

# **A sketch of the central nervous system and its origins**

G. E. Schneider 2014

**Part 1: Introduction**

## **MIT 9.14 Class 2**

Methods for mapping pathways and  
interconnections that enable the integrative activity  
of the CNS

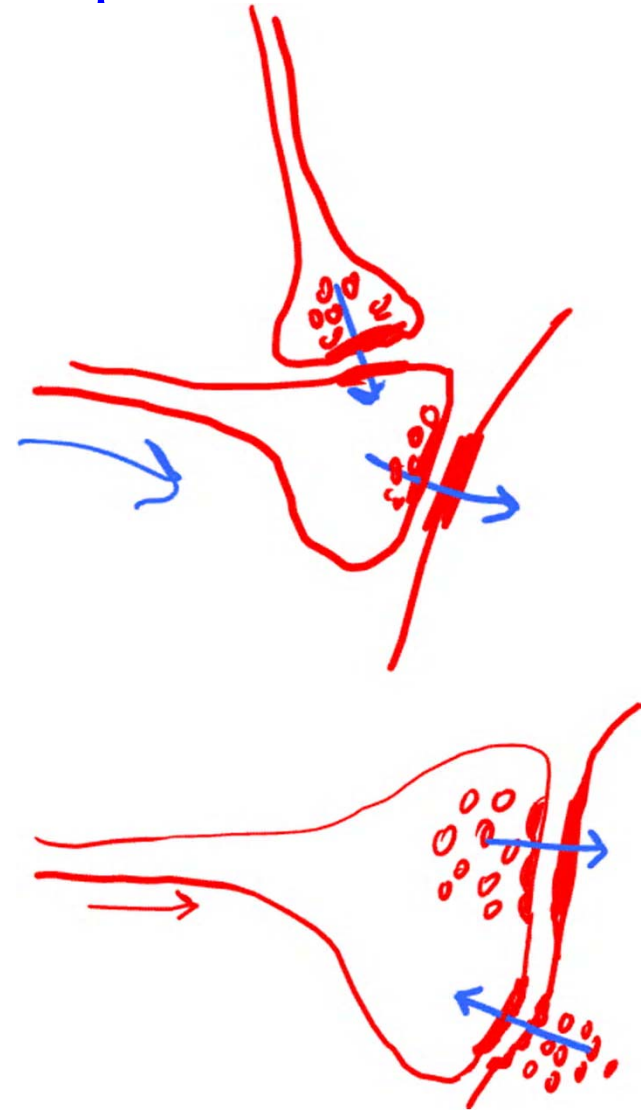
# Questions from class 1

- Presynaptic inhibition
- Presynaptic facilitation
- Other questions?

[-- asked individually by several students after class ended]

# Synapses: varied structural arrangements: Consider the functional possibilities

3. **Axo-axonal**  
*Presynaptic inhibition and facilitation*
4. (Also: dendro-dendritic, dendro-axonal...)
5. Reciprocal synapses



**Fig 1-13**

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Schneider, G. E. *Brain structure and its Origins: In the Development and in Evolution of Behavior and the Mind*. MIT Press, 2014. ISBN: 9780262026734.

# The need for integrative action in multi cellular organisms

- Problems that increase with greater size and complexity of the organism:
  - How does one end influence the other end?
  - How does one side coordinate with the other side?
  - With multiple inputs and multiple outputs, how can conflicts be avoided (often, if not always!)?
- Hence, the **evolution of interconnections** among multiple subsystems of the central nervous system necessarily had to occur as the CNS enlarged.

# How can such connections be studied?

- **The methods of neuroanatomy (neuromorphology):** Obtaining data for making sense of this “lump of porridge”.
- We can make much more sense of it when we use multiple methods to study the same brain. *E.g.*, in addition to neuroanatomical methods we can use:
  - **Neurophysiology**: electrical stimulation and recording
  - **Neurochemistry**; neuropharmacology
  - **Behavioral studies** in conjunction with brain studies
- In recent years, **various imaging methods** have also been used, with the advantage of being able to study the brains of humans, cetaceans and other animals without cutting them up. However, these methods are very limited for the study of pathways and connections in the CNS.

# A look at neuroanatomical methods

- Initial steps (often after an experimental procedure involving surgery):
  - Fixation
  - Embedding or freezing
  - Cutting into thin sections

# Sectioning and mounting on glass slides

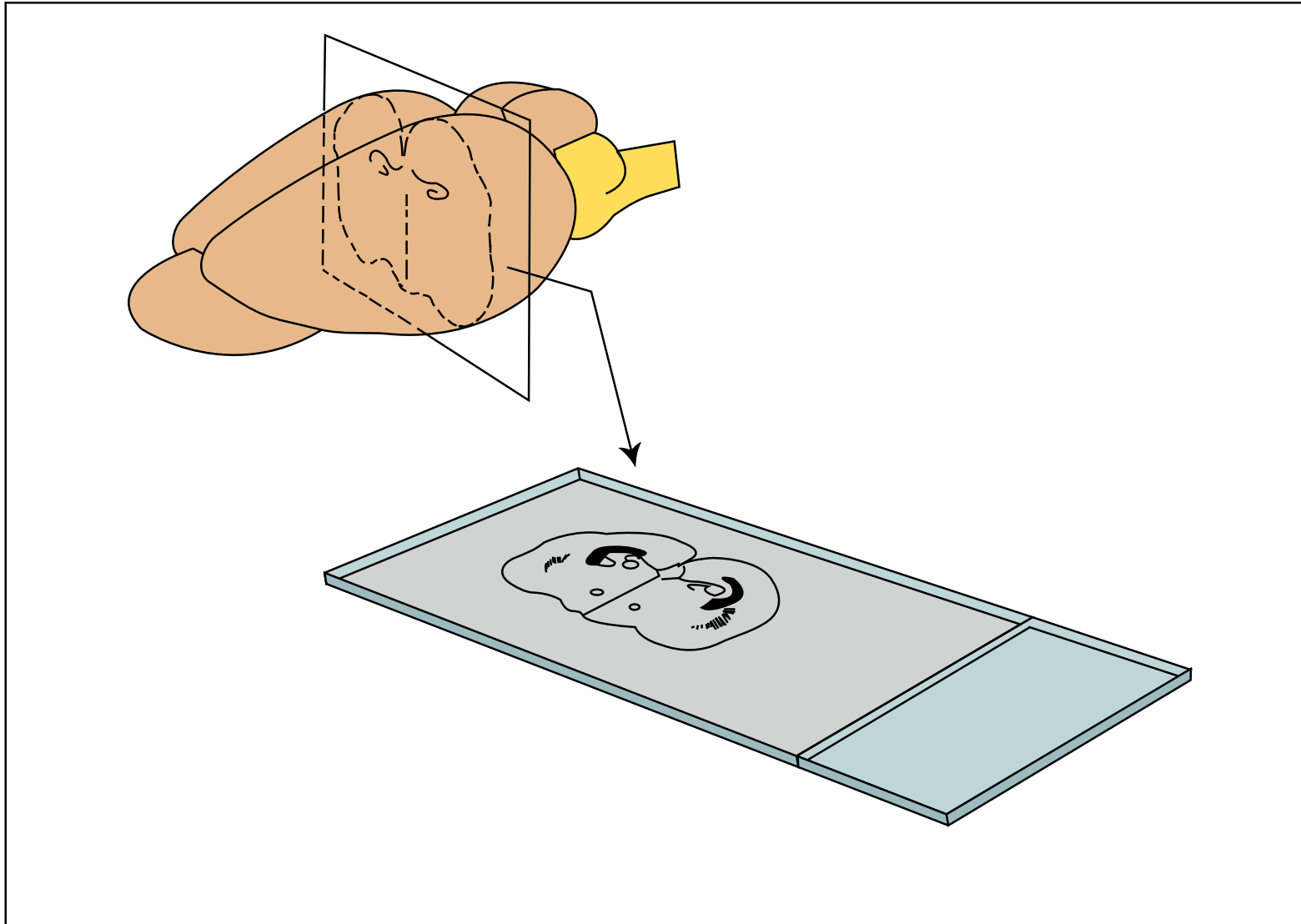


Image by MIT OpenCourseWare.

**Fig 2-1**

## Questions, chapter 2

1. What are some techniques used to prepare brain tissue for cytoarchitectural studies? Describe some findings of such studies.

-- Nissl stains for cell bodies

Next slide

-- Eosin used by pathologists, but not very good for cytoarchitectural analysis

2. What are some techniques used to prepare brain tissue for fiber-architectural studies? Describe some findings of such studies.

-- Silver stains for normal axons

-- Myelin stains [e.g., Weigert stain; iron-hemotoxylin]

Slide 14: myelogenesis studies revealed the axonal pathways of the visual radiations from thalamus to neocortex in humans, also the auditory radiations and the somatosensory radiations.



## Cytoarchitecture:

Using dyes to bind  
components of the  
tissue selectively:  
Example of stain for  
cell bodies

Figure removed due to copyright restrictions.

Please see course textbook or:

Swanson, L. W. "Brain Maps: Structure of the Rat Brain." *Elsevier*, 1992.

# Another example of cytoarchitecture: The area 17-18 border, Nissl stained section, neocortex of human brain

Figure removed due to copyright restrictions.

Please see course textbook or:

Nauta, Walle J. H., and Michael Feirtag. *Fundamental Neuroanatomy*.

Freeman, 1986. ISBN: 9780716717232.

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Freeman, 1986. ISBN: 9780716717232.

# Fiber architecture

Example: visualizing  
a chemical that binds  
to myelin

Myelo-  
architecture of  
human midbrain

Figure removed due to copyright restrictions.  
Please see course textbook or:  
Nolte, John. *The Human Brain in Photographs and Diagrams*.  
Elsevier Health Sciences, 2013.

Figure removed due to copyright restrictions.

Please see course textbook or:

Jhaveri, Sonal, Reha S. Erzurumlu, et al. "Oligodendrocytes and Myelin Formation along the Optic Tract of the Developing Hamster: An Immunohistochemical Study using the Rip Antibody." *Glia* 6, no. 2 (1992): 138-48.

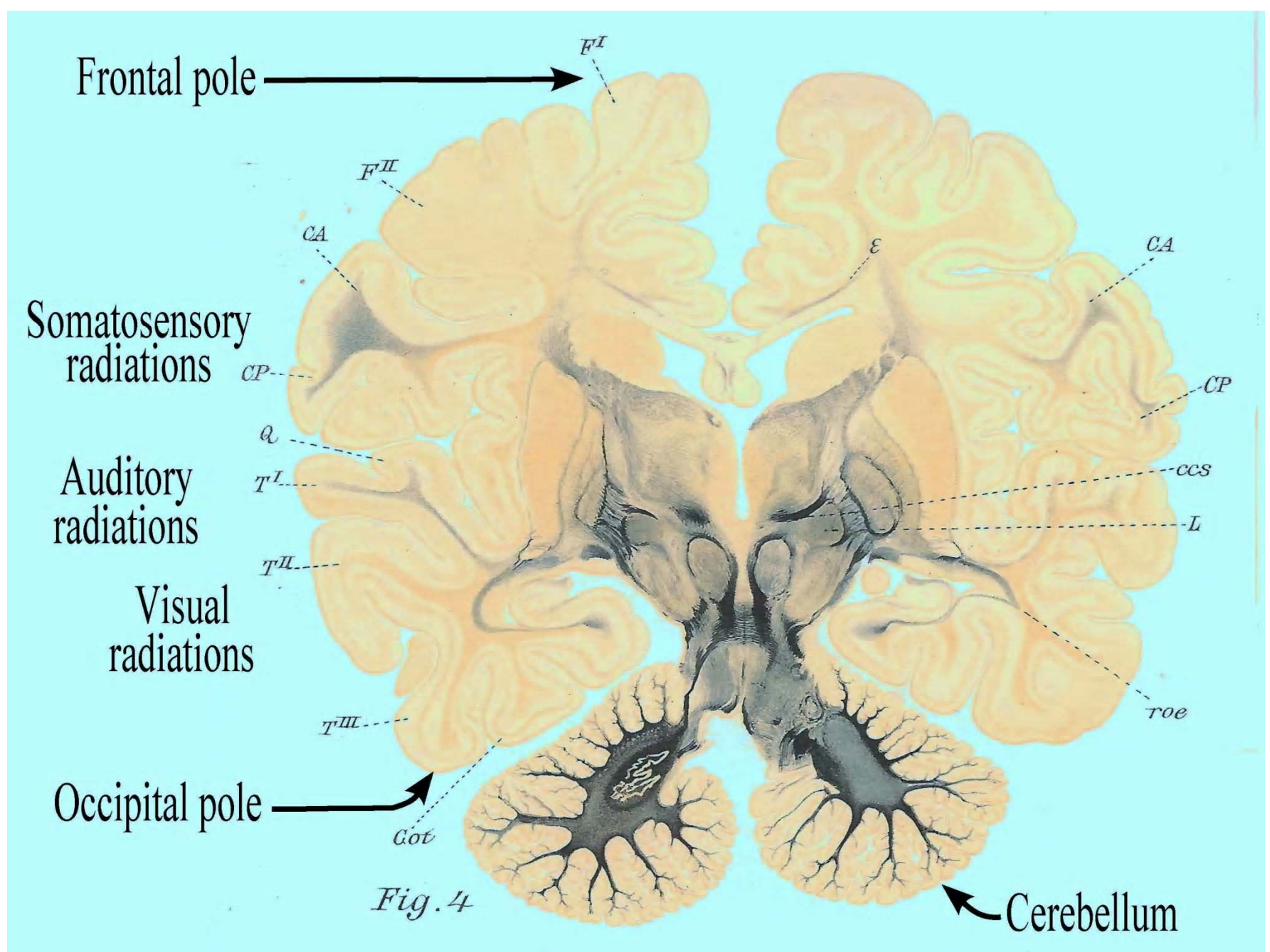


Image is in public domain. Flechsig, Paul. 1920. Anatomie des menschlichen Gehirns und Rückenmarks auf myelogenetischer Grundlage. Leipzig: G. Thieme.

## Fig 2-5 Myelinating Pathways, 7-wk human (Paul Flechsig)

## Questions, chapter 2

3. Describe a use of immunohistochemistry for neuroanatomical studies, after defining what immunohistochemistry is.
4. How was histochemistry used for help in identifying comparable forebrain structures in mammals and birds?

Immuno-  
histochemistry,  
example:  
  
Opiate receptor  
localization in  
rat brain  
(horizontal section)

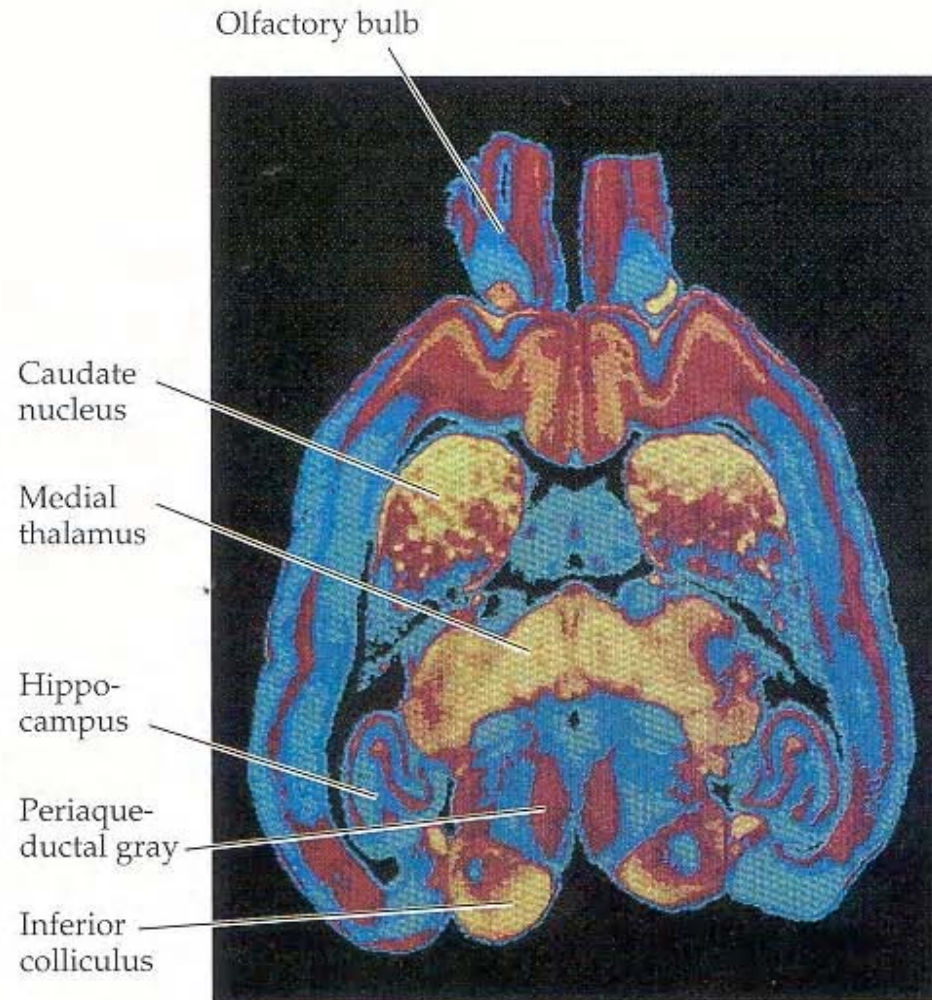


Figure 9 from Herkenham, M., and C. B. Pert. "Light Microscopic Localization of Brain Opiate Receptors: A General Autoradiographic Method which Preserves Tissue Quality." *The Journal of Neuroscience* 2, no. 8 (1982): 1129-49. Available under Creative Commons BY-NC-SA.

**Fig 2-8a**





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Source: Graybiel, A. M. "A Stereometric Pattern of Distribution of Acetylthiocholinesterase in the Deep Layers of the Superior Colliculus." *Nature* 272, no. 5653 (1978): 539. © 1978.2026734.

## Chemoarchitecture, example:

Acetylcholinesterase stain reveals layers and patches in rat midbrain

**Fig 2-6**

Figure removed due to copyright restrictions.

Please see course textbook or:

MacLean, Paul D. "Cerebral Evolution and Emotional Processes: New Findings on the Striatal Complex." *Annals of the New York Academy of Sciences* 193, no. 1 (1972): 137-49.

More chemo-architecture: AChE Histochemistry applied to comparative neuroanatomy of the forebrain

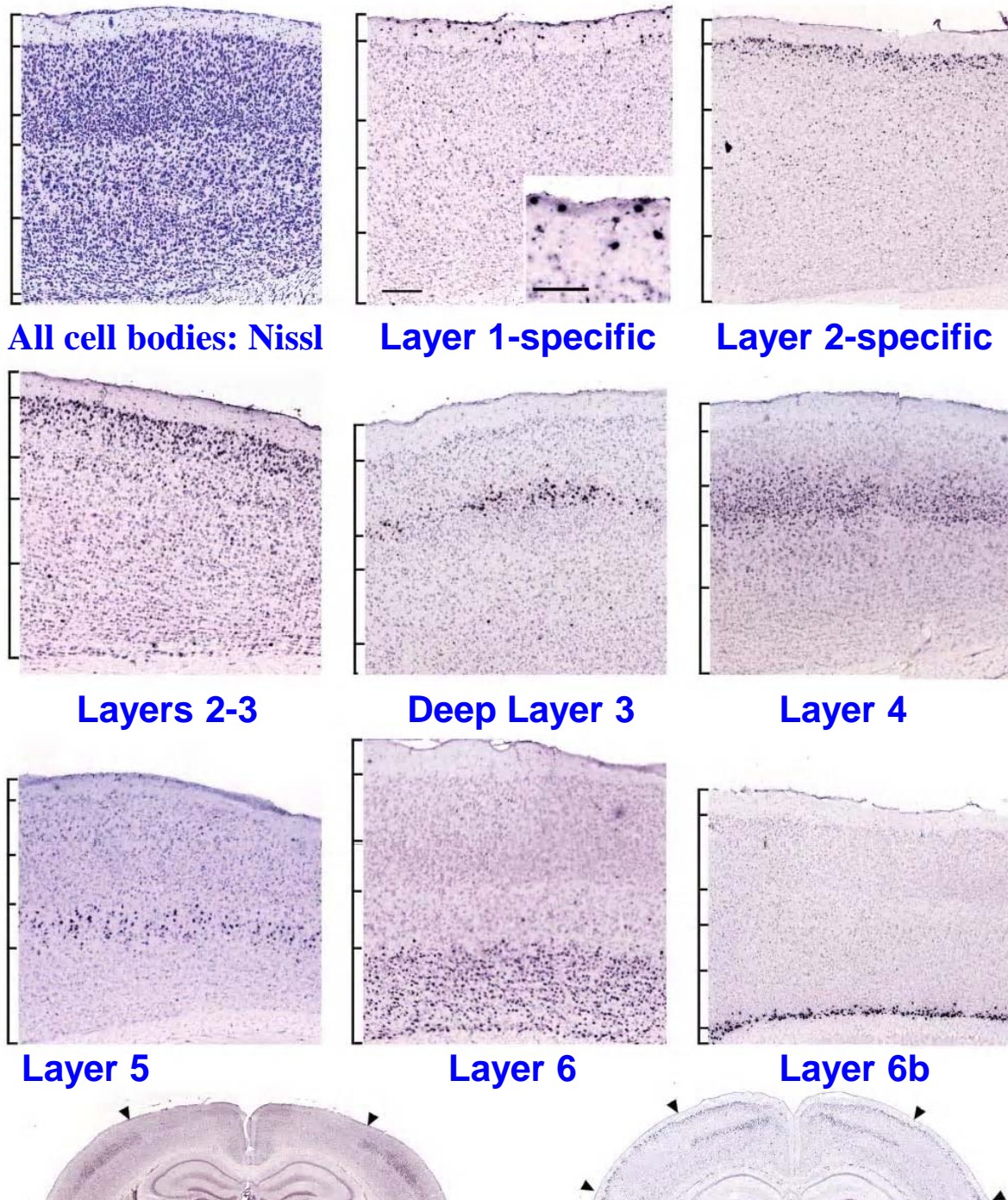
# Newer technology: gene expression patterns in the CNS

Figure from recent *Nature*: *see next slide*

Genome-wide atlas of gene expression in the adult mouse brain, *Nature*, 11 January 2007, 168-176 (with 108 authors)

*Expression patterns of about 200,000 genes are available online.*

Figure 6 of that paper:  
Laminar and region-specific neocortical gene expression.



Reprinted by permission from Macmillan Publishers Ltd: Nature.  
Source: Lein, Ed S., Michael J. Hawrylycz, et al. "Genome-wide Atlas of Gene Expression in the Adult Mouse Brain." *Nature* 445, no. 7124 (2006): 168-76. © 2006.

## Questions, chapter 2

5. Describe advantages and disadvantages of using the Golgi method for tracing interconnections of structures in the CNS.
6. Who was Ramon y Cajal?

# Golgi Stain:

Used by  
Ramon y Cajal to  
study connectivity  
of the brain and  
spinal cord

Image removed due to copyright restrictions.

Figure removed due to copyright restrictions.

Please see course textbook or:

Glaser, Edmund M., and Hendrik Van der Loos. "Analysis of Thick Brain Sections by Obverse—Reverse Computer Microscopy: Application of a New, High Carity Golgi—Nissl Stain." *Journal of Neuroscience Methods* 4, no. 2 (1981): 117-25.

Santiago  
Ramon y Cajal,  
drawing at his  
microscope



**Fig 1-8**

Image is in public domain. Courtesy of [www.wikimedia.org](http://www.wikimedia.org).



# Golgi method: axons in spinal cord (Ramon y Cajal)

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HISTOLOGY OF THE NERVOUS SYSTEM

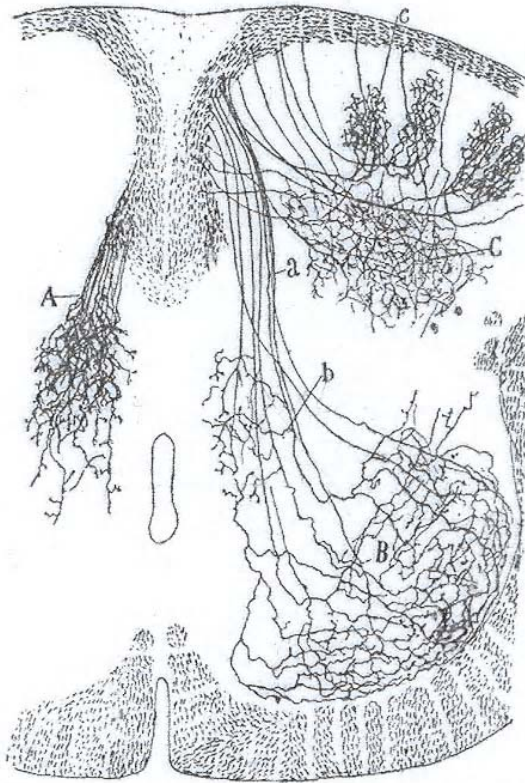


FIG. 113. Major sensory collaterals in the newborn-rat. Golgi method.

A, collaterals in the intermediate nucleus; B, arborizations in the motor nucleus; C, extended ramifications in the head of the sensory-motor bundle; a, sensory-motor bundle; b, collateral to the intermediate nucleus; c, deep collaterals in the substantia gelatinosa.

**Axons from dorsal root,  
carrying sensory  
information from body  
surface into spinal cord**

Courtesy of MIT Press. Used with permission.

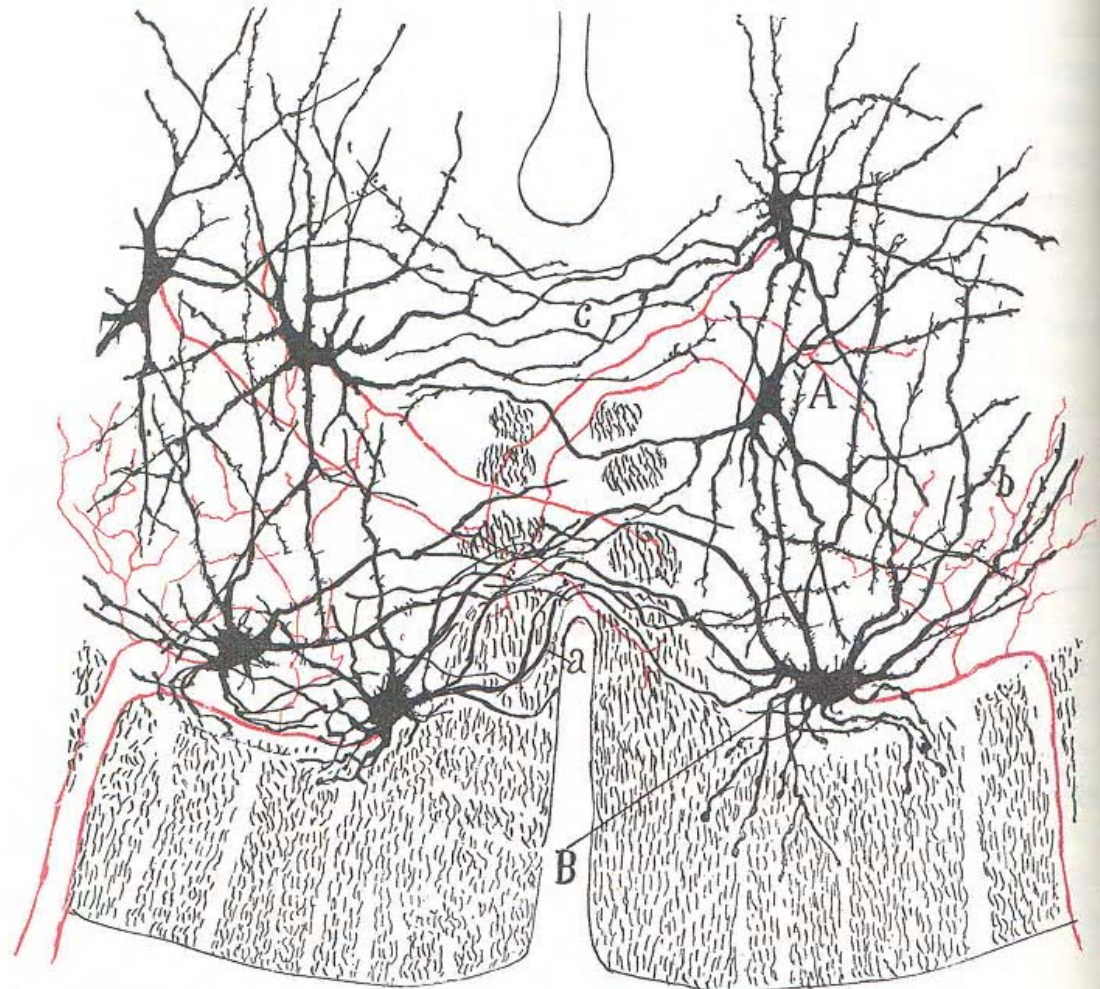
Schneider, G. E. *Brain structure and its Origins: In the Development and in Evolution of Behavior and the Mind*. MIT Press, 2014. ISBN: 9780262026734.

**Fig 2-10**

# Neurons of spinal cord (Ramon y Cajal)

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HISTOLOGY OF THE NERVOUS SYSTEM



**Motor neurons, with →  
axons (shown in red)  
going to striated  
muscles**

FIG. 129. Ventral root and commissural neurons in the thoracic spinal cord of the cat fetus. Golgi method.

A, commissural neuron; B, motor neuron in the medial nucleus; a, commissural dendrites; b, dorsal dendrites; c, commissure that is formed by the dendrites of funicular neurons. Red fibers are axons.

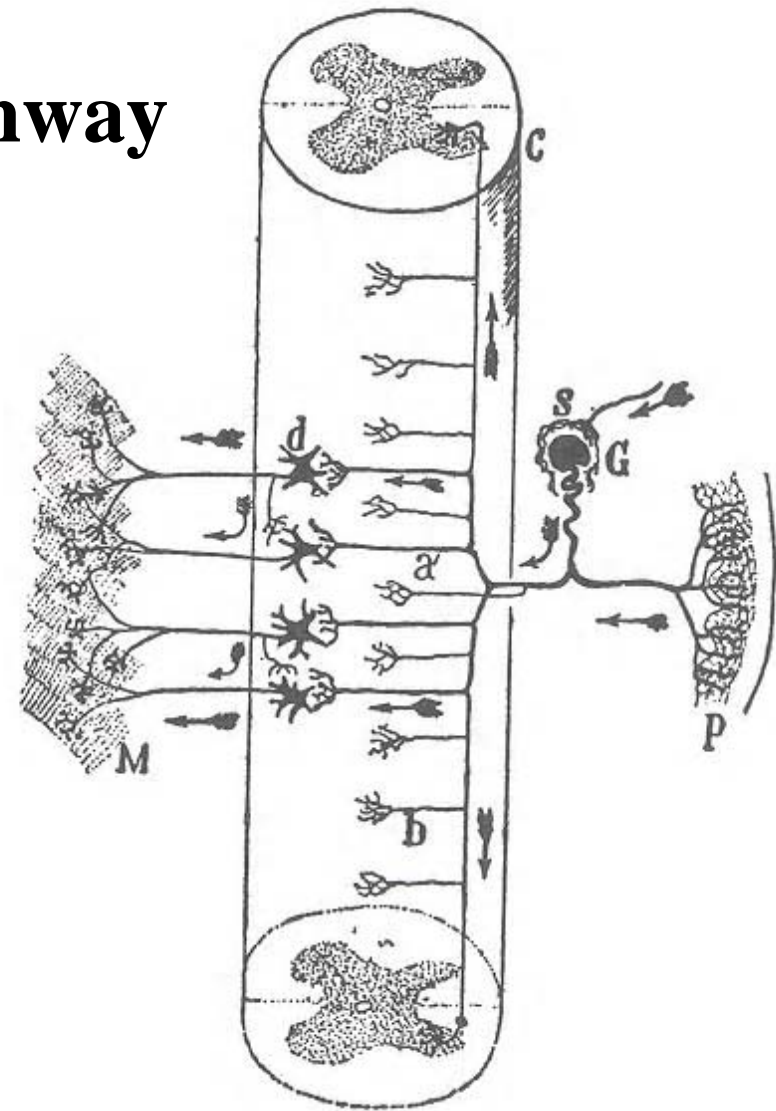
**Fig 2-11**

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# Diagram of local reflex pathway by Ramon y Cajal

Cajal saw an entire S-R pathway for the first time: a pathway beginning with a Stimulus which activates a sensory neuron, and ending with a Response, the movement caused by the activity of motor neurons.



as shown here

**Fig 2-12**

With Golgi methods, tracing long axons is very difficult or impossible, but local connections can be seen clearly.

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## Questions, chapter 2

7. How did Ivan Pavlov's discoveries make the S-R model of behavior more comprehensive? **Reflex connections can be plastic, thus accounting for [some] learning**
8. Describe an argument made by Karl Lashley against the adequacy of an S-R model for explaining all human behavior.

# Brain connections and behavior

- The story of Karl Lashley's encounter, as a young student, with slides of a frog brain: *“If I could use this kind of material to see all of the connections, it would be possible to explain the frog's behavior.”*
- Assumption: the S-R model, originating with René Descartes—who described a reflex arc for the first time. It was championed by the philosopher LaMettrie in the following century, and boosted by the Russians Ivan Sechenov and his famous student, Ivan Pavlov. (*Pavlov showed how reflexes could be plastic—changed by learning.*)

# Brain connections and behavior

- Arguments against the adequacy of S-R models as explanations of behavior:
  - In the first half of the 20<sup>th</sup> century, Karl Lashley argued against the adequacy of S-R theory for explaining temporal order in rapid sequences of behavior: ~~reaction times are too slow.~~ \*
  - Also, the role of **endogenous** activity does not fit the S-R model. (More about that later.)
  - Motivational systems can initiate behavior independent of external stimuli.
- Nevertheless, the S-R model remains a common assumption among neuroscientists.
- In any case, a knowledge of brain organization and interconnections is basic for understanding how behavior is produced and controlled.

\* Thus, there must be central programs controlling the movements.

## Questions, chapter 2

9. How was the phenomenon of retrograde degeneration used in experiments on animals to establish the existence of a major pathway taken by visual information to the neocortex? What belief was destroyed by the experiments? (explained in chapter 2)

## Questions, chapter 2

10. What electrophysiological method can be used to verify the existence of a direct axonal pathway from one location to another in the CNS? It was used by Sherrington and others.

See next slide.



# Connectivities: How do we know about them?

- ✓ Dissection
- ✓ Staining techniques: cell bodies, fibers; uniqueness of Golgi methods
- ✓ Complexity problem: How to be sure of a connection? (What is the big limitation of the Golgi method?)
  - ✓ Historical example (late 19<sup>th</sup> century): How does info get from eye to neocortex?
- ✓ Electrophysiology: “antidromic” stimulation and recording (following Sherrington)
- **Experimental methods of tracing pathways by selective labeling**

## Questions, chapter 2

11. What was a major difference between the tract-tracing methods of Marchi and Nauta?
  
12. Fink and Heimer at MIT in the 1960s altered the Nauta method to make it much more sensitive for the tracing of axons. For many pathways, a group of axons could be traced to their terminal enlargements—the boutons where synapses are made. Describe another technique that can be used for such tract tracing from neuronal cell bodies to their endings. What problem with methods using axon degeneration did the new method overcome?

# Walle J. H. Nauta

## 1916-1994

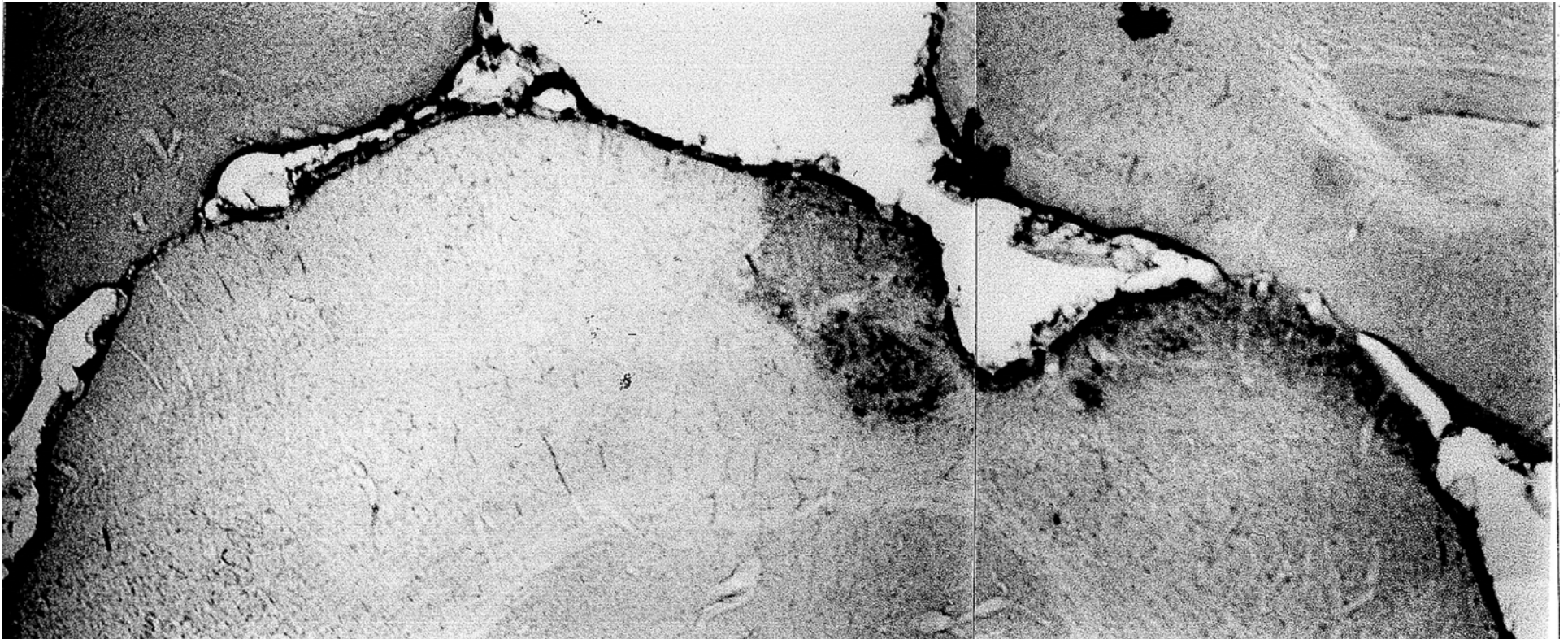
- From the Netherlands, he came to USA *via* Switzerland
- Father of modern experimental neuroanatomy
- M.I.T. Professor, 1964-1986 (Institute Professor from 1973) in what became BCS
- First neuroanatomist to be appointed to the faculty of a psychology department (1964, MIT). This move by Hans-Lukas Teuber presaged the development of modern neuroscience.

Walle Nauta  
M.I.T.  
Institute Professor

Photograph of Walle J. H. Nauta removed due to copyright restrictions.

*Example:*

Hamster with unilateral lesion of midbrain surface on first postnatal day:  
tracing of retinal projections from left eye, using a modified Nauta silver  
stain for degenerating axons



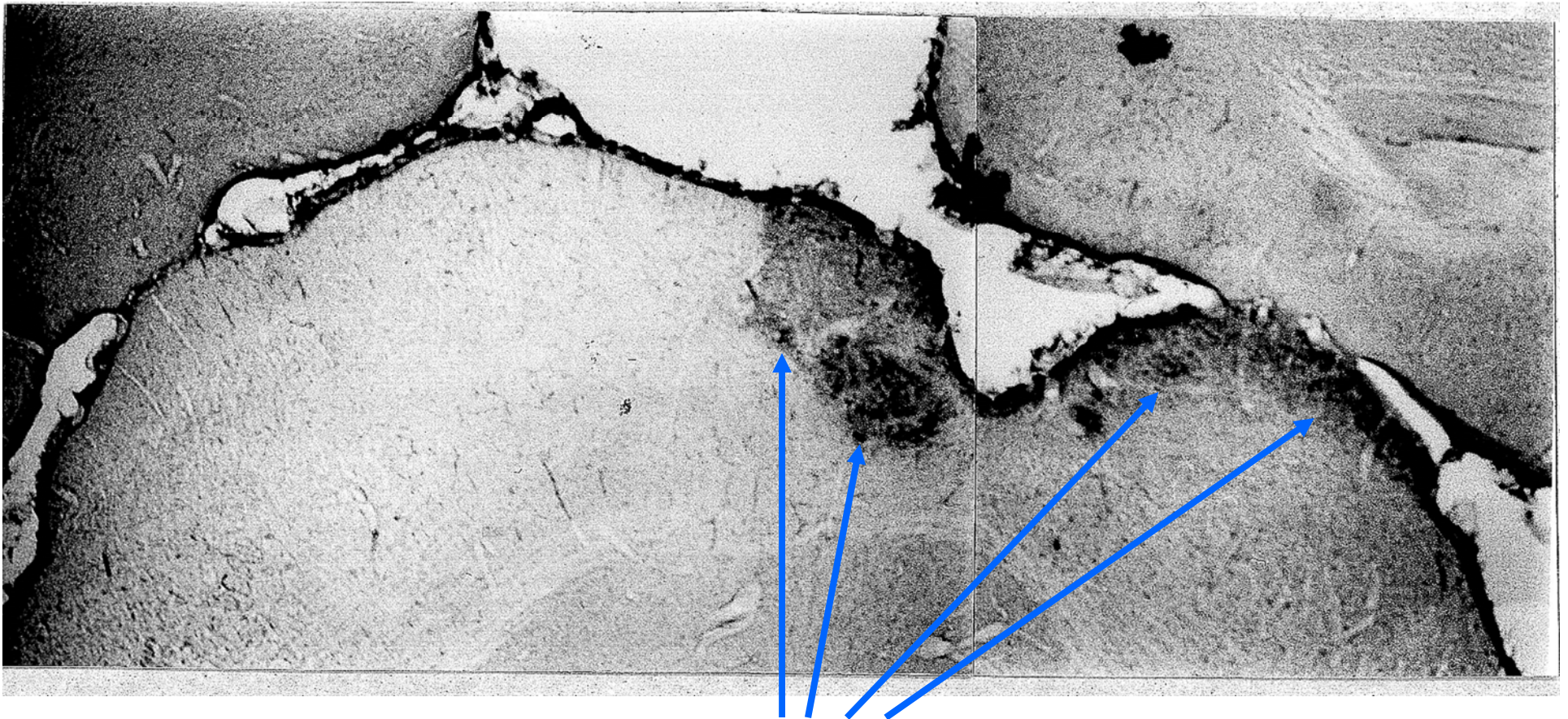
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**Fig 2-14**

## *Example:*

Hamster with unilateral lesion of midbrain surface on first postnatal day:  
tracing of retinal projections from left eye, using a modified Nauta silver  
stain for degenerating axons



**Silver-stained degenerating axon terminals  
(boutons)**

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Evolution of Behavior and the Mind*. MIT Press, 2014. ISBN: 9780262026734.

## Questions, chapter 2

13. What is HRP, and how can it be used for tract tracing?

**\_taken up and transported both anterogradely and retrogradely**

14. Describe advantages of using fluorescent molecules for tract tracing. They have become increasingly used as the sensitivity of fluorescence microscopy has improved.

**Sections are mounted but not stained.**

HRP staining, after anterograde transport from retina of hamster pup: labeled axons seen in diencephalon using dark-field microscopy

**Terminating axons**

**Optic tract at surface**

**(Dorsal is up)**

**Fig 2-15**

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# Retrograde tracer: Fluorogold (transport from SC to retina; labeled retinal ganglion cells seen in whole mount)



**Fig 2-17**

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# Double Labeling: Nuclear Yellow and HRP (retrograde transport from optic tract to retina, seen in retinal whole mount)



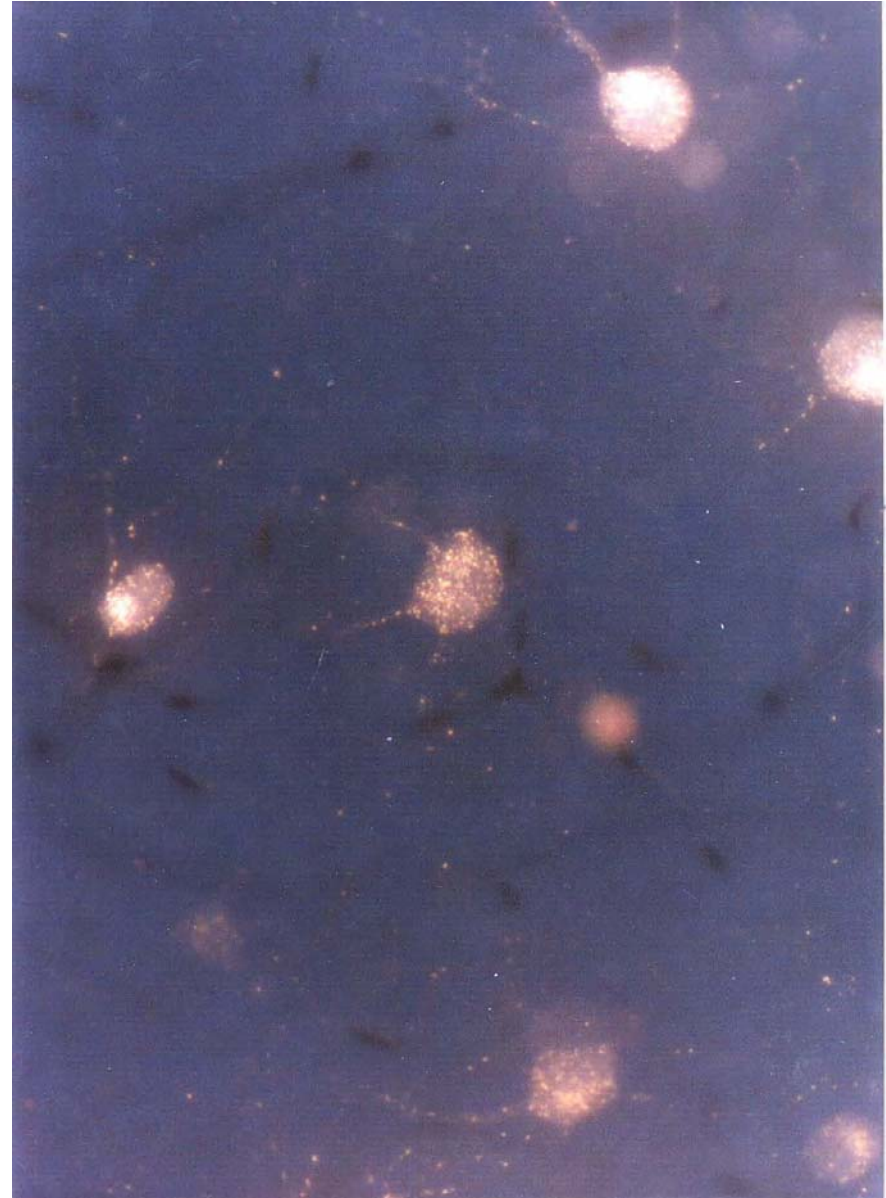
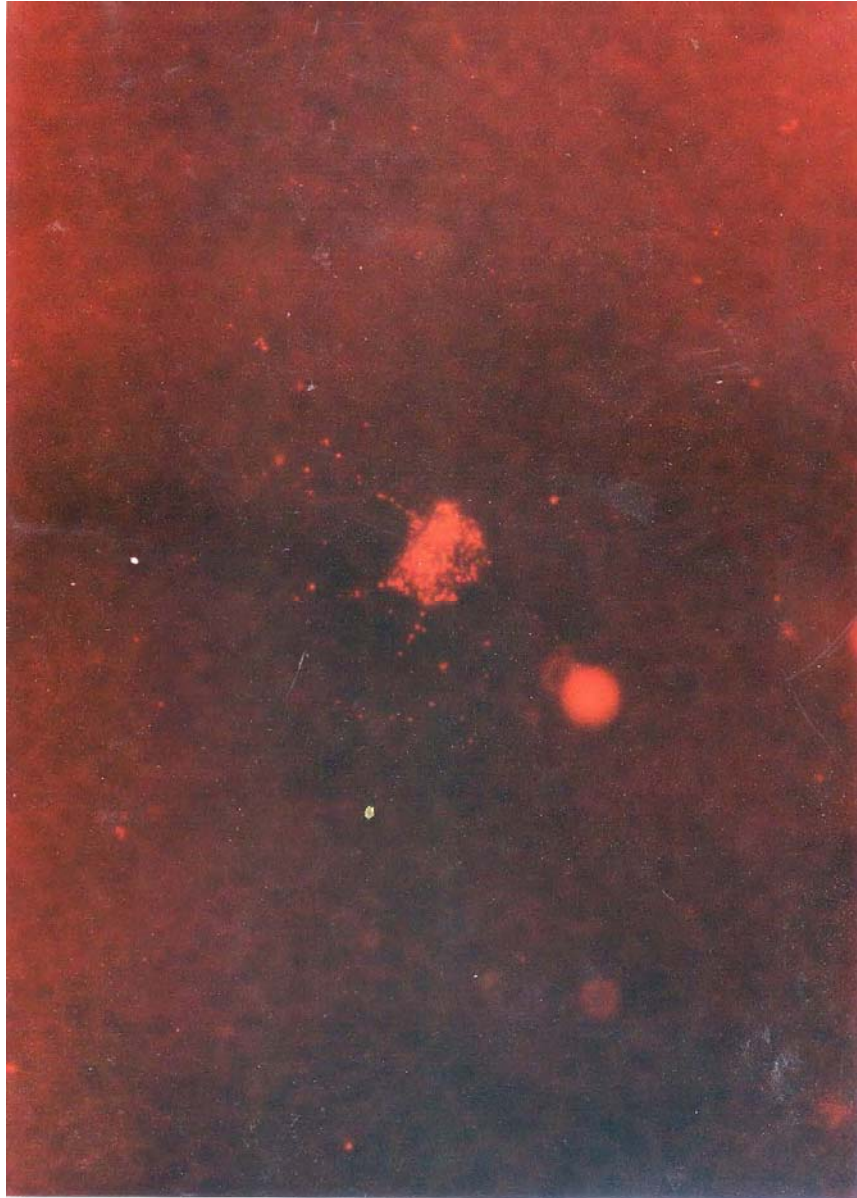
**Fig 2-18**

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# Co-localization: fluorogold & fluorescent beads

(matched views of retina illuminated with different wavelengths of light)



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Note the double labeling of two cells

Connectivities: How do we know about them?

## Experimental methods of tracing pathways:

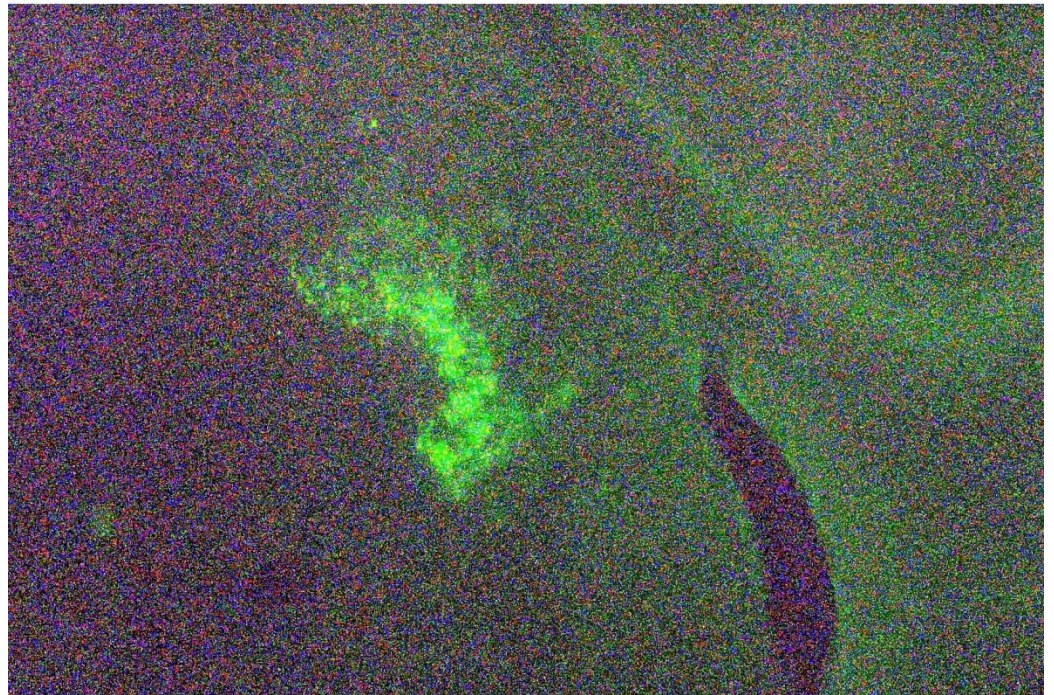
- ✓ **Marchi method**: an experimental neuroanatomical technique for selective staining of degenerating myelin
- ✓ **Nauta methods** for silver staining of degenerating axons
  - ✓ Not limited to myelinated axons [*see next slides*]
- ✓ **Labeled amino acids** and autoradiography
- ✓ **HRP histochemistry**: 2-way transport utilized
- ✓ **Fluorescent tracers**
- **Immunohistochemistry**: not only for chemoarchitecture, but also for detecting **various axonal tracers**, *e.g.* **CT-B** (cholera toxin fragment B) [to be illustrated later]

## *Bright field*

Immunohistochemical  
staining for Cholera Toxin,  
subunit B  
(anterograde transport from  
part of retina to lateral  
geniculate nucleus)



## *Dark field*



**Fig 2-16**

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## Questions, chapter 2

15. What is the method of diffusion tensor imaging?  
What are its advantages and its limitations?

# Gross dissection of human brain after fixation

Figure removed due to copyright restrictions.

Please see course textbook or:

Jellison, Brian J., Aaron S. Field, et al. "Diffusion Tensor Imaging of Cerebral White Matter: A Pictorial Review of Physics, Fiber Tract Anatomy, and Tumor Imaging Patterns." *American Journal of Neuroradiology* 25, no. 3 (2004): 356-69.

# Diffusion Tensor Imaging of brain of ~~alive~~ human living

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Spring 2014

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