## Development and evolution of connectivity

### Development of connectivity

- **Mechanisms of axon guidance**

  ![Axon guidance mechanism](image.png)

- **Development of cortical and thalamocortical connections**

  ![Cortical and thalamocortical connections](image.png)

### Principles of evolution of connectivity

- **Epigenetic population mapping**

- **Parcellation hypothesis**

- **Connectional invasion and displacement hypothesis**

- **Principles of network design**
This was state of the art in 1996 … but a lot has been done since then. I will try to incorporate some more recent information and will give more up to date references.
Mechanisms of axon guidance – some definitions

- **Growth cone**: at the tip of an extending axon is a flattened, fan-shaped structure called a growth cone, with many long spikes that radiate outward

- **Molecular guidance cues**

- **Intermediate targets**: small clusters of “guidepost cells’ or large groups of functionally specialized cells.

- **Stepwise process**

- **“Pioneer” axons**

- **Four guidance forces**: attractive and repulsive cues, which can be either short-range or long-range.
Development of connectivity

Cells secrete diffusible chemoattractant or chemorepulsion substances that attract or repel axons at a distance.

Short-range or contact-mediated mechanisms involving nondiffusible molecules.
Development of connectivity

For a more up to date reviews of these molecules, see:

- Four major families of guidance cues ("canonical" guidance-cues):
  
  (1) Netrins
  
  (2) Slits
  
  (3) Semaphorins
  
  (4) Ephrins

- Morphogens and growth factors

- Cell-adhesion molecules of the immunoglobulin and cadherin super families
Some general principles

(1) Axons are guided through the combined operation of four guidance mechanisms (short- and long-range attraction, and short- and long-range repulsion).

(2) Multifunctional nature of guidance cues.

(3) These mechanisms appear to operate in all types of decisions: linear growth, sharp turns, axons fasciculation and defasciculation, and target invasion and selection.

(4) The four guidance mechanisms are mechanistically related and phylogenetically conserved. There are parallels between pathfinding events in nematodes, insects, and vertebrates.

(5) There is redundancy of guidance cues.

(6) A single growth cone may respond to the same cue in different ways at different points along its “journey”.
Example 1: Long-range guidance to and from the midline & local guidance at midline

- Axons that link the two sides of the nervous system project toward and across the midline, forming axon commissures.

- Long-range chemoattractants emanating from the midline – the netrins.

Dixon (2002)

For a more recent review, see Dixon & Zou (2011)
Development of connections

Dixon & Zou (2011)
Example 2: Regulation of axon fasciculation
- Growth cones often extend along the surface of other axons in axon fascicles to initiate the next leg of their trajectory.
Example 3: Target selection

- **Invading the target region** is regulated by both pathway- and target- derived cues:
  - (1) ‘Upward” gradient mechanisms: target invasion can be regulated by members of the nerve growth factor family of neurotrophins.
  - (2) “Downward” gradient mechanisms: axons “skirt” the target.

- **Generating topographic projections**: Topographically organized patterns of neuronal connections, in which neighboring neurons project to neighboring sites in the target, occur throughout the nervous system. – the connections from retina to optic tectum/superior colliculus are the leading model (next slides)

- **Selecting discrete targets**: After reaching their topographically appropriate sites retinal axons turn to seek their appropriate laminar termination site within the tectum, presumably in response to laminar-specific guidance cues.
Development of connectivity

Sperry’s (1963) chemoaffinity hypothesis
- The molecular tags on projecting axons and their target cells are distributed in complementary gradients that mark corresponding points in both the projecting and target neural populations.
  - Each point in optic tectum has a unique molecular address determined by the graded distribution of the topographic guidance molecules along its two axes.
  - Each retinal ganglion cell has a unique profile of receptors for those molecules that would result in a position-dependent, differential response to them by axons.
Development of connectivity

Topographic projections

Feldheim & O’Leary (2011)
See also McLaughlin & O’Leary (2005)
# Development and evolution of connectivity

## Development of connectivity

- **Mechanisms of axon guidance**
  - ![Axon guidance mechanism](image)
- **Development of cortical and thalamocortical connections**
  - ![Cortical and thalamocortical connections](image)

## Principles of evolution of connectivity

- **Epigenetic population mapping**
- **Parcellation hypothesis**
- **Connectional invasion and displacement hypothesis**
- **Principles of network design**
THALAMOCORTICAL DEVELOPMENT: HOW ARE WE GOING TO GET THERE?

Guillermina López-Bendito and Zoltán Molnár

The arealization of the mammalian cortex is believed to be controlled by a combination of intrinsic factors that are expressed in the cortex, and external signals, some of which are mediated through thalamic input. Recent studies on transgenic mice have identified families of molecules that are involved in thalamic axon growth, pathfinding and cortical target selection, and we are beginning to understand how thalamic projections impose cytoarchitectonic differentiation on the developing cortex. By unravelling these mechanisms further, we should be able to increase our understanding of the principles of cortical organization.
1. Most neocortical neurons, including all projection neurons are generated within ventricular zone (VZ) and subventricular zone (SVZ — between VZ and IZ — intermediate zone) in the lateral ventricle.

2. The first postmitotic neurons accumulate on the top of VZ, forming the preplate (PP), positioned just beneath the pial surface.

3. Neurons subsequently generated in the VZ migrate along radial glia, aggregate within the PP, and form the cortical plate (CP), which splits the PP into a superficial marginal zone (MZ) and a deep subplate (SP).

4. The CP gradually differentiates in a deep to superficial pattern, forming layers 6 though 2 of the adult neocortex.

5. The MZ contains Cajal-Retzius neurons that express reelin, a large secreted protein required for radial migration of CP neurons and their formation of layers.

6. The SP contains local and long-distance projection neurons, proposed to serve a number of critical roles in cortical development, among them the pioneering of the internal capsule and the formation of major input and output projections paths between the cortex and the rest of the central nervous system.
Rakic et al, 2009
Monkey fetal cerebral wall

CC: Cortico-cortical connection

TR: thalamic radiation

http://rakiclab.med.yale.edu/research/index.aspx
- Thalamus and cortex develop synchronously.
- Most thalamic neurons in the rat are born between embryonic day (E) 13 and E19.
- Second and third week of gestation: neocortex and dorsal thalamus start to link each other through reciprocal connections.

Emerging boundary zones with distinct molecular properties:
- DTB – diencephalic-telencephalic boundary
- PSPB – pallial-subpallial boundary
Factors that affect the growth of thalamocortical axons:

1. Guidance molecules in the thalamocortical pathway — see slide
2. Forebrain patterning and transcription factors — see slide
3. Interactions with other cells and fibers
4. Interactions between thalamic and cortical axons — "handshake hypothesis"
Development of connections

(1) Guidance molecules in the thalamocortical pathway

- Netrin 1
  - Chemoattractant signal for thalamic axons
  - Chemoattractant signal for corticofugal axons
  - Chemorepellent signal for thalamic axons
  - Chemorepellent signal for corticofugal axons
- Thalamocortical axons
- Corticofugal axons

- LAMP
  - Limbic thalamic axons
  - Chemoattractant LAMP signal for limbic thalamic axons
  - Chemorepellent LAMP signal for non-limbic thalamic axons

- Ephrin-A5 expression
  + Chemoattractant Ephrin-A5 signal for thalamic axons

- Ephrin-A4 expression

- Ephrin-B3 expression
  - Chemorepellent Ephrin-B3 signal for lateral thalamic axons

- LGN thalamic axons
(2) Forebrain patterning and thalamocortical development

- Guidance molecules that modulate pathfinding of thalamocortical axons are in turn controlled by regulatory genes and transcription factors in the forebrain.

- Mutants lacking transcription factors that are expressed along the axons’ route of navigation cause abnormalities of thalamocortical development.
Development of connections

First communication with cortex

Embryonic period

Thalamocortical Axons (red lines)

Early postnatal period

E11
PP
SVZ
VZ

E15
MZ
DCP
SP
IZ
SVZ
VZ

E18
MZ
CP
V
VI
SP
IZ
SVZ
VZ

P0
MZ
CP
V
VI
SP
IZ
SVZ
VZ
WM

P2
MZ
CP
V
VI
SP
IZ
SVZ
VZ
WM

P8
MZ
CP
V
VI
SP
IZ
SVZ
VZ
WM

E11
PP
SVZ
VZ

E15
MZ
DCP
SP
IZ
SVZ
VZ

E18
MZ
CP
V
VI
SP
IZ
SVZ
VZ

P0
MZ
CP
V
VI
SP
IZ
SVZ
VZ
WM

P2
MZ
CP
V
VI
SP
IZ
SVZ
VZ
WM

P8
MZ
CP
V
VI
SP
IZ
SVZ
VZ
WM
Development of connections

Do thalamocortical axons contribute to cortical patterning?

- The initial expression of region-specific and lamina-specific markers is probably independent of thalamic innervation.

- But the differentiation of many of the anatomical features that distinguish different cortical areas depends to a large extent on the input of thalamocortical axons.

- Cortical regionalization is initially created by the graded expression of various genes, and thalamic input controls the later stages of area subdivision through activity-dependent or independent mechanisms.

- But there is also some evidence that thalamic afferents release a diffusable factor that promotes proliferation of neurons and glia by cortical VZ – Dehay et al. (2001)
Development of connections

Do thalamocortical axons contribute to cortical patterning?

Somatosensory cortex of rodents - BARREL cortex

a  P0
Birth

b  Nissl
Cells in layer IV shown through Nissl staining.

NG

Dendrites in layer IV shown through cytochrome oxidase (CO) staining.

c  P8
Second postnatal week

D
Nissl

CO
Development of connections

Does neural activity play a part in thalamocortical development?

a. Outgrowth
   Spontaneous exocytosis and NT release?
   - Membrane addition
   - Pathway selection and guidance

b. First contact
   Spontaneous NT release
   — Absent in Munc13 and Munc18 KO
   - Differentiation of pre- and postsynaptic structures

(c. Communication
   Evoked, synchronous NT release
   — Absent in Snap25 KO
   - Synaptic stability and elimination (pruning)
   - NT selection/reassignment

Trophic factors?

Activity dependent
Development of connections

More readings:

**Decision by division: making cortical maps**

Pasko Rakic, Albert E. Ayoub, Joshua J. Breunig and Martin H. Dominguez

**THE SUBPLATE, A TRANSIENT NEOCORTICAL STRUCTURE: Its Role in the Development of Connections between Thalamus and Cortex**

Karen L. Allendoerfer

**Slit2 Activity in the Migration of Guidepost Neurons Shapes Thalamic Projections during Development and Evolution**

Franck Bielle, Paula Marcos-Mondejar, Maryama Keita, Caroline Mailhes, Catherine Verney, Kim Nguyen Ba-Charvet, Marc Tessier-Lavigne, Guillermna Lopez-Bendito, and Sonia Garel
Development and evolution of connectivity

Development of connectivity

- Mechanisms of axon guidance
- Development of cortical and thalamocortical connections

Principles of evolution of connectivity

- Epigenetic population mapping
- Parcellation hypothesis
- Connectional invasion and displacement hypothesis
- Principles of network design
Epigenetic population mapping – Katz and Lasek 1978

- Most brain regions contain 20-80% more neurons at the end of neurogenesis than in adulthood. The excess is eliminated by naturally occurring cell death.

- Motor neuron loss is regulated, at least in part, by the amount of target issue that is available for innervation.

- Developing motor neurons compete with one another for some “trophic factor” that is produced by muscle fibers, taken up by the axon, and required for neuron’s survival. As a result for this competition for trophic support, the number of projection neurons is effectively matched to the number of available target cells.
Parcellation hypothesis – Ebbesson 1980

- “Nervous system becomes more complex by a process of parcellation that involves the selective loss of connections of the newly formed daughter aggregates and subsystems”.

![Diagram showing the parcellation process from ancestor to intermediate to descendant stages.](image-url)
Evolution of connectivity

Striedter 2005

Connectional invasion

- Evolution of projections to unusual targets, i.e., phylogenetic appearance of projections to targets that did not ancestrally receive homologous inputs

- Connectional invasion has occurred repeatedly as brains evolved.

- Deacon (1990) – displacement hypothesis: connectional invasion generally occurs when a brain region becomes disproportionately large in evolution.
Evolution of connectivity

Developmental exuberance

- **Exuberant development of connections**: overproduction of axons, axonal branches and synapses, followed by selection

- Macroscopic exuberance: formation of transient projections between macroscopic brain parts.

- Microscopic exuberance: formation of transient structures that are involved in communication between neurons within a restricted cortical territory.
Evolution of connectivity

![Diagram showing the evolution of connectivity in the brain with different regions and labeling such as Dorsal, Ventral, Neocortex, LGE, MGE, VT, Th, PT, and HT. Additionally, the diagram highlights transient projections, thalamocortical axons, and transient subplate cells.](image-url)
Evolution of connectivity
Functions of exuberant connections

- Construction of cortical circuitry – case of thalamocortical development

- Provide a high degree of flexibility in the formation of cortical circuits — important for evolution?
Evolution of connectivity
Evolution of connectivity

1. Subcortical branching
2. Cortical ingrowth
3. Intracortical branching
4. Synaptogenesis
5. Synaptic reduction

Legend:
- Growth cones
- Synapses
- Stable projections
- Transient projections
Evolution of connectivity

Pathway selection → Near-target selection → Target selection
Evolution of connectivity

Factors that affect axonal selection

(1) Input from the periphery
(2) Axon-axonal competition
(3) Thyroid hormones, alcohol syndrome
(4) Markers of targets for persistent innervation
Evolution of connectivity

Striedter 2005

General principles of network design

- The number of connections in a fully interconnected network increase exponentially with neuron number, which means that such networks quickly become axon dominated as they increase in size – Deacon 1990, Ringo 1991.

- Real brains do not scale like that. The average neuron in real brains projects to roughly the same number of other neurons, no matter how large the brain – this helps to keep down the metabolic and physical costs of axonal “wiring”. Therefore, real brains become sparsely interconnected as they increase in size.

- Minimum-wire principle: connections lengths are minimized.
General principles of network design

- Problem: a network’s average “degrees of separation” increases as connection lengths are minimized.

- Answer: not all connections are minimized— “small-world architecture”.

- Visual cortex as small world architecture: Young (1992), Sporns et al. (2000) – did anyone go to the presentation?
```
The Geometric Structure of the Brain Fiber Pathways
Van J. Wedeen et al.
Science 335, 1628 (2012);
DOI: 10.1126/science.1215280
```