LECTURE 11
CNS Pathfinding in Vertebrates and Invertebrates

Introduction
Pathfinding is a central unsolved problem in developmental neurobiology

The Limb Bud
The Grasshopper Limb System - Bentley
Diagram of limb
Ti1 cells emerge from the neuroepithelium
Growth cone orientation toward CNS is intrinsic
Pathfind along guideposts - Fe1, Tr1, Cx1
Later sensory nerves follow the Ti1 pathway
Pioneers and guideposts die after establishing pathway

Experimental Questions
Do Fe1 need Ti1 pioneers?
No! - ablations of Ti1s
Do pioneers need guideposts?
Yes, to an extent. - kill Cx1: Ti1 stuck at Tr/Cx boundary
Kill Fe1 or Tr1: Ti1 slows down, but ultimately finds its way
Axons react to guideposts - make close contact, dye couple
Single filopodia touch a guidepost (Cx1) reorient whole growth cone
Remove filopodia with cytochalasin - Ti1s get lost

Filleted limb prep
What do Ti1 axons follow between Ti1 and Tr1?
Not signal from CNS - filleted limb separated from CNS
Not signal on mesoderm - can dissect it off
Not signal on ECM - can digest it away
Signal must be on remaining epithelium

CNS pathways: first seven axons
MP1, dMP2, vMP2; pCC, aCC; U1, U2 make first three longitudinal fascicles
dMP2/MP1 and pCC interact/fasciculate
aCC follows/fasciculates on U’s
ablate Us - aCC gets lost

Motor axon pathfinding SNa versus SNb axon projections to ventral muscles

What molecules are involved?
The search for Fasciclins with mAbs
Fasl - novel membrane protein (Fasl on aCC not pCC)
FasII - Ig superfamily - homolog to NCAM (FasII on MP1, vMP2 axons)
FasIII and Neuroglian - also Ig family members
Neuroglian - homolog of L-1
Netrin (= worm unc6) and Netrin receptors (attractive receptor: human DCC = deleted in colorectal cancer = fly Frazzled = worm unc40, repulsive receptor = worm unc-5
Roundabout (Robo)-Slit and Commisureless (Comm)
Axonin
(See Figure 7a)

Midline Axon Guidance
Netrin = midline attractant for most axons and repellent for others
Attraction mediated by DCC = Frz = Unc40 -> ↑cAMP
Repulsion mediated by unc-5 -> ↓cAMP

Robo = receptor for repulsive Slit ligand expressed in midline cells
Comm expressed in midline -> ↓ Robo
robo-; comm- double mutants = robo- (don’t need to downregulate Robo if it isn’t there)
Moderate over-expression of comm = robo- phenotype
Strong over-expression of comm = slit phenotype -> all axons collapse onto midline
(reason that slit- mutant is stronger than robo- due to existence of multiple Robos: Robo, Robo2, and Robo3).
- Inner track axons express only robo
- Intermediate track axons express robo and robo2
- Outer track axons express robo, robo2, and robo3
  ↑ #robo genes expressed (e.g. ↑ levels) -> axon tracks further from midline.
(See Figure 7a)

Robo-Slit also mediate repulsion for migrating fly muscle cells and for vertebrate leukocytes -> similar mechanisms underlie migration and pathfinding.

Axonin: Binds attractant NrCAM at midline. Binding is required to cross midline.

Motoneuron Axon Guidance
Three primary motoneuron = motor neuron paths: SNb -> ventral muscles,
SNa -> lateral muscles, and ISN -> dorsal muscles
Goodman screened for mutants with abnormal muscle innervation -> mutant affecting
different steps in pathfinding: early: beaten path; middle: short stop; late: stranded;
target recognition: walkabout, clueless
Fasciclins: act as homophillic cell adhesion molecules - when expressed in S2 cells
fasII- mutants – complete or partial loss of vMP2 and MP1 fasciculation
FasII misexpression: SNb axons fail to defasciculate with SNa axons
beaten path: required for FasII down-regulation in SNb axons
beaten path mutant = FasII mis-expression
Overall Evolutionary Conservation of Developmental Mechanisms

D/V axis:
  Neural vs. non-neural ectoderm: = Dpp/Sog = BMP-4/Chd
  Proneural domains -> Lateral Inhibition: AS-C-> Delta->->N -> Su(H) -> E(spl)
  Subdivision of neuroectoderm into ventral, medial, and lateral domains:

A/P axis:
  Segment identity: Homeotic genes = HOX genes: expression and function

Appendages
  A/P patterning: Hh (= ZPA factor) –I ptc –I Ci-> Dpp
  D/V patterning = Notch signaling center at D/V border (vert AER)
  Appendage outgrowth? apterous

Basic tissue type specification:
  Mesoderm formation: sna twi ?, Sog –I Dpp -> Neural induction
  Heart specification: tinman/mef2
  Eye development: eyeless/pax-6

(see Figure 6.6)

Early pathfinding:
  Netrins (unc)6 -> Receptors = (positive) DCC/frz/unc40 and (neg.) unc5
  Robo/Slit

Basic Acute Phase Immunity

What might have allowed the common ancestor to displace other life forms??
  Evolution of position and tissue-type identity genes (e.g., Hox genes along A/P
  axis that determine segmental identity and neural identity genes along D/V
  axis determining cell type identity)??
  Evolution of mechanisms for extracting low levels of atmospheric oxygen which
  were present at that time allowing growth of more complex body forms??