Cortical organization: neuroanatomical approaches

Study of neuronal circuitry and microstructure

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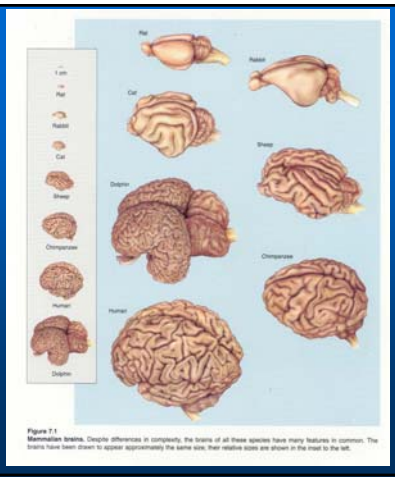
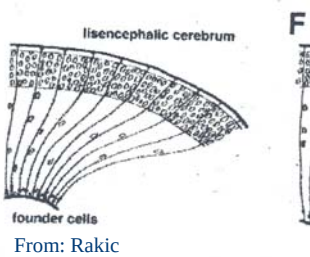
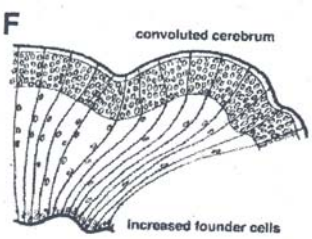
Neuroinformatics Aug., 2009

Changes in characteristics seen through phylogeny

- Increase in the absolute and particularly relative mass of the brain compared to body size
- Comparatively larger increase in telencephalic structures particularly the cerebral cortex
- Expansion in number of radial units







From: Rakic

Neoteny: prolongation of fetal growth rate into postnatal times results in increase in the size of the human brain

Evolution of the Nervous System

How does a larger or reorganized brain come about?

Environmental factors

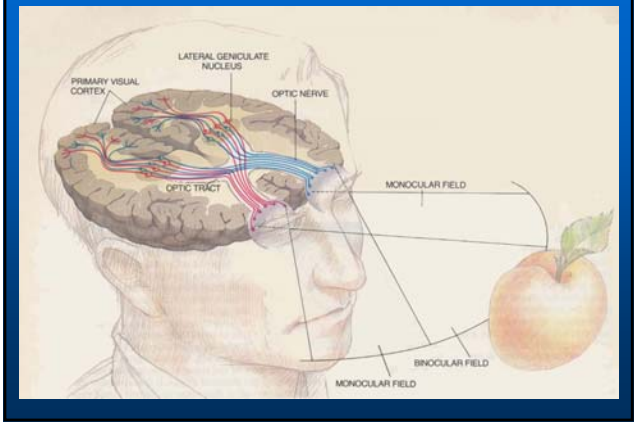
Internal factors – change in posture

- freedom of the hands
- movement of eyes from a lateral to central position

Crossing at the optic chiasm is complete in animals with small degree of binocular vision

FREEDOM OF THE HANDS

- Direct projection to the pyramidal tract in animals with fine use of the hands



How did these changes come about in evolution?

By what mechanism?

Distinct modes of radial migration:

Inside-out: characterizes pattern in mammalian cortex

Outside-in: characterizes reptilian cortex

Consequences of differential migration: differences in cortical thickness, synaptic interactions

reelin (glycoprotein)
mice deficient in reelin have inverted cortical lamination

cdk5 (cyclin-dependent protein kinase)
mice lacking cdk5 or its activator p35 show inverted cortical lamination

Differences in the two mutants:
in cdk5/p35 mutants the marginal zone and subplate are properly differentiated;
in the reeler they are not

Addition of new cells to the primate CNS

Example from humans
Spindle cells in cingulate gyrus

GABAergic cells in the dorsal thalamus (DT)

Large expansion of ‘association’ areas

Progressive change from the general to the specific; loss of some connections

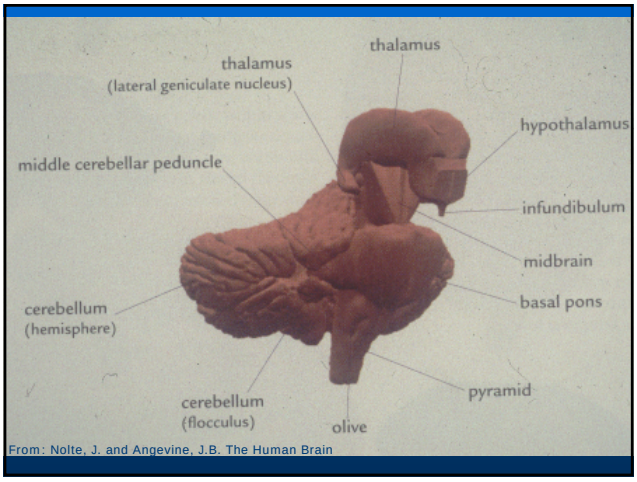
Differentiation of cortical layers

Structures that are large relative to overall brain size have late birthdays

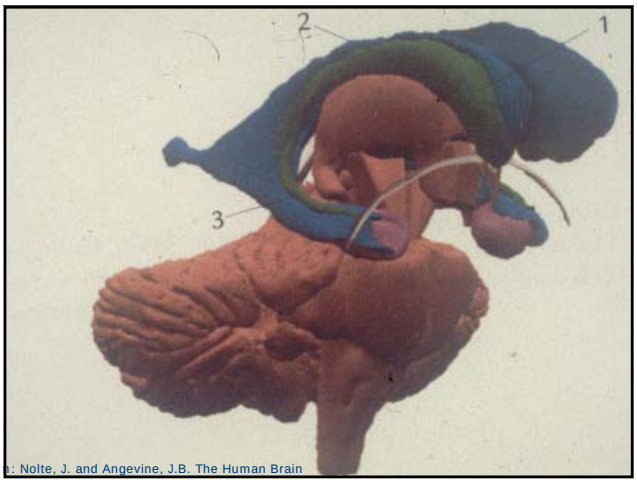
Why?

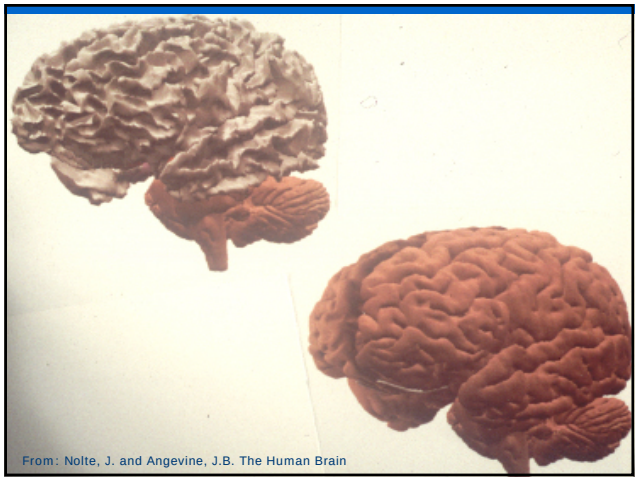
Building the Brain

- Brainstem
- Thalamus and hypothalamus
- Ventricles
- Basal Ganglia
- Amygdala
- Hippocampus
- Pathways



From: Nolte, J. and Angevine, J.B. The Human Brain





MAJOR INFLUENCES ON CORTEX

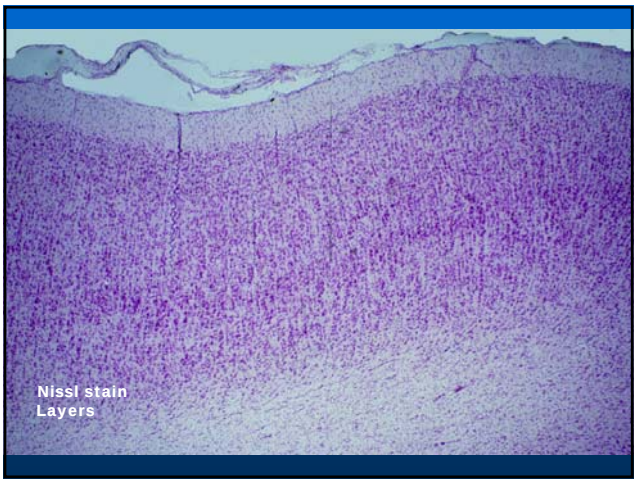
Neurotransmitter- specific projection systems: Cholinergic; Adrenergic; serotonergic; Dopaminergic
arise from subcortical structures

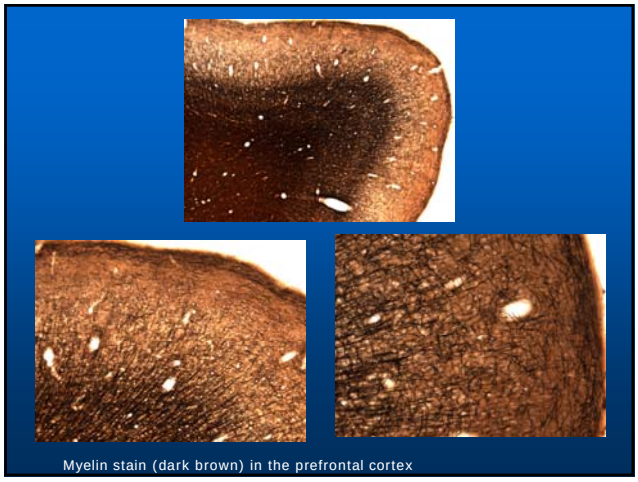
Thalamus/hypothalamus

Specialized projections:
- Hippocampal formation
- Amygdala

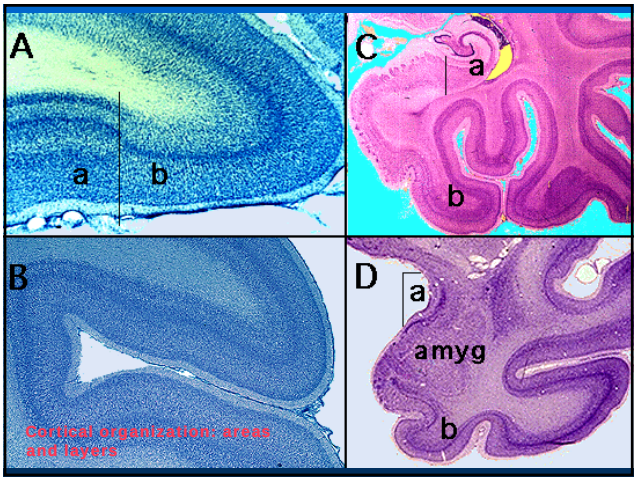
Methods to study cortical architecture

Global structure: classical methods
- Nissl stain (stains all neurons and glia)
- myelin stain

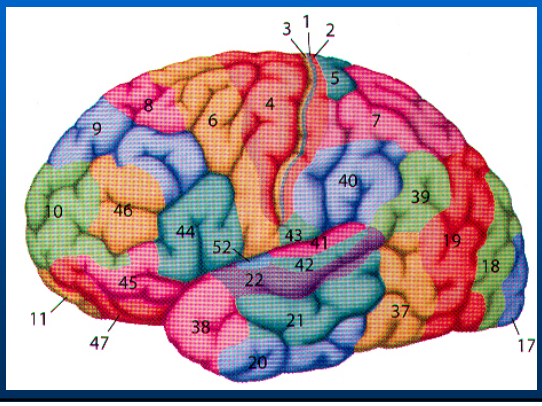




Myelin stain (dark brown) in the prefrontal cortex



Cerebral Cortex: Brodmann map



Classical stains:

Nissl stain
myelin

Are these methods useful today?

Applications
stereological analysis to estimate density or overall number of neurons

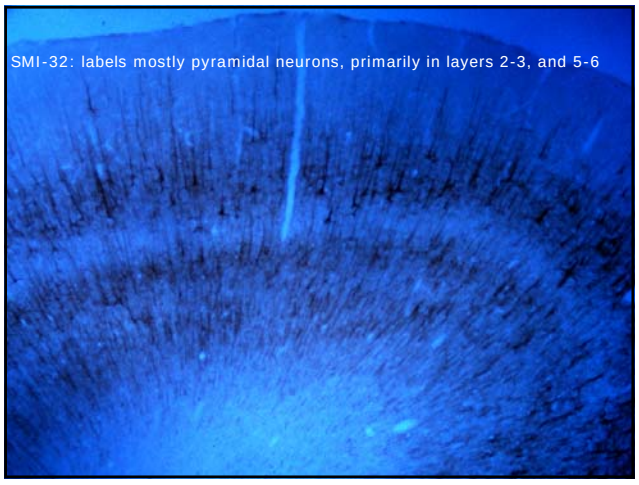
Myelinogenesis: The method was introduced by Flechsig; it makes use of the fact that different fiber tracts become myelinated at different times in their development. Thus, study of the nervous system in embryos and in early neonatal life often provides information about the existence and locality of the different fiber tracts.

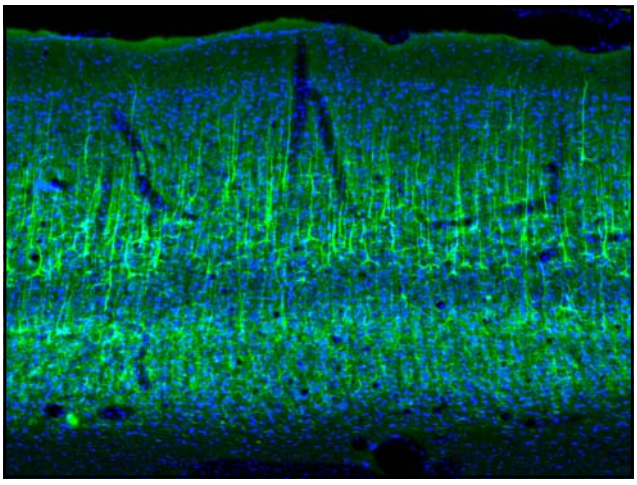
Myelination occurs throughout development, and up to the fifth decade of life, and can be used to show development of pathways, particularly in children and adolescents.

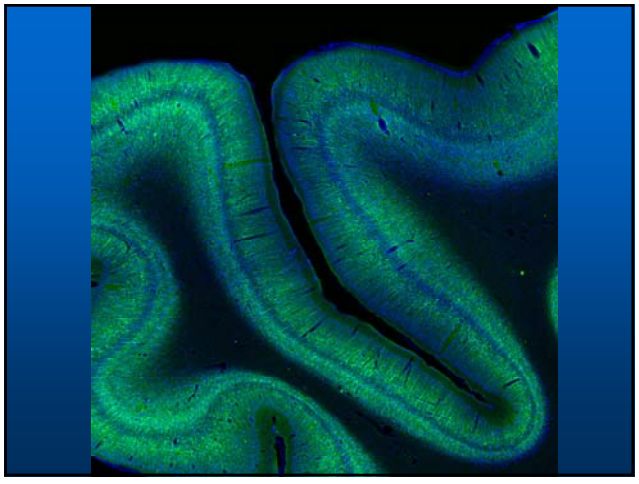
Selective labeling of classes of neurons

Histochemistry

Immunohistochemistry


SMI-32: labels mostly pyramidal neurons, primarily in layers 2-3, and 5-6






Cortical architecture: organization into:
layers, columns, and modules
Cortical architecture:
modules seen by anterograde labeling

MAJOR CORTICAL CELL TYPES:

Principal or Projection neurons:

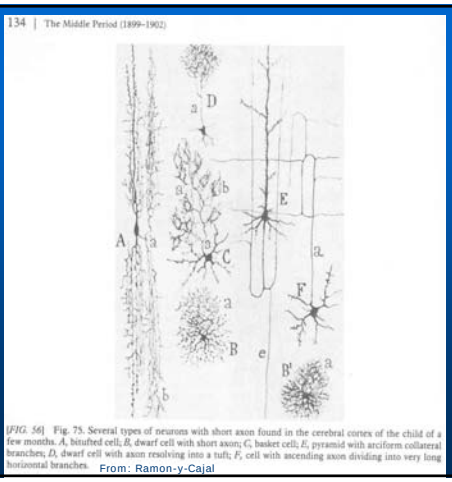
Pyramidal: project outside of immediate area to other cortices or to subcortical structures

Other shapes (e.g., fusiform)

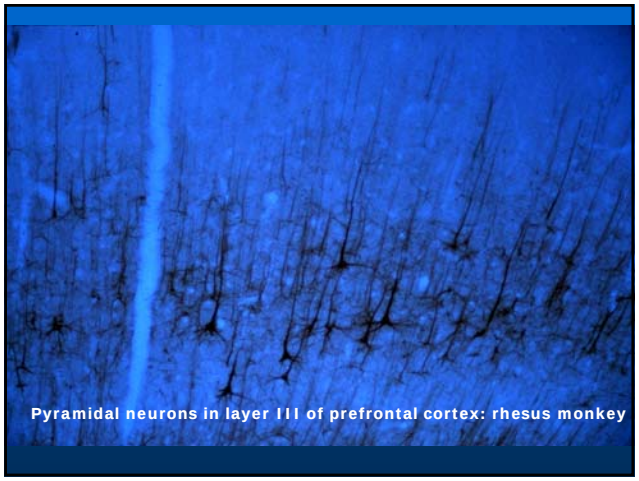
Interneurons:

Excitatory: stellate: small local neurons

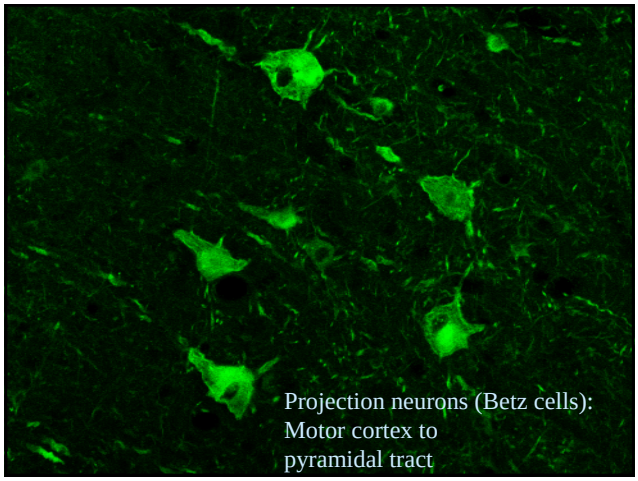
Inhibitory: (many different shapes)



[FIG. 56] Fig. 75. Several types of neurons with short axon found in the cerebral cortex of the child of a few months. *A*, bitufted cell; *B*, dwarf cell with short axon; *C*, basket cell; *E*, pyramid with arciform collateral branches; *D*, dwarf cell with axon resolving into a tuft; *F*, cell with ascending axon dividing into very long horizontal branches.



Pyramidal neurons in layer III of prefrontal cortex: rhesus monkey



Projection neurons (Betz cells): Motor cortex to pyramidal tract

Neurochemical markers selectively label distinct classes of neurons in the brain:

Labeling of inhibitory neurons in the brain

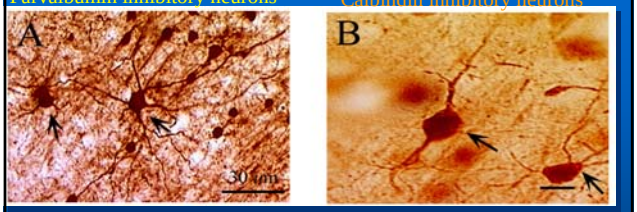
e.g., GABA (GAD)

Neurochemical classes of inhibitory neurons labeled with the calcium binding proteins: parvalbumin; calbindin; calretinin (label inhibitory neurons in the cortex and amygdala but not in thalamus)

Differences in neurochemical classes of inhibitory interneurons

Parvalbumin inhibitory neurons

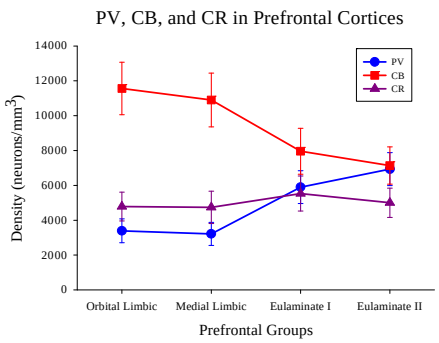
Calbindin inhibitory neurons



Parvalbumin positive neurons predominate in the middle cortical layers; they are basket or chandelier type inhibitory neurons, targeting the proximal dendrite or axon initial segment of other neurons.

Calbindin positive neurons predominate in the superficial cortical layers; they are double bouquet type inhibitory neurons, targeting the distal dendrite of other neurons.

Regional distribtution of neurochemical classes of inhibitory neurons



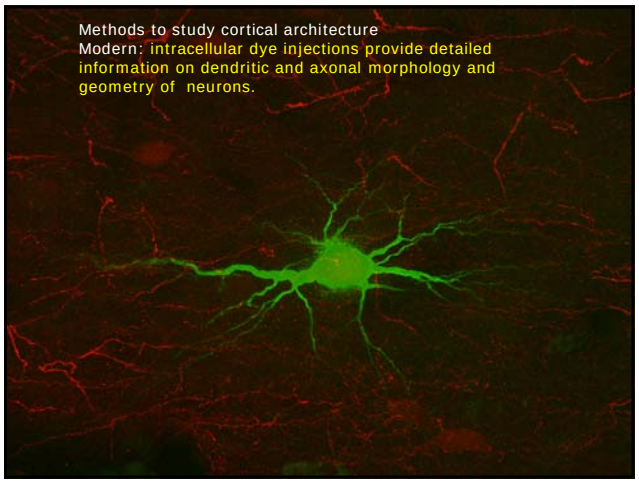
Methods to study cellular architecture

Classical:

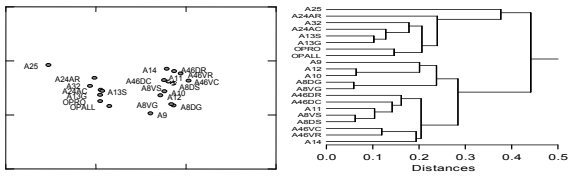
Single cell structure: Golgi method

The Golgi method provides detailed information on dendritic and axonal morphology and geometry of neurons.

Drawback of method: labeling is random and unpredictable



What do architectonic methods tell us about cortical organization?



Methods to study connections

Classical:

Anterograde degeneration: After a lesion axon terminals and the surrounding myelin undergo degeneration and degenerating axons and terminals can be stained selectively.

Retrograde degeneration: After a lesion cell bodies that lose their postsynaptic input degenerate: e.g., LGn neurons degenerate after lesion of V1 (in some species).

Classical:

Efferent connections: degenerating axons

After a lesion has been produced in animals or humans and sufficient time has elapsed for anterograde degeneration to set in, the brain can be studied, and degenerated tracts can be localized by one of the following methods:

•***Methods that stain degenerating axons (Nauta-Gygax, Fink-Heimer, De Olmos):*** Silver impregnation techniques that stain degenerating axons and pre-terminals (Nauta-Gygax) or terminals (Fink-Heimer, De Olmos).

Modern methods to study connections

Incorporation of tracers into neurons: Passive and active processes (uptake, transport, etc.). Anterograde, retrograde, and transneuronal transport of the tracers can take place depending on the tracer and methods.

Modern methods of tract tracing:

Retrograde tracers: used to study the entire input to an area injected with a tracer.

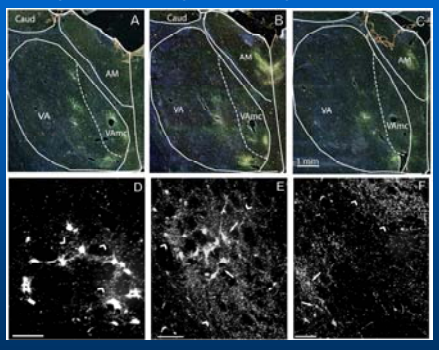
This method replaced the old retrograde degeneration method, which had significant disadvantages.

Some retrograde tracers: fluorescent dyes (e.g., fast blue, diamidino yellow); Fluoro-Gold; Cholera toxin, β subunit

•Enzymatic method: When the enzyme horseradish peroxidase (HRP) is injected at the site of termination of nerve fibers, it is taken up by the nerve terminals and transported retrogradely to the perikaryon where it is visualized by an enzyme histochernical technique as blue or brown granules in the soma and dendrites.

•HRP reacts with its substrate, hydrogen peroxide, in the presence of an electron donor (i.e. diamino-benzidine, or tetramethyl benzidine) to yield a brown or blue reaction product, seen by light microscopy; or reacted with osmium tetroxide to yield an electron-dense marker for ultrastructural studies. The HRP marker is widely used for tracing neuroanatomical pathways in the brain.

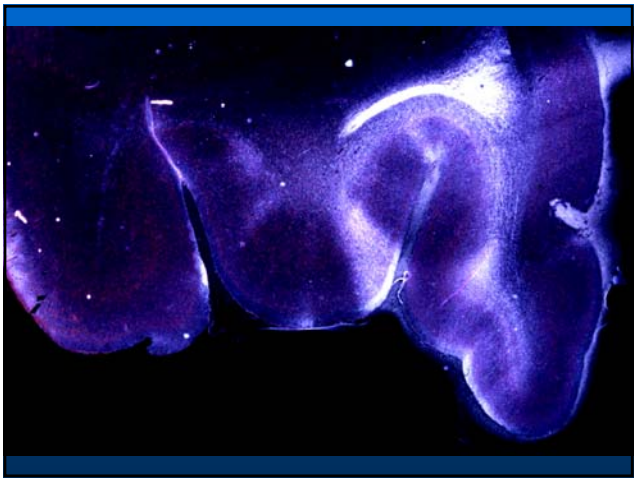
Bidirctional tracers: HRP-WGA, showing both anterograde and retrograde labeling

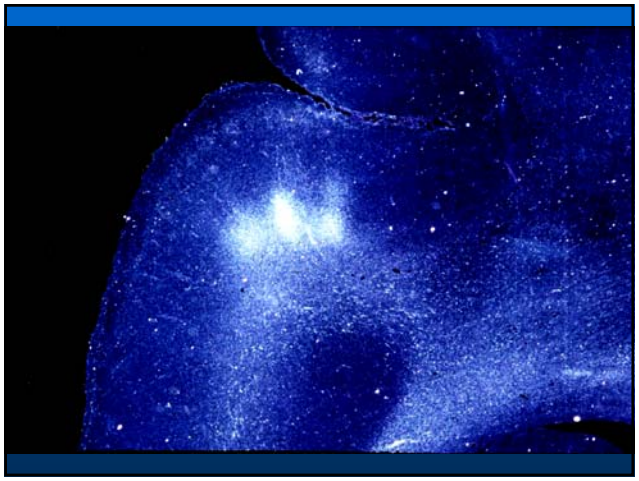


From: Xiao and Barbas, Thal. and Related Systems, 2004.

•Modern anterograde tracing (efferent connections)

- **•Autoradiography:** A method introduced in the 70s, and based on the principle that radioactive amino acids injected in the vicinity of neurons are taken up by the neurons, incorporated into macromolecules, and transported from the cell body down the axon (anterograde transport) to the axon terminal.
- •After a finite time following injection, the radioactive amino acid can be demonstrated by autoradiography. By this method, the path of a neural tract can be traced from its origin to its termination.
- •This method replaced the old ablation-degeneration methods (Nauta, Fink-Heimer, etc).
- •Some anterograde tracers: ^{3}H amino acids; BDA

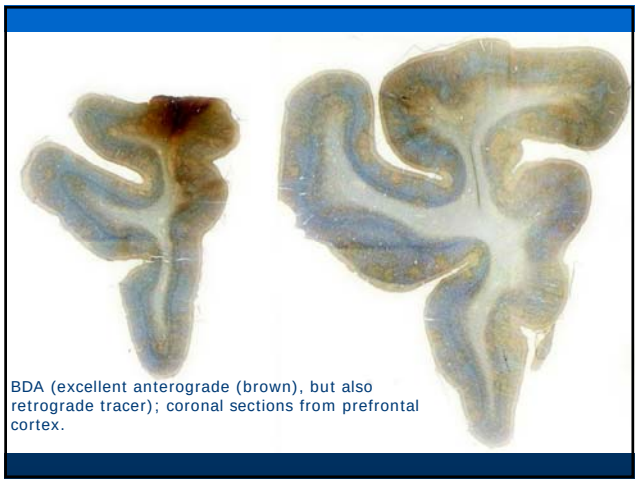




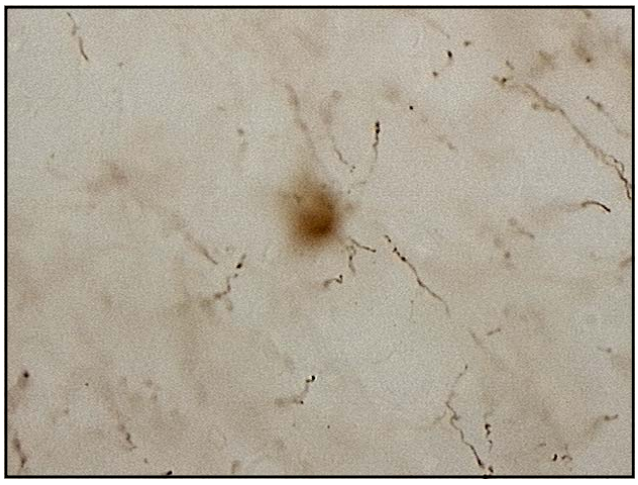
•Bidirectional tracers

•Dextran amines are bidirectional tracers: Introduced in the late 1980s, the method is highly sensitive, and widely used for anterograde and retrograde pathway tracing studies of the nervous system.

•Dextran amines can be reliably delivered into the nervous system by iontophoresis or pressure injection and visualized with fluorophores or an avidin-biotinylated HRP (ABC) procedure, followed by a standard or metal-enhanced diaminobenzidine (DAB) reaction.



BDA (excellent anterograde (brown), but also retrograde tracer); coronal sections from prefrontal cortex.



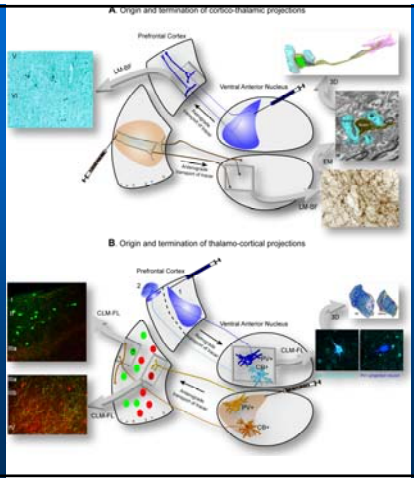
Anterograde and retrograde tracers that work in fixed tissue:

Carbocyanines (DiI, DiO, DiA) for tracing studies in fixed brain tissue

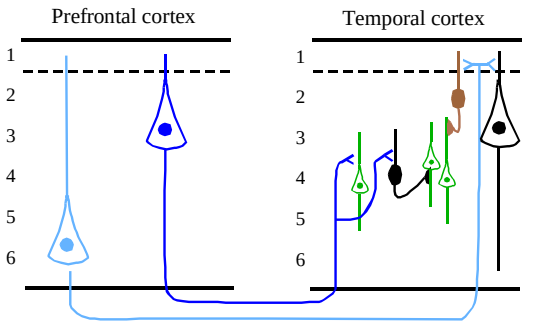
Disadvantage: very long periods are required, especially for human tissue.

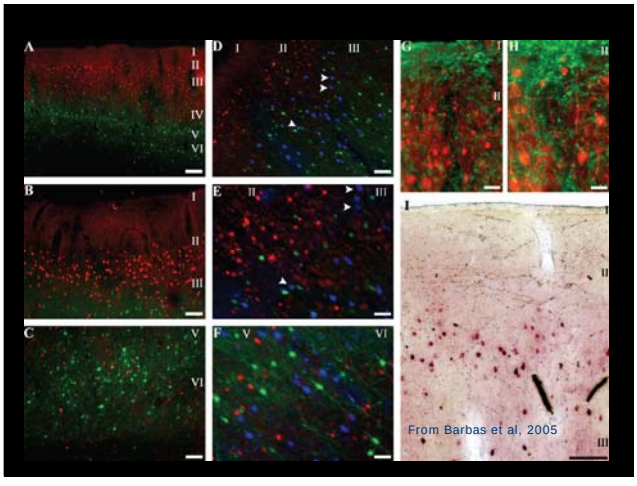
- Trans-synaptic transport
  - Anterograde tracers injected in the eye (e.g., ^{3}H amino acids, HRP-WGA; these dyes work only in this system and were used to show the ocular dominance columns in the primary visual cortex (V1) after injection in the eye.
  - Neurotropic viruses for the study of chains of lilnked neurons; transsynaptic tracing in the retrograde direction (pseudorabies virus)

Combining tract tracing and neurochemical markers can provide information on the microenvironment of the origin or termination of distinct pathways



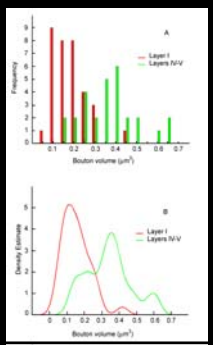
From: Zikopoulos and Barbas, 2007.



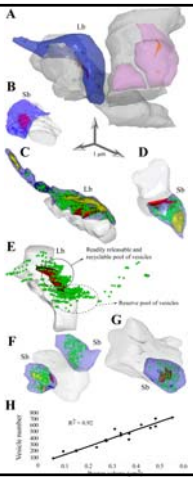


From Barbas et al, 2005

Prefrontal pathways at the synaptic level: axonal boutons terminating in the middle layers are larger than boutons terminating in layer I of superior temporal auditory association cortex



From Germuska et al, Cererbal Cortex, 2006



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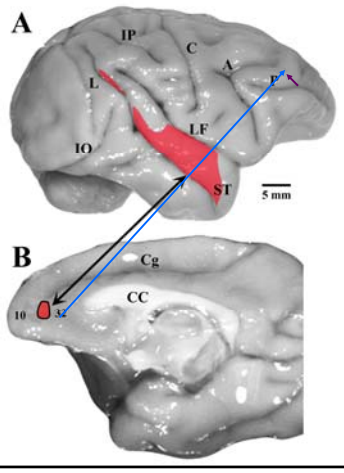
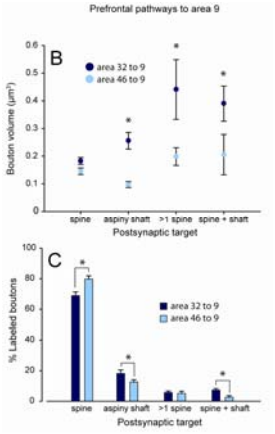
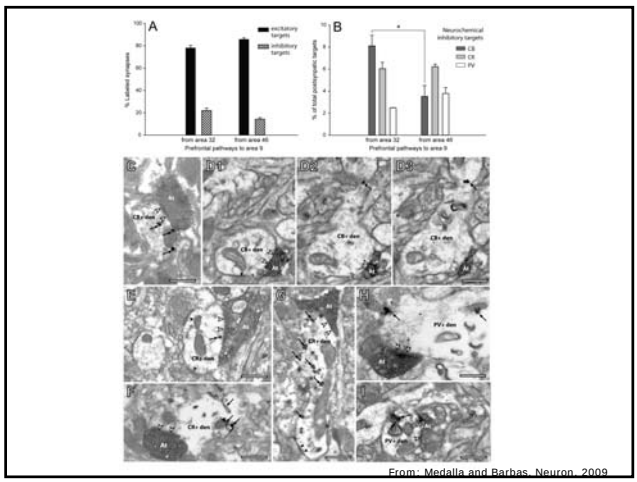




From: Medalla and Barbas, Neuron, 2009



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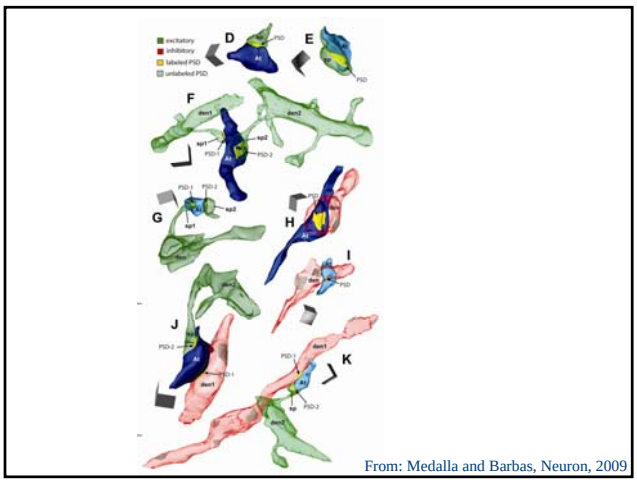

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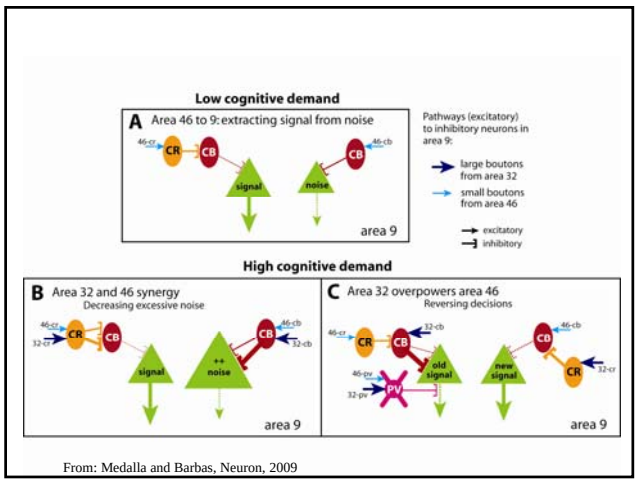


From: Medalla and Barbas, Neuron, 2009

What can pathways tell us about normal function and pathology?

Pathology in schizophrenia: Perspective from pathways

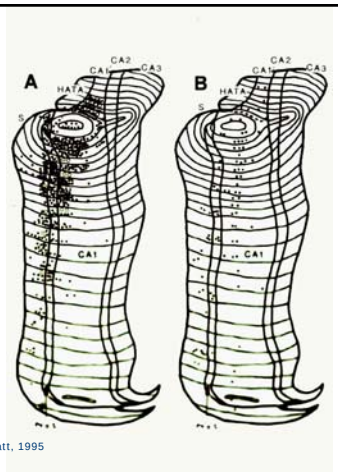
Pathology in schizophrenia

The roots of the disease are in development, affecting the delicate balance of neuronal migration, architecture and ultimately connections

Pathology in schizophrenia affecting cingulate and interconnected structures

Hippocampal formation; the rostral half is affected preferentially in schizophrenia

The rostral half of the hippocampal formation is the principal source of projections to the anterior cingulate



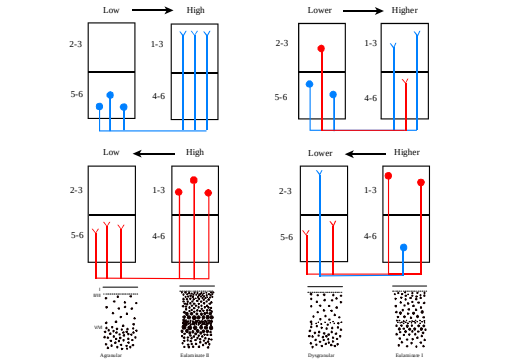
From: Barbas and Blatt, 1995

Pathology in schizophrenia

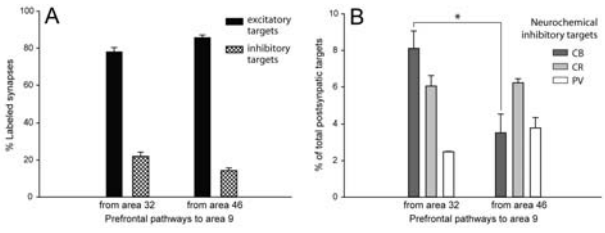
The number of pyramidal (excitatory) neurons is reduced in the deep layers of the anterior cingulate cortex (ACC) in schizophrenia (Benes et al., Biol. Psych., 50, 2001).

The deep layers of ACC project to the upper layers of dorsolateral prefrontal cortex.

Linking cortical architecture to corticocortical connections



Adapted from: Barbas and Rempel-Clower, 1997



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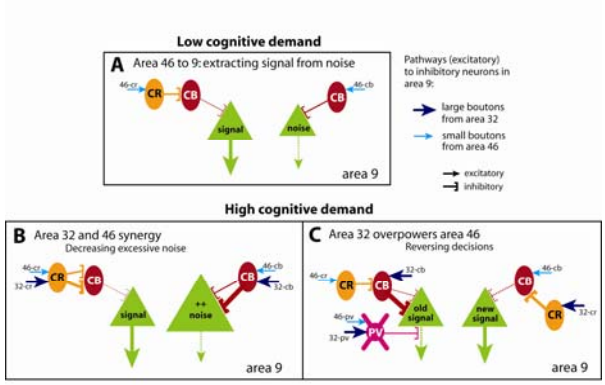

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