A sketch of the central nervous system and its origins

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Part 5: Differentiation of the brain vesicles

MIT 9.14 Classes 13-14

Developmental differentiation: growth of axons, and some phenomena of CNS plasticity
Major stages of nervous system development

- **Proliferation** of cells
- **Migration** from birth places to destinations
- **Differentiation** of neurons and cell groups
  - **Growth of extensions** (axons, dendrites, spines)
  - Sculpting by branch loss and cell death
  - Maturation
- **Plasticity**
Nerve fiber development (Cajal)

How did he observe such developmental dynamics?

*Only in fixed tissues, using Golgi stain*
Membrane incorporation in the growing axon

- What do Purves & Lichtman say about this? Where is new membrane added, and how does it occur? *(Chapter 4b, p 98-99)*

*What was the experiment that showed where membrane was being added?*
Developmental dynamics:
More questions from Purves & Lichtman (chapter 4b)

What technical advances in neuroembryology can be attributed to Ross G. Harrison (p. 96)?

- tissue culture

How did Speidel's method (p. 105) differ from Harrison's?

He used living tadpoles.

How?
Growth of dendrites and axons

• The growth cone
  – Motile filopodia (plural of filopodium) containing actin filaments

• Selective adhesion by filopodial tips;
  – CAMs (cell adhesion molecules, like N-CAM)
  – Contraction of filopodia due to contractile proteins (mostly actin).
Axon growth cone, scanning e.m. picture

Figure removed due to copyright restrictions.
From Wessells and Nuttall, 1978 (reproduced in Zigmond et al., 1999).

From Wessells and Nuttall, 1978 (reproduced in Zigmond et al., 1999)
Growth of axons in tissue culture: NGF Assay

NEXT: Living growth cones in tissue culture; growth factors
Large growth cone in tissue culture, video clip

Figure removed due to copyright restrictions.
Growing axons in culture: chick retina and DRG (video clip)

Figure removed due to copyright restrictions.
Three growth cones \textit{in vitro} (video clip)

Figure removed due to copyright restrictions.
Axonal growth cone of sympathetic ganglion neuron and fibroblast *in vitro* (video clip)

Figure removed due to copyright restrictions.
Using tissue culture:

**NGF Assay**
First developed by Rita Levi-Montalcini

“Neurotrophins”: a family of growth factors
Growth of dendrites and axons

• The growth cone
  – Motile filopodia (*plural of filopodium*)

• Selective adhesion by filopodial tips
  – CAMs (cell adhesion molecules, like N-CAM)

• Contraction of filopodia due to contractile proteins (mostly actin)

**Schematic Illustration:**
Growth cone (a model)

Central Domain
Peripheral Domain

F-actin
Filopodia
Lamellipodia

Figure by MIT OpenCourseWare.

Singular terms (Latin):
Filopodium,
lamellipodium

Zigmond et al., ‘99
An experiment on the growth of sensory axons in the developing grasshopper leg

- How can the axons be observed?
- How does an axon find its way to its target ganglion?

*From Purves & Lichtman (’85); Zigmond et al., ’99.*
Evidence for “Guidepost cells”

From Zigmond et al (’99), based on Bentley & Caudy (’83)

Figure by MIT OpenCourseWare.
Grasshopper leg vs. mammalian optic tract

• Grasshopper leg
  – *Pioneer axon*: The role of "guidepost cells" and long filopodia of growth cones of the primary sensory neurons in the epithelium.
  – Later growing axons *follow* the first one.

• Mammalian optic tract
  – What happens in the grasshopper leg is not what happens in the mammalian optic tract
  – Later-growing axons do not grow along the surfaces of the earlier ones, but rather space themselves between them.
An experiment on specificity of axon growth

What was the major result in Hibbard's experiment on transplanted amphibian Mauthner cells, and what does it mean?

(Purves & Lichtman, pp.118-119)
Hibbard's experiment on transplanted amphibian Mauthner cells:

He used grafted medullary segments, placed just rostral to the normal position in either normal or reversed orientation (published in 1965).

Results indicate that cues in the tissue guide axons growing through it.

From *Purves & Lichtman* p 118
Guidance Mechanisms for axon outgrowth: Four mechanisms as seen in 1985

- Stereotropism
- Galvanotropism
- Tropism based on differential adhesion
- Chemotropism
  - Membrane contact
  - Diffusible signals

(Purves & Lichtman pp 119-129)
More recent studies have supplemented this picture considerably

They have distinguished four types of chemical guidance, adding new detail to the above. What are they?

(Summarized in Zigmond et al. ’99)
Semaphorins (Secreted)
Netrins

Eph Ligands
Semaphorins
(Transmembrane)
ECM (for example, tenascins)

Long-Range Cues

Chemorepulsion
Chemoattraction

Short-Range Cues

Contact Repulsion
Contact Attraction

Ig CAMs
Cadherins
ECM (for example, Laminins)

Growth Cone

Figure by MIT OpenCourseWare.
Thus, chemical specificity depends on two different kinds of effects:

1. Attraction effects
2. Repulsion effects, or inhibition of growth

Each of these can involve effects of
a) diffusing chemicals, or
b) effects of contact
More detail:

Chemical specificity 1: attraction effects

• Cell-cell adhesion (CAMs; ECM molecules like the laminins and cadherins)

• Growth factors:
  – Contact (Semaphorins)
  – Diffusible (Netrins; NGF and other neurotrophins; other families of GFs)
Chemical specificity 2: barriers; inhibition of growth

• Midline barriers
  – by contact repulsion, e.g., by certain proteoglycans secreted by midline radial glia

• Oligodendrocyte factors
  – A membrane protein, *Nogo*, which inhibits axon growth

• Secreted and transmembrane proteins (Semaphorins; neurotrophins and other GFs; ephrins)
More about diffusible growth factors: Contrasting “trophic” and “tropic” effects of NGF

**Tropic**: Influencing the direction of growth

**Trophic 1**: Survival promotion

**Trophic 2**: Growth promoting. Greater growth vigor increases the ability of axons to compete with other axons.
Guidance mechanisms are not fixed:

- Modulation by intrinsic metabolic factors (discoveries by Mu-ming Poo’s group)

“Conversion of neuronal growth cone responses from repulsion to attraction by cyclic nucleotides”

Sema III* and cGMP  (M. Poo ‘98)

Figure removed due to copyright restrictions.
A study of Xenopus spinal neuron axon growth in tissue culture:

Growth cone turning in a gradient of Sema III (Song et al. 1998):
Effects of manipulating cGMP (A), cAMP (B), …

{8-bromo-cGMP: a membrane permeable agonist of cGMP signalling pathways.}
An apparent role of Semaphorin III (collapsin) in the innervation of the spinal cord by dorsal root axons:
A molecular sieve? Semaphorin III produced in the ventral half of the embryonic spinal cord (dark tan) may repel axons of temperature- and pain-sensory neurons while allowing in those of Ia afferent neurons that respond to muscle stretch.

Similarly, we can describe a role of the netrin molecules in the formation of the spinothalamic tract decussations

• Netrins diffuse from floor plate region
  – Discovery of the netrin molecules by Tom Jessell and co-workers at Columbia

• They have tropic effects on axons of dorsal horn cells which form the spinothalamic tract.

• *If they attract the axons growing from the dorsal horn, how can this result in a decussation?* Why don’t they stop there? The attraction must change to repulsion, as in the experiments of Par.
Developing axons do much more than simply find their path to a target

- We have been considering the elongation mode of axonal growth.
- In this mode, they grow much faster, and branch much less, than during the subsequent arborization mode of growth.
- The following picture is taken from a study of optic-tract axon growth.
Two modes of axon growth

Figure by MIT OpenCourseWare.

5 stages of development
Topics in the study of optic-tract development & plasticity: these apply also to other axonal systems

- Embryonic formation; 2 modes of growth
  - *Optic tract*: geniculostriate pathway; other connections

- **Map formation; chemoaffinity**

- Map plasticity: lesions

- Collateral sprouting; competitive interactions in axonal growth

- Roles of cell death

- Regeneration in development and adulthood
Map formation; chemoaffinity

Ephrins and Eph receptors are responsible for the naso-temporal retinal axis representation in the tectum (superior colliculus). How does it work?

Discovery of specific mechanisms at the cell-molecular level came many years after Roger Sperry formulated his chemoafffinity theory, based on studies of regeneration in fish and amphibians (by himself and a few others)
Distribution of Eph receptors and ephrin ligands in the developing chick retinotectal system related to retinotopic projections

From O’Leary, Yates & McLaughlin, 1999

After discoveries by Flanagan et al (at Harvard), and by Bonhoeffer et al (in Germany)
The mechanism: selective repulsion.
Response of temporal and nasal RGC axons to a gradient of tectal membranes, from purely anterior to purely posterior

Figures removed due to copyright restrictions.
Such chemical specificity does not prevent plasticity of the developing maps

- Map compression
- Map expansion

What factors other than chemospecific factors are active?
- Evidence for other factors has been obtained from studies of effects of damage during development.
“Collateral sprouting“ in development

• Can cause developing axons to violate the normal rules of regional specificity:
  – e.g., in developing hamster or ferret, the optic tract can be induced to grow into the medial geniculate body of thalamus (normally part of auditory system) or the ventrobasal nucleus (normally in receipt of somatosensory system axons from spinal cord).
Effects of early ablation of SC:
Lesion in newborn hamsters; studies of adults using axonal tracing with Nauta silver stains for degenerating axons

Note the sprouting in LP and LGv as well as in the remaining SC.

Figure by MIT OpenCourseWare.
Sprouting phenomena: axonal competition and spreading

Figure by MIT OpenCourseWare.
Competition among axons: What is it?

• Competition for terminal space
  – for growth factors
  – for occupancy of synaptic sites

• Axon-axon contact interactions
  – Retraction reactions; "collapsin" molecules causing a contact inhibition of extension
Modulation of "competitive growth vigor"

The more growth vigor an axon has, the more it grows and the better it competes for terminal space.

• **By chemical factors**: more growth with more growth factor
  – E.g., NGF (see figure).
  – There are also molecular factors intrinsic to the cells which determine growth capacity, in either elongation or arborization.

• **By activity**: 
  – More growth by more active axons, as in formation of ocular dominance columns in visual cortex

• **By "pruning"**: 
  – Sprouting in one region due to blockage of or damage to an axon in another region (see figure)
NGF: effects on growth vigor in DRG axons

Figure removed due to copyright restrictions.
Intrinsic, competitive vigor of axon growth

Changes during development:
Intrinsic, competitive vigor of axon growth

After Development: Sprouting Response to a Pruning Lesion

Growth Potency:
- Low
- High
- Low

Pruning effect: Effects of pruning lesion on growth vigor. Such phenomena provide evidence for “Conservation of Terminal arbor size”, discovered in studies of developing optic and olfactory tracts.
Thus, we have two types of factors that could play roles in both development and evolutionary change:

1) Extrinsic factors in axon-axon competition
2) Intrinsic factors in “conservation of terminal quantity”
Structural changes, or “plasticity”, occur naturally during major stages of nervous system development:

- Proliferation of cells
- Migration from birth places to destinations
- Differentiation of neurons and cell groups
  - Growth of extensions (axons, dendrites, spines)
  - Sculpting by branch loss and **cell death**
  - Maturation
- Plasticity after damage or due to learning
Phenomena of neuronal death & survival; roles of neurotrophic factors and intrinsic factors

• Many neurons depend on axon target contact for survival. The target tissue gives them trophic factors.

• Without sufficient trophic factor (growth factor), they undergo apoptosis (cell suicide, or “programmed cell death”) unless protected by intrinsic factors.
Evidence of the role of cell death during development:

Example:
CNS effects of limb-bud extirpation vs. grafting of a supernumerary limb in the embryo

- Greater than normal motor neuron death after limb-bud extirpation
- Less than normal motor neuron death after grafting of supernumerary limb in the embryo

*Purves & Lichtman, ch. 6 pp 144f*
Experimental grafting of supernumerary limbs in the embryo

(Purves & Lichtman)

Figure removed due to copyright restrictions.

Please see:

Two major possible purposes in naturally occurring neuronal death

- Population size matching
- Error correction

Purves & Lichtman, ch. 6 pp 144-149
Neurotrophins are important trophic factors that prevent cell death. There are additional roles for neurotrophins

- Activity-induced plasticity
  - E.g., in visual cortex
- Learning
  - BDNF: associated with phosphorylation of specific subunits of the NMDA receptor.
- New neurons in adult brain (BDNF)
Axon regeneration studies: 

*a very brief introduction*

- Developmental changes in re-growth capacity
- Need to **Preserve** the damaged cells. (Dying cells don’t re-grow axons.)
- The mature tissue environment contains many inhibitory factors that may be overcome by the right procedures to **permit** axon growth.
- **Promotion** of growth vigor may be needed after the early period of development.
- **Plasticity** of the regenerated connections can play an important role in functional recovery.

These are the 4 P’s of regeneration, from Ph.D. thesis by Rutledge Ellis-Behnke at MIT.
Recent and current research:

- **PN bridges.** Alternatives: Schwann cells; olfactory ensheathing cells; stem cells.
- **New materials** used to promote healing and tissue bridge formation (work with bioengineers using nanotechnology)
- Chemical methods for inhibiting scar formation or for breaking it up
- Genetic transfections

Examples of experimental bridges used to obtain axon regeneration:
Optic tract entering midbrain is the "Brachium of the Superior Colliculus"
Brachium Bridge

parasagittal section of midbrain surface
Brachium Bridges

midbrain

caudal

rostral

top view

optic tract

Thalamus

MGB

LGB

LP

PT

IC

SC
Brachium Bridge Video Clip

Hamster HBSC-99-5

Surgery at age 5 ½ months:
  - Transection of right BSC,
  - Bridges over lesion by PN homografts.

Behavioral testing at age 6 ½ months:
  - Orienting to stimuli in left visual field (vs. right)
Brachium Bridge
Anatomy

Figures removed due to copyright restrictions.
Brachium
Bridge
Anatomy

5.5
months
old

Figures removed due to copyright restrictions.
Experiments with injection of self-assembling peptides in lesions of the optic tract

Location of surgery and SAP injection

IC
Knife cut in SC
1 month post lesion:
2 Controls  2 SAP alone

Figures removed due to copyright restrictions.
Normal Animal

Retina to Contralateral SC
Hamster Brain with the cortex removed

1 mm Grid

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Brachium Transection
• Regenerated axons in the middle of the lesion site

Figures removed due to copyright restrictions.
Re-innervation of the SC by axons from retina

Figures removed due to copyright restrictions.
Blind Control

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SAP Brachium Bridge,
Functional return of vision

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