Axon guidance

Wiring the brain:
How specific connections are established between neurons and their target cells

Axon guidance outline

• The growth cone
  – motor for axon growth
  – structure and dynamics

• Navigational cues
  – attractants and repellents, gradients

• Dorsal-ventral guidance
  – Commissural axons

• Anterior-posterior guidance
  – Longitudinal axons

• Wiring the eye: retinotectal axon projections
  – topographic mapping
  – Eye regeneration experiments by Sperry
  – Chemoaffinity hypothesis
  – Ephrins as tectal guidance cues
Wiring the brain

• Connectivity map of the monkey visual system
• Each line represents $10^6+$ axons

• How do axons navigate to find targets?
• Can connections be re-formed following injury or disease?

Fellman and Van Essen, 1991

Growth cone:
– growing tip of axon
– “Amoeba on a string”

Functions of:
– microtubules
– actin-myosin
– membrane vesicles
– filopodia

Fig 19.11
The growth cone: senses and responds to environment

- Analogy: amoeba on a string; Fig 11.20
- Growth cones navigate long distances through the developing brain (and body)
- Capabilities determined by receptors on its surface
  - Receptors allow growth cone to respond to cues
  - Responds by altering cytoskeleton
  - Filopodia: “string-feet” actively sense and respond to environment

Four classes of guidance cues

- Cues can act:
  - At a distance
  - Locally by contact
- Cues can act
  - Positively
  - negatively

(Adapted from Tessier-Lavigne and Goodman, 1996)
Two axon directions

- **Commissural**
  - Axons grow toward and across midline
  - Link sides of brain
  - Guidance: floor plate is key source of cues

- **Longitudinal**
  - Axons grow along length of neural tube
  - Link brain regions
  - Guidance: little is known

Crossing the midline: commissural axons

- **Floor plate is a local source of diverse cues**
- **Local adhesive cues**
- **Secreted attractants**
  - Netrin, Sonic hedgehog
- **Secreted repellents**
  - Slits

- **Axon trajectory**:
  - Repelled by roof plate
  - Attracted by floor plate
  - Adhesion promotes crossing
  - Then, repellent pushes axons away
Gradients guide axons

- **A. Dorsal commissural neurons**
  - cell bodies in dorsal position
  - axons grow down to cross floor plate

- **B. Floor plate is attractive**
  - culture experiment
  - piece of floor plate secretes chemoattractant in gradient
  - high ventral, low dorsal

- **C. Netrin expressed in floor plate**
  - secreted protein
  - Is netrin sufficient?
  - Transfect netrin plasmid into generic cells
  - Result: attracts axons

Axons are lost when chemoattractant is missing

- **Netrin:** secreted by floor plate cells
- **Purified netrin protein attracts axons**
- **Netrin knock out mice:** commissural axons are misguided

(Fig 11.30, Wolpert, Principles of Development)
The problem of longitudinal axon guidance

- Two hypotheses:
  1. Local cues define pathways for longitudinal axons to follow
  2. Gradients of cues from midline guide longitudinal axons

Shh: a morphogen-cue that sets dorsal-ventral patterns of cells

Morphogens: DV patterns

- Morphogen: patterning signal in a gradient
- Gradients in neural tissue
- Sonic hedgehog (Shh)
  - Produced by floor plate
  - Different [Shh] induce gene patterns and neuron patterns
- Shh sets DV code
- BMPs: dorsal morphogen
Morphogens: also cues for axons?

- But, surprisingly!
- Shh can directly guide commissural axons
  - Axons need floor plate to grow ventrally
  - Cultured axons grow toward Shh source

- Could Shh influence longitudinal axons?
  - Axons follow longitudinal columns of cells?
  - Or, Shh directly guides?

**Initial test of Shh role in longitudinal axon guidance:**
- Expand Shh into axon pathway
- Important: protect neuron from being altered

**Approach: electroporation in early chick embryos**
- Embryos accessible by windowing egg
- Can zap plasmid DNA into patch of cells
- See patch by green fluorescent protein (GFP)
- Test for Shh expression by antibody labeling
- Label axons with fluorescent antibody
• Axon pathway diverts around patches of ectopic Shh
  – Suggests repellent effect (?)
• Targeting different longitudinal tracts causes similar effects

**Shh: sets DV position of axons?**

**The Big Question:** Does Shh act directly or indirectly?
– Direct: axons somehow navigate using Shh gradient
– Indirect: Shh acts by inducing a direct cue
– Could act in both ways
– Strategy: change Shh gradient or DV patterning, independently
To see the light: connecting the eye and brain

- **Retina:** photoreceptors respond to light
  - send signals to neuron network in retina
  - optic axons: integrate and relay signals from many photoreceptors to brain

- **Tectum:** region of midbrain that receives optic input (fish, frog, bird)
  - diencephalon in mammals: lateral geniculate nucleus (LGN)

- **Retino-tectal map:** optic axons have patterned connections, or “map” to the tectum
- **Best studied example of axon guidance**
  - eye is exposed part of brain, so experimental access
  - easy to give controlled stimuli (spots of light)
  - several different ways to measure response

**Overview of eye wiring in humans**

- **Fig 16.1, Matthews**
- **Crossing inputs**
  - retinal axons project from eye into optic nerve
  - cross at optic chiasm
  - targets in thalamus (and other locations)
  - Thalamic neurons project to visual cortex
Visual fields and retinal projections

• Lateral eyes see separate fields
  – examples: frogs, horses
  – axons project to opposite side of brain
  – complete crossing

• Frontally placed eyes have overlapping fields of vision
  – binocular vision
  – axons from one eye have a split projection
  – part of visual field is projected across, part is sent to same side

• Important
  – projections retain topographical map

The retina maps onto the tectum

• Image on retina is represented in tectum
• Neighbor relationships maintained, but inverted
• Two ways to determine map
  – anatomical: label axons and follow projection to tectum
  – functional: stimulate part of retina, and get signal in tectum
Retinal axon connections make map in tectum

- Fig 11.25, Wolpert
- Eye coordinates
  - dorsal-ventral
  - nasal-temporal
- Tectum coordinates
  - dorsal-ventral
  - anterior-posterior

Q: How are these wiring patterns set up?

Frog eyes can regenerate connections

- Roger Sperry, 1943
- Optic nerve cut, then eye rotated
- Optic axons regenerate connections to tectum (a few months)
- Behavioral defect:
  - frog strikes opposite direction of fly, and never learns to compensate
How is retino-tectal map generated?

- Competing hypotheses:
  - 1. Functional molding or adaptation:
      - connections are set up randomly, and adapt themselves through experience (learning)
  - 2. Roger Sperry: chemoaffinity hypothesis
      - Observation: Axons discriminate between different areas of tectum, and match up with appropriate targets
      - Implies chemoaffinity: axon ability to detect chemical cues in the tectum (lock and key specificity)

Rotating the frog eye: map regenerates, but upside down

- Frogs never learn to adapt: argues against functional molding hypothesis
- Chemoaffinity hypothesis
  - Axons return to their original targets in the tectum
  - Conclusion: position within retina determines target in tectum

![Diagram showing the process of map regeneration](image)
Support for chemoaffinity hypothesis

- In vitro assay: retinal axons prefer to grow on cell membranes isolated from their tectal target (Fig 11.27, Wolpert)
  - striped carpet assay
  - cell membranes painted in stripes
  - axons grow out of piece of retina
- Strong repellent in posterior tectum
  - only temporal retinal axons are repelled by it
  - nasal retinal axons do not react

Eph ligands repel axons

- Eph receptors are expressed by retinal axons
- Ephrin ligands are expressed in gradient in tectum
- Why do temporal axons prefer anterior tectum?
  - Hypothesis: Because they express high levels of receptor, so project to anterior tectum, where there is less ligand to repel them
Axons reach target and form synapse

- **Synaptogenesis**
  - recognition of proper target cells
  - interactions between axons and target cells
    - signals sent by both axon and target
  - synthesis and targeting of components of specialized synaptic structures
  - initiation of signaling