**Principles of Axon Guidance**

- **Chemoaffinity Hypothesis** – the specificity of wiring is based on recognition of chemical cues
- Axons reach their targets in a series of discrete steps
- Different cells respond to the same guidance cues in different ways
- Chemical cues exist at many points along the axon guidance pathway e.g. the optic tectum and optic tract

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**How are axons guided to their targets?**

- **Long-range cues**
  - **Chemorepulsion**
  - **Chemotraction**
- **Short-range cues**
  - **Contact repulsion**
  - **Contact attraction**

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**How does an axon find its way? Signposts are everywhere**

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An axon senses short and long-range attractant and repellent cues

Four conserved families of axon guidance cues
- netrins
- slits
- semaphorins
- Ephrins (last time)

- Netrins, slits and some semaphorins are secreted
- Ephrins and some semaphorins are membrane bound
- Netrins can act as primarily as attractants
- Slits, semaphorins and ephrins act primarily as repellants
- For each cue there is one or more transmembrane receptor

Extracellular matrix adhesion: short-range attraction
Growth cones 'adhere' to extracellular matrix proteins

Cell-surface adhesion: short range attraction (includes fasciculation)

Cadherins and immunoglobulins mediate cell adhesion

1. Dissociate neurons will aggregate together
2. Cells expressing cadherins will aggregate together
3. Cadherins and immunoglobulins have one transmembrane domain
Properties of cadherin (immunoglobin) recognition

1) Molecules on different cells recognize each other
2) Different combinations can recognize each other
3) Some cadherins bind in a calcium-dependent manner
4) 100s of molecules in these families

Chemoattraction: Short or long range attractant

Commissural neurons in the spinal cord are attracted to the floorplate

Commissural neurons extend ventrally and then toward floor plate, if within 250mm from the floor plate
Dissect 1000s of chick brains, fractionate proteins.

What protein makes axons extend toward it?

Identified a molecule called netrin that is secreted by the floorplate.

Netrins are bifunctional molecules, attracting some axons and repelling others.

The receptors that mediate the attractive and repulsive effects of netrins are also highly conserved.

Growth cone attraction involves the transmembrane receptors of the DCC family.

Repulsion involves the transmembrane receptors of the UNC-5 family.

PKA mediates attraction

Netrins at the midline guide axons in worms, flies and vertebrates

Attractive and repulsive

PKA activation modifies growth cone responses to netrins.
Three families of axon guidance molecules that mediate repulsion

1) Ephrins (last time)
2) Semaphorins
3) Slits

Semaphorins

- Family contains over 20 members
- Best characterised function of semaphorins is in axon repulsion
- 2 distinct classes of semaphorin receptors identified
  - Neuropilins
  - Plexins
Semaphorins cause repulsion and growth cone collapse
Semaphorins can be secreted or membrane-bound

Semaphorins can be repulsive or attractive
Expt: Neurons extend their axons toward or away from guidance cues
SEMA is ligand that causes repulsion, but SEMA + cGMP causes attraction
Combinations of factors can produce novel responses

Central Nervous system of Drosophila embryo
- Neurons make railroad tracks
- Some neurons are ipsilateral.
- Others cross the midline and are contralateral
Identifying an axon guidance molecule and its receptor

Expt: screen mutant embryos
abnormal CNS patterning

Results:
Robo and slit are necessary
to repel axons from the midline

Many expts later...
Slit is a repulsive guidance cue
Secreted at the midline
Robo is receptor on axon that recognizes slit

Slits

• Slit is repellent ligand
• Slit has a role in axon guidance at the midline.
• Receptor (Robo) is expressed by axons that run longitudinally
  and never cross the midline.
• In Robo mutants axons freely cross the midline.
• Commisural axons up-regulate Robo after they have crossed the midline

Summary of axon guidance molecules and their receptors
<table>
<thead>
<tr>
<th>Principles</th>
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<tbody>
<tr>
<td>• Axon guidance molecules can be secreted or membrane-tethered</td>
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<tr>
<td>• Receptors for guidance cues have one transmembrane domain</td>
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<tr>
<td>• The line between receptor and ligand is blurry</td>
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<tr>
<td>“bi-directional signaling”</td>
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<tr>
<td>• The same guidance cue can mediate attraction or repulsion</td>
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<tr>
<td>• Same guidance cues are used over and over for targeting axons</td>
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<td>• Guidance molecules are conserved in many organisms</td>
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