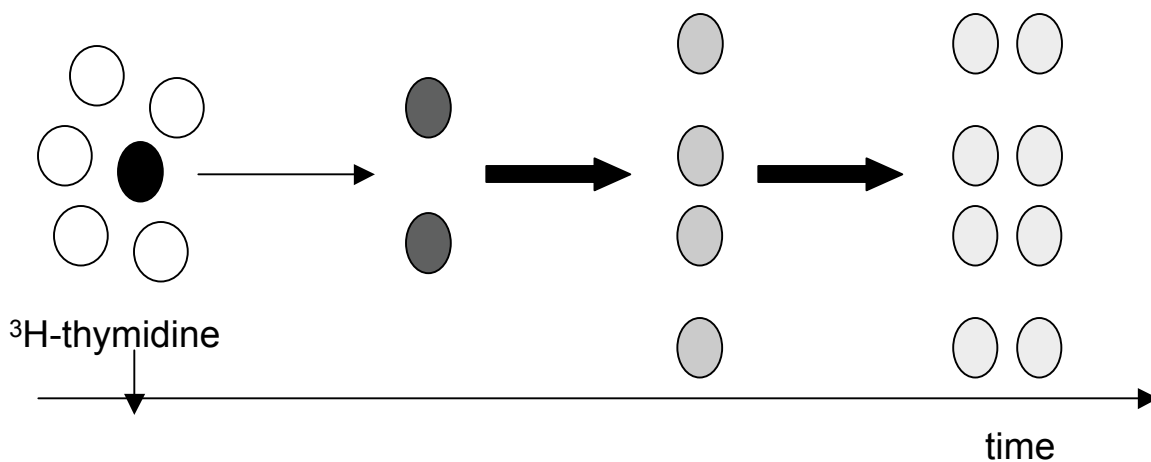


Formation of the Cortex

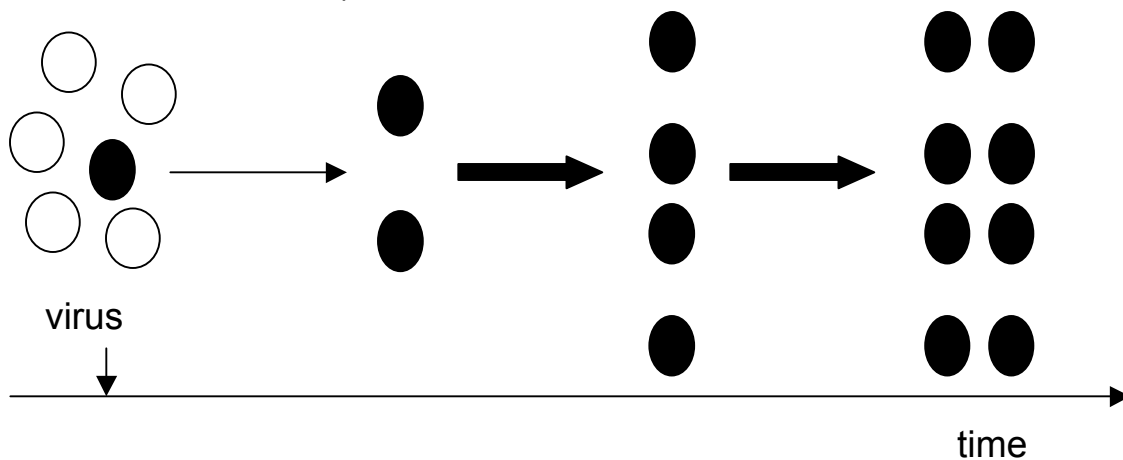
Neuronal Birthdating with ^3H -thymidine

- ^3H -thymidine is incorporated into the DNA during the S-phase (replication of DNA).
- It marks all mitotic cells
- Quantitative technique. (you can distinguish mother from daughter cells)
- If only applied in a single pulse, it will disappear in ~2-4 generations. You can detect 1st and 2nd, but then it is less clear.



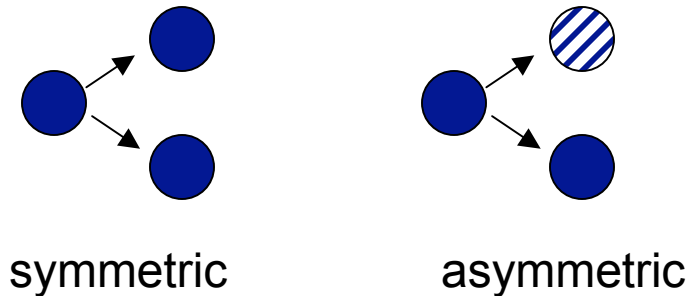
Lineage Tracing

- to look at layer specific lineage
- Use **replication incompetent retrovirus** (cannot replicate and infect other cells, but can still be incorporated into a replicating cell's DNA)
- The virus DNA will be inherited by all the daughter cells in the same amount. (it doesn't become diluted)



Formation of the Cortex

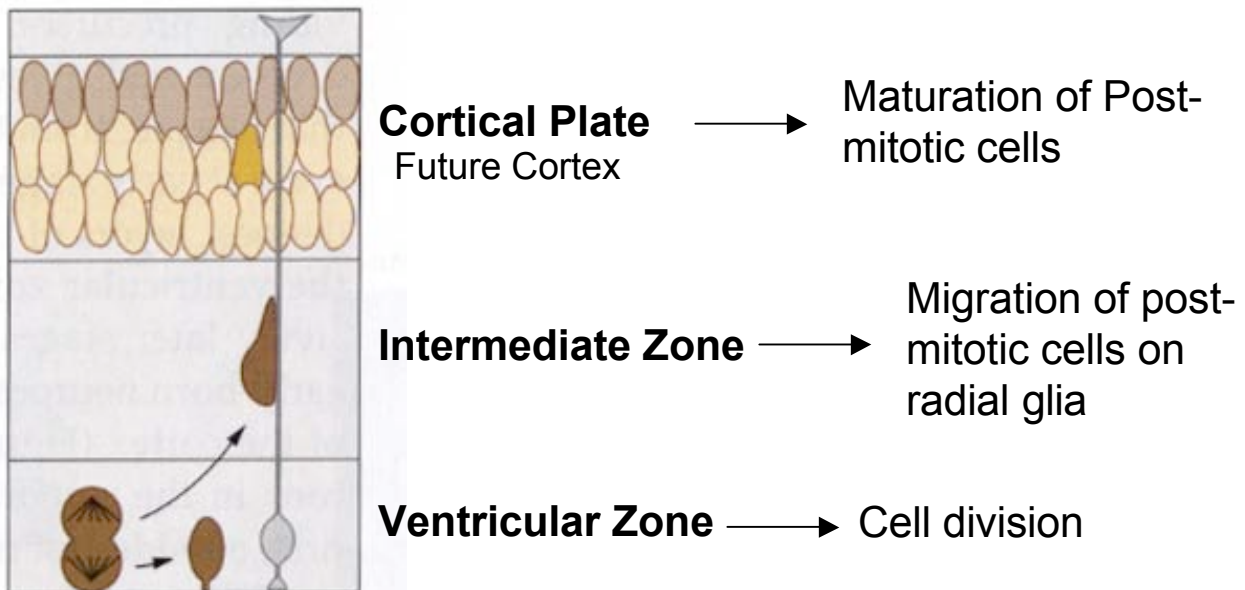
Symmetric vs Asymmetric Cell Division



The plane of division of the progenitor cells in the ventricular zone of the cerebral cortex influences their fates (fig 53.9). The reason for this is not known.

Looking at the early cortex:

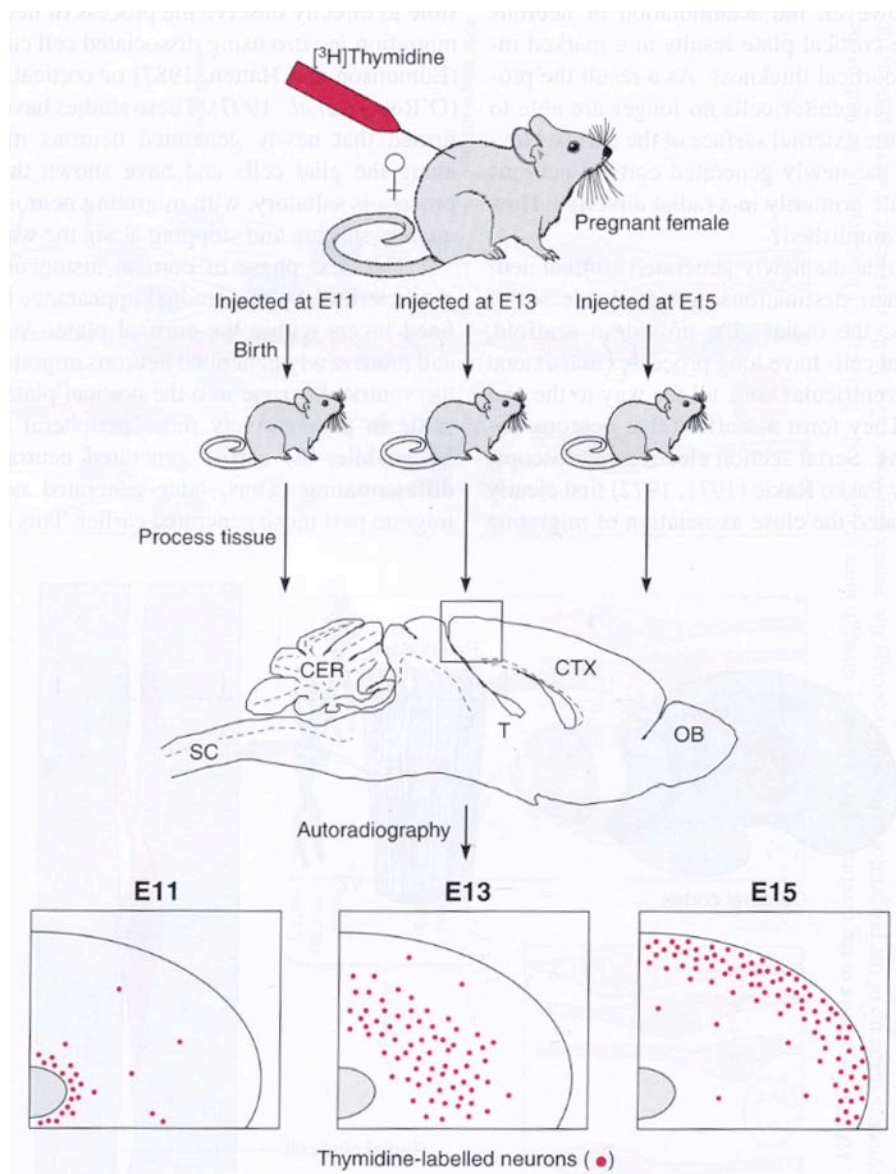
Neurons from the cerebral cortex are generated in the **ventricular zone**, which consists of epithelial layers of progenitor cells that line the lateral ventricles



Formation of the Cortex

Question: are cells in different layers specified in any order?

Experiment: Label embryos with ^3H -thymidine at different times in development.



The later the injection of thymidine, the more superficial the labeling. This suggests that there is an **inside-out** pattern of cortical neurogenesis. Each cortical layer, had a relatively restricted period of developmental time, over which it is normally generated.

Formation of the Cortex

What differentiates layer 2/3 cells from layer 6 cells?

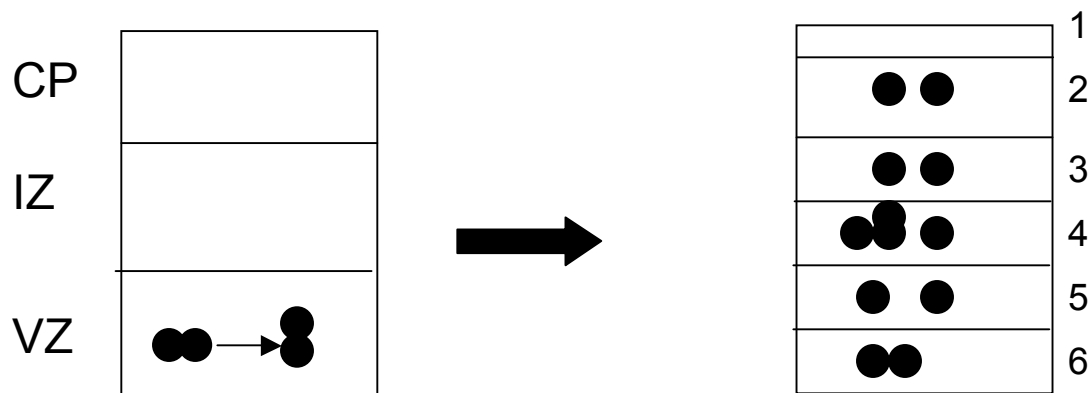
Lineage Model

There are layer specific progenitors (cells that only give origin to cells in one layer)

Instructional Model

There is a common progenitor for cells of all layers. The fate of each particular cell is determined by the environment.

Experiment: Use lineage tracing to label single neurons and follow their progenies.



Single cells could give origin to neurons in different layers. Thus, this results disqualifies the lineage model and supports the instructional model, although it doesn't prove it.

Formation of the Cortex

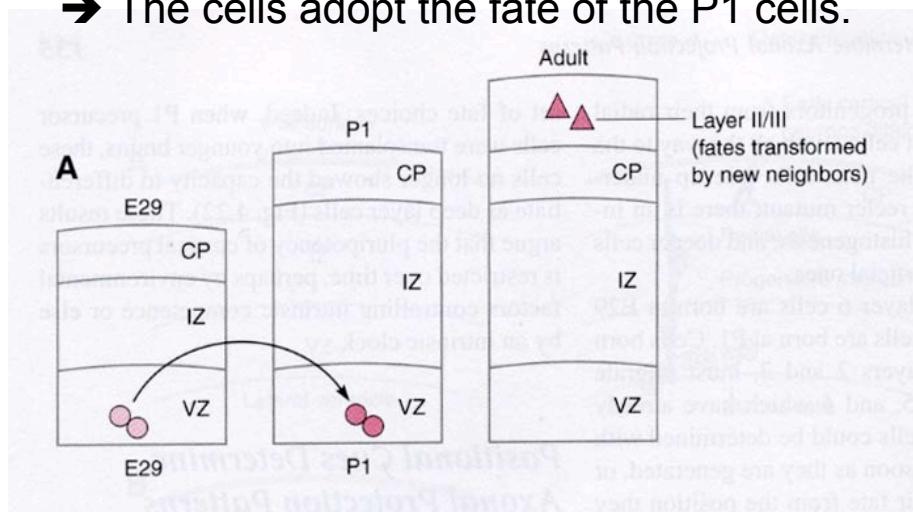
What differentiates layer 2/3 cells from layer 6 cells?

Experiments: Heterochronic transplants (McConnell)

In ferret, layer 6 cells are born at E29 and layer 2/3 cells are born at P1.

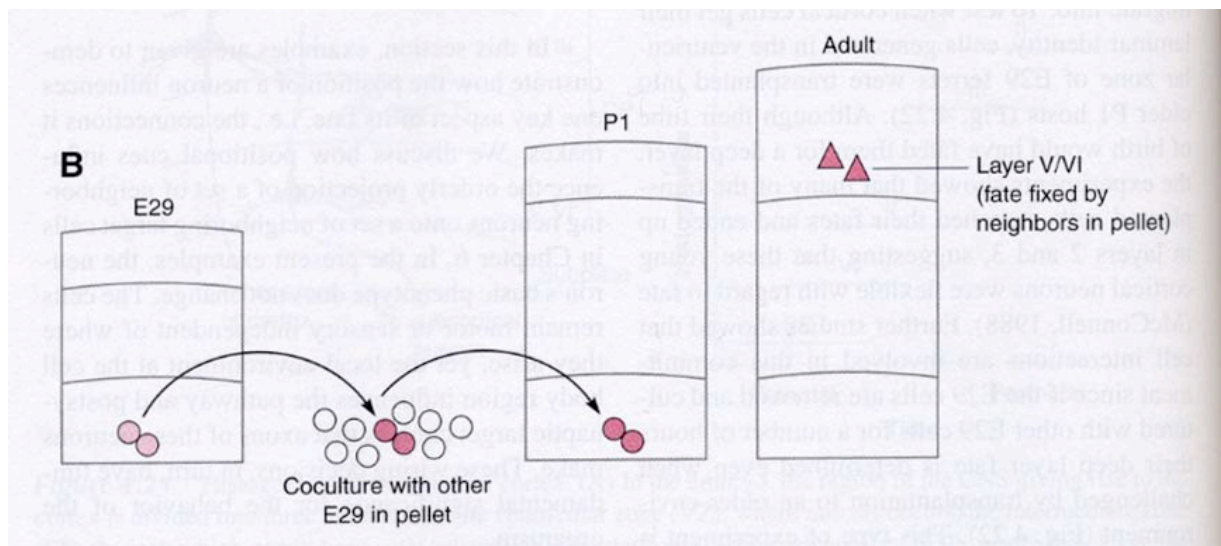
First: Label with mitotic cells with 3H-thymidine. Transplant E29 cells into P1 animal.

→ The cells adopt the fate of the P1 cells.



Second: Label and isolate E29 cells, cultivate them apart for a while and then place them in the ventricular zone of the P1 animal.

→ These cells adopt the fate that they had in the E29 animal



Formation of the Cortex

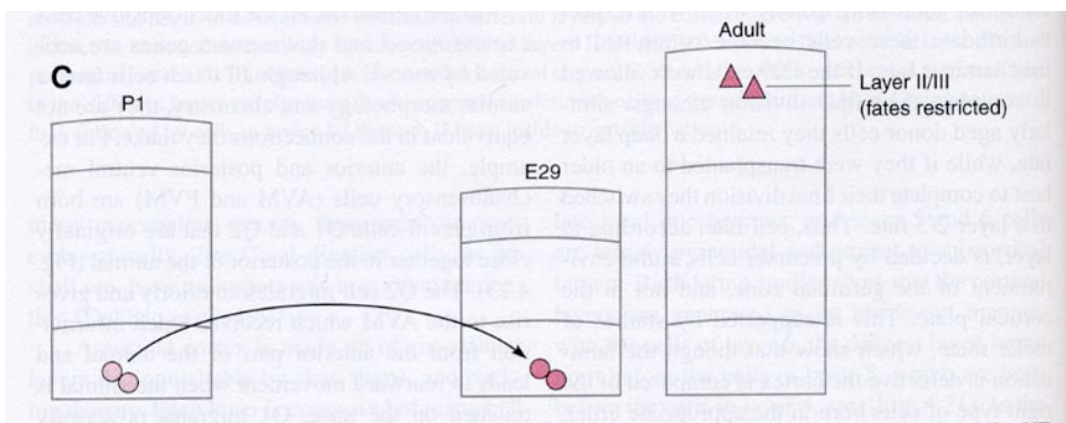
What differentiates layer 2/3 cells from layer 6 cells?

Experiment: Heterochronic transplants (McConnell)

In ferret, layer 6 cells are born in E29 and layer 2/3 cells are born in P1

Third: Label with ³H-thymidine. Transplant P1 cells into E29 animal.

→ The cells adopt the fate of the P1 cells. This suggests that pluripotency of cortical precursors could be restricted over time. (**competence** is lost)



→ **The cells seem to adopt the fate of the environment in which they were located during their last mitotic division.** (the specific phase of the cell cycle the cell is at the time of the transplantation is crucial)

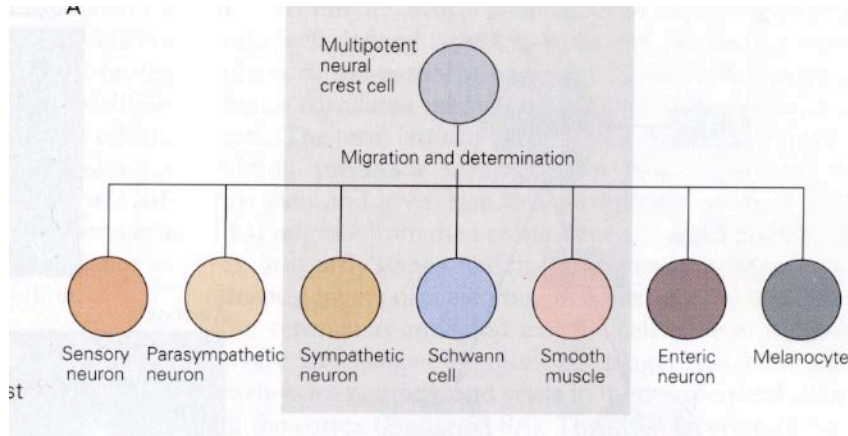


Evidence for the instructional model

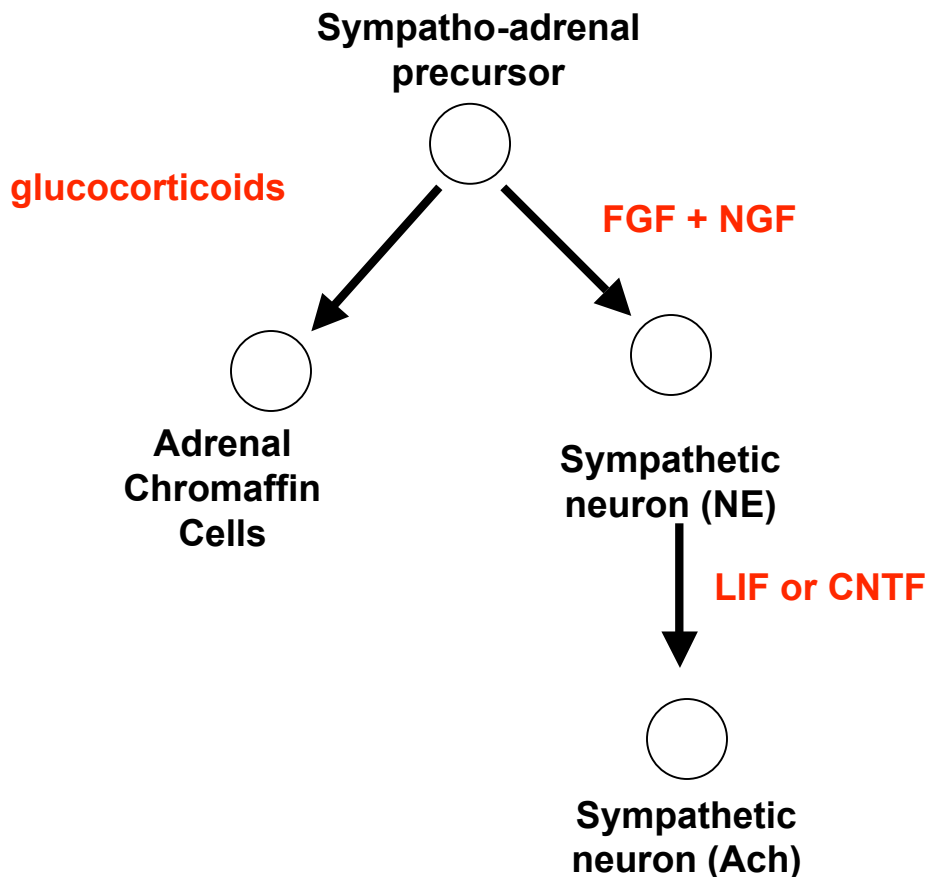
Determination of Neural Phenotype

Neural crest cells:

- They give rise to melanocytes, neurons and Schwann cells.
- The type of cell they give origin to depends on the signals that the neural crest cell receives.



In class we saw: (fig53.10)



Determination of Neural Phenotype

*Postganglionic Sympathetic neurons are adrenergic except for those innervating the exocrine sweat glands in the foot pad, which are cholinergic. **What determines whether the sympathetic neuron will be cholinergic or adrenergic?***

Experiment: Transplant the sweat gland from the foot pad from a new born rat into a site of the skin that usually receives adrenergic sympathetic innervation.



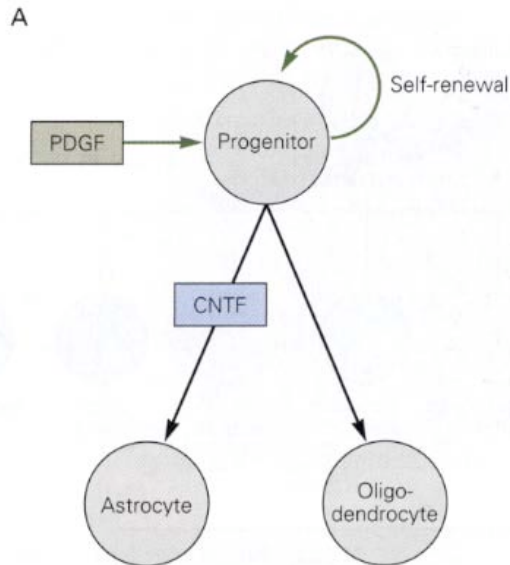
The sympathetic neuron becomes cholinergic.

→ The **target** releases some factor/s that induce cholinergic properties in the neurons that would otherwise be adrenergic. (interleukin 6 like molecules)

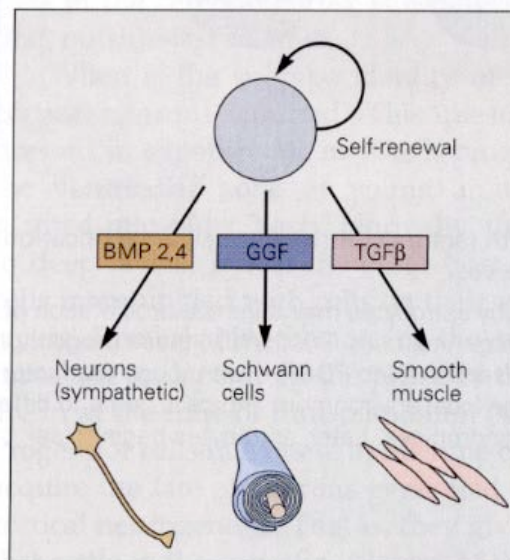
Determination of Neural Phenotype

Two other examples of local signaling

First example: Growth factors control glial cell diversification in the rat optic nerve.



A second example: Looking at the **cells from the neural crest** that migrate and form the autonomic ganglia. As the first cells differentiate into neurons, they express GGF. This acts on nearby neural crest cells to prevent neurogenesis.

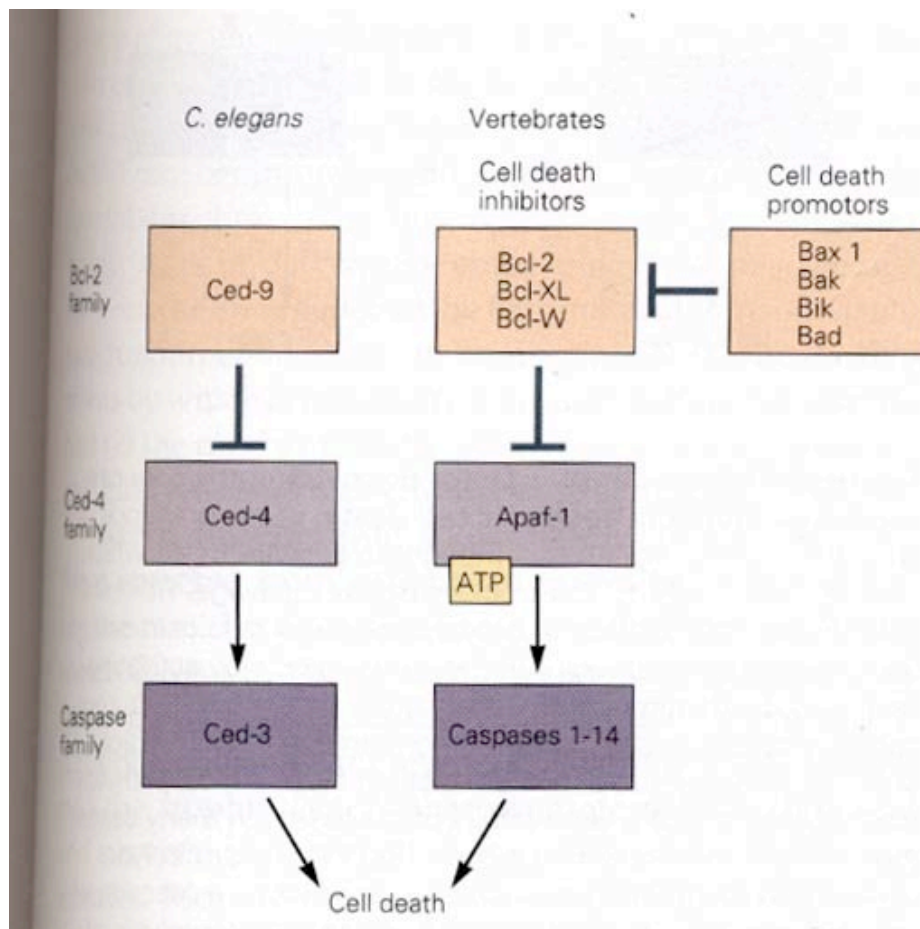


Neuronal Survival

Apoptosis vs Necrosis

Apoptosis	Necrosis
<ul style="list-style-type: none"> • Genetically programmed • Cell Shrinkage • Chromatin Condensation • Cellular Fragmentation • Phagocytosis 	<ul style="list-style-type: none"> • Trauma/Injury • Messy • Lytic • Inflammatory Response

Molecules involved in apoptosis:

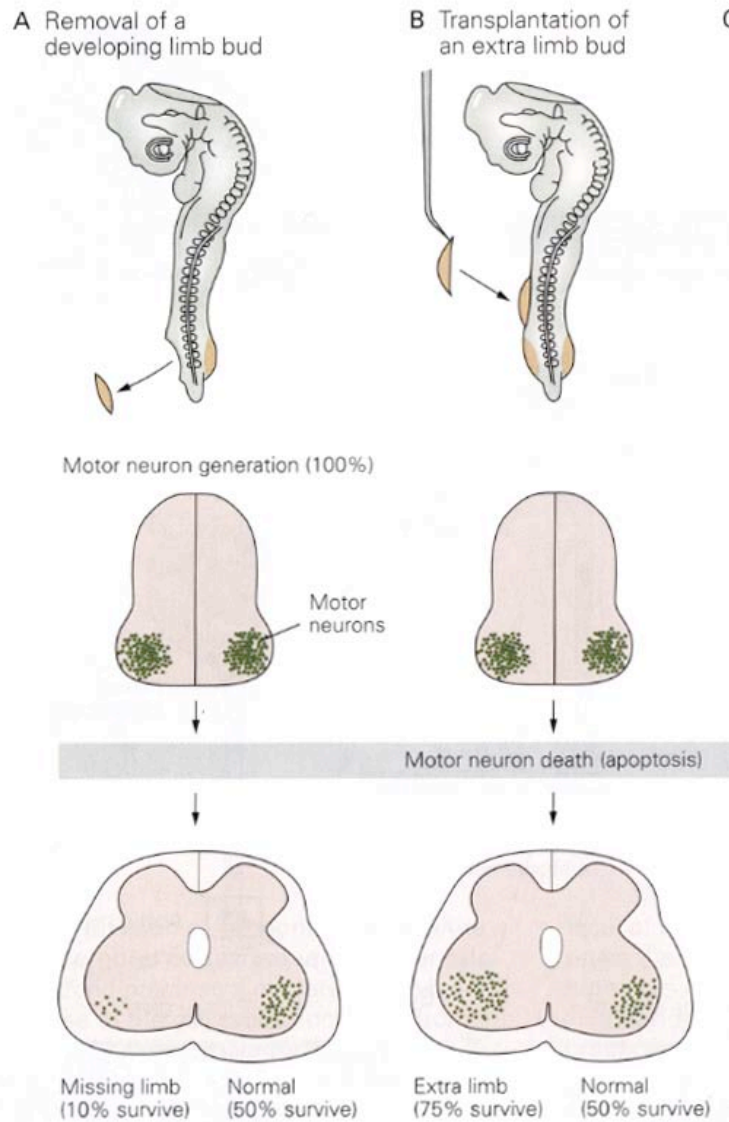


Neuronal Survival

Role of the Target

Experiment: Viktor Hamburger

Look at the effect of removing or adding a limb on motor neuron survival in the spinal cord.



Adding a limb increases neuronal survival, while removing one reduces it. → **The target is making something in limited quantities that promotes cell survival.**

Neuronal Survival

Neurotrophic Hypothesis

The experiments by Hamburger suggested the existence of factor/s released by the target cell that:

- Promote cell survival
- Are in limiting quantities
- Generates competition between the innervating cells

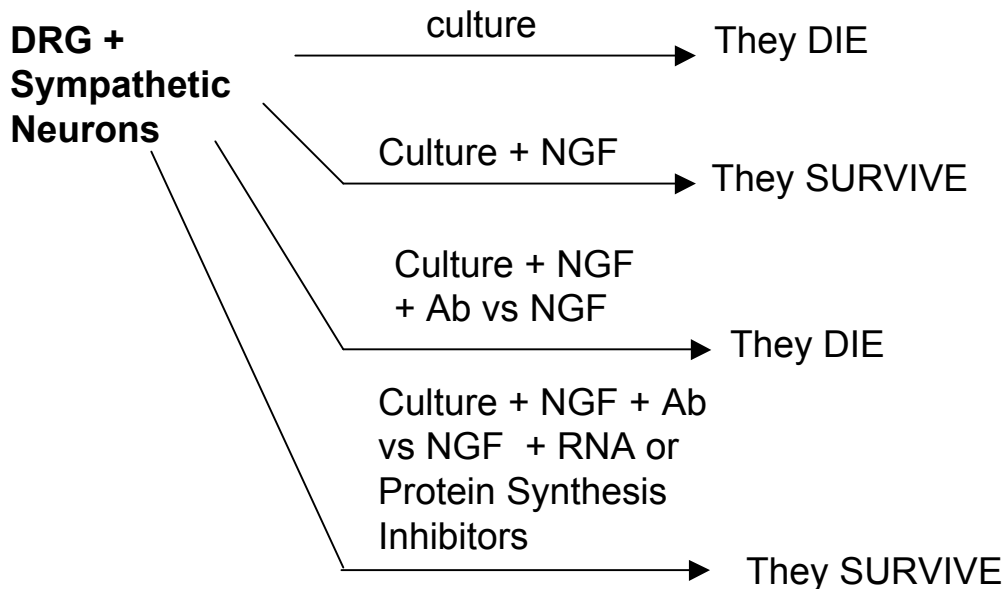
→ This is known as the **neurotrophic hypothesis**

Experiment 1: Rita Levi Montalcini/ Stan Cohen

They used mouse sarcoma tumors (the animals presented enlarged DRGs). These released some factors that promoted survival of DRGs and sympathetic neurons.

→ They isolated the factor and called it **nerve growth factor (NGF)**.

Experiment 2: Rita Levi Montalcini/ Stan Cohen

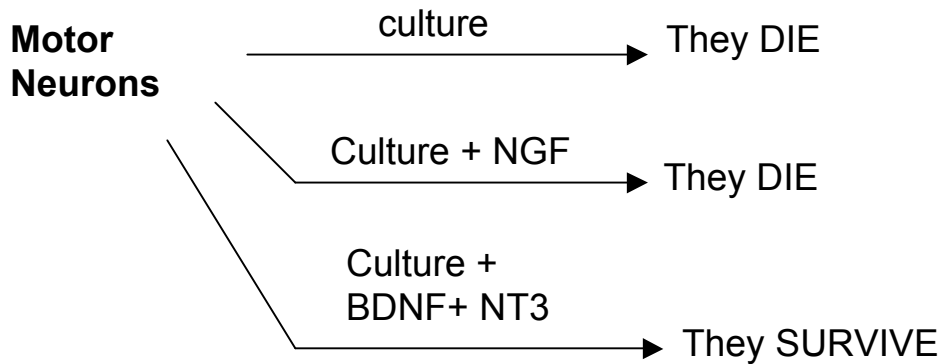


→ **Removal of the trophic factors leads to cell death.**
NGF is made by the target neuron

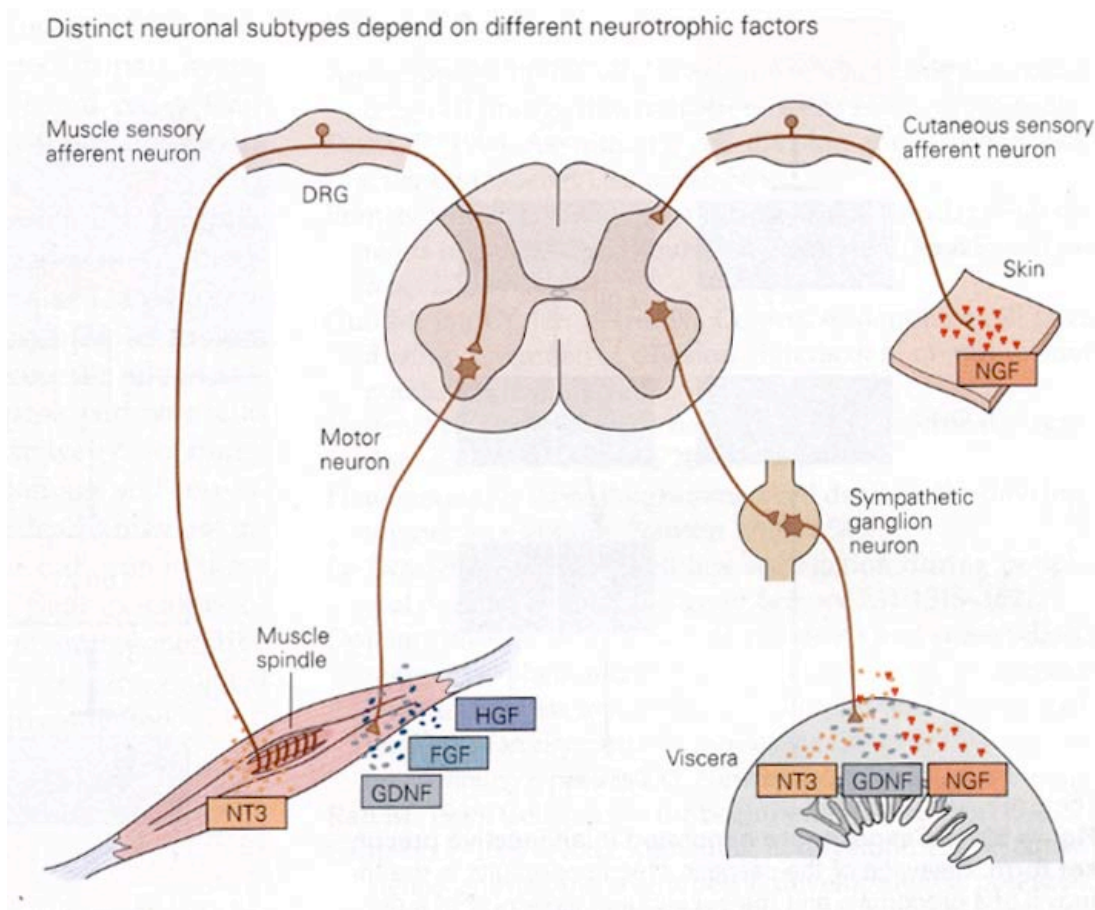
Neuronal Survival

Neurotrophic Hypothesis

Experiment 3: Looking back on Hamburger's experiments



→ Different kinds of neurons require different type of neurotrophins to promote their survival



Neuronal Survival

Neurotrophins

Trophic Factors:

There are many kinds of trophic factors:

- **Neurotrophins**

- **NGF** → TrkA Receptor
- **BDNF** → TrkB Receptor
- **NT3** → TrkC Receptor
- **NT4/5** → TrkB Receptor

They all also bind **p75** receptors. And they can bind the other receptors but with lower affinities

- **TGF-Beta Family**

- **Interleukin – 6 related cytokines**

- **FGFs**

A Neurotrophin receptor interactions

