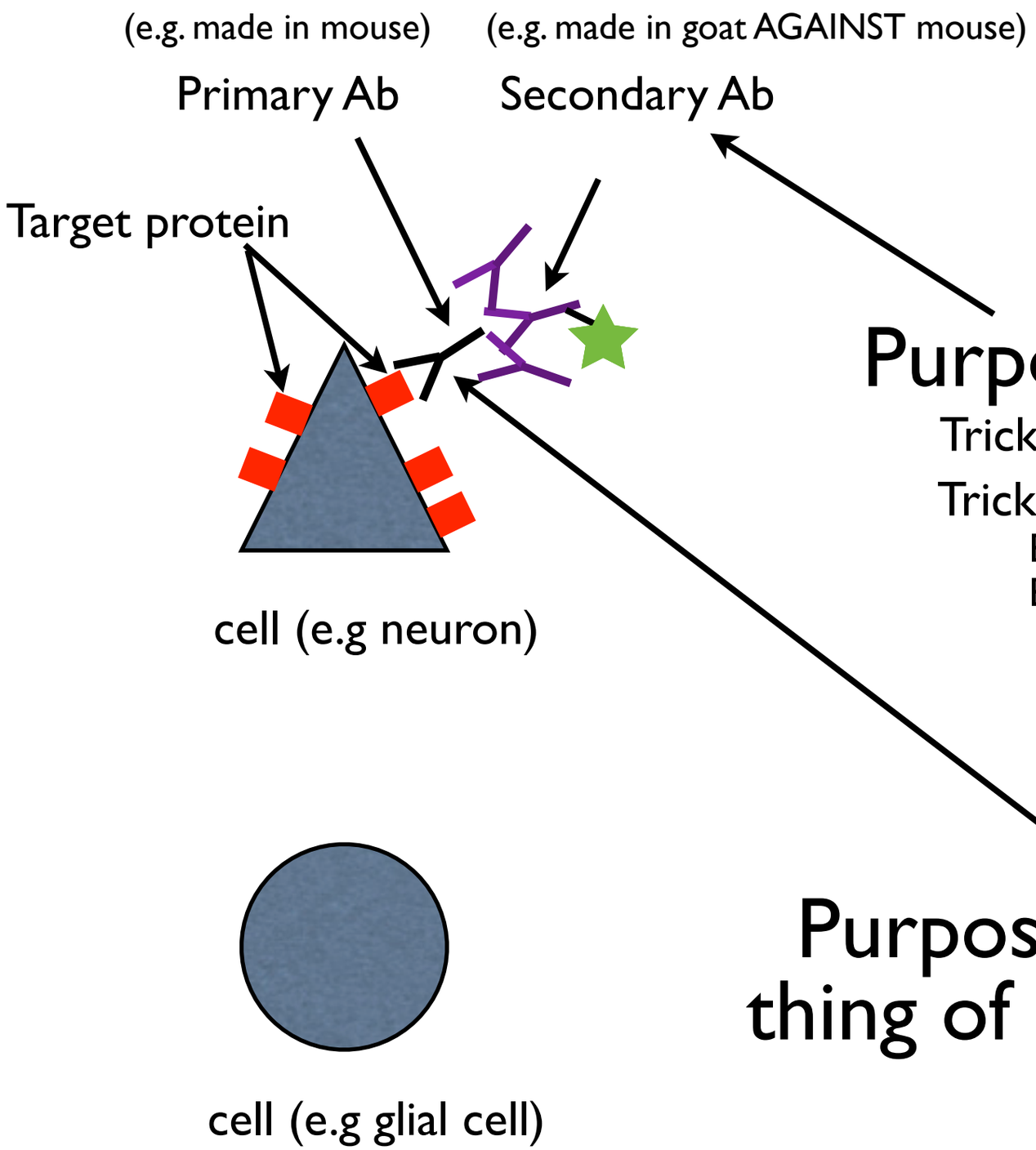
Cytochrome oxidase stain

(mitochondria)

barrel cortex (somatosensory)

Staining: immunohistochemistry

Ab = antibody (protein)



Purpose: visualize the Primary

Trick 1: attach (only) to Primary Ab

Trick 2: Carry something we can see!

Example: fluorescent molecule

Example: a chemical reaction product

Quiz question: why not simply attach the visualization agent to the primary Ab?

Purpose: attach (only) to the thing of interest (target protein)

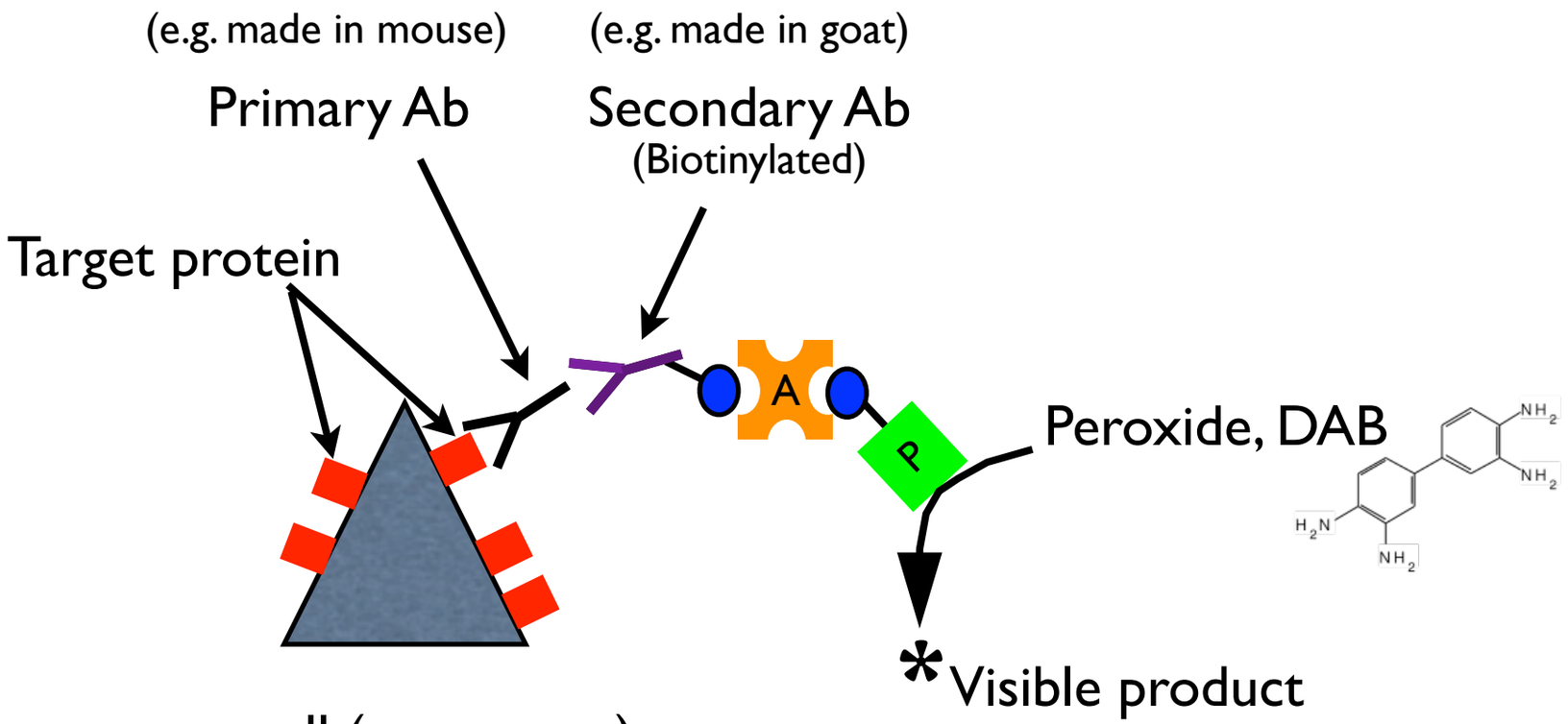
Staining: immunohistochemistry

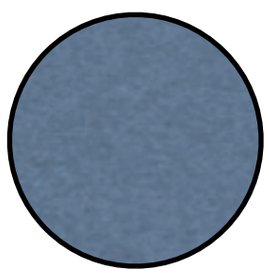
Ab = antibody (protein)

Biotin

A Avidin (protein)

P peroxidase





Staining: immunohistochemistry

Ab = antibody (protein)

Biotin

A Avidin (protein)

P peroxidase

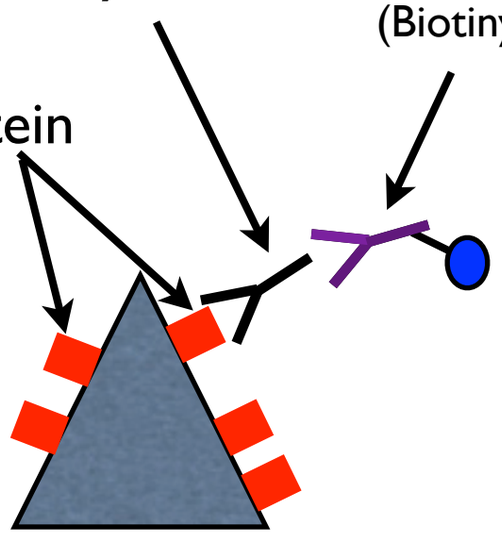
(e.g. made in mouse)

Primary Ab

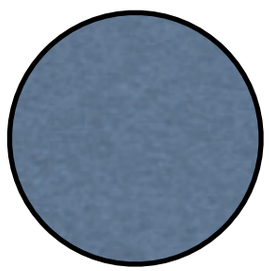
(e.g. made in goat)

Secondary Ab
(Biotinylated)

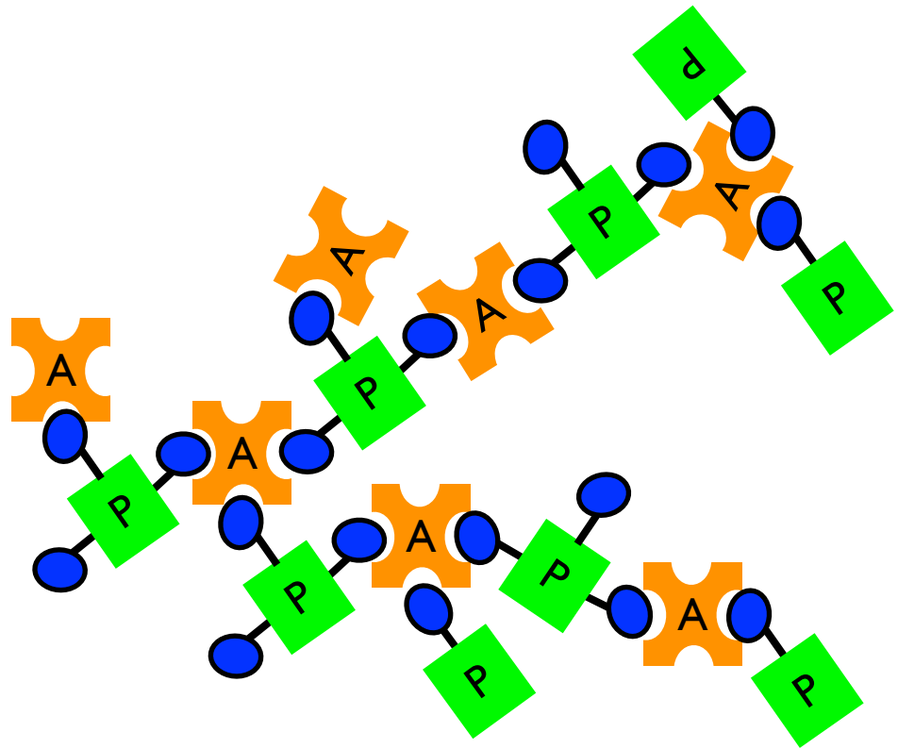
Target protein



cell (e.g neuron)



cell (e.g glial cell)



"AB complex"
(Avidin, Biotin, Peroxidase)

Staining: immunohistochemistry

Ab = antibody (protein)

Biotin

A Avidin (protein)

P peroxidase

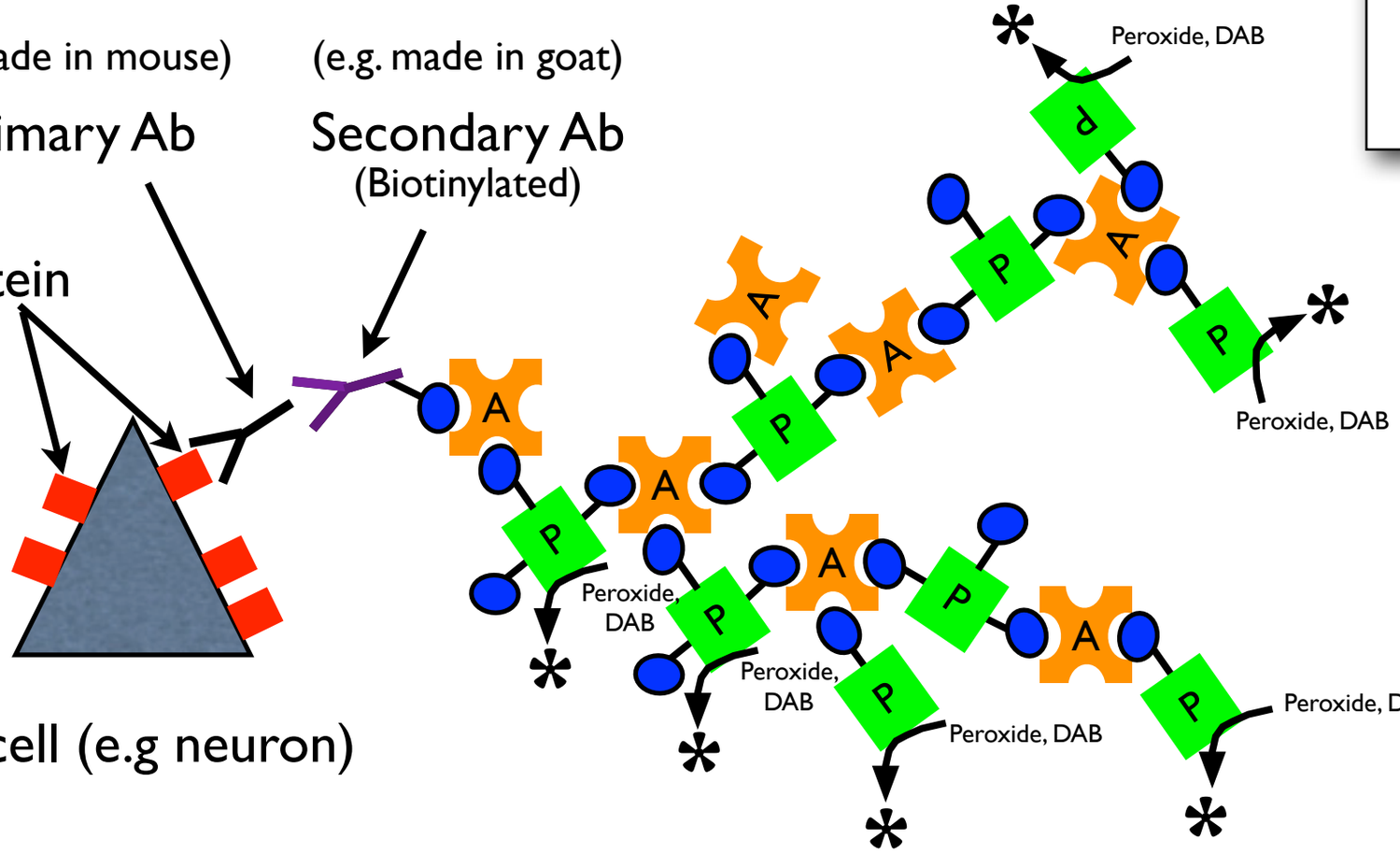
(e.g. made in mouse)

Primary Ab

Target protein

(e.g. made in goat)

Secondary Ab
(Biotinylated)



cell (e.g neuron)

* Visible product

cell (e.g glial cell)

"AB complex"
(Avidin, Biotin, Peroxidase)

Staining: immunohistochemistry

In lab this week: ***NeuN*** "stain"

NeuN is the target protein (the primary antibody binds strongly to *NeuN*)

NeuN stands for "Neuronal nuclei" because ...

... it was found in the nucleus of (most) neurons. *Mullen et al. (1992)*

Fig. 1. Immunohistochemical staining of adult CNS with mAb A60 removed due to copyright restrictions. See Mullen RJ, CR Buck, et al. "NeuN, A Neuronal Specific Nuclear Protein in Vertebrates." *Development* 116 (1992): 201–11.

Parasagital section of mouse, immunostaining for NeuN. Mullen et al. (1992)

mouse neocortex

Basic neurohistology method sequence

Experimental manipulation e.g. rat whisker removal (sensory deprivation)
e.g. electrode marking

1. Euthanasia / perfusion / fixation
2. Brain extraction
3. Photograph / cut blocks (large brains, optional)
4. Cut sections
5. Staining (if needed)
6. Mount sections (for some stains, we mount BEFORE staining)
7. Coverslip (if needed)
8. Microscopy / documentation

Goal of this Lab: learn basic neurohistology <u>methods</u>

Prepare for lab/quiz: - Review section 3 of the handbook
- review lecture notes

Lab Notebook: divide page in two parts with vertical line

Name and date (each page)

LEFT: outline the sequence of neurohistology
(IN THE ORDER THAT THEY ARE TYPICALLY DONE)

Provide extra detail on procedures you will do (esp. immunocytochemistry)

RIGHT: in the lab, you will fill in changes and observations next to each planned section on the left

9.17 Systems Neuroscience Laboratory

Neurohistology

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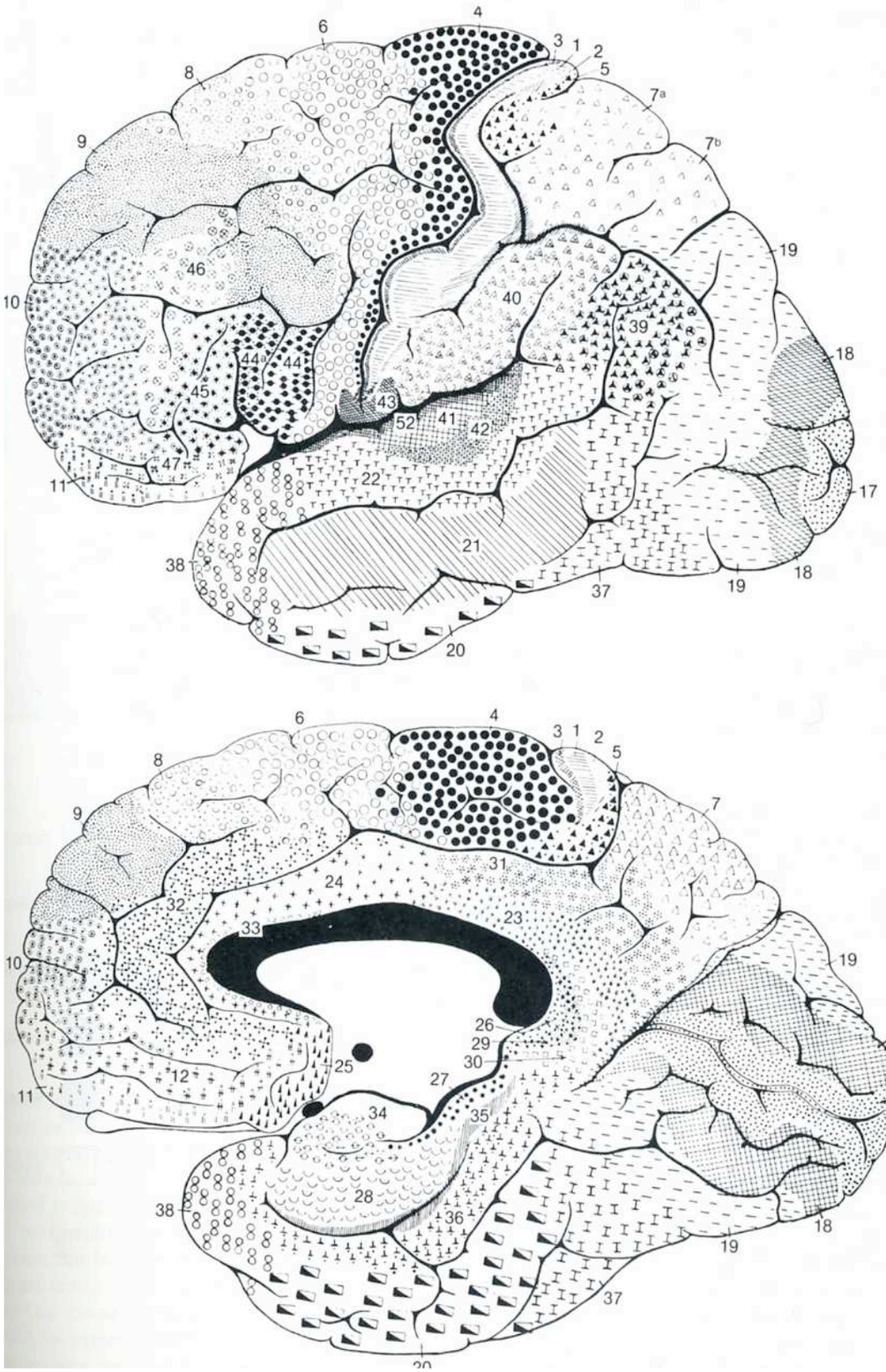
The neocortex



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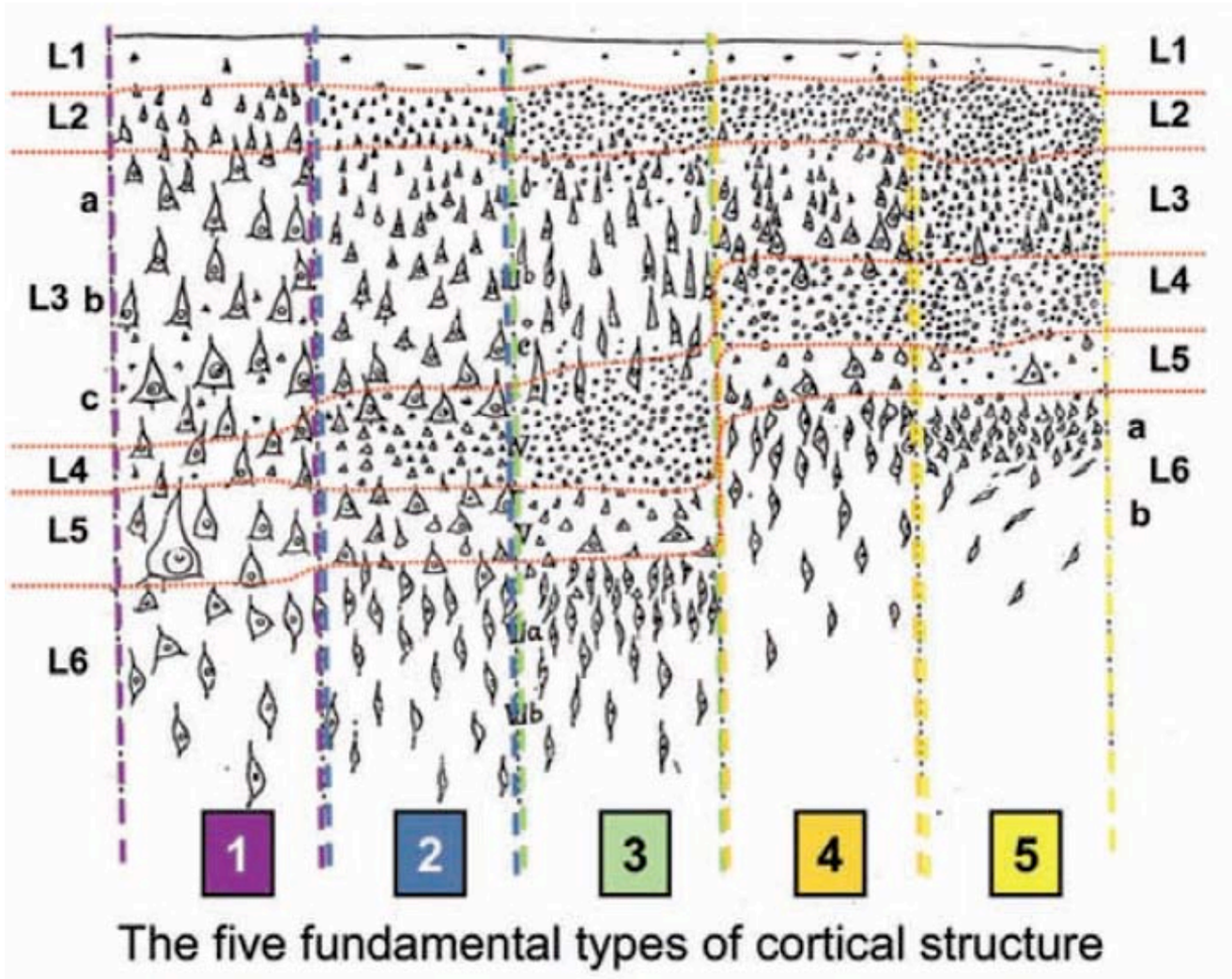
Motor cortex

Sensory cortex



Courtesy of Soren Van Hout Solari and Rich Stoner. Used with permission. CC BY-NC. "Cognitive Consilience: Primate Non-primary Neuroanatomical Circuits Underlying Cognition." *Frontiers in Neuroanatomy* 5, no. 65 (2011). doi: 10.3389/fnana.2011.00065.

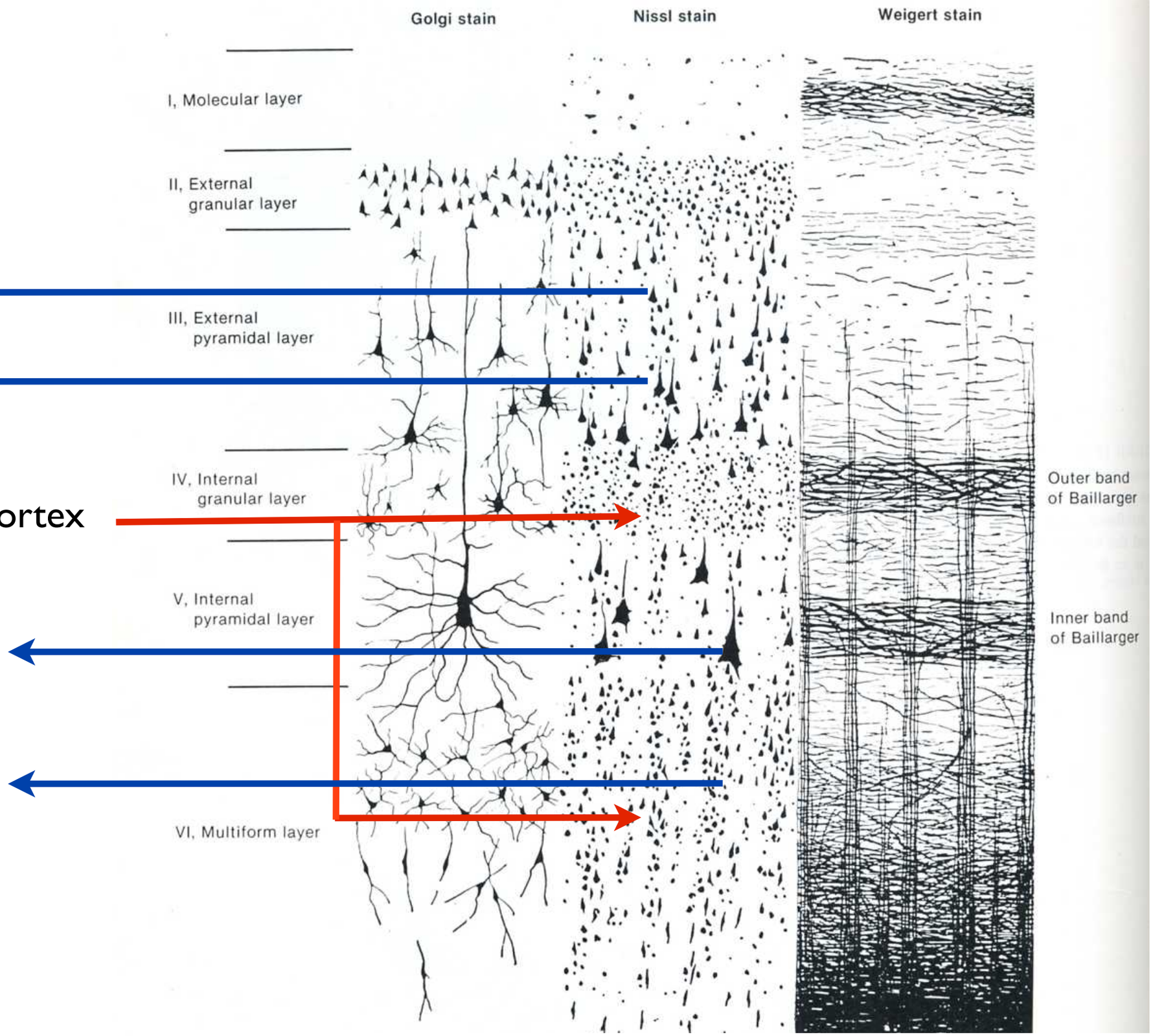
The neocortex

six layers

output layers:
to "higher" cortex
(axons <u>from</u> layers 2 and 3)

input layers:
from thalamus or "lower" cortex
(axons <u>into</u> layers 4 and (6))

output layers:
to subcortical targets
and "lower" cortex
(axons <u>from</u> layers 5 and 6)



9.17 Systems Neuroscience Lab

Spring 2013