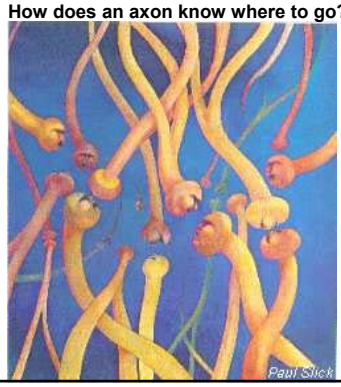
Axon Guidance
How does an axon find the right target?

- 100,000,000,000 neurons in the brain
- 1000 synapses per neuron
- 100,000,000,000,000 connections in the brain

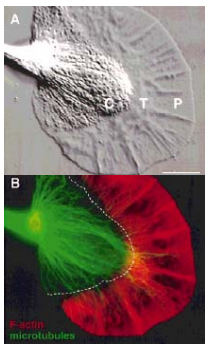
1

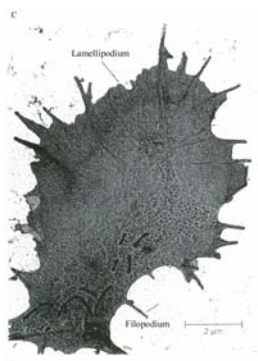
How does an axon move?
How does an axon know where to go?



2

The Growth Cone is the expanding tip of the axon





3

Properties of Growth Cones

- **A growth cone is an enlargement at the end of a growing axon.**

- **Contains several finger-like projections that are called filopodia and sheet-like projections called lamellipodia.**

- **Filopodia and lamellipodia contain actin-filaments.**

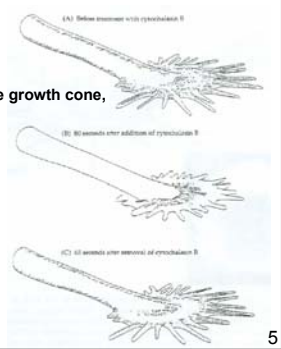
- **The growth cone core or central domain contains microtubules, mitochondria and vesicles.**

4

Axons require actin at the growth cone to extend

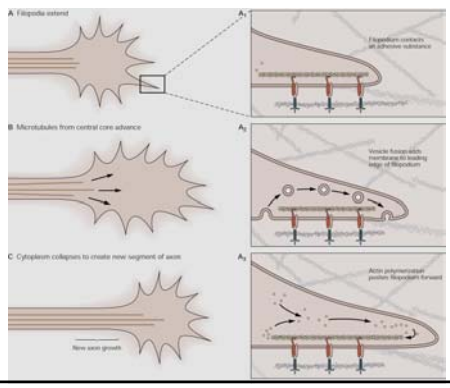
1) **cytochalasin B is a drug that binds to actin filaments and prevents their polymerization.**

2) **Add cytocholasin B locally to the growth cone, quickly inhibit movement**



5

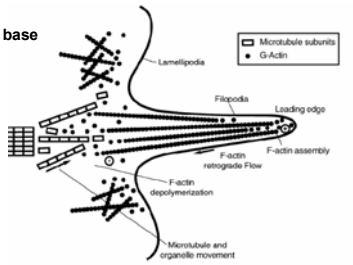
Movement of the Growth Cone



6

Movement of the Growth Cone

1. Actin polymerizes at filopodia tip and depolymerizes at base (provides directionality)
2. Microtubules extend from the growth cone base (central core)



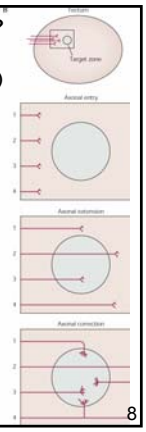
7

How does a growth cone know where to go?

Retinal ganglia cells synapse in the optic tectum (LGN)

1. Most axons stop at the right target
2. Some overshoot, turn around and come back

Model: There is a signpost that the axon recognizes



8

The pathways that developing axons take are very accurate

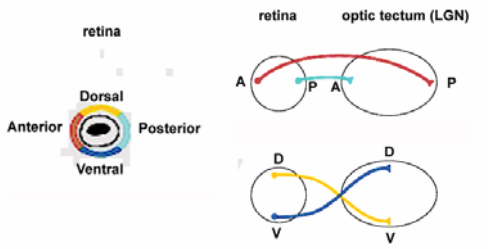
Ramon y Cajal (1890s)
- growth cones move in an ordered and directed manner (Cajal won the Nobel Prize in 1906 for this work).

Ross Harrison (1930s)
- first observed growth cone movement of neurons growing in tissue culture

Roger Sperry (1940s-1950s)
- formation of neural pathways in the brain is very precise

9

Retinal ganglion cells project to optic tectum
There is a topographic map

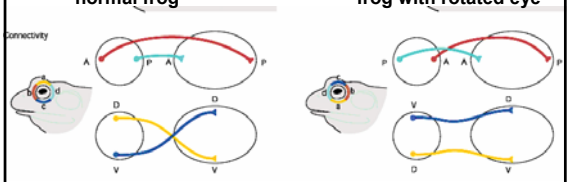


10

Roger Sperry

Regenerating retinal ganglion neurons project to their appropriate position

normal frog **frog with rotated eye**



Axons know where to go

11

Sperry's Classic Experiment: axons know where to go

Normal frog

Frog with rotated eyes

12

Chemoaffinity Hypothesis

Specificity of wiring is based on chemical tags. Individual neurons express distinct molecular markers during development. The formation of appropriate synaptic connections depends on the matching of complementary molecules on pre- and postsynaptic neurons

3 tenets of the hypothesis

1. **neurons are intrinsically different from one another**
2. **Differences in position are biochemical in nature**
3. **Differences are acquired early in development**

13

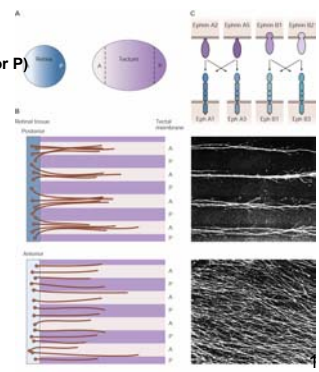
What are the molecules in the optic tectum that guide retinal ganglion cells?

How is the topographic map of retinal axons established?

14

How to isolate molecules involved in guidance of retinal axons

Develop an assay:
culture retinal neurons (A or P) with tectum (A and P)
"stripe assay"



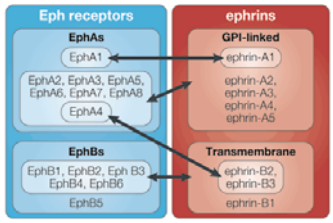
Posterior retinal axons only grow on anterior tectum

Anterior retinal axons grow on both

15

Ephrins are guidance cues (ligands) in the optic tectum
Ephrin receptors (receptors) are in the retina axons

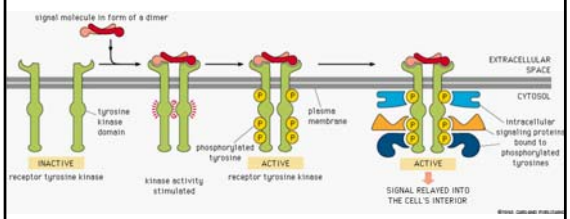
Many receptors, many ligands



Nature Reviews | Neuroscience

16

Ephrin receptors are receptor tyrosine kinases

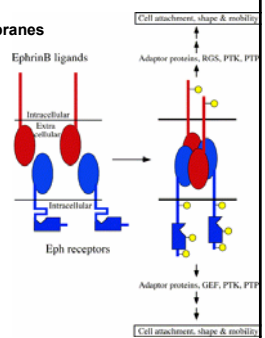


17

Ephrin receptors are receptor tyrosine kinases

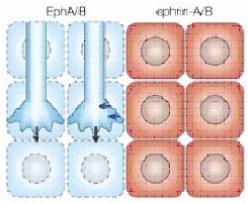
ephrin ligands are tethered to membranes

Both ephrins and Ephrin receptors can activate intracellular signaling=== Bidirectional signaling



18

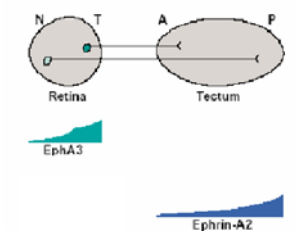
Eph receptors and ephrins restrict growth-cone migration by repulsion



Boundaries of ephrin A protein restrict growth cones of neurons expressing Ephrin-A receptor.

19

Gradients of Ephrin receptors in the retina and ephrins in the tectum set up the topographic map



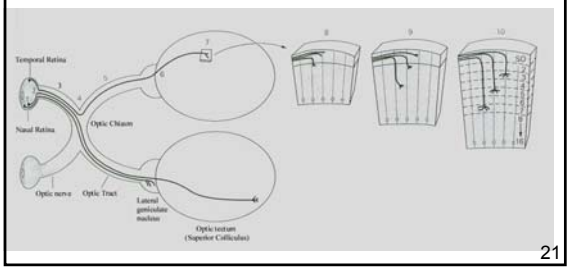
Anterior retinal axons project to posterior tectum:
Low levels Ephrin receptor read only high levels of ephrin

Posterior retinal axons project to anterior tectum:
High levels Ephrin receptor read low levels of ephrin

20

Axons Reach their Destination in a Series of Discrete Steps

- **Axons reach distant targets in a series of discrete steps**
- **Make decisions at frequent intervals along the path**
- **e.g. retinal axon path to the optic tectum**
- **At least 10 steps on the journey**



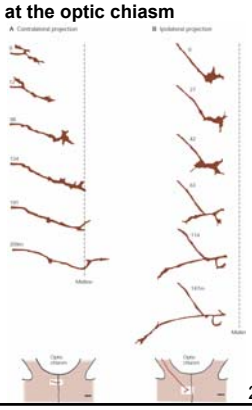
21

Ganglion cells cross (or not) at the optic chiasm

Time course of retinal axon growth

Some axons cross (contralateral projections)

Others do not cross (ipsilateral projections)

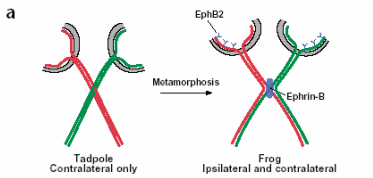


22

How do axons decide whether or not to cross?

Early in development all projections are contralateral

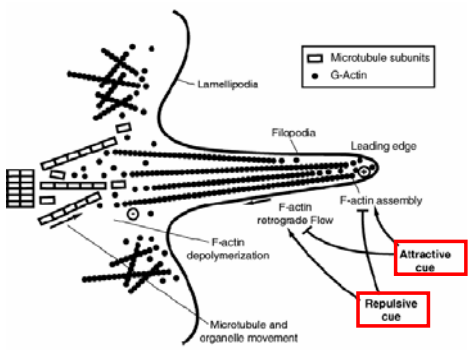
At the time ipsilateral projections are formed, Ephrin B receptors are expressed in posterior (temporal) retina ephrin B becomes expressed in the optic chiasm



Ephrins act at multiple steps to guide axons to their targets by steering them away from inappropriate targets

23

Activation of axon guidance signaling pathways influences actin polymerization



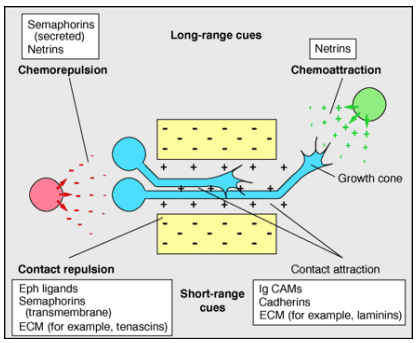
24

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**

25

Next time: Axons recognize short-range, long-range attractive and repulsive cues



26