SYAPTIC PLASTICITY AND AXONAL GUIDANCE

1. The Neuron: basic Mechanisms of Action
2. Axon Guidance and Nerve Growth: Basic Principles
3. Short Range Guidance: Eph-Ephrins / Semaphorins
4. Long Range Cues: Semaphorins / Netrins / Nogo / Other
5. Gene Expression Switch and Regulation of Pathways
6. Learning and Memory - Guidance and Neuronal Adaptation in the Adult

AXON GUIDANCE AND NERVE GROWTH

During development, axon will grow until they reach their target area. They will be guided by four different guidance forces:

a) long-range, soluble trophic factors, eg NGF (nerve growth factor), neurotrophins (BDNF, etc) which can be either repulsive (eg soluble semaphorins) or attractive (neurotrophic factors)

b) local, membrane bound cues (receptors, Eph, Ephrins, Semaphorins, etc), expressed ectopically by « guiding cells » all along the pathway; these cues again can be either attractive or repulsive. At the target area, repulsive guidance cues will induce the growth cone collapse and synapse formation.
1. Pathway selection
2. Target selection
3. Address selection

Overall Process

<table>
<thead>
<tr>
<th>Birth of neurons</th>
<th>Outgrowth of axons and dendrites</th>
<th>Synaptic connections made</th>
<th>Refinement of synaptic connections</th>
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<td><img src="image1.png" alt="Birth of neurons" /></td>
<td><img src="image2.png" alt="Outgrowth of axons and dendrites" /></td>
<td><img src="image3.png" alt="Synaptic connections made" /></td>
<td><img src="image4.png" alt="Refinement of synaptic connections" /></td>
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Axon guidance mechanisms

- Axonal growth is led by growth cones
  - Filopodia are able to sense the environment ahead for chemical markers and cues.
  - Mechanisms are fairly old in evolutionary terms.
- Intermediate chemical markers
  - Guideposts studied in invertebrates
- Short and long range cues
  - Short range chemoattraction and chemorepulsion
  - Long range chemoattraction and chemorepulsion
- Gradient effects

AXON GROWTH and CYTOSKELETON

The cytoskeleton of the growth cone continuously changes during outgrowth and navigation.

Fig 147
(A and B) A model showing one way in which a growth cone might turn toward an attractant (green).
Fig. 154. Stepwise guidance of axons in the grasshopper limb. (A) Characteristic trajectory of the axons. It is broken into 7 segments (a-g in small drawing). (B, C) segment f ends at the Cx1 cell, which is required for guidance and axons fail to progress forward when Cx1 is ablated.

Fig. 155. Axons are guided by the simultaneous and coordinate actions of four types of guidance mechanisms: - contact attraction, - contact repulsion and - chemorepulsion.

Individual growth cones might be:
- "pushed" from behind by a chemorepellent,
- "pulled" from in front by a chemorepellant,
- and "hemmed in" by attractive and repulsive local cues (cell surface or extracellular matrix molecules).

Push, pull and hem: these forces act together to ensure accurate guidance.
GROWTH CONES OF PERIPHERAL NEURONS rely on guidepost cells to navigate through the limb of the grasshopper. In normal embryos, the axon of the Ti1 neuron encounters a series of guidepost cells on its route to the central nervous system: F1, F2, and two CT1 cells. If the CT1 cells are killed early in development, the Ti1 neuron forms several axonal branches at the site of cell F2, with growth cones extending in abnormal directions. (After Bentley and Caudy, 1983.)

Axon guidance cues can be either attractive or repulsive

Fig.167

Fig.168
Four families of axon guidance molecules and their receptors

- Netrins (DCC, Unc5)
- Slits (Robo)
- Semaphorins (plexin, neuropilin)
- Ephrins/Eph (Eph/ephrin)
Growth cone behavior depends on resting $[\text{Ca}^{++}]_i$

Fig. 150. Members of Ig superfamily. These molecules are characterized by different Ig domains and fibronectin type III domains in their extracellular portions.

Fig. 151. The Ig superfamily member Fasciclin II mediates fasciculation of subsets of axons in *Drosophila*. FasciclinII is a homophilic adhesion molecule. Axon of neurons MP1, dMP2, vMP2 and pCC express FasciclinII and course together and fasciculate a portion of their trajectory in wt flies (A) but not in ko flies.

Fig. 149. Examples of extracellular matrix molecules that modulate axon growth. Laminin-1 and Fibronectin can stimulate axonal outgrowth. Tenascin-C is *bi-functional* affecting different classes of axons in opposite ways. Motifs include EGF-like domains and fibronectin Type III domains.

Fig. 152. The Ig Superfamily members of Axonin-1 and Nr-CAM are required for the crossing the floor plate by commissural axons. (A) normal trajectory: axons grow from their cell bodies of origin in the spinal chord to the floor plate (fp) at the ventral midline; they cross the midline, then turn longitudinally to grow alongside the floor plate. Commisural axons express Axonin-1 on their surface, whereas floor plate axons express Nr-CAM. (B) Reagents that disrupt Axonin-1-NrCAM interaction (antibodies) impair commisural crossing.
Fig. 153. Switching sensitivity at the midline. As they cross the floor plate, vertebrate commissural axons lose sensitivity to the midline attractant, netrin, and acquire sensitivity to Slit and semaphorin repellents. This switch may be mediated in part by silencing of netrin attraction by Slit.

Drosophila commissural axons also become sensitive to Slit only after crossing. This appears to reflect Comm’s role in regulating the intracellular trafficking of Robo.

Crossing the midline: Molecules and mechanisms

Crossing the midline:
A smooth journey controlled by dynamic receptor interactions

Local protein synthesis is required for axon guidance beyond the midline
Midline Crossing: the Action of Chemoattractive and Chemorepulsive cues

Three questions for midline crossing

- Why ipsilateral axons do not cross?
- Why contralateral axons cross?
- Why contralateral axons never go back after initially crossing the midline?

Robo Is the Answer to Question 1

-Slit expressed by midline cells is a ligand for Robo

- Ipsilateral axons express high level of Roundabout (Robo), a cell surface receptor for Slit.
- Contralateral axons express low level of Robo prior to crossing and up-regulate the level of Robo after crossing the midline.
- Why do contralateral axons migrate towards the midline?

Reason 1: Down-regulation of Robo-mediated repulsive response

Reason 2: attractive forces induced by attractive cues produced by midline cells

- Down-regulation of Robo-mediated repulsive response in Drosophila

Commissureless (Comm) downregulates the level of Robo in contralateral axons prior to crossing in Drosophila.

- How can contralateral axons leave the midline?

- Netrin, an attractive cue from midline cells

- Contralateral axons express DCC/Frazzled, a cell surface receptor for netrin.

- The binding between netrin and DCC induces an attractive response, thus guiding contralateral axons towards the midline.
These rules encompass both gradients and selective stabilization. A summary version of several selective-stabilization models, proposed by Changeux and Vaughn, consider the cell–cell interactions that occur once contact is initiated. The synapse is initially labile (L), and becomes stabilized (S) or destabilized (and regresses, R) by differences in activity, by the presence or absence of secreted neurotrophins or elimination factors, and according to the extent of contact-mediated molecular matching between cells. Stabilized synapses would then progress to the next level of stabilization, the ultimate result being a stable, appropriately localized synapse.

**What is a gradient?**

The Merriam–Webster dictionary defines a gradient as “a gradual difference in physiologic activity along an axis”, but the definition does not reveal the very different mechanisms that can be used to generate gradients. During early development, diffusible gradients are used to generate polarity, & attract and repel migrating cells and growing processes. The extracellular space is relatively broad, and molecules that are synthesized and secreted by cells can migrate extensively, generating true, diffusible gradients. However, as development proceeds and the brain parenchyma grows in volume, cell-packing density increases, extracellular space decreases, and distances between structures become greater, making it unlikely (with a few notable exceptions) that diffusible gradients are efficiently generated and maintained. By the time of synaptogenesis, neuronal and astrocytic processes crowd local environments, their surfaces are studded with proteins, and the only way to establish a lasting and stable gradient for pathfinding and recognition is to generate a standing gradient of cell-surface molecules, such as that proposed by Sperrey, by a progressive manipulation of gene expression on a cell by cell basis, across a broad region.

**What are the gradients?**

- **Position-dependent adhesive interactions**
  - Cell-surface molecules mediate adhesion.
  - Adhesion molecules can be expressed on cell bodies, leading to adhesion between cells.
  - Adhesive interactions can be mediated by integrins, cadherins, and other cell-surface proteins.

- **Contact-mediated mechanical forces**
  - Direct mechanical forces can be transmitted between cells.

- **Contact-mediated electrical interactions**
  - Electrical signals can be transmitted between cells.

- **Contact-mediated molecular interactivity**
  - Molecular interactions can be mediated by adhesion molecules and other cell-surface proteins.

- **Cell migration**
  - Cells can migrate in response to gradients.

- **Cell differentiation**
  - Cells can differentiate in response to gradients.

- **Cell death**
  - Cells can die in response to gradients.

**Multiple constraints**

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Fraser and Perkel’s rules as a test for a ‘multiple constraints’ model serves as an example of the multiple interactions that would act to achieve synapse specificity. These rules encompass both gradients and selective stabilization.