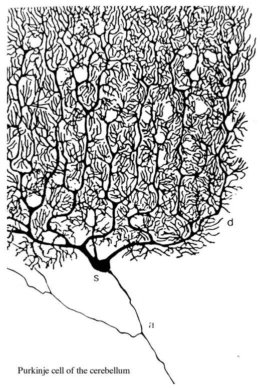
Like the entomologist in search of brightly colored butterflies, my attention hunted, in the garden of gray matter, cells with delicate and elegant forms, the mysterious butterflies of the soul.

Santiago Ramon y Cajal (1937)



Dorsal-Ventral Pattern in the Vertebrate Spinal Cord

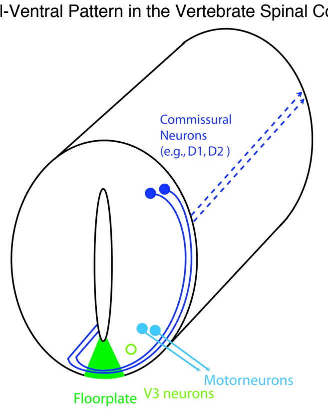


Figure 12.13 ***Cascade of Inductions Initiated by Notochord in Ventral Neural Tube***

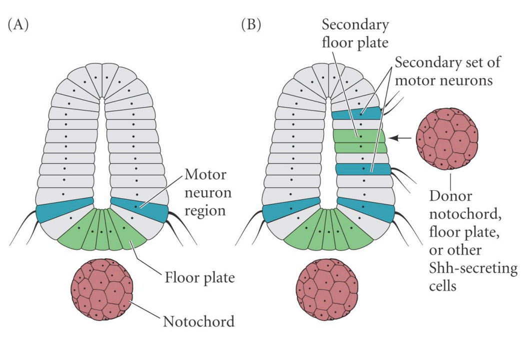


Figure 12.12(1) ***Dorsal-Ventral Specification of the Neural Tube***

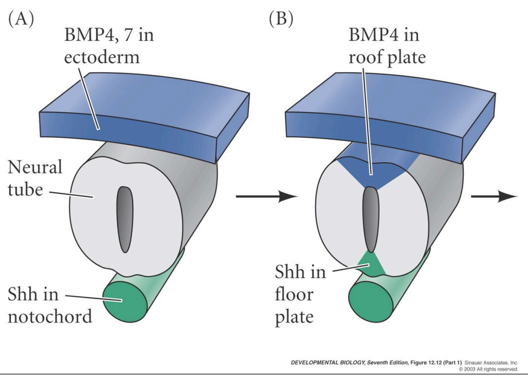


Figure 12.12(2) ***Dorsal-Ventral Specification of the Neural Tube***

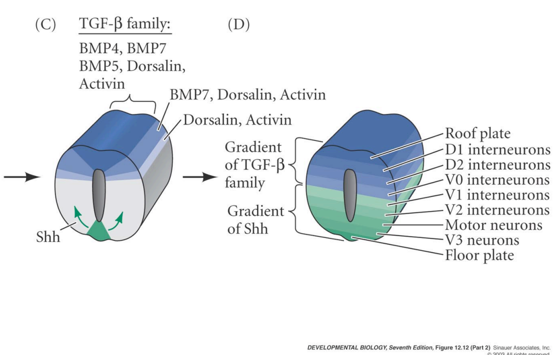


Figure 12.12

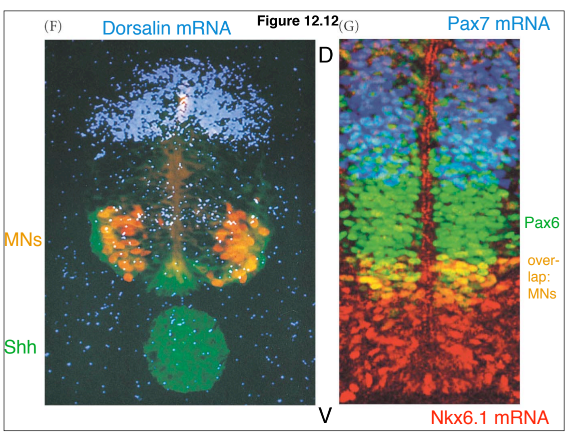


Figure 12.12 (E)

Shh (and BMP's) gradient determines TF expression, specifying cell types

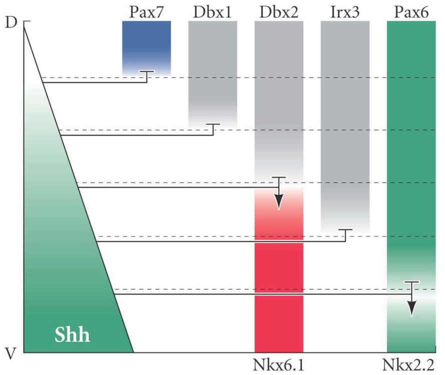
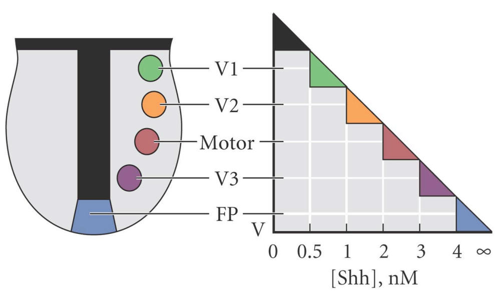


Figure 12.13(2) ***Cascade of Inductions Initiated by Notochord in Ventral Neural Tube***

(C)



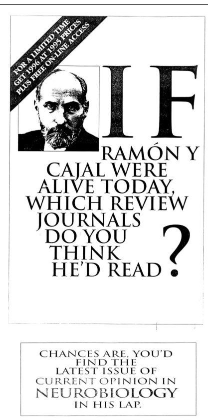
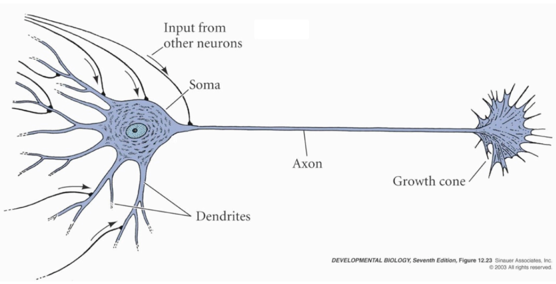
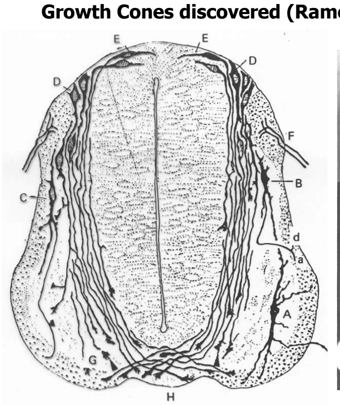
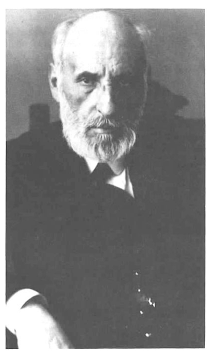


Figure 12.23 (modified) ***Diagram of a Motor Neuron***



Growth Cones discovered (Ramon y Cajal, 1890)

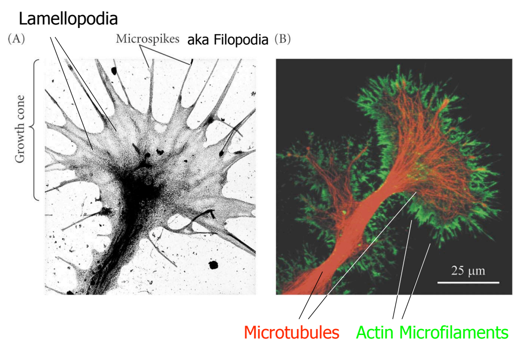


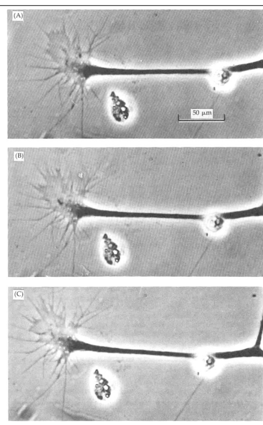


The inventor of tissue culture and first observer of live growth cones (1910) with the discoverer of the "organizer."

Hans Spemann & Ross Harrison

Figure 12.24 *Growth Cone structure*





Growth Cone extending *in vitro*

Mechanisms of Growth Cone Guidance

Contact-mediated (short-range)
- requiring direct cell-cell or cell-substrate contact

Contact attraction

Contact repulsion

Diffusible (‘ long-range’)

Chemoattraction

Chemorepulsion

(Tessier-Lavigne & Goodman, 1996 “Molecular Biology of Axon Guidance”)

Mechanisms of Growth Cone Guidance

Contact mediated (short-range) - requiring direct cell-cell or cell-substrate contact

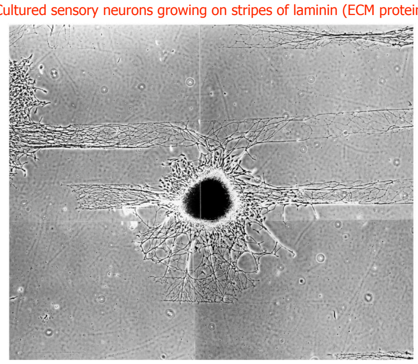
Contact attraction - mediated by two classes of proteins

Direct Cell-Cell Adhesion: Cell Adhesion Molecules (CAMs)

Cell - Substrate Adhesion: Extracellular (ECM) Matrix proteins and their cellular receptors

Figure 13.18 ***Outgrowth of Sensory Neurons***

Cultured sensory neurons growing on stripes of laminin (ECM protein)



Mechanisms of Growth Cone Guidance

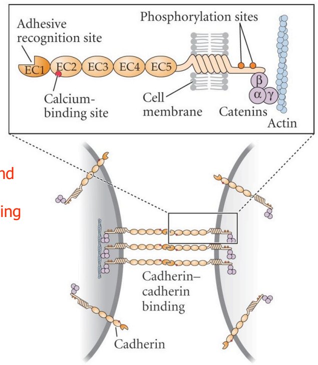
Cell Adhesion Molecules (CAMs)

Major classes:

Calcium-dependent Adhesion proteins: Cadherins
e.g. N-cadherin, E-cadherin, P-cadherin

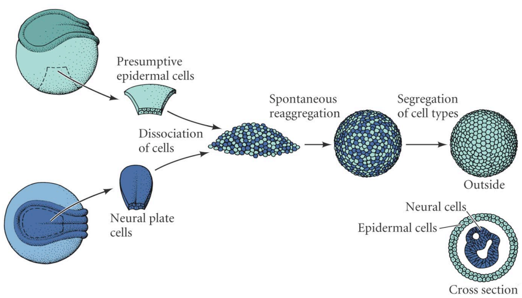
Immunoglobulin Superfamily Adhesion proteins: Ig-CAMs
e.g. NCAM, L1, Fasciclin II (FasII), etc.

Figure 3.28 ***Cadherin-Mediated Cell Adhesion***



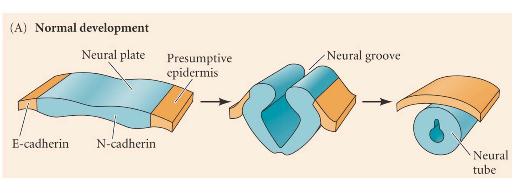
Both Cadherins and IgCAMs mediate **homophilic** binding (like to like).

Figure 3.23 ***Reaggregation of Cells From Amphibian Neurulae***



Cadherins mediate tissue-specific adhesion

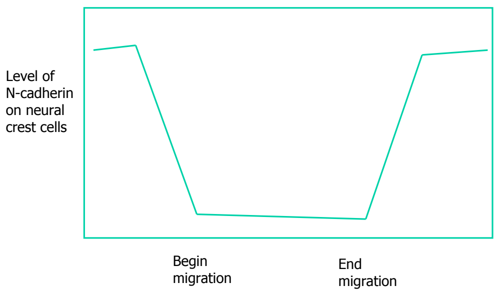
Fig 12.6 ***Expression of N- and E-cadherin Adhesion Proteins During Neurulation***



Cadherins aid separation of epidermal and neural ectoderm

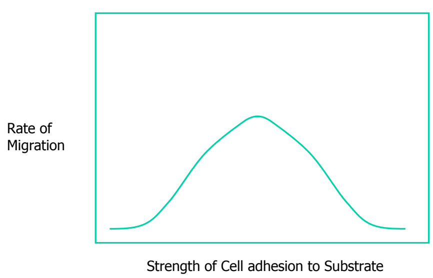
N-cadherin plays a role in neural crest cell migration

N-Cadherin level is high prior to neural crest cell migration, low during migration, and high again after migration ends.



Cell Adhesion

Cell adhesion can promote or inhibit migration



Mechanisms of Growth Cone Guidance

Cell Adhesion Molecules (CAMs)

Immunoglobulin Superfamily Adhesion proteins: Ig-CAMs
NCAM, L1, Fasciclin II, etc.

NCAM - more general nervous system "glue"

- used to direct retinal growth cone outgrowth (1 function)
- two different forms based on glycosylation:
  - high SA (lower adhesion)
  - low SA (higher adhesion)
    [SA = sialic acid]
- high SA found during growth, low after reaching target

Mechanisms of Growth Cone Guidance

Cell Adhesion Molecules (CAMs)

Immunoglobulin Superfamily Adhesion proteins: Ig-CAMs
NCAM, L1, Fasciclin II, etc.

L1 - more specific vertebrate CNS CAM
- found in limited number of tracts, regions of brain & s.c.

Human mutations cause neurological disorders such as X-linked hydrocephalus, MASA syndrome

L1 disruption by ethanol may cause part of Fetal Alcohol Syndrome birth defects

Figure 21.13(2) ***Possible Mechanisms Producing Fetal Alcohol Syndrome (FAS)***

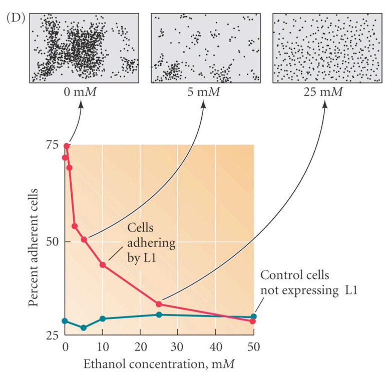


Figure 21.12 ***Comparison of a Brain from an Infant with Fetal Alcohol Syndrome (FAS, Left) With a Brain From a Normal Infant of the Same Age (Right)***

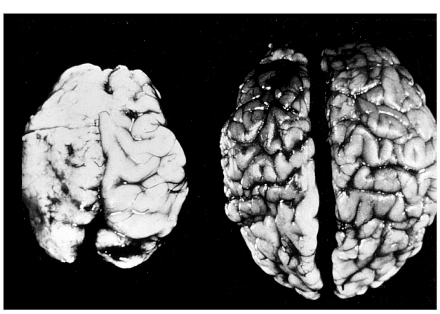
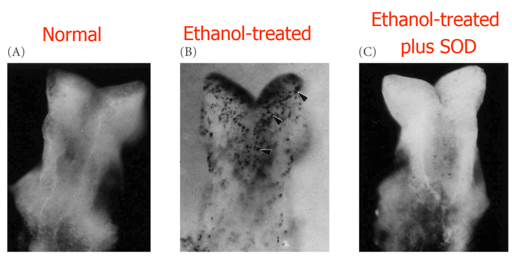


Figure 21.13(1) ***Possible Mechanisms Producing Fetal Alcohol Syndrome (FAS)***



Nile-Blue stained dying cells
in 9d mouse embryo brain

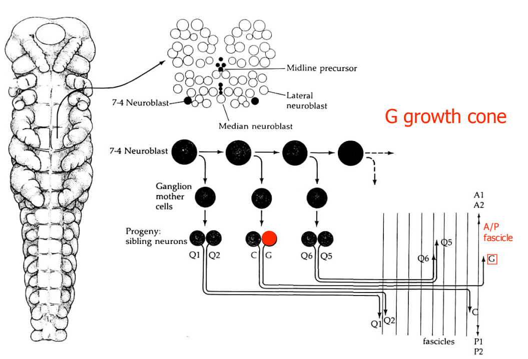
Mechanisms of Growth Cone Guidance

Contact attraction by CAMs

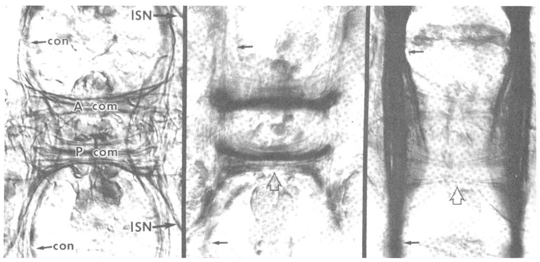
The "Labeled Pathways" Hypothesis -

Most growth cones are guided along axons already laid down (by "pioneer neurons") - each with a unique molecular marker or set of markers

Identified Growth Cone Guidance in Grasshopper Embryo



A Grid of Pioneer Axon Bundles Guides Later Growth Cones, such as 'G'

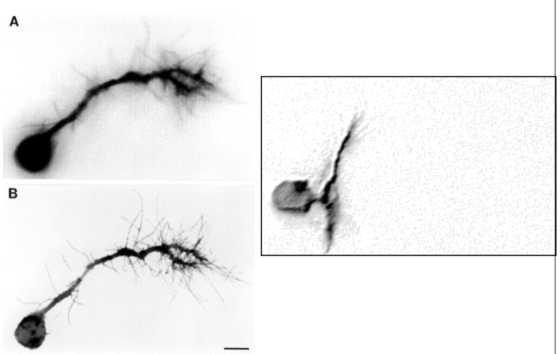


Grid of axon bundles (fascicles) in a single ganglion

Expression of Fas I in Commissures extending R-L

Expression of Fas II in Connectives Extending A-P

A Grid of Pioneer Axon Bundles Guides Later Growth Cones, such as 'G'



A Grid of Pioneer Axon Bundles Guides Later Growth Cones, such as 'G'

Experiments:

Kill cells that make A/P bundle:
G growth cone guidance disrupted

Block function of Fas II (found on A/P bundle):
G growth cone guidance disrupted

Mechanisms of Growth Cone Guidance

Contact attraction by CAMs

Intermediate cell recognition by pioneer neurons:
"guidepost cells" in grasshopper limb bud

Pioneer Neurons in the Grasshopper Limb Bud

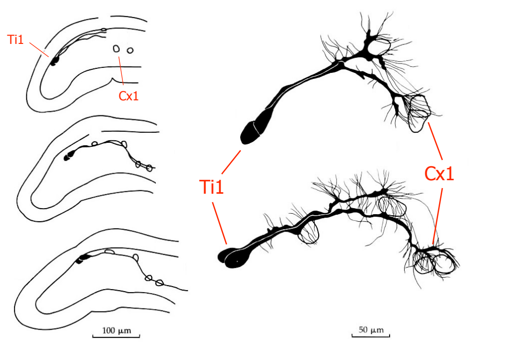
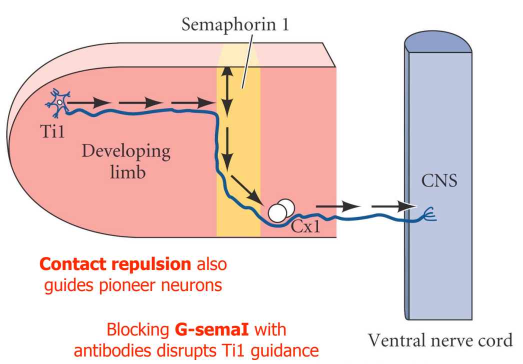
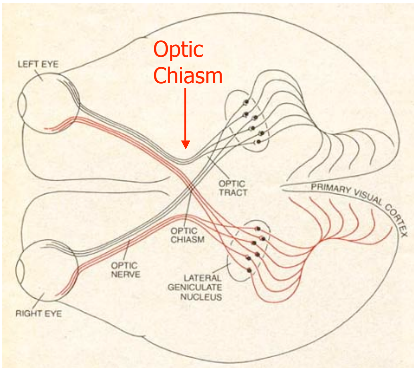


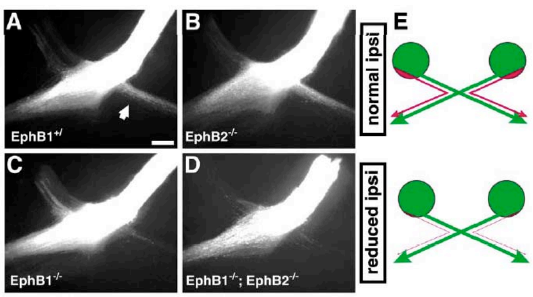
Fig. 13.20 ***The Action of Semaphorin 1 in the Developing Grasshopper Limb***



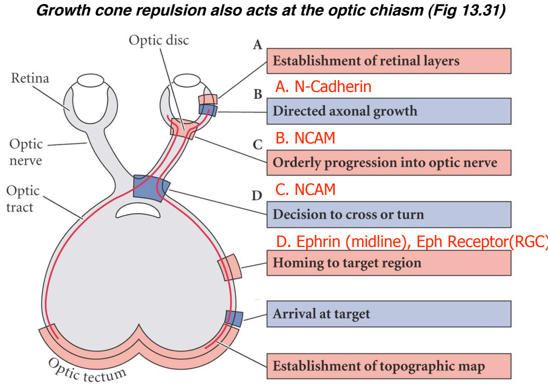
Crossing of retinal axons at the optic chiasm



Ephrins act in contact repulsion at the optic chiasm



Growth cone repulsion also acts at the optic chiasm (Fig 13.31)



Mechanisms of Growth Cone Guidance

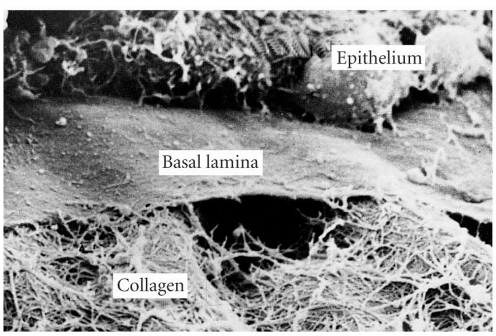
Extracellular Matrix proteins:

Collagen, Fibronectin, Laminin, etc.

ECM Receptors:

Integrins (alpha and beta subunits)

Fig. 6.33 - SEM of Extracellular Matrix



DEVELOPMENTAL BIOLOGY, Seventh Edition, Figure 6.33 Sinauer Associates, Inc.

There are many different types of Collagen (at least 16)

80-90% of collagen is types I-III

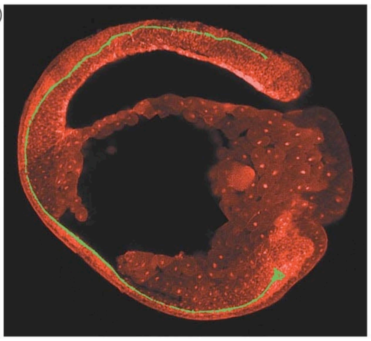
Table 12–2 Types of Collagen and Their Properties

Type	Molecular Formula*	Polymerized Form	Distinctive Features	Tissue Distribution
I	$[\alpha1(I)]_2\alpha2(I)$	fibril	low hydroxylysine low carbohydrate broad fibrils	skin, tendon, bone, ligaments, cornea, internal organs (accounts for 90% of body collagen)
II	$[\alpha1(II)]_3$	fibril	high hydroxylysine high carbohydrate usually thinner fibrils than type I	cartilage, intervertebral disc, notochord, vitreous body of eye
III	$[\alpha1(III)]_3$	fibril	high hydroxyproline low hydroxylysine low carbohydrate	skin, blood vessels, internal organs
IV	$[\alpha1(IV)]_3$ (controversial)	basal lamina	very high hydroxylysine high carbohydrate probably retains procollagen extension peptides	basal laminae
V	$[\alpha1(V)]_2\alpha2(V)$	unknown	high hydroxylysine high carbohydrate	widespread (in small amounts)

*The seven different α-chains are designated α1(I) through α1(V), α2(I), and α2(V).

Fig. 6.23(A - rotated) - Fibronectin in the ECM of Xenopus *Gastrula*

(A)



DEVELOPMENTAL BIOLOGY, Seventh Edition, Figure 6.32 (Part 1)

Mechanisms of Growth Cone Guidance

ECM Receptors:

Integrins (α and β subunits)

Different combinations of α and β subunits make receptors that bind different ECM proteins:

Examples -

$\alpha_1\beta_1$ integrin binds collagen, laminin

$\alpha_5\beta_1$ integrin binds fibronectin

Mechanisms of Growth Cone Guidance

Chemoattraction by diffusible substances

Chemoattraction of growth cones first shown with NGF

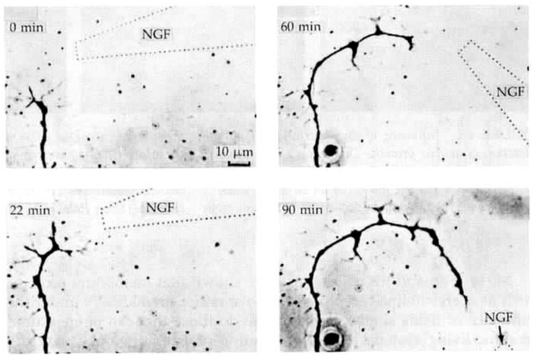


Fig 13.27 ***Embryonic Axon From a Rat Dorsal Root Ganglion Turn in Response to NT-3***

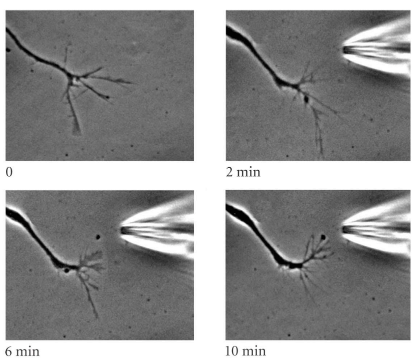
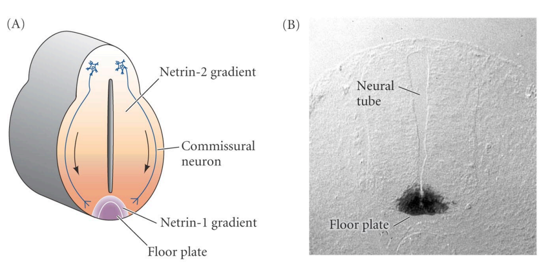


Figure 13.22 ***Trajectory of the Commissural Axons in the Rat Spinal Cord***



Netrin-1 mRNA expression

Fig. 13.23 ***Transformed Chick Fibroblast (COS) Cells Secreting Netrins Elicit Axon Outgrowth of Commissural Neurons from 11-d Emb. Rat Dorsal Spinal Cord Explants***

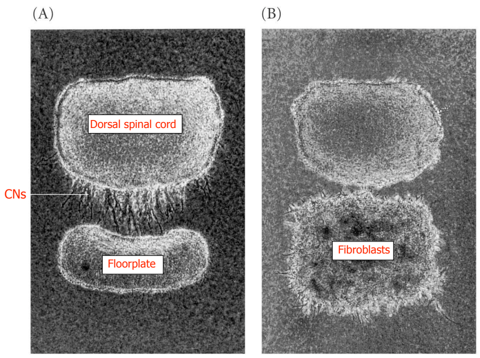
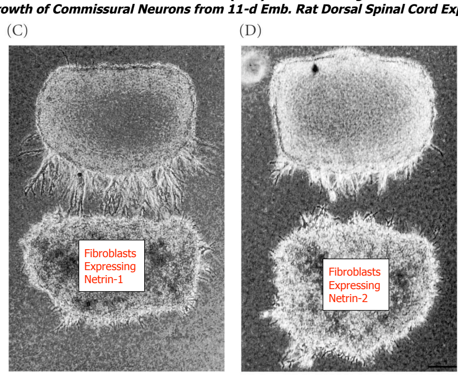


Fig. 13.23 ***Transformed Chick Fibroblast (COS) Cells Secreting Netrins Elicit Axon Outgrowth of Commissural Neurons from 11-d Emb. Rat Dorsal Spinal Cord Explants***



Signals can be attractive or repulsive, depending on the receptor

Netrin-mediated dorsal/ventral signaling is an ancient feature of animal nervous systems.

Netrin signaling also patterns growth cone guidance and cell migration in the nematode *C. elegans.*

Mutants with abnormal nervous system function ("unc" - uncoordinated) were found to be affected in netrin-mediated growth cone guidance.

The first example of a netrin receptor mediating *chemorepulsion* was discovered in *C. elegans* (the *unc-5* gene).

Chemorepulsion by netrins is also found in the vertebrates.

Figure 13.24 C.e. *Netrin Expression And Function in Axonal Guidance*

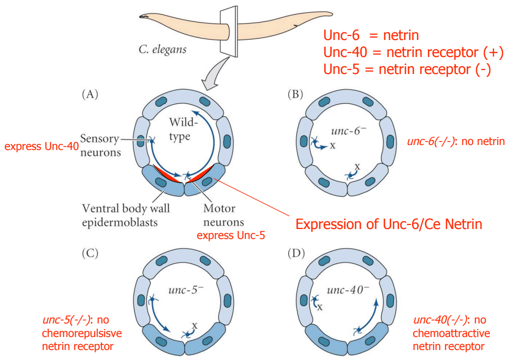
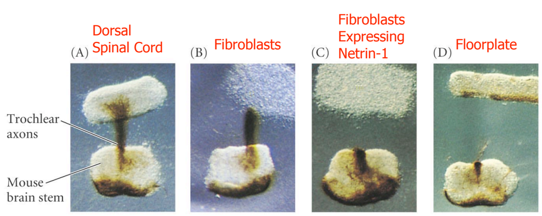
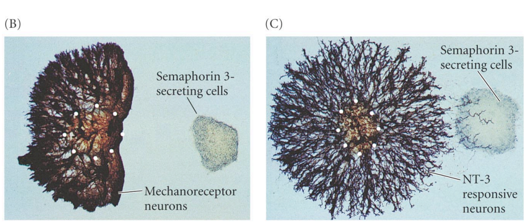


Figure 13.25 *Netrins inhibit Outgrowth of Trochlear Axons From Explants of the Mouse Brain Stem*

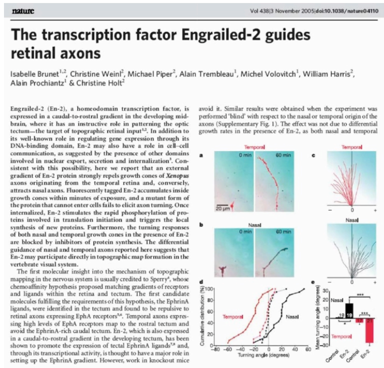


A vertebrate example of Chemorepulsion by a netrin

Figure 13.21(2) *Semaphorin 3 as a Selective Inhibitor of Axonal Projections into the Ventral Spinal Cord*



Engrailed-2 as a secreted chemorepulsive and chemoattractive agent for *Xenopus* Retinal Neurons


nature Vol 438|3 November 2005|doi:10.1038/nature04110

The transcription factor Engrailed-2 guides retinal axons

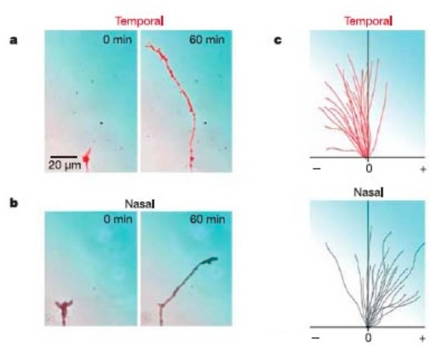
Isabelle Brunet[1,2], Christine Weinl[2], Michael Piper[2], Alain Trembleau[1], Michel Volovitch[1], William Harris[2], Alain Prochiantz[1] & Christine Holt[2]

Engrailed-2 (En-2), a homeodomain transcription factor, is expressed in a caudal-to-rostral gradient in the developing midbrain, where it has an instructive role in patterning the optic tectum—the target of topographic retinal input[1,2]. In addition to its well-known role in regulating gene expression through its DNA-binding domain, En-2 may also have a role in cell–cell communication, as suggested by the presence of other domains involved in nuclear export, secretion and internalization[3]. Consistent with this possibility, here we report that an external gradient of En-2 protein strongly repels growth cones of *Xenopus* axons originating from the temporal retina and, conversely, attracts nasal axons. Fluorescently tagged En-2 accumulates inside growth cones within minutes of exposure, and a mutant form of the protein that cannot enter cells fails to elicit axon turning. Once internalized, En-2 stimulates the rapid phosphorylation of proteins involved in translation initiation and triggers the local synthesis of new proteins. Furthermore, the turning responses of both nasal and temporal growth cones in the presence of En-2 are blocked by inhibitors of protein synthesis. The differential guidance of nasal and temporal axons reported here suggests that En-2 may participate directly in topographic map formation in the vertebrate visual system.

The first molecular insight into the mechanism of topographic mapping in the nervous system is usually credited to Sperry[4], whose chemoaffinity hypothesis proposed matching gradients of receptors and ligands within the retina and tectum. The first candidate molecules fulfilling the requirements of this hypothesis, the EphrinA ligands, were identified in the tectum and found to be repulsive to retinal axons expressing EphA receptors[5,6]. Temporal axons expressing high levels of EphA receptors map to the rostral tectum and avoid the EphrinA-rich caudal tectum. En-2, which is also expressed in a caudal-to-rostral gradient in the developing tectum, has been shown to promote the expression of tectal EphrinA ligands[7,8] and, through its transcriptional activity, is thought to have a major role in setting up the EphrinA gradient. However, work in knockout mice

avoid it. Similar results were obtained when the experiment was performed 'blind' with respect to the nasal or temporal origin of the axons (Supplementary Fig. 1). The effect was not due to differential growth rates in the presence of En-2, as both nasal and temporal

Temporal *Xenopus* Retinal Neurons are repulsed by gradient of En-2; Nasal Retinal Neurons are attracted.



Engrailed-2 can function as a secreted signaling protein

Fluorescently-labelled En-2 taken up by retinal growth cone.

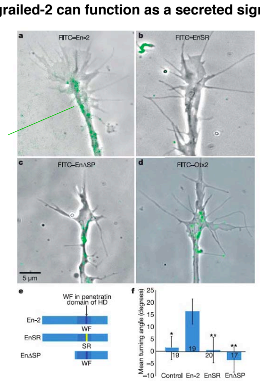


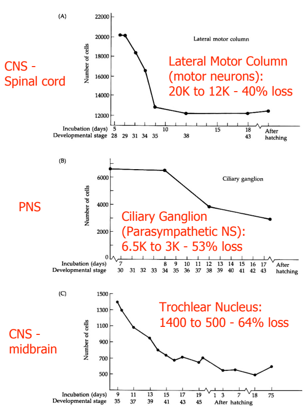
Figure 2 | En-2 is internalized in the growth cone and guidance depends on internalization. a–c, Live retinal growth cones following 5 min exposure to

Engrailed-2 is secreted and can be internalized by growth cones.

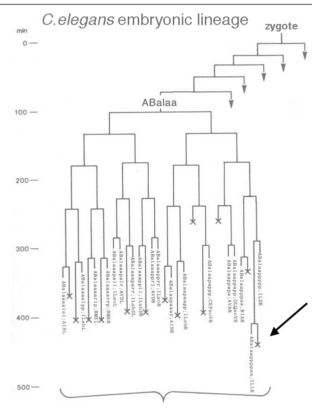
Internalization is essential for En-2's guidance function

Neuronal Development

Neuronal cell death is a normal part of development in all nervous systems.



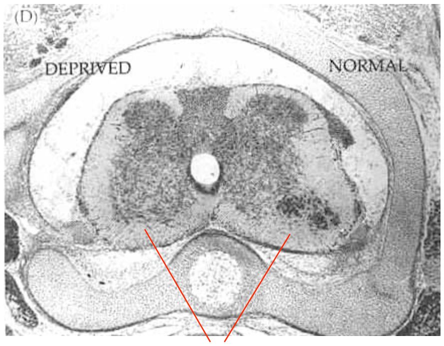
Many neurons in the vertebrate nervous system (CNS and PNS) normally die during development



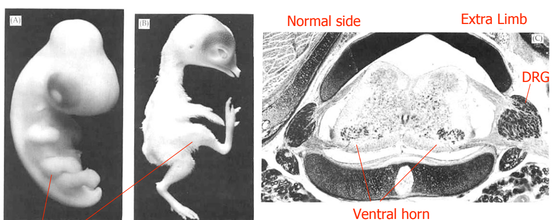
Many cells die in neuronal lineages in simpler animals like the nematode *C. elegans.*

(x = death)

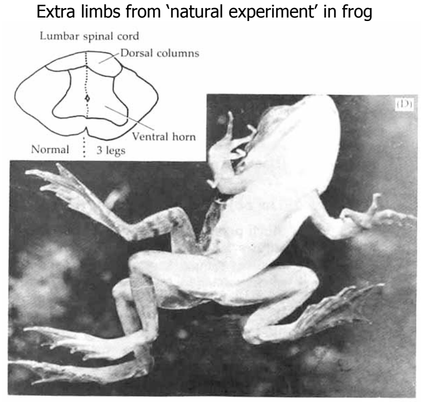
Loss of target tissue causes increased cell death among neurons



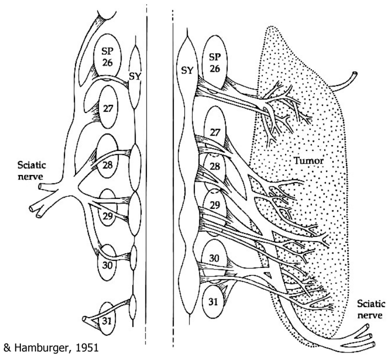
Extra target tissue supports the survival of additional neurons



Extra limbs from ‘natural experiment’ in frog



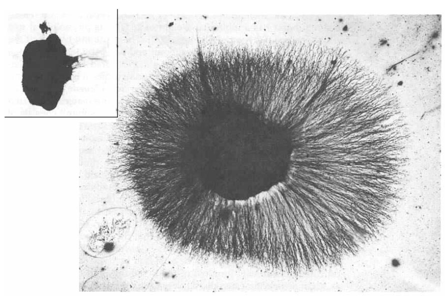
Extra tissue also supported growth and survival of neurons distant from the transplant - indicating a *diffusible* factor



Levi-Montalcini & Hamburger, 1951

Extracts of peripheral tissues support PNS neurons *in vitro*

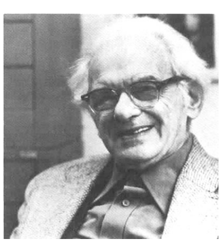
Cultured PNS without NGF



Cultured PNS with NGF

Chick 8d embryo DRG, 24 hr in culture

Discoverers of Nerve Growth Factor (NGF)

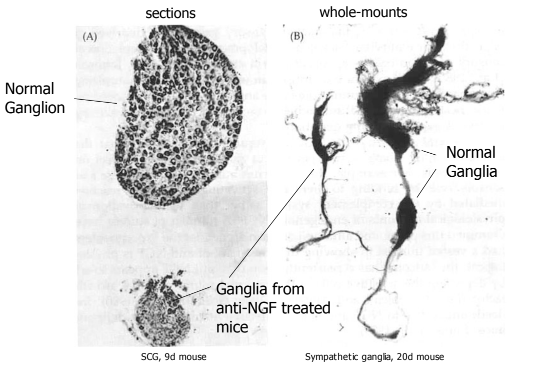


Viktor Hamburger

Rita Levi-Montalcini

Stanley Cohen and Rita Levi-Montalcini awarded Nobel Prize for their work on NGF in 1986

NGF function is essential for neuronal survival *in vivo*

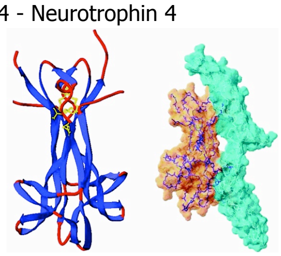


Levi-Montalcini, 1972

Neurotrophic Factors support the survival of specific types of neurons

Neurotrophins - family of NGF-related proteins
- NGF - Nerve Growth Factor
- BDNF - Brain-derived neurotrophic factor
- NT3 - Neurotrophin 3
- NT4 - Neurotrophin 4

NGF homodimer



NGF homodimer bound to part of p75 NGF Receptor

Neurotrophic Factors support the survival of specific types of neurons

Neurotrophins - family of NGF-related proteins
- NGF - Nerve Growth Factor
- BDNF - Brain-derived neurotrophic factor
- NT-3 - Neurotrophin 3
- NT-4 - Neurotrophin 4

13 kDa secreted glycoproteins (as homodimer)

Bind to receptor tyrosine kinases: TrkA, TrkB, TrkC

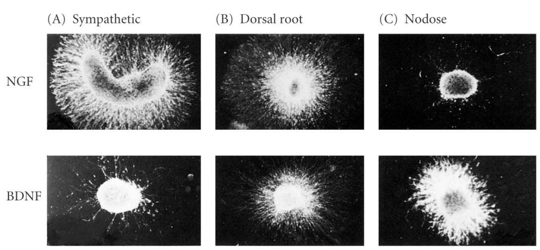
e.g., NGF receptors - TrkA
BDNF, NT-4 receptor - TrkB
NT-3 receptor - TrkC
(NT-3 also binds to TrkA, TrkB)

Neurotrophic Factors support the survival of specific types of neurons

Neurotrophins - family of NGF-related proteins
- NGF - Nerve Growth Factor
- BDNF - Brain-derived neurotrophic factor
- NT3 - Neurotrophin 3
- NT4/5 - Neurotrophin 4/5

Other Neurotrophic and Differentiation Factors:
- GDNF- Glial-derived neurotrophic factor
- CDF/LIF - Cholinergic differentiation factor/ Leukemia inhibiting factor

Figure 13.29 *Effects of NGF And BDNF On Axonal Outgrowth*



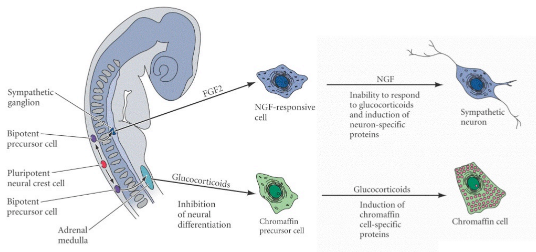
Neurotrophic Factors support the survival of specific types of neurons

Three potential functions of neurotrophic and related factors
1. *Neurotrophic* factor
2. *Chemoattractive* factor
3. *Instructive* (inductive/signaling) factor

NGF shows all three functions

Experiment showing NGF's instructive role:
NGF can convert presumptive adrenal medulla cells (would become chromaffin cells) into neurons.

Figure 13.7 Differentiation of trunk neural crest cells



Neuronal Differentiation Factors

Example: CDF/LIF

CDF is made by heart muscle cells.

Some NC-derived neurons that innervate heart initially make norepinephrine (NE).

Interaction with heart converts cells to use ACh.

CDF has other roles in development:

- LIF (immune system)
- Essential for implantation of blastocyst