Cortical organization: neuroanatomical approaches

Study of neuronal circuitry and microstructure

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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

**Factors contributing to the inside-out pattern**
- **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)**
Specificity of Evolutionary Changes

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
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
Classical stains:
- Nissl stain
- Myelin

Are these methods useful today?

Applications
- Stereological analysis to estimate density or overall number of neurons

Myelogenesis: 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: modules seen by anterograde labeling

Cortical architecture: organization into: layers, columns, and modules
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)
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** 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.

### 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.
Methods to study cortical architecture
Modern: intracellular dye injections provide detailed information on dendritic and axonal morphology and geometry of neurons.

What do architectonic methods tell us about cortical organization?

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).

Methods to study connections

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 histochemical 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.

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

• 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 linked 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.

The microenvironment of the origin and termination of 'forward' and 'feedback' connections is different.


From: Barbas, 2006

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.

What can pathways tell us about normal function and pathology?
Pathology in schizophrenia: Perspective from pathways

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.
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.