



Nerve Cell Differentiation & Developmental Factors

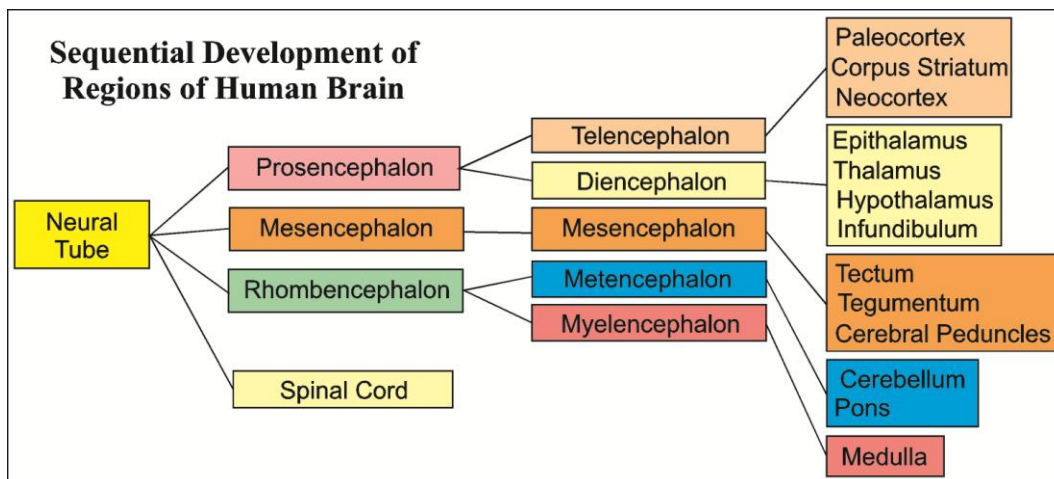
Three Stages of CNS Development

- Neurogenesis
- Axon & Dendrite Outgrowth
- Synapse Refinement (Not covered)

Brain & Spinal Cord Development

3 Brain regions are evident at 3½ wks:

- Prosencephalon (forebrain), will give rise to telencephalon and diencephalon
- Mesencephalon (midbrain),
- Rhombencephalon (hindbrain), will give rise to metencephalon and myelencephalon



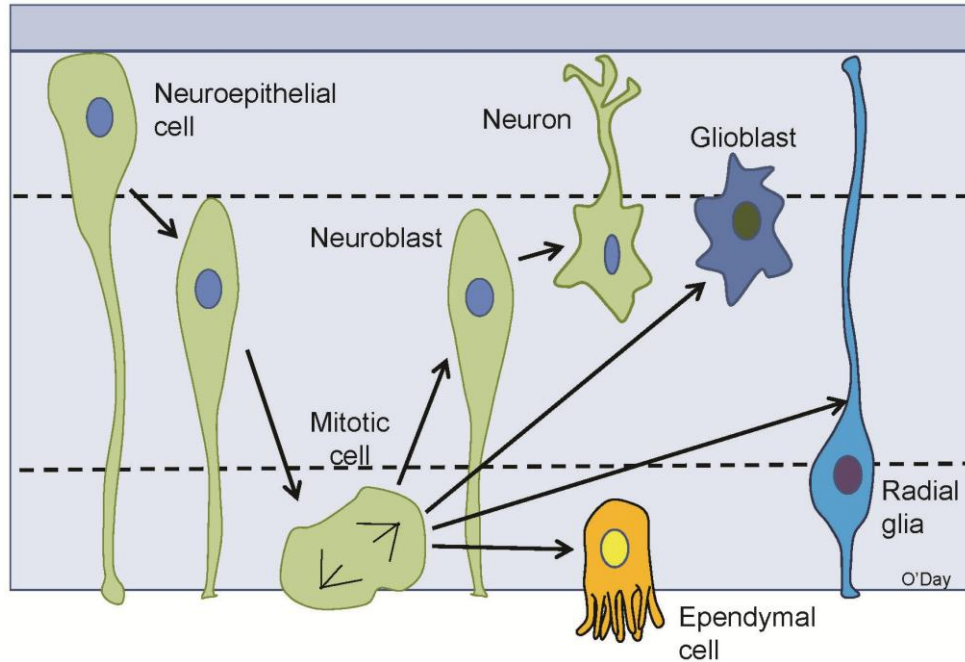
Two Main Cell Types: Neurons (Nerve cells) & Glia

- 1×10^{11} neurons in CNS: more neurons in embryo than adult!
- Glia serve supportive function
- More recently glia shown serve other critical roles: intercellular communication with neurons
- Glia outnumber neurons; 1×10^{12} in CNS
- Outgrowths for both occur at "Growth Cones"

Stratification of the Neural Tube

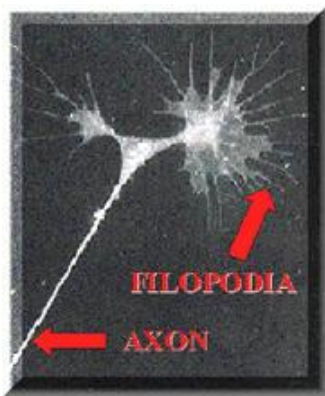
- Neuroepithelium of neural tube is originally one cell layer thick
- Neuroepithelial cells of neural tube are Stem Cells
- Neuroepithelial stem cells divide to form Neuroblasts (stem cells committed to neuron formation) and glioblasts (stem cells committed to glial cell formation)
- Neuroblasts & glioblasts move radially away from lumen leading to stratification of CNS that is characteristic for each region
- Ependymal cells are epithelial cells that secrete cerebrospinal fluid (CSF)

Nerve Cell Differentiation & Developmental Factors

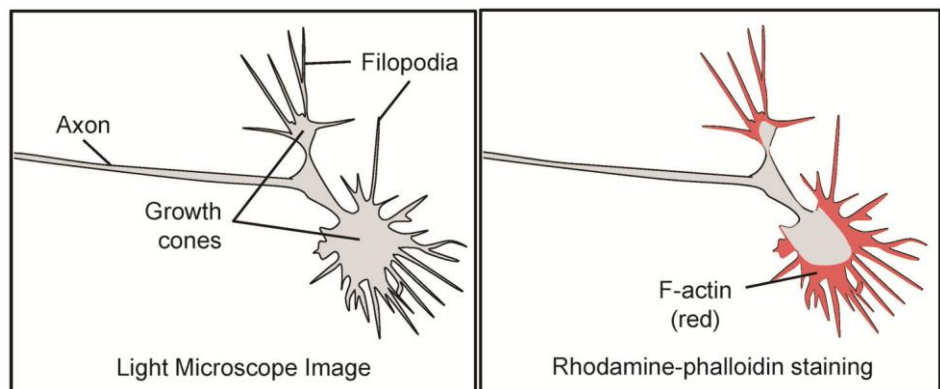


Nerve Outgrowth

- Neurons grow by extending "Growth Cones"
- "Growth Cone" looks just like the leading edge of moving leukocyte (white blood cell)
- Rich in actin filaments
- Extends "ruffling membrane" & filopodia
- Movement directed by cellular contacts & ECM
- Growth is promoted by "Neurotrophic Factors"

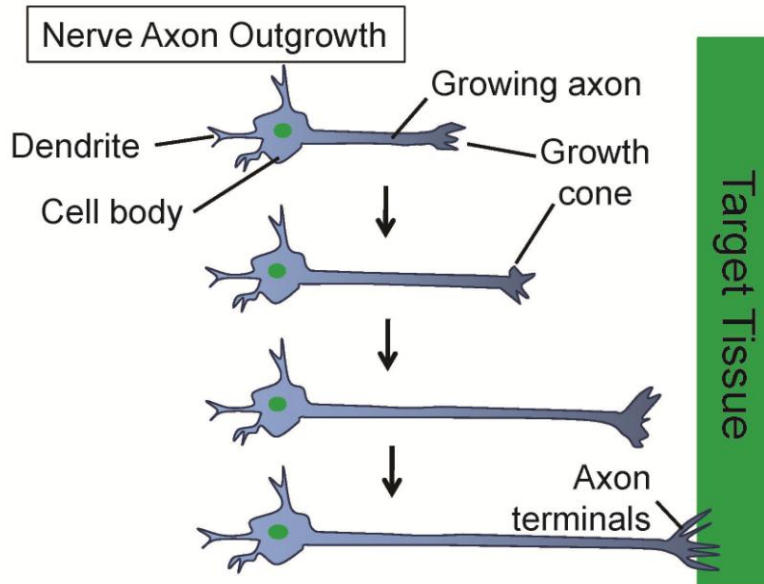


Light Microscope

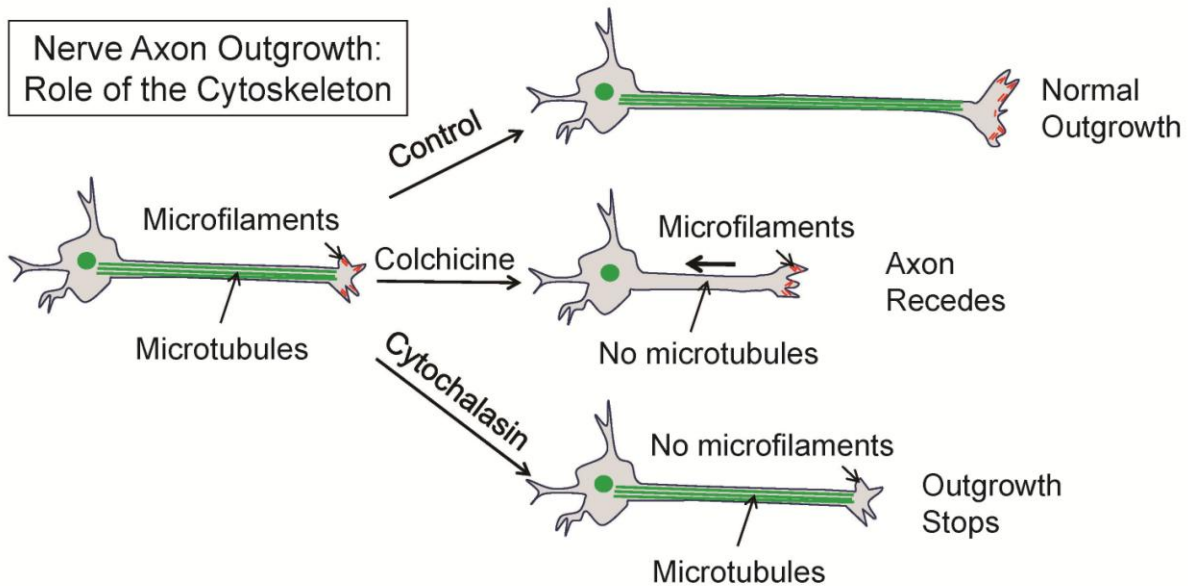


Nerve Cell Differentiation & Developmental Factors

The Outgrowth of the Nerve Axon Towards Its Target Tissue



Experiments Showing the Role of the Cytoskeleton in Axonal Outgrowth

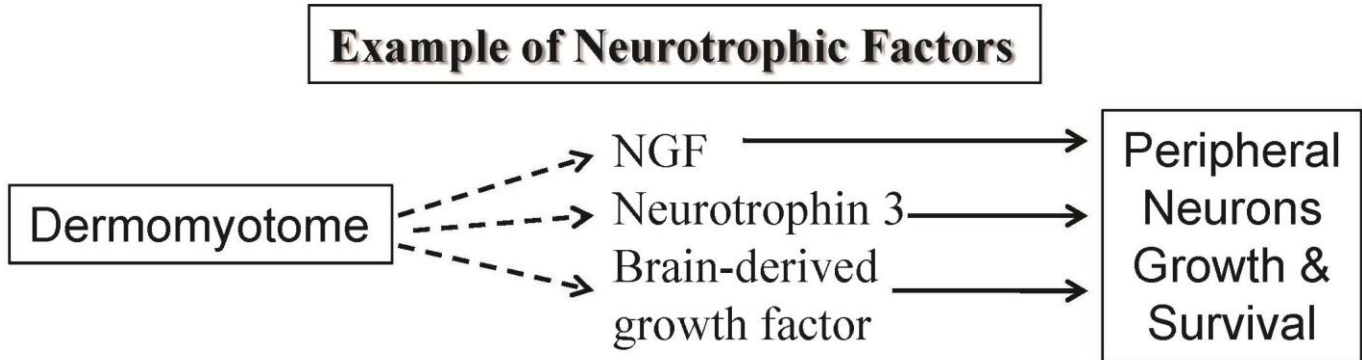


- Microtubules are essential for neuronal outgrowth: growth of axon
- Microfilaments are essential for neuronal outgrowth: motility of growth cone

Nerve Cell Differentiation & Developmental Factors

Neurotrophic Factors

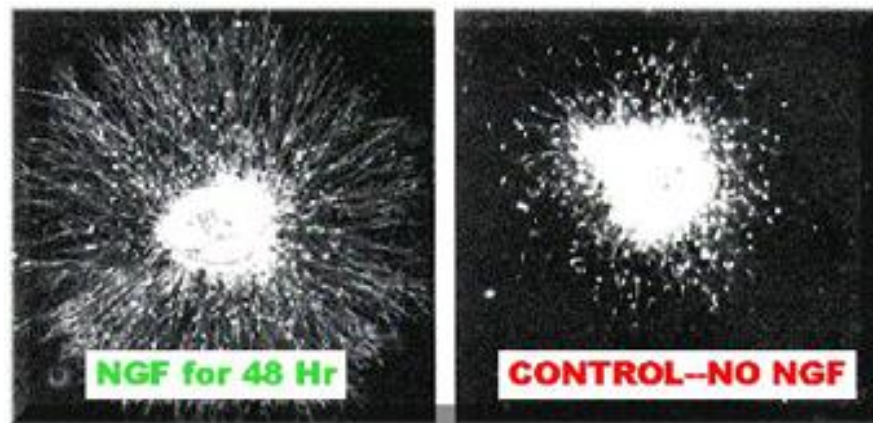
Neurotrophic Factors are growth factors that act on neurons to support their differentiation, growth and survival.



- NGF Superfamily: NGF, Neurotrophins, brain-derived growth/neurotrophic factors
- Secreted proteins that stimulate neuron differentiation, outgrowth & survival
- Nerve Growth Factor (NGF) was first known & is most studied
- Rita Levi-Montalcini was awarded Nobel Prize for her pioneering work on NGF

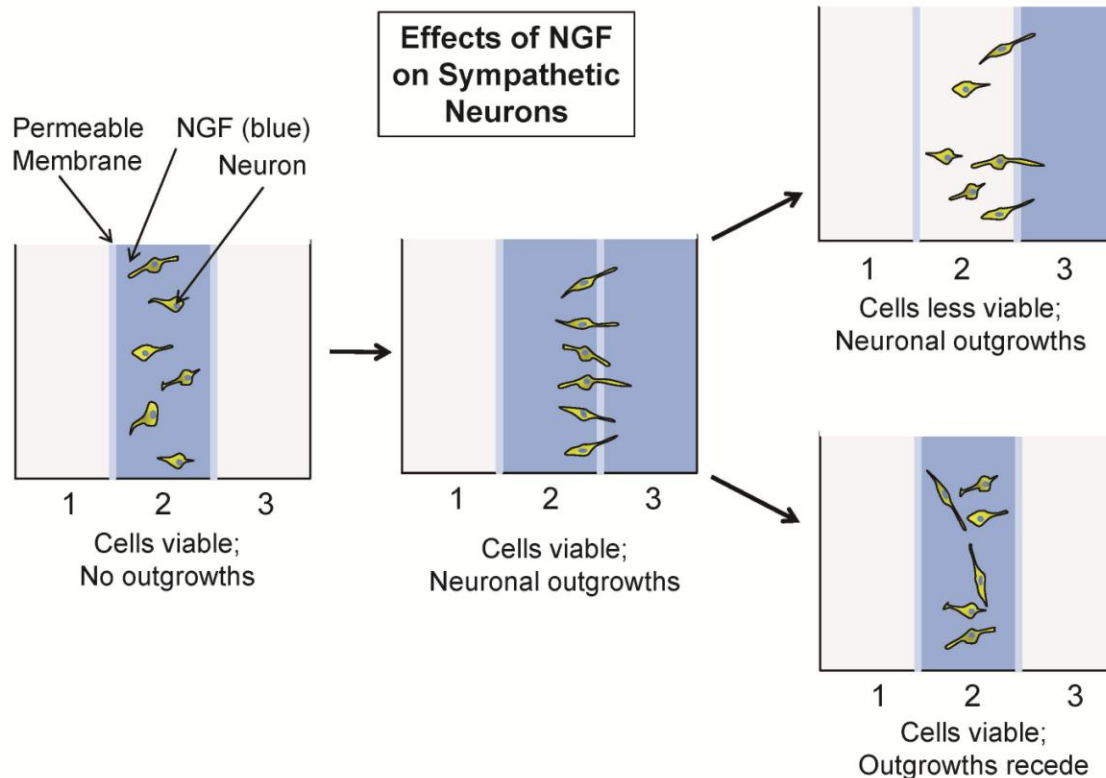
Experiments to Show NGF Promotes Neuron Outgrowth & Survival

Effects of NGF on Cell Outgrowths: Tissue Culture of Sympathetic Ganglia



In the presence of sufficient nerve growth factor, extensive axonal outgrowths occur within 48 hours after explanting sympathetic ganglia into tissue culture medium. In the absence of added NGF, the explanted ganglia show little outgrowths *in vitro*.

Nerve Cell Differentiation & Developmental Factors



As shown in the above figure, more insight into the roles of NGF was obtained by culturing sympathetic neurons in multi-well chambers which were separated by membranes through which the axons could extend. While the axons can extend through the small pores in the membrane and NGF can diffuse easily between the chambers, the cell bodies are too big to move from one chamber to the next. In the first experiment (NGF in chamber 2 only; left panel), the cells are viable but no outgrowths occur. When NGF is also present in the adjacent chamber 3 (center panel), neuronal outgrowths extend towards chamber 3. Removal of NGF from chamber 3 (lower right panel), the cells retract their axons but remain viable. If NGF is removed from the central chamber, then the neuronal outgrowths remain extended but viability drops due to the low level of NGF surrounding them. These and other experiments verified that high levels of NGF are needed for survival and a gradient of NGF is required for neuronal outgrowths.

Differentiation Factors

We've already discussed some of the factors that regulate embryonic development and in the next few lectures we'll get more details. Since this is an introductory course and due to time considerations, the areas of differentiation factors and subsequent signal transduction events have not been covered in detail. At this point, we'll look at the nature of differentiation and growth factors and list the main groups and where they function. This will give you a guide on where to look should this topic be of interest to you. This is one of the hottest areas of biomedical science right now, so finding papers on the subject is very easy.

Nerve Cell Differentiation & Developmental Factors

Differentiation factors and growth factors are also generally referred to as paracrine factors because they diffuse from a cell or group of cells to affect adjacent cells. Thus they are different from true hormones (endocrine factors) that diffuse through the body via the blood system. The historical inducing factors of embryologists are mainly paracrine factors. They can be classed into several major groups. The roles have not been all verified for humans but likely will in the future.

Fibroblast Growth Factors (FGF)

- Over 12 genes encode the FGF family of proteins; splicing allows for more variants.
- FGF binds to FGF Receptors (FGFRs) which are receptor tyrosine kinases
- Binding of FGF to the FGFR leads to a well established sequence of events: dimerization of the receptor followed by activation of the kinase activity, leading to autophosphorylation of the FGFR and phosphorylation of other signaling components in the cell
- FGFs function in axon extension, blood vessel formation (angiogenesis), and mesoderm formation

Hedgehog Proteins

(Note: many terms used in cell and developmental biology originally originated from studies on *Drosophila* where scientists often show a weird sense of humour. The hedgehog gene was discovered when a mutation in the fruit fly produced a mutant with a lot of spines making it resemble the body of a hedgehog. When multiple copies of the gene were discovered one was named after one of the central characters in an electronic game.)

- Vertebrates have at least three genes for hedgehog proteins: sonic hedgehog (shh; yes, this was the gene named after the Sega Genesis character); indian hedgehog (ihh) and desert hedgehog (dhh)
- Sonic hedgehog--has the most diverse roles of all three especially in the patterning of the embryo; functions in the patterning of the neural tube and somites, mediates formation of L-R axis of the embryo and of limbs, induction of differentiation of gut regions
- Indian hedgehog--operates in gut and cartilage development
- Desert hedgehog--functions in the Sertoli cells

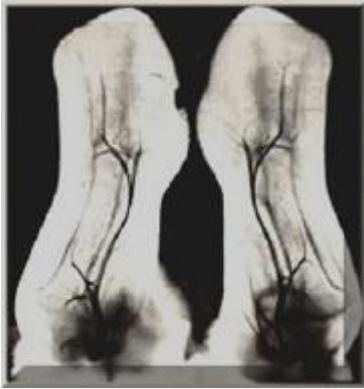
Wnt Family

- The Wnt Family consists of of at least 15 different cysteine-rich secreted glycoproteins.
- They are involved in inducing dorsal cells of somite to form muscle; in establishing polarity of limb and in development of the urogenital system, etc.

Transforming Growth Factor Beta (TGF- β) Superfamily

- This Family contains the TGF- β family, activin family, bone morphogenetic proteins (BMPs), VG1 family and more (e.g., Nodal).
- TGF- β proteins regulate matrix formation and cell division
- BMPs were 1st discovered as bone morphogens also regulate cell division, apoptosis, cell migration and differentiation.

Nerve Pathways are Precisely Defined



- Figure Shows nerve pathways in left & right chick limbs
- Near perfect mirror-image symmetry
- Thus, neurons follow precisely defined paths

References

- Cirulli et al, 2009. The NGF saga. *Frontiers in Neuroendocrinology* 30: 379-395.
- Jiang et al, 2009. Hedgehog signalling in development and cancer. *Mol. Cell* 15: 801-812.
- Krejci et al, 2009. Molecular pathology of the fibroblast growth factor family. *Human mutation* 30: 1245-1255.
- MacDonald et al, 2009. Wnt/ β -catenin signalling: components, mechanisms and diseases. *Mol. Cell* 17: 9-26.
- Simpson et al, 2009. Trafficking, development and hedgehog. *Mech. of Dev.* 126:279-288.
- Wu & Hill, 2009. TGF- β Superfamily signalling in embryonic development and homeostasis. *Mol. Cell* 16: 329-343.



© Copyright 1998-2011 Danton H. O'Day