Cell Death & Trophic Factors I

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Progressive Events in Nervous System Development

- proliferation
- migration
- process growth (axons & dendrites)
- synapse formation
- process arborization

Regressive Events in Nervous System Development

- programmed cell death (PCD)
- process elimination

Types of Cell Death

- Apoptosis (nuclear)
  - chromatin condenses
  - cell shrinks (pyknosis)
  - DNA fragments (cut by endonucleases into 180bp fragments)
  - membrane bound blebs form

- Necrosis (cytoplasmic)
  - cell swells
  - lysosomal enzymes breakdown organelles
  - cell membrane breaks apart

Types of Cell Death in the Nervous System

- Trauma or vascular insult induced cell death is typically via necrosis.

- Normal developmental cell death (= programmed cell death or PCD) is typically via apoptosis.

Methods used to Detect Apoptotic Cells Histologically

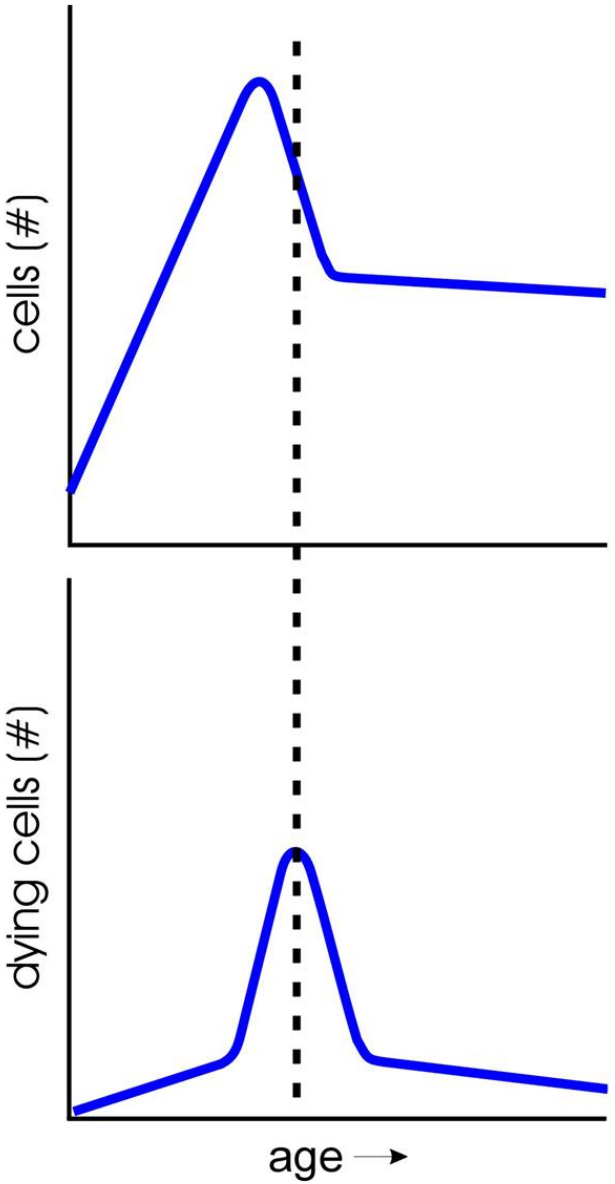
- Basic dyes – stain dying cells darkly compared to healthy cells (pyknotic cells)
- Terminal transferase UTP Nick End Labeling (TUNEL) – labels fragmented DNA
- Caspase immunohistochemistry – labels cells with activated caspase

Programmed Cell Death

Hamburger showed…

- Normally, there is a ~50% reduction in the number of dorsal root ganglion neurons and spinal motor neurons late in development.

- This decrease in cell number correlates with a period of massive cell death.



Programmed Cell Death

- Interneurons and projection neurons die.

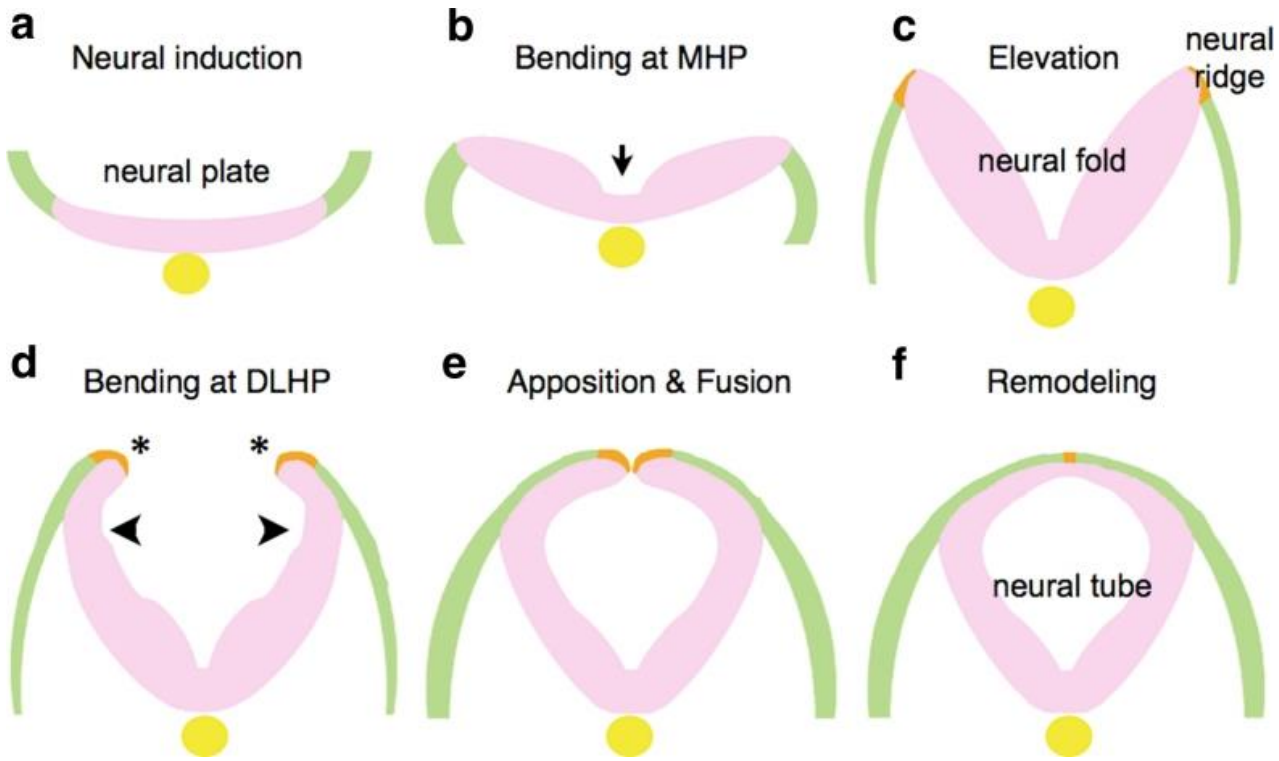
- Glia die.
  ~50% of optic nerve cells die (only glia)

- Most neuronal populations exhibit PCD but the magnitude varies:

rohon-beard cells	100%	cell loss
mesencephalic nucleus	85%	
retinal ganglion cells	50%	
cochlear nuclei	15%	
locus coeruleus	0%	?
typical	50%	

Morphogenetic Cell Death (e.g. during Neural Tube Closure)

- Cells die at the junction between neural and non-neural ectoderm during tube closure, which separates the tube from the surface ectoderm.
- This death is required for complete tube closure.



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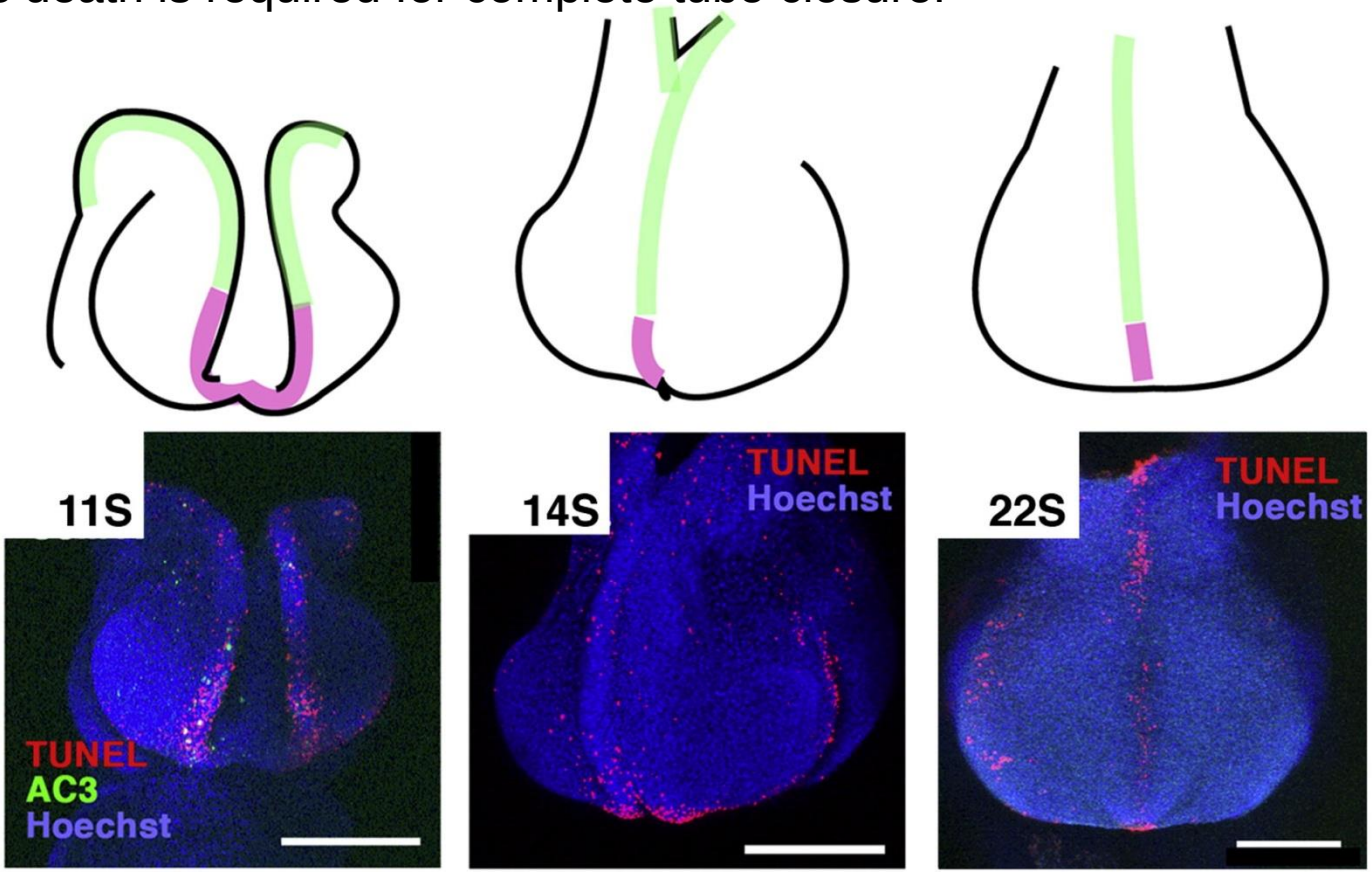


Yamaguchi & Miura, 2013

Two Waves of Cell Death in Developing Nervous System

- Early cell death:
  - during the period in which cells are dividing and becoming postmitotic
  - 15% of the DRG neurons die within 2 hrs of birth
  - early cell death in retina is required for axons to exit eye

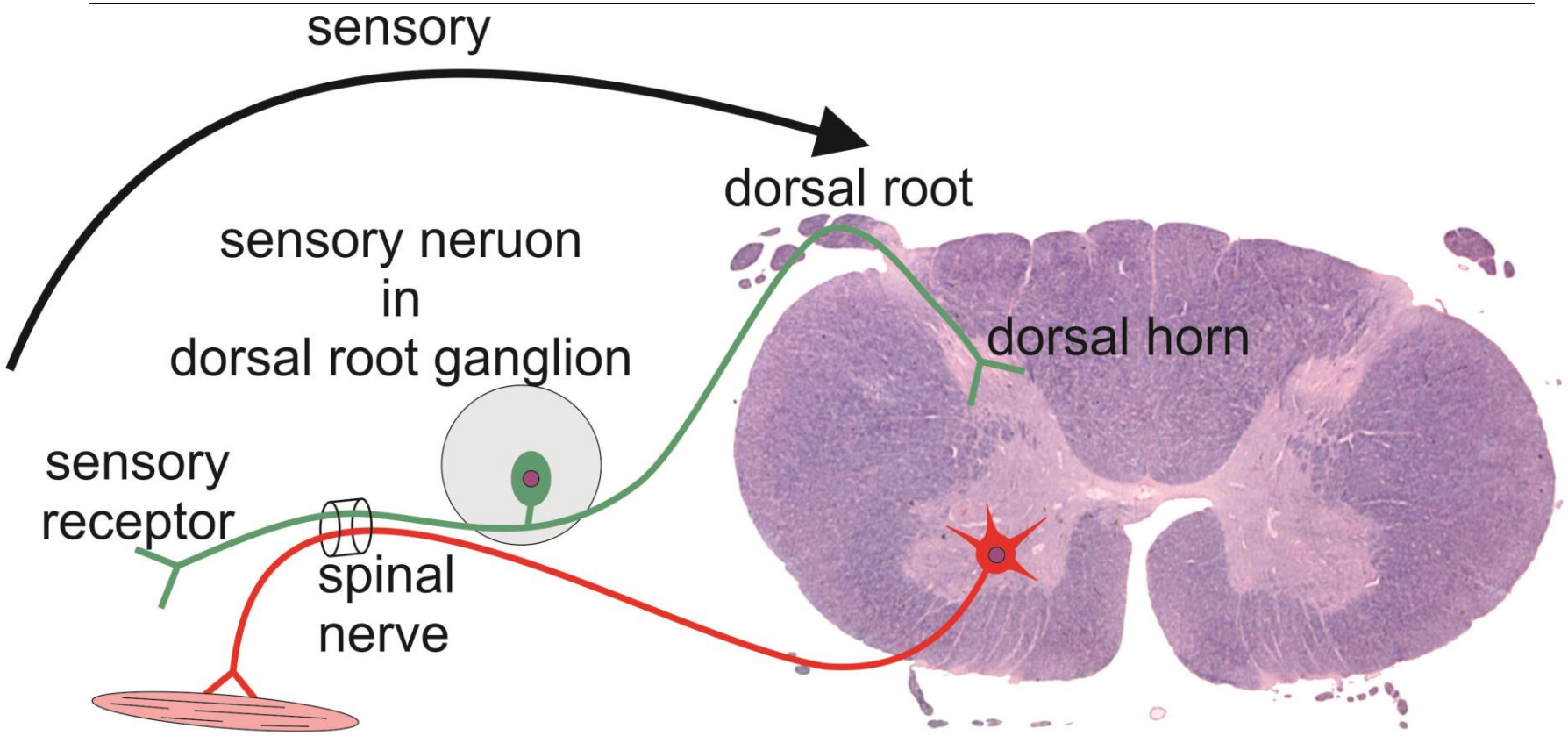
    (triggered by proneurotrophin & $p75^{NTR}$)

- Late cell death:
  - during the period in which axons arborize and synapses are forming
  - typically ~50% of the neurons die in this wave of death
  - this death is most studied

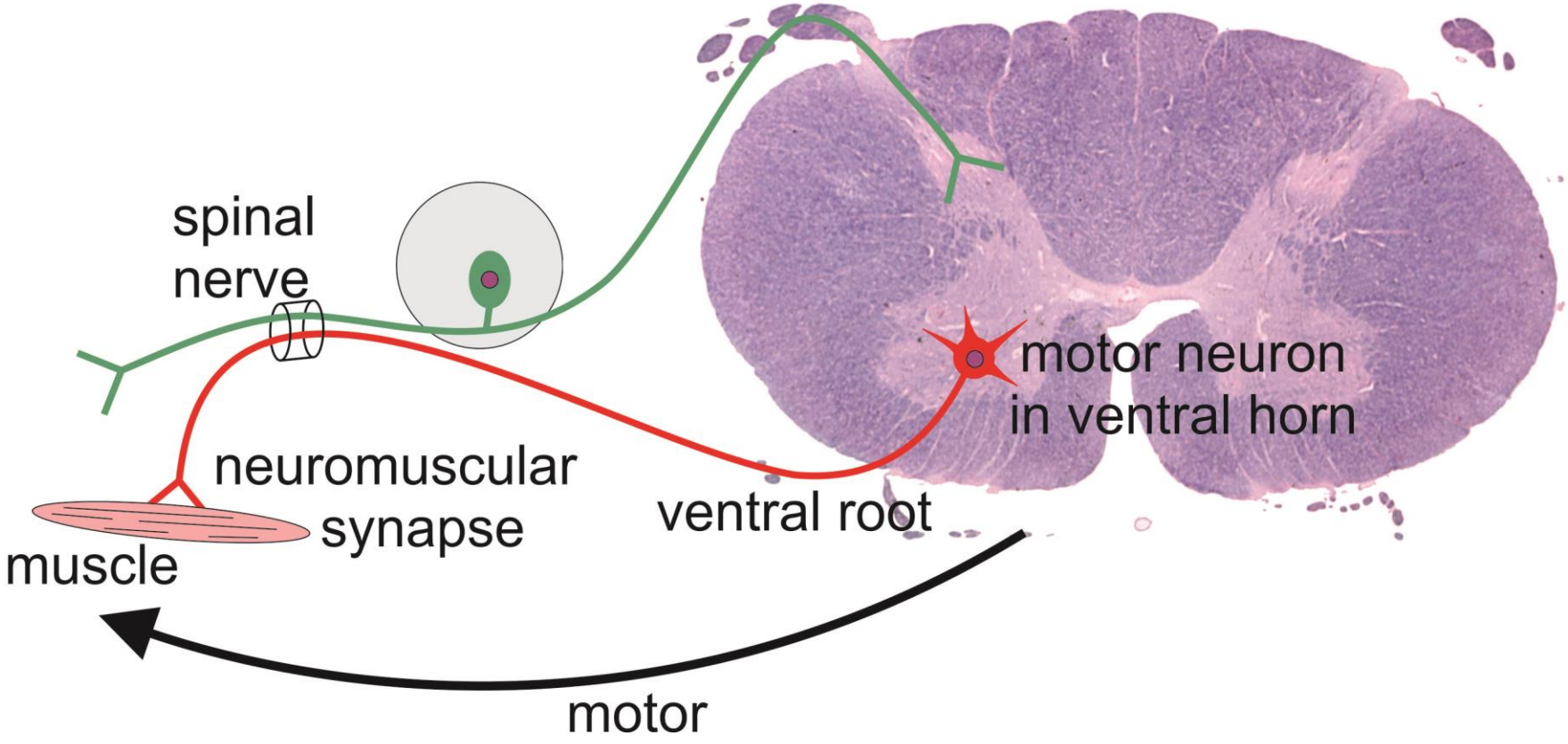
Hey!

About half the neurons generated die during normal development!

Sensory Input to the Spinal Cord



Motor Output from the Spinal Cord



Evidence for Axon-Target Cell Interactions Regulating PCD

- Shorey (1909) removed a chick embryo limb bud prior to the ingrowth of axons and observed fewer DRG neurons late in development.

- Detwiler (1920) grafted an additional limb onto a chick embryo early in development and observed more DRG neurons late in development

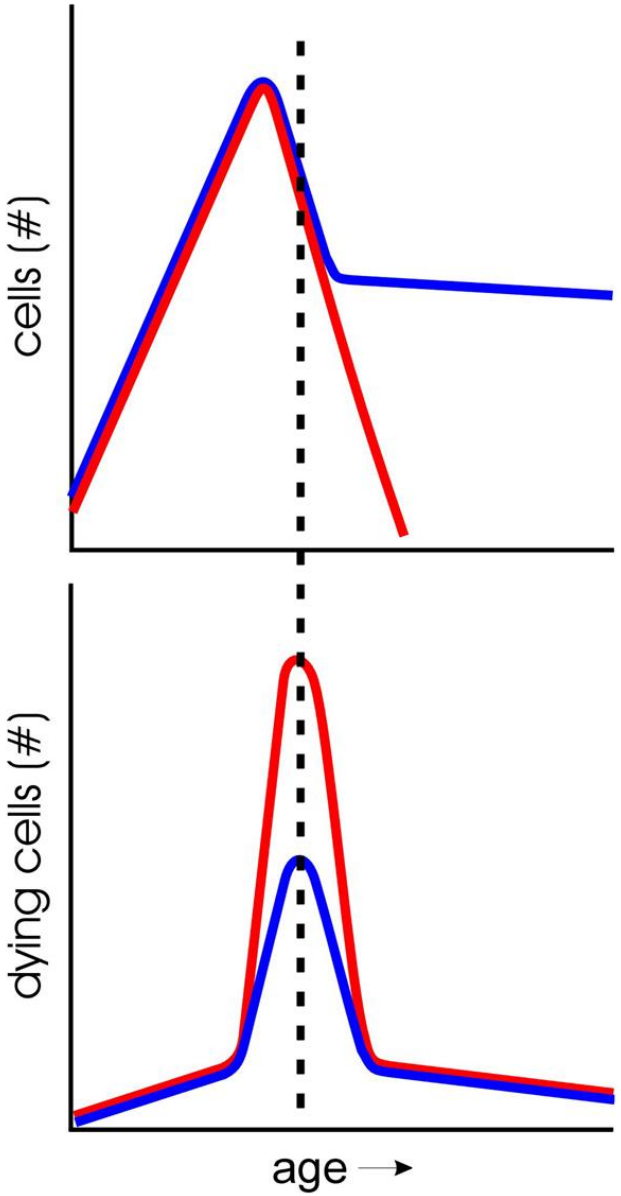
It was assumed that the target of the axons in some way altered cell division and thus the genesis of the neurons.

Evidence for Axon-Target Cell Interactions Regulating PCD

Hamburger showed…

- Experimental augmentation of the limb size altered the ratio of dying & surviving cells in DRG and spinal motor neurons. (e.g. complete limb removal)

- The presence or absence of the limb had minimal effect on cell genesis (shown using thymidine labeling).



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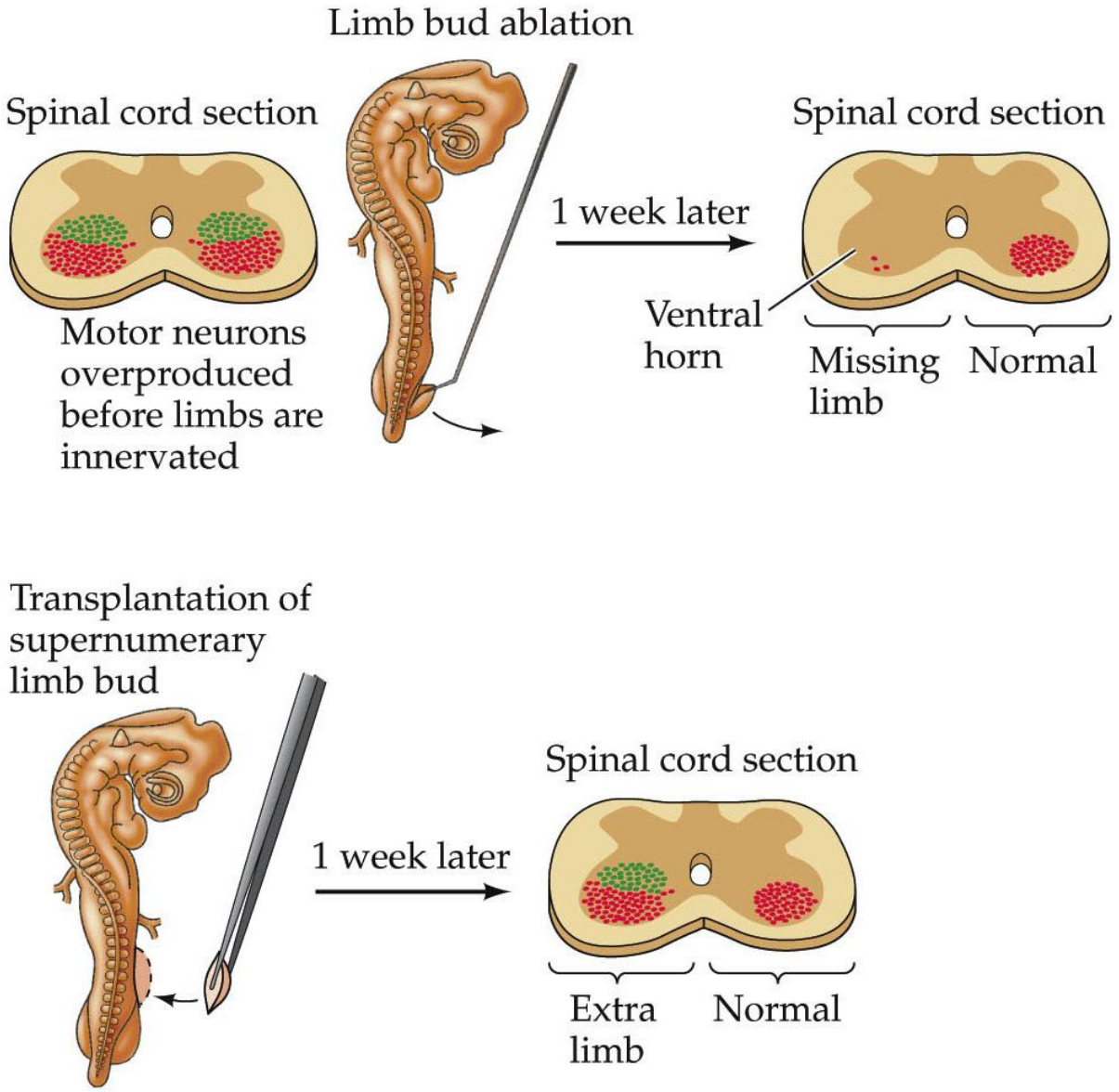
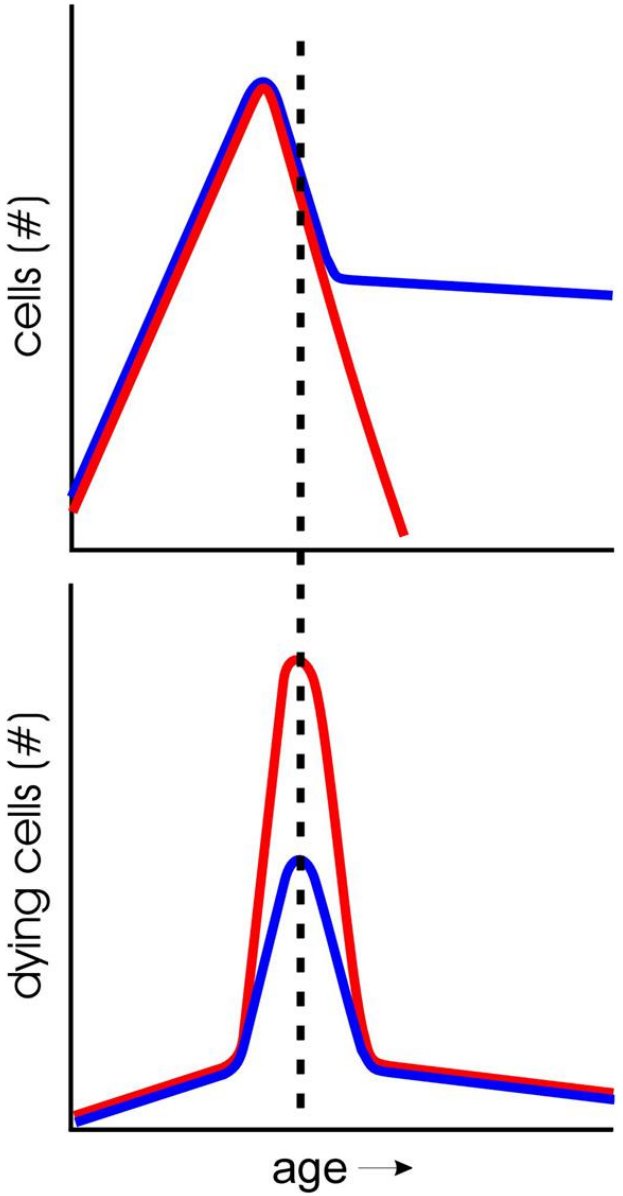


Critical Period of Development

- The target has the greatest influence on cell death/survival during a discrete period of development, a critical period.
- This period is when the neurons are forming synaptic connections.
- In the adult, most neurons are not dependent on synaptic connections for survival.



Hey!

Most normal developmental cell death takes place during a discrete period of development!

This developmental period for a given neuronal population correlates with the time in which they are forming synaptic connections!

The size of the target for a given neuronal population is proportional to survival & death of those cells!

What does programmed cell death accomplish?

- Eliminates defective neurons -- probably not!
  - Motor neurons that die appear normal by EM, express choline acetyltransferase and acetylcholinesterase, receive synapses, have axons that reach muscle.

  - Most cells can be rescued from death by administering additional neurotrophin.

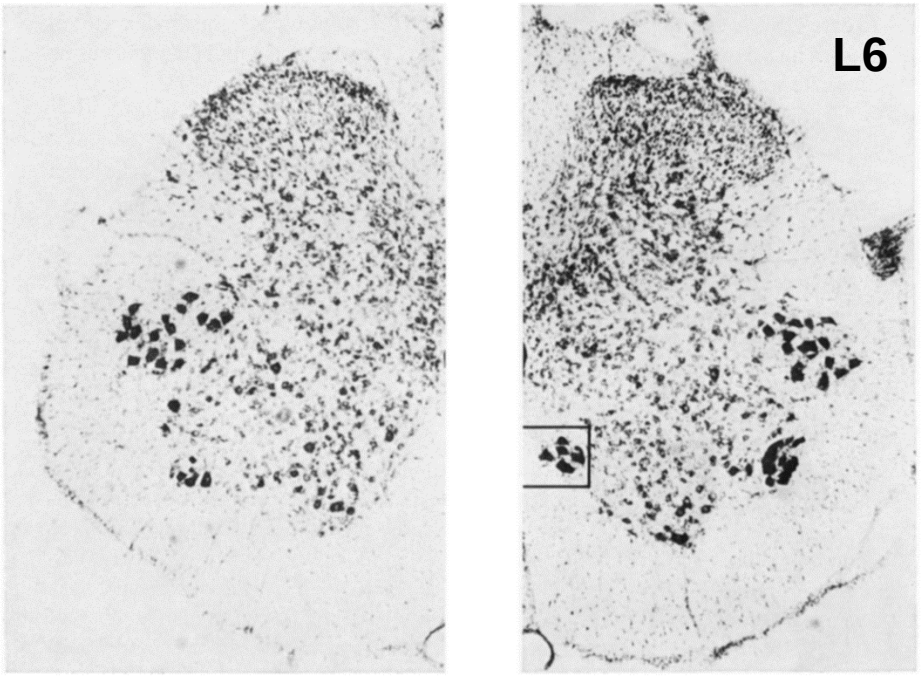
What does programmed cell death accomplish?

- Eliminates extra neurons - systems matching
  - i.e. results in 'optimal' number of each cell type

Systems Matching by Cell Death

- Spinal motor nuclei that innervate the genitalia are larger in males than in females.

- They start with the same number of cells, but more cells are eliminated by cell death in females.



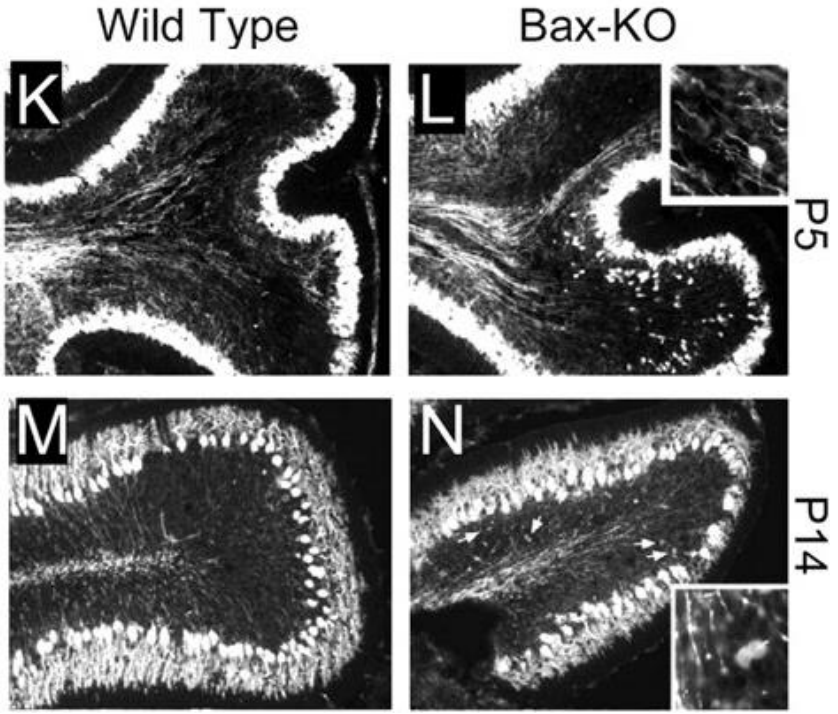
female male (rats)

spinal nucleus of the bulbocavernosus muscle

Breedlove & Arnold 1981

What does programmed cell death accomplish?

- Eliminates neurons that migrated incompletely or incorrectly.
  - Blocking cell death by knock-out of Bax resulted in persistent misplaced Purkinje cells (calbindin+).



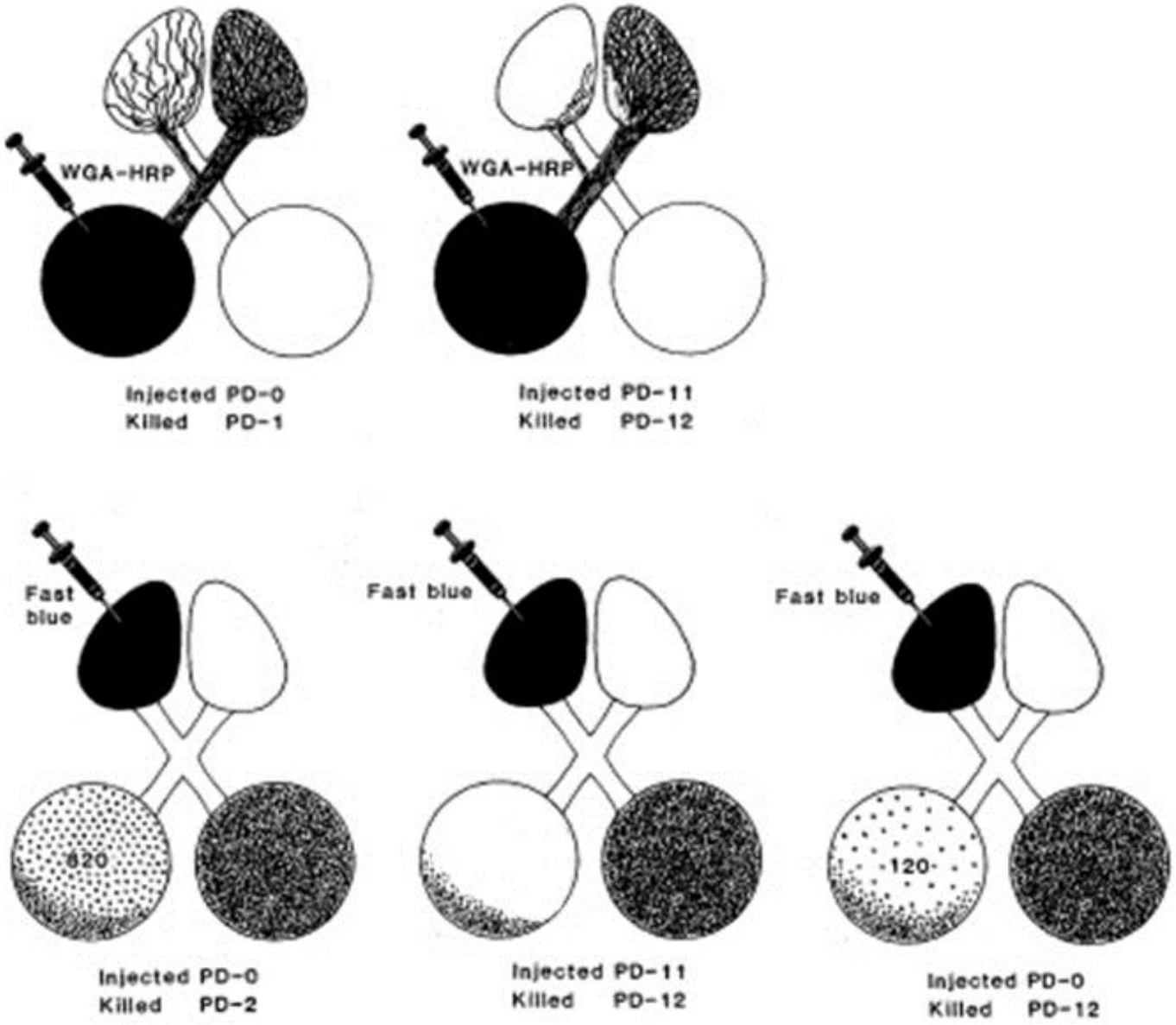
(Jung AR et al. 2008)

What does programmed cell death accomplish?

- Eliminates neurons with inappropriate connections (i.e. refinement)
  - Developing retinal ganglion cells transiently project to:
    opposite eye
    wrong side of brain
    wrong nuclei
    wrong topographic position within target

    These errors are partially corrected by cell death.

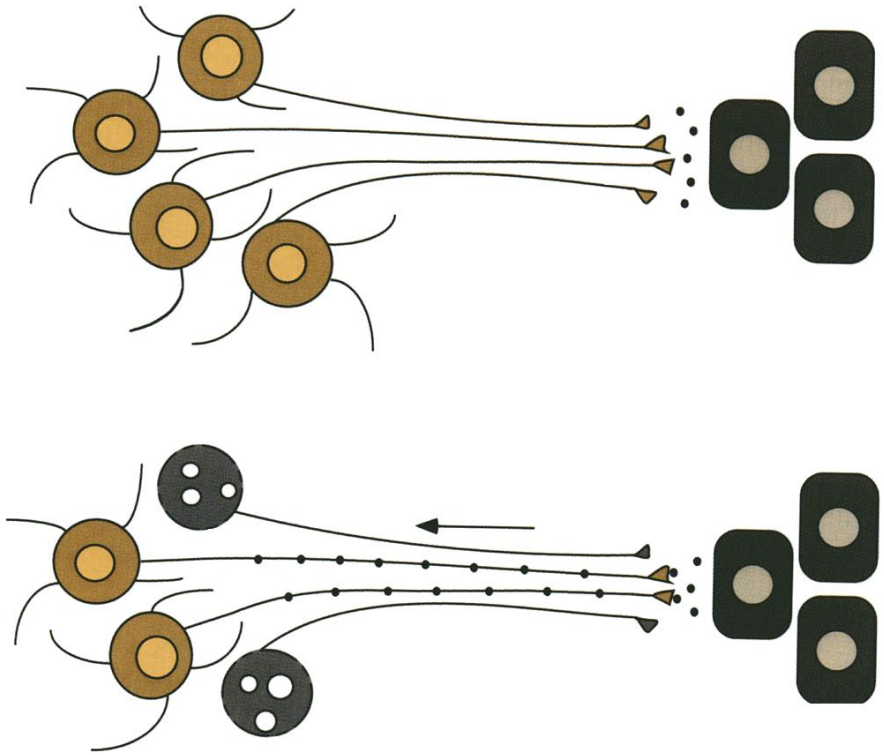
Refinement of Connections by Cell Death



Trophic Theory of Cell Survival and Death

- Thus, the number of neurons in a given population that live is proportional to the size of the target field innervated by the axons from that population.

- This suggests that the axons compete for a cell survival factor (trophic factor or neurotrophin) obtained from their target.



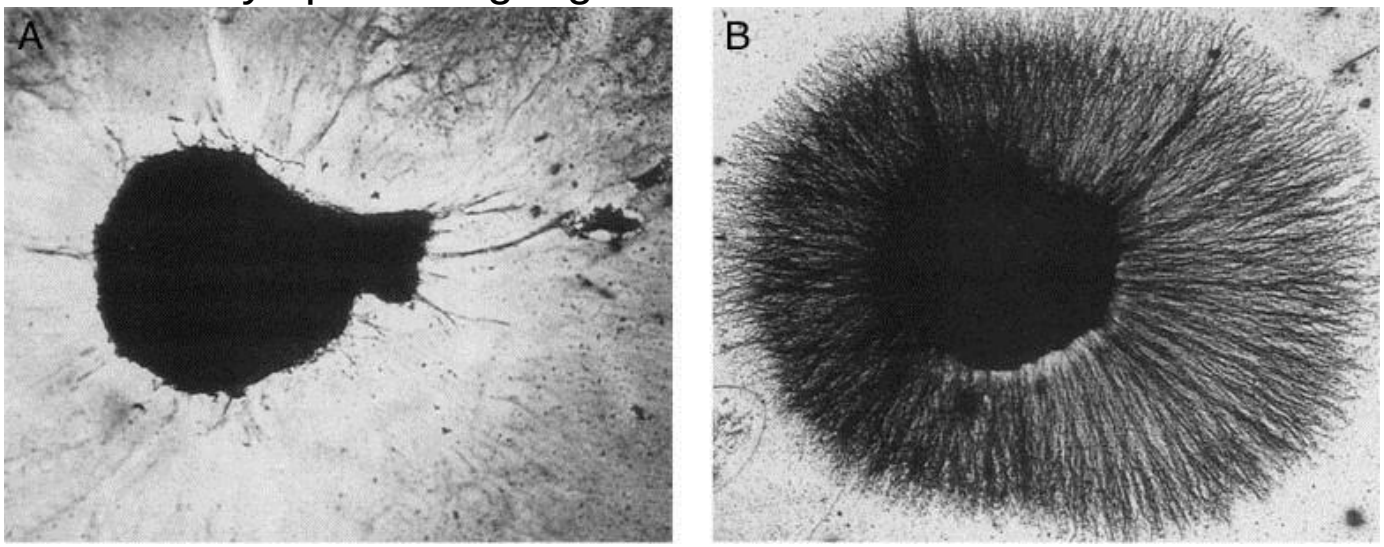
What is competed for? … a trophic factor.

- Nerve Growth Factor (NGF) was the first neurotrophin identified and is the best understood.
  (Hamburger, Cohen, Levi-Montalcini)

- NGF acts on sympathetic and certain sensory ganglion neurons in the PNS and cholinergic neurons in the CNS.

- NGF is expressed by the target cells of these neurons.

Cultured sympathetic ganglia

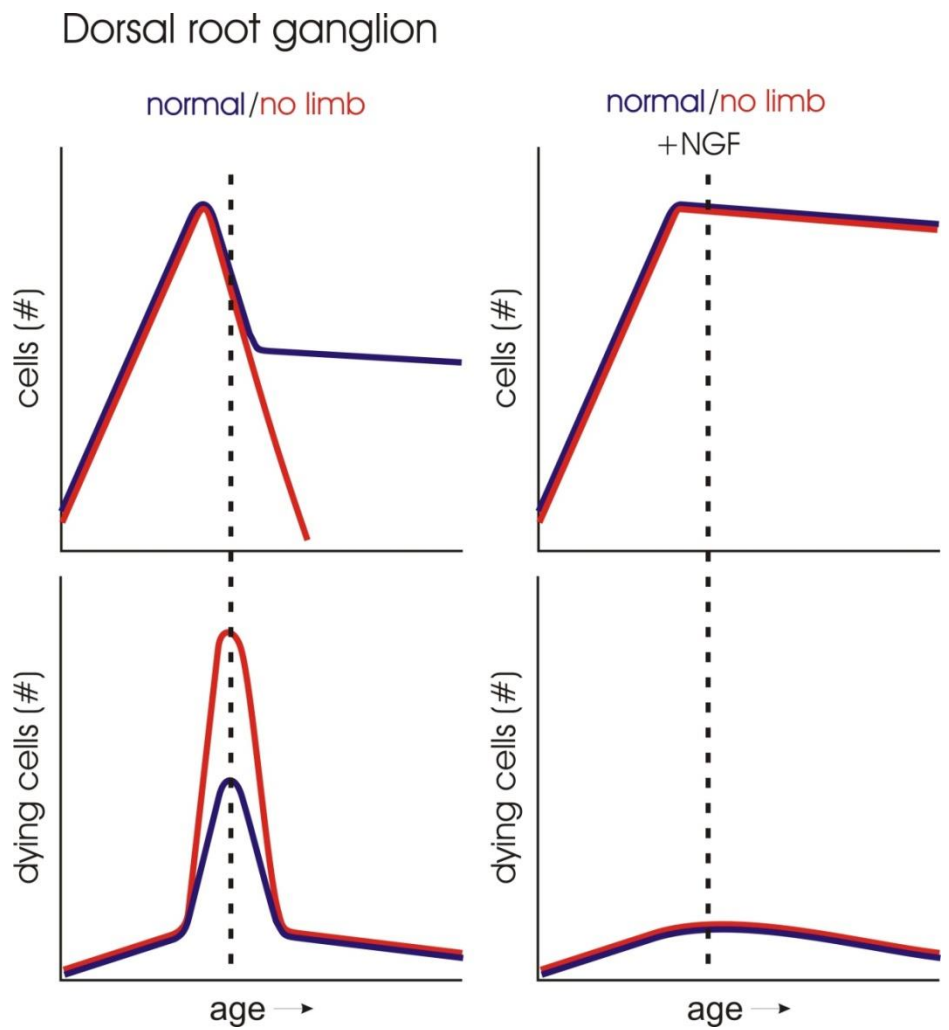


- NGF

+ NGF

What is competed for? … a trophic factor.

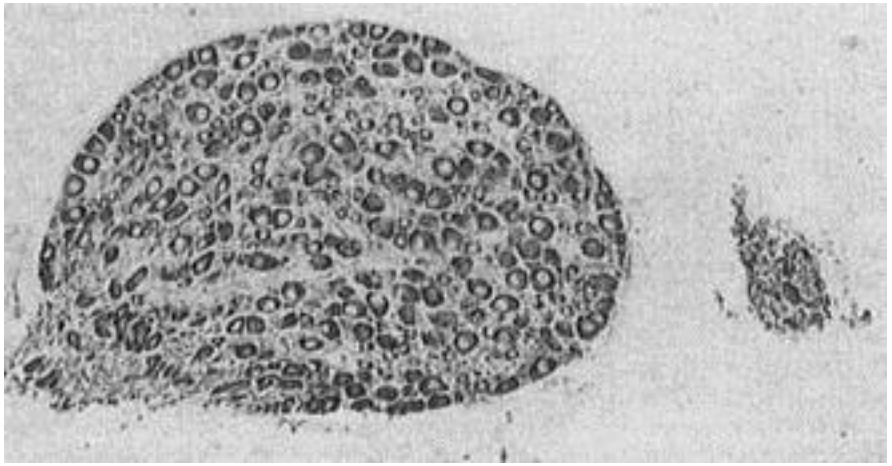
- Neurons can be rescued from normal or induced cell death by administration of the appropriate neurotrophin.



What is competed for? … a trophic factor.

- Newborn mice were injected daily with antibodies to NGF. Sympathetic ganglion were almost eliminated in these animals.

Superior cervical sympathetic ganglia



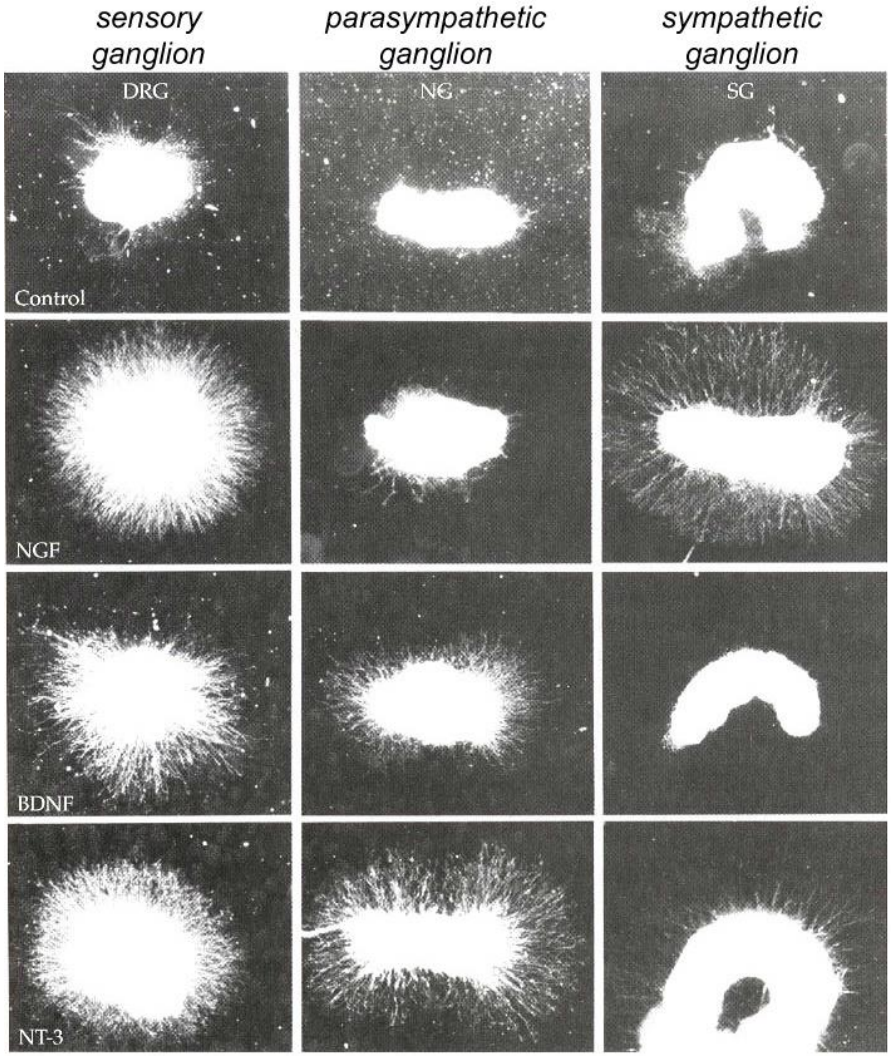
normal anti-NGF treated

What is competed for? … a trophic factor.

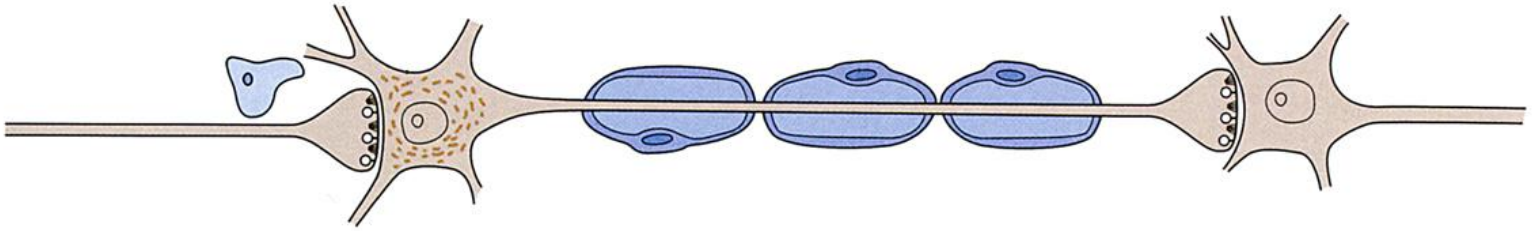
- NGF is a member of a family of neural trophic factors, the neurotrophins.

- Multiple neurotrophins have been identified:
  - nerve growth factor (NGF)
  - brain derived neurotrophic factor (BDNF)
  - neurotrophin 3 (NT3)
  - neurotrophin 4/5 (NT4/5)
  - ciliary neurotrophic factor (CNTF)
  - glial derived neurotrophic factor (GDNF)

- Different neurons respond to different neurotrophins.



Hey!

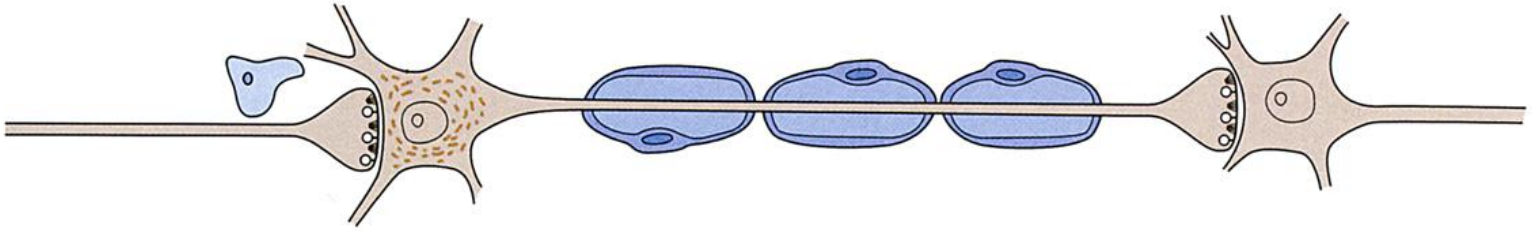


Neurotrophins are cell survival factors that neurons get from their target cells!

There is a family of neurotrophins, and different types of neurons are sensitive to different neurotrophins!

Cells that receive too little of the appropriate neurotrophin die!

Cell survival requires afferent input .



- Developing lateral geniculate nucleus and superior colliculus neurons die in the absence of retinal ganglion cells when they reach a certain stage in development.

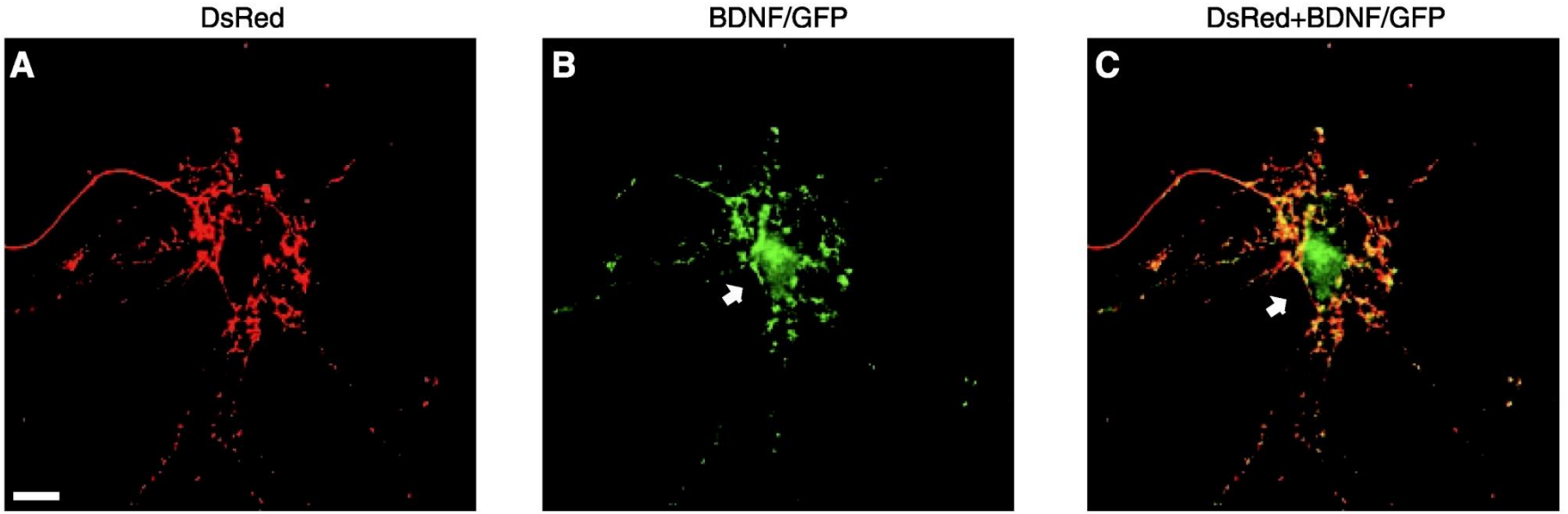
- Retinal ganglion cells express BDNF and anterogradely transport BDNF.

- Injecting an antibody to BDNF into the colliculus increased death of colliculus neurons.

$\therefore$ suggests that BDNF released by retinal ganglion cell axons acts as a survival factor for retinorecipient neurons

Cell survival requires afferent input .

- Neurotrophin can transfer from axon to the postsynaptic neuron.



Neurotrophins

- Neurons require neurotrophin for maturation, as well as for cell survival.