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COLLEGE ON NEUROPHYSICS:
"DEVELOPMENT AND ORGANIZATION OF THE BRAIN"
7 November - 2 December 1988

"Neuronal Histogenesis"

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Please note: These are preliminary notes intended for internal distribution only.

Neuronal Histogenesis

E.W Rubel

I. INTRODUCTION

. . Growth of Human Brain

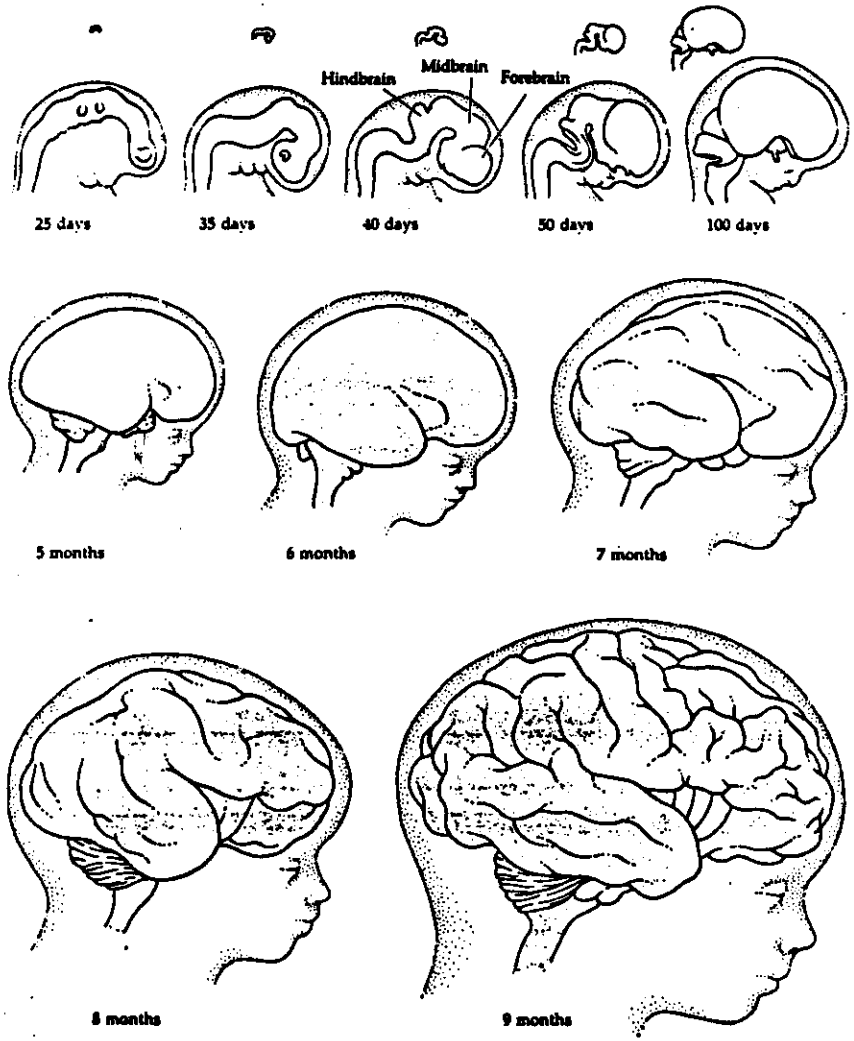


FIGURE 1. *Development of the human brain. The diagrams in this sequence are about four-fifths life-size; the lower diagrams in the first row are enlarged for clarity. Assuming that the fully developed human brain these do not divide after birth, the developing brain must add an average of 250,000 neurons per minute of early development. Note the exceptional growth of the fore-*

2. Ontogeny and Phylogeny

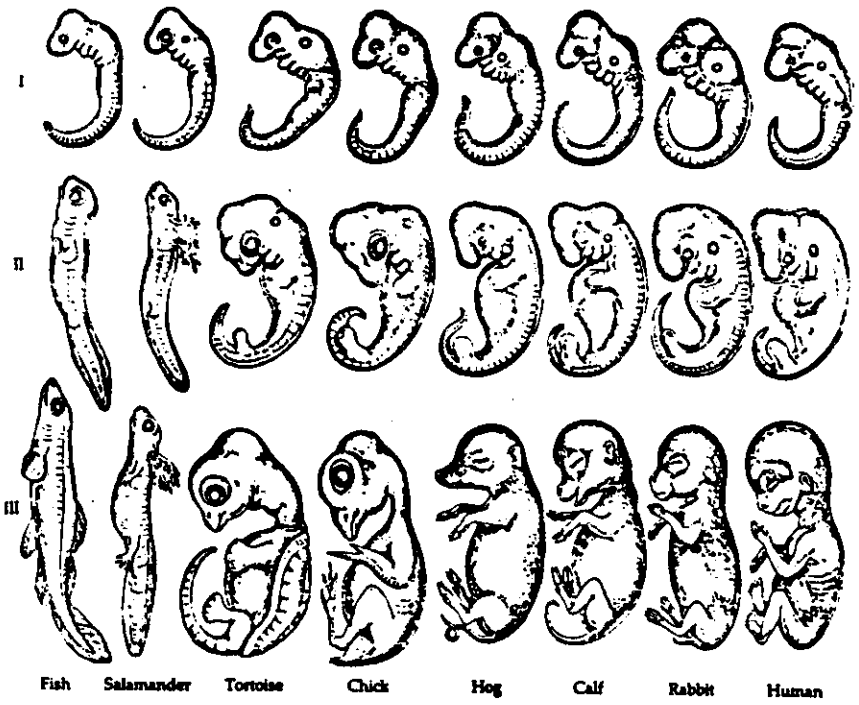


FIGURE 2. *The appearance of vertebrate embryos at various stages of development. The similarity of different embryos during early development is striking; this fundamental observation suggests that these different animals share both a common ancestor and the same basic mechanisms of development. (From Romanes, 1901.)*

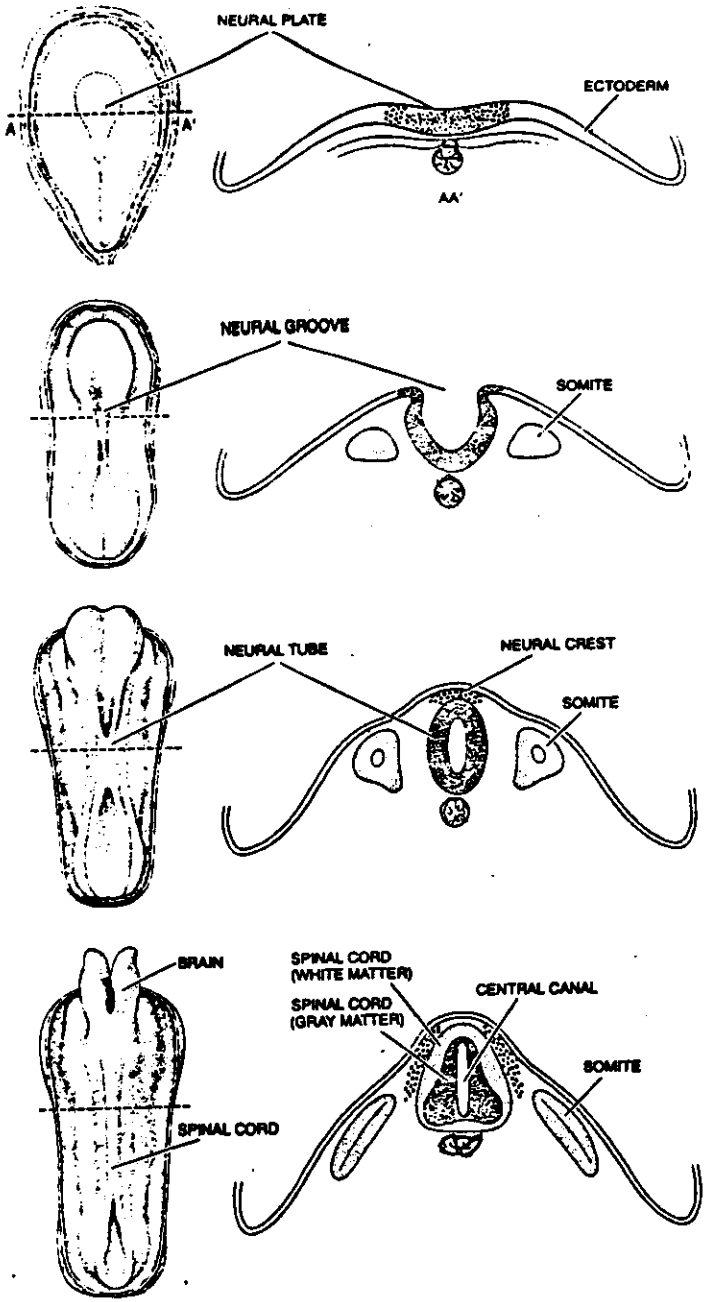
II. FORMATION OF NEURAL ECTODERM

A. Gastrula - Creation of 3 germ layers by invagination

B. Neurulation

1. Neural plate stage

2. Gradual formation of Neural tube by closure from anterior to posterior.



GENESIS OF THE NERVOUS SYSTEM from the ectoderm, or outer cell layer, of a human embryo during the third and fourth weeks after conception is represented in these four pairs of drawings, which show both an external view of the developing embryo (*left*) and a corresponding cross-sectional view at about the middle of the future spinal cord (*right*). The central nervous system begins as the neural plate, a flat sheet of ectodermal cells on the dorsal surface of the embryo. The plate subsequently folds into a hollow structure called the neural tube. The head end of the central canal widens to form the ventricles, or cavities, of the brain. The peripheral nervous system is derived largely from the cells of the neural crest and from motor- [illegible] that leave the lower part of the brain at each segment of the future spinal cord.

3. Mesodermal derivatives

   Notochord

   Somites - vertebrae, ribs, skeletal muscle, dermis

C. Sources of Neural Tissue

1. Neural Tube

   Brain and spinal cord

2. Neural Crest - arises from neural plate but separates as the tube closes to form a band lying along the length of the tube, dorsal and lateral to the neural tube. Derivatives of neural crest:

   (1) Most of the peripheral nervous system including spinal ganglia and autonomic (sympathetic and parasympathetic) ganglia; (2) variety of non-neural tissues including melanocytes, chromafin cells of adrenal medulla, blood forming cells, dura and arachnoid, parts of the facial skeleton including the parts of the ossicles.

3. Epidermal Placodes - arising from non-neural ectoderm. There are 9 or 10 pairs. The most important are the nasal, otic, epibranchial and dorsolateral. They give rise to several sense organs cranial ganglia and cranial ganglia and cranial nerves.

D. Organizer Experiment - Primary Embryonic Induction

Question - What determines that a part of the embryo will develop into the nervous system? Are the "fates" of tissues determined in the blastula or do they depend on cellular interactions from surrounding tissues?

E. Spemann and H Mangold (1920's) transplanted pieces of blastula from one embryo to an atypical portion of host.

1. Found dorsal lip of blastopore could induce atypical regions to form neural plate and tube.

2. Found that mesoderm including notocord was the critical region responsible for inducing host ectoderm to become neural tissue.

Organizer experiments demonstrate epigenetic nature of neural development. Phyenotypic expression results from genes working in environments throughout development.

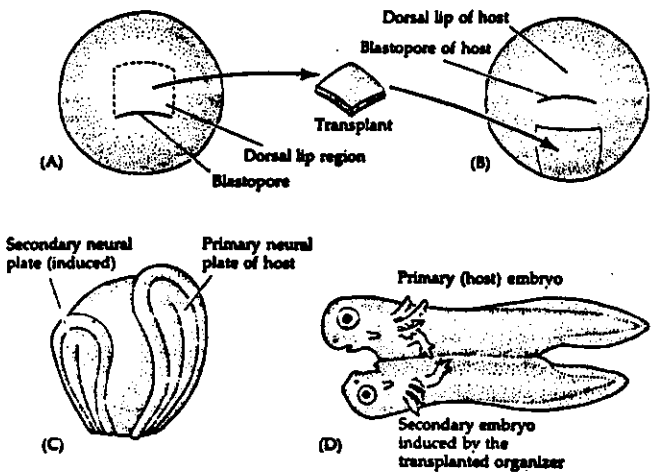


FIGURE 4. *The "organizer" experiment carried out in the early 1920s by H. Spemann and H. Mangold. Transplantation of the upper lip of the blastopore from one amphibian gastrula to another (A,B) often led to the induction of a second neural plate (C). In some instances an entire secondary embryo formed on the flank of the host (D). The ability of the upper lip of the blastopore to induce the formation of a secondary embryo suggested that this piece of tissue organized development in some fairly general way. (After Hamburger, 1963.)*

F. Origins of Neurons and Glia within the CNS

The majority of cells in the central nervous system arise by active mitosis in the ventricular zone of the neural tube (portion next to the central canal). It is now thought that quite early in development two populations of precursor cells exist and are intermixed. At the neural tube stage specific glia cell makers such as glial fibrillary acidic protein (GFAP) have been shown to label some dividing cells.

In general, glia cell proliferation is later the neuronal proliferation. In addition, glia can proliferate throughout life, whereas 99.9% (or more) neuronal proliferation occurs during he prenatal or early postnatal period.

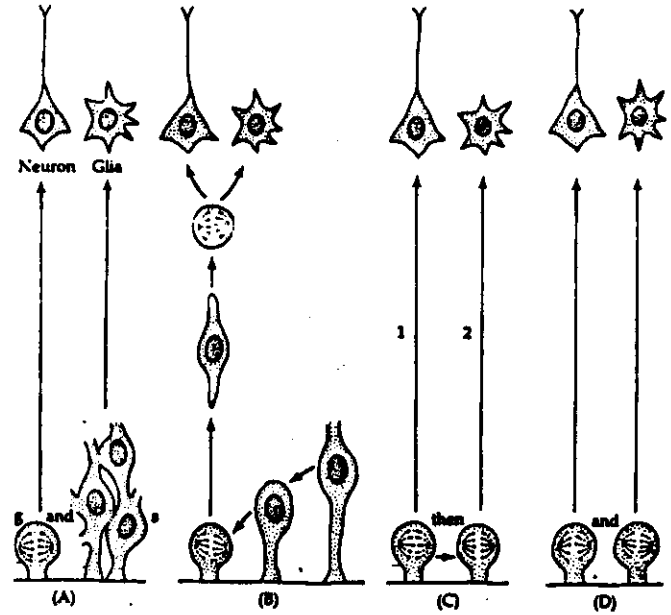
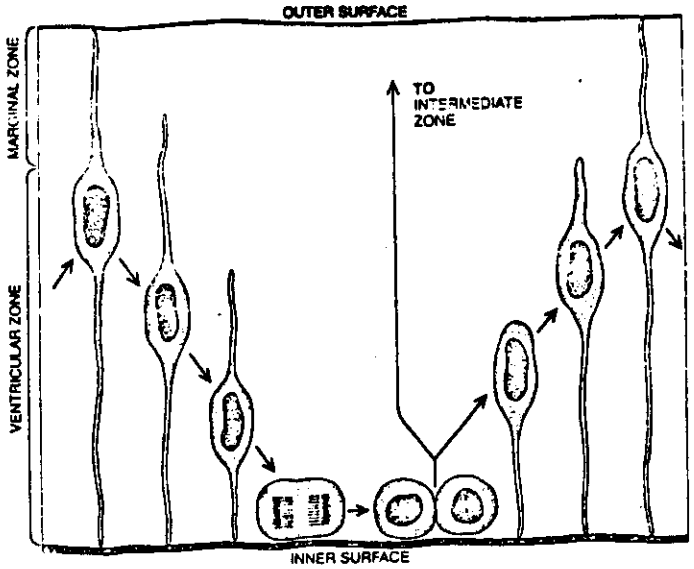


FIGURE 5. *Theories about the origin of neuronal and glial cell lines. (A) W. His originally proposed about a century ago that neural and glial lines were entirely separate. g, Germinal cell; s, spongioblast. (B) An alternative view, suggested by A. Schaper, was that these two cell types arise from a single class of precursors that divide after migrating away from the ventricular zone. (C) The introduction of thymidine autoradiography led S. Fujita to conclude in the 1960s that dividing cells first give rise to neurons (1) and then, after neurogenesis has ceased, produce glial cells (2). (D) The ability to demonstrate glial fibrillary acidic protein, a specific glial marker, provides evidence for the current view that nerve and glial cell precursors coexist in the ventricular zone from very early stages. (After Rakic, 1981.)*

III. NEURONAL PROLIFERATION

A. The neural tube is a pseudostratified epithelium. Precursor neurons form a single layer with protoplasmic processes extending from the ventricular surface to the outer surface. Movement of the nucleus within the processes is described below. After leaving the mitotic cycle neurons migrate from the ventricular region.



NUCLEI OF NERVE CELLS MIGRATE in the layer of epithelial tissue that forms the wall of the neural tube in the developing embryo, as this multistage schematic diagram shows. When the cells in this layer, called the neuroepithelium or ventricular zone, replicate their DNA, their nuclei migrate toward the inner surface of the epithelium, their peripheral processes become detached from the outermost layer and the cells become rounded before dividing. After mitosis (cell division) the daughter cells either extend a new process so that their nuclei can migrate back to the middle level of the epithelium, or (if the cells have stopped dividing) they migrate out of the epithelium to form part of the intermediate zone in the wall of the brain.

Cell cycle times and cell "birthdays" are typically studied using tritiated (^{3}H) thymidine. This method is based on the fact that thymidine is incorporated into cells only during DNA replication. By introducing radioactively labelled thymidine as a pulse (followed by large amounts of "cold" thymidine) cells which are replicating DNA during the pulse can be labelled. The cell cycle times can be studied by observing which cells are heavily labelled at short intervals. Since postmitotic neurons no longer incorporate thymidine and incorporated thymidine will be divided among daughter cells the time at which neurons become postmitotic (leave the proliferative stage) can be studied. Labelled and unlabelled neurons are visualized by autoradiography.

Conclusions:

1. Cell cycle times for neurons range from 5 to 24 hours. There is a rough relationship between age and cell cycle times. Cycle times tend to be shorter early, and longer later in development.

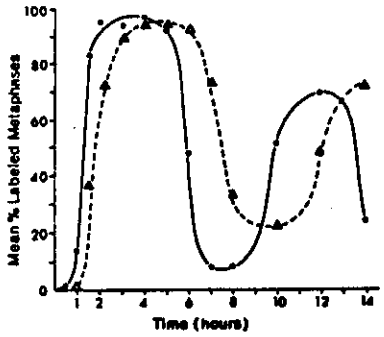


Figure 7. Comparison of the generation cycle of neuroepithelial germinal cells in the mouse fetus at 10 days of gestation (solid line) and 11 days of gestation (broken line). The mean percentage of labeled mitotic figures in the neural tube at different times after an injection of tritiated thymidine (1 μCi/g) to pregnant mice. From S. L. Kauffman, *Exp. Cell Res. 49*:420–424 (1968), copyright Academic Press, Inc.

2. Spatial and temporal gradients relate time of terminal mitosis to final position of neurons. In a given neural structure cells may assemble by either "stacking" or "packing".

   a. Most laminar structures form by stacking, following an "inside-out" pattern: later formed neurons migrate past (or through) earlier formed neurons, e.g. cerebral cortex.

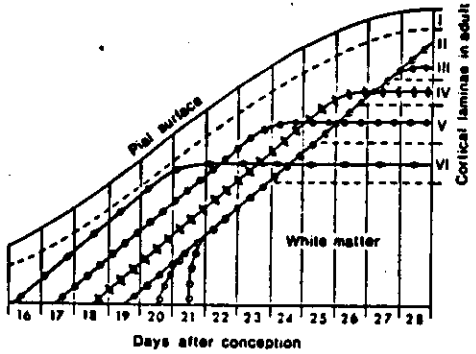


Figure 8. Time of origin and pattern of migration of young neurons in the cerebral isocortex of the rat as revealed by labeling of the cells with tritiated thymidine injected on the 16th, 17th, 18th, and 19th–21st days of gestation. From M. Berry, A. W. Rogers, and J. T. Eayrs, *Nature 203*:591–593 (1964).

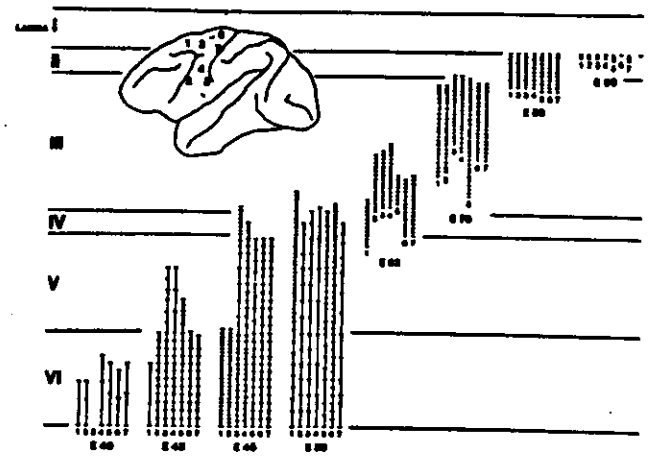
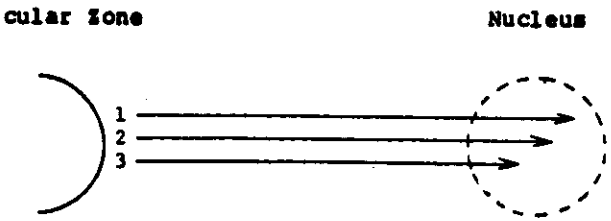


Figure 9. Diagrammatic representation of the positions of heavily labeled neurons in the motor cortex of eight monkeys that were exposed to ^{3}H-TdR at various embryonic (E) days indicated at the bottom of each vertical line. Arabic numerals 1 to 7 indicate the area of the motor cortex sampled, while the Roman numerals I to IV indicate cortical layers. Note that motor efferent cells of layer V that subserve the arm (4) and those subserving the leg (6) are generated simultaneously although these two classes of cells project to cervical and lumbar levels of the spinal cord, respectively. [J. Dekker and P. Rakic]

Cerebellar cortex presents a variant on this scheme with proliferation of granular cells in the external granular layer and then migration past the purkinje cells.

b. Most nuclear structures form by packing. This is "outside-in".



3. Large neurons tend to be born before small neurons. An extreme example of this is provided by granule cells of the cerebellum and hippocampus. They continue proliferating well after birth.

4. Regions which differ in cell structure (cytoarchitecture) tend to have different birthdays.

5. In any region neurons tend to have proliferated prior to glia.

6. Phylogenically older parts of the brain tend to be born before newer parts.

B. Subventricular Zone

Late in gestation a proliferative zone forms under the lateral ventricles. It persists throughout life, is thought to produce both glia and neurons initially and then mostly glia. It is also thought to be responsible for glial tumors; in animals it is one of the commonest sites for glial tumors induced by chemical carcinogens.

C. Regulation of Neuronal Proliferation

The final number of neurons in any brain area is a complex function of proliferation, migration and cell death (see below). Normally the number of neurons that initially make up a brain region varies from animal to animal by less than 10%, which indicates that proliferation of neurons destined for any site is

highly regulated. However, during the proliferative and migratory phases neurons are extremely sensitive to alterations in their environment. Some examples are:

1. Effects of irradiation on proliferation in external granule cell layer.

2. Viruses and toxins on external granule cell layer, cortex and hippocampus. In this and the above case brief and incomplete destruction can cause a prolongation of proliferation which partially makes up for the cell loss.

3. Hypothermia - as little as a 3-4 ^{o}C increase in female guinea pig temperature for 1 hour can cause a 10% change in brain weight. Longer exposusres have been shown to cause damage to the ventricular germinal cells of the forebrain.

4. Insufficiency or excess in fetal hormones.

   Thyroxin: Deficiency during critical period produces multitude of direct and indirect effects on brain development. Mechanisms only partially known but mostly not due to changes in total amount of total proliferation. Timing of proliferation with respect to other events is disturbed, leading to cell loss.

5. Malnutrition: Effects on brain development and cell number are clear. It is not clear what cells are affected or whether this is a continuous or threshold type function.

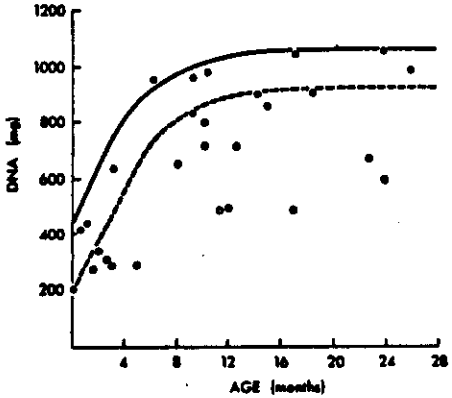


Figure 10. DNA content of the cerebrum of normal children (•) and marasmic children (○). From M. Winick, P. Rosso, and J. Waterlow, *Exp. Neurol.* 26:393–400 [1970], copyright Academic Press, Inc.

Indirect effects: Effects on subsequent generations.

IV. MIGRATION OF NEURONS

There are several mechanisms for cell migration which we will only mention.

Passive migration: pulled or pushed.

Active movements: Musclelike proteins associated with cell polarity or growth cones.

Cues: Chemotaxis, differential substrate adhesion.

A. Central Nervous System

1. Migration along radial glia

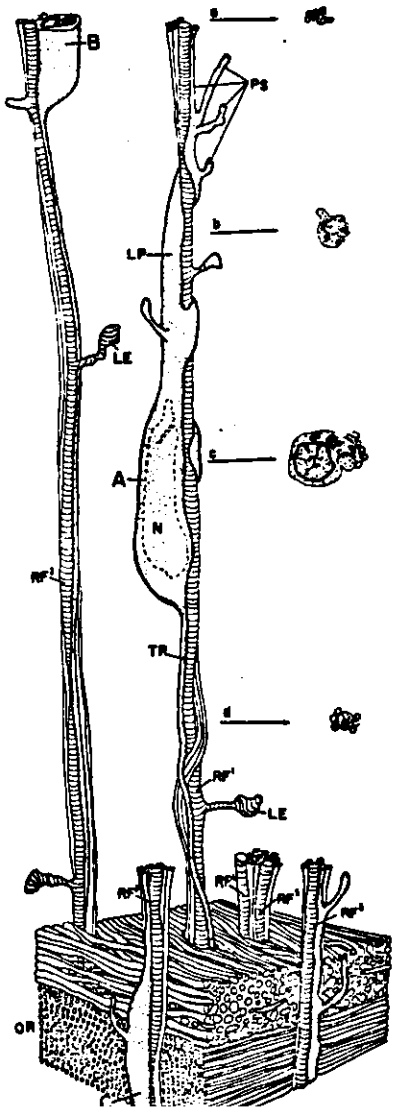


Figure 11. Relationships between migrating cells and radial fibers in the intermediate zone of the developing cerebral neocortex of the fetal monkey. The subventricular zone lies some distance below the area selected for reconstruction, whereas the cortex is more than 1000 μm above it. The lower portion of the diagram contains uniform, parallel fibers of the optic radiation (OR) and the remainder is occupied by more variable and irregularly disposed fiber systems; the border between the two systems is easily recognized. Except at the lower portion of the figure, most of these fibers are deleted from the diagram in order to expose the radial fibers (striped vertical shafts RF^{1-3}) and their relationships to the migrating cells (A, B, and C) and to other vertical processes. The soma of migrating cell A, with its nucleus (N) and voluminous leading process (LP), is within the reconstructed space, except for the terminal part of the attenuated trailing process and the tip of the vertical ascending pseudopodium. Crossections of cell A in relation to the several vertical fibers in the fascicle are drawn at levels "a" to "d" at the right side of the figure. The perikaryon of cell B is cut off at the top of the reconstructed space, whereas the leading process of cell C is shown just penetrating between fibers of the optic radiation (OR) on its way across the

2. Translocation of cell body within peripheral process

3. Other? Migration may follow rather than preceed establishment of peripheral contacts.

B. Neural Crest

Migration of neurons and the influence of the migratory route on subsequent differentiation has been extensively studied here.

1. Normal Migration

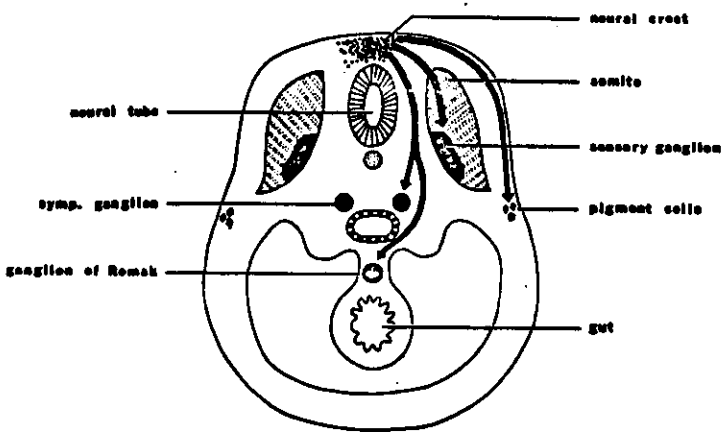


FIGURE 12. This cross section of the developing chick embryo illustrates the migration pathways of some neural-crest derivatives.

2. Epigenetic factors:

a. Undifferientiated crest cells transplanted to new region will assume migratory route dictated by new environment.

b. Transmitter development

Parasympathetic ganglia normally migrate to target tissue and produce acetylcholine. Most are derived from cranial crest. Sympathetic ganglia form sympathetic chain, produce norepinephrine and are not derived from cranial region. Transplantation of premigratory crest cells to new region will result in production of transmitter appropriate for new region.

Postmigratory sympathetic cells can change their transmitter type of culturing in conditioned medium.

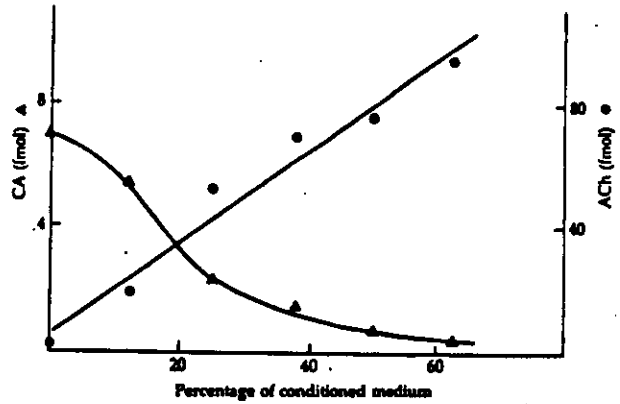


FIGURE 13. *The effect of heart-conditioned medium on the choice of neurotransmitter in sympathetic neurons maintained in culture. Sympathetic ganglion cells from late embryonic rats were maintained in culture for 20 days. On the second day, and every second day thereafter, fresh medium was added to the cultures. The amount of conditioned medium mixed with fresh medium varied from about 10 to about 62 percent (as indicated on the abscissa). On day 20, the cultures were incubated with labeled choline and tyrosine to determine their ability to synthesize radioactive acetylcholine (ACh) and catecholamines (CA). Those cultures maintained in high concentrations of conditioned medium synthesized smaller amounts of catecholamines (▲) and larger amounts of acetylcholine (●) than cultures maintained in lower concentrations of conditioned medium. The number of surviving cells does not vary as a function of the amount of conditioned medium supplied. (After Patterson and Chun, 1977.)*

3. Cessation of Migration:

Big? Some aspect of target tissue or input.

V. CELL DEATH - V. Hamburger

A. Normal sculpting of Neuronal numbers through overproliferation and subsequent loss of neurons.

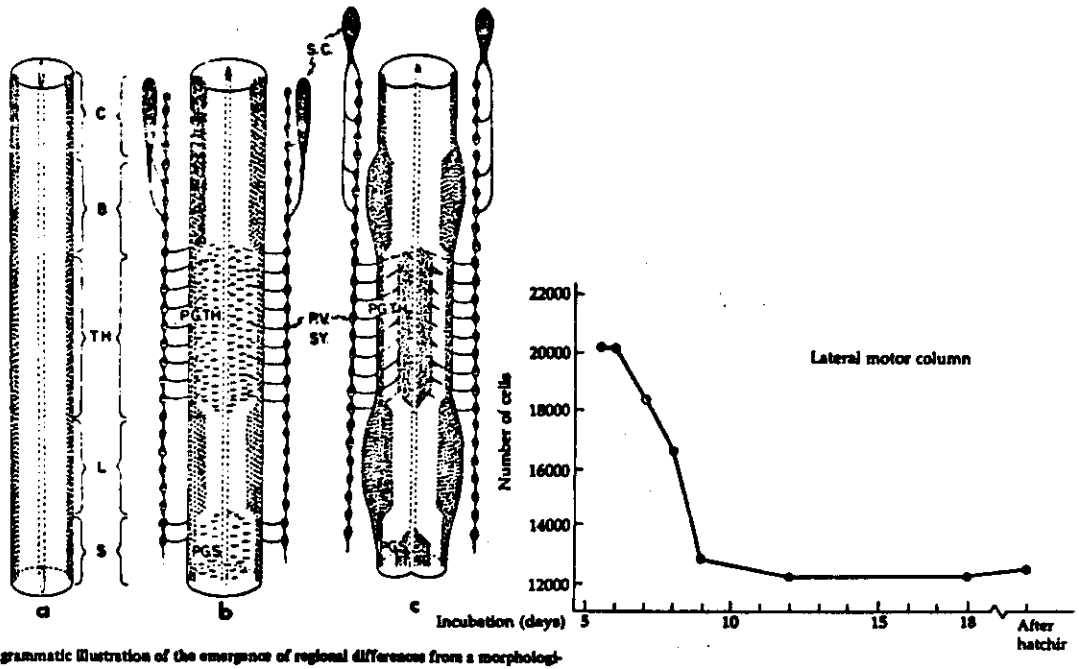
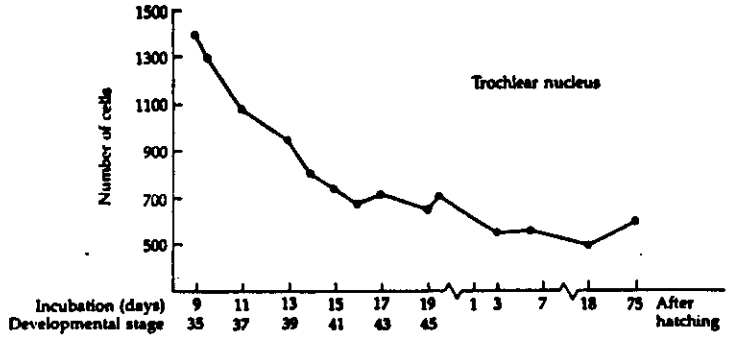


Figure 14 Diagrammatic illustration of the emergence of regional differences from a morphologically uniform system in the spinal motor column of the chick embryo. a, 3-day embryo: The motor column is of uniform width from the cervical to the sacral level. b, 5-day embryo: The majority of the differentiating neuroblasts in the cervical segment of the motor column undergo degeneration. They are represented as solid black circles. In the thoracic and sacral segments the migration of the preganglionic columns is under way. c, 8-day embryo: The degenerated nerve cells in the cervical segment of the motor column have disappeared. The remaining nerve cells in this segment form the slender medial motor columns. Note the size increase of the brachial and lumbar motor columns innervating the limbs.



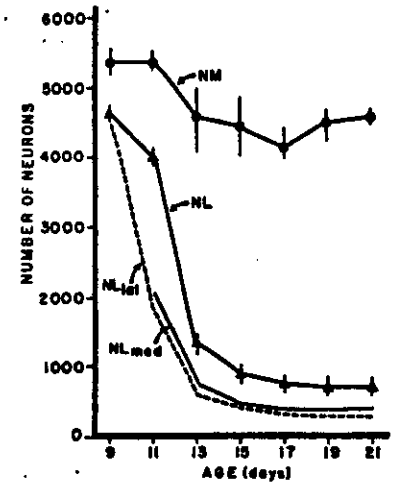
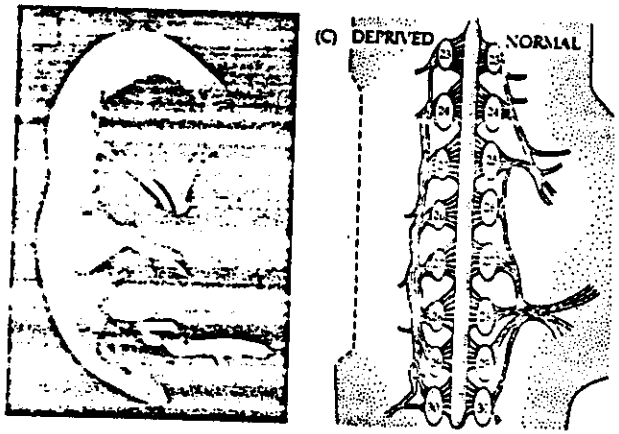


Figure 15 Cell counts in nucleus magnocellularis and nucleus laminaris in the chick embryo from 9 days to hatching. Points are means for each time, and vertical bars show ranges. Lines showing counts for the divisions of nucleus laminaris are mean values. NM, Nucleus magnocellularis; NL, n. laminaris (total); NL_{lat}, lateral division of n. laminaris; NL_{med}, medial division of n. laminaris. From E. W. Rubel, D. J. Smith, and L. C. Miller, *J. Comp. Neurol. 166*:469–490 (1976).

B. Epigenetic Influences

1. Target removal enhances cell death.



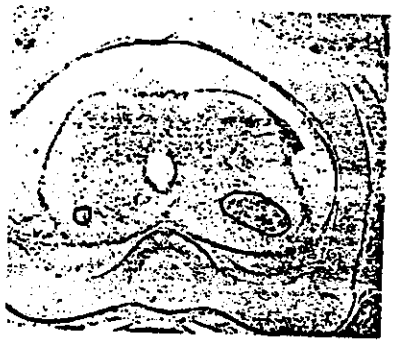
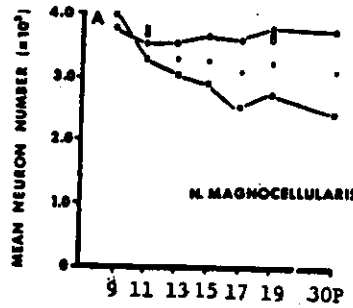


FIGURE 16. *The effects of limb bud ablation in chick embryos. At approximately 2½ days of incubation, a leg primordium can be excised with a fine glass needle. (B) A 10-day chick embryo whose right leg primordium was extirpated as in A. (C) Reconstruction of the lumbosacral spinal cord, spinal ganglia, and peripheral nerves in a 6½-day embryo, one of whose legs was extirpated at 2½ days. Note the smaller size of the relevant spinal ganglia on the operated side. (D) Cross section of the lumbar spinal cord from a 9-day chick embryo, showing the appearance of the lateral motor column on the normal side and the contralateral side on which the leg primordium was extirpated at 2½ days of incubation. The lateral motor column on the deprived side is virtually absent. B from Hamburger, 1977; C after Hamburger, 1958; D from Hamburger, 1958.)*

2. Afferent Regulation: Removal of afferent input enhances cell death.



3. Enlargement of peripheral target decreases cell death - (more neurons).

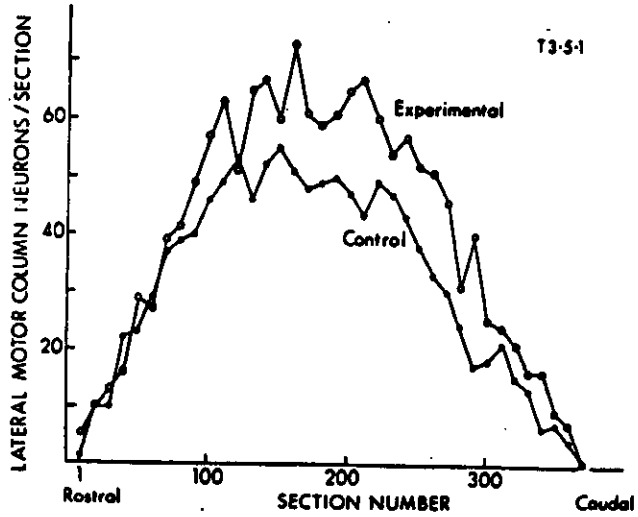


Figure 18 Graph of lateral motor cell counts in lumbar spinal cord of embryo of Figure 13B, on right (experimental) and left (control) ime side. Note that the extra cells on the transplant side are distributed along the entire rostrocaudal extent of the lim. [Hollyday and Hamburger, 1976]

4. Correlation with synaptic formation

   a. Afferent regulation of cell death corresponds to timing of functional synaptic input.

   b. Elimination of muscle activity (which causes reduction from polyinervation to monoinervation) delays cell death of spinal motor neurons.

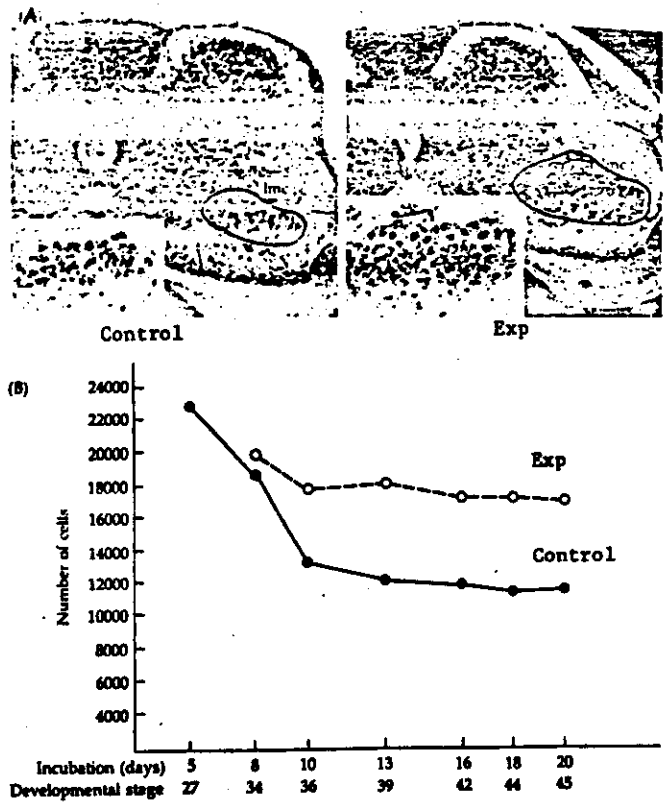


FIGURE 19. *The effect of neural activity on nerve cell survival. (A) The number of motor neurons persisting in the lateral motor column of the developing chick spinal cord can be influenced by neuromuscular blocking agents. A cross section of the lumbar cord of a normal 10-day chick embryo (left) is compared to a similar section from a curare-treated embryo at the same age. Insets are enlargements of the lateral motor column (lmc; ×110). (B) Graphic summary of the effects of curare treatment. The solid line shows the normal course of motor neuron death in control animals (cf. Figure 4A). Neuromuscular blockade with curare for about 3 days during this period (days 6 through 9 of incubation) reduces the degree of nerve cell degeneration. When motility returns, the surplus motor neurons die (not shown). Each point represents the average of 2 to 10 embryos. (A from Pittmann and Oppenheim, 1979; B after Pittmann and Oppenheim, 1979.)*

C. Nerve Growth Factor: Rita Levi-Montalcini

Discovery: Mouse sarcoma tumors inplanted into periphery to enlarge periphery with rapidly growing neoplastic tissue.

Effects on sensory ganglia

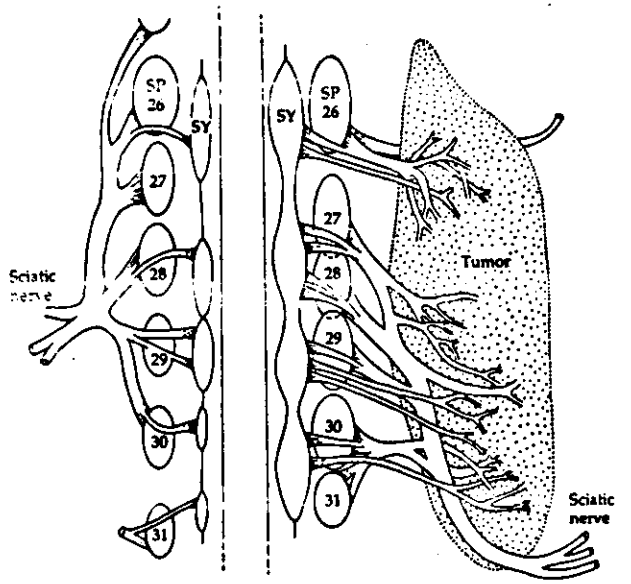


FIGURE 20. *The discovery of nerve growth factor was based on the effects of a piece of mouse sarcoma tumor on the developing nervous system of chick embryos. This drawing shows the lumbosacral region of a 15-day-old chick embryo into which a tumor (dotted line) was implanted a few days earlier. The sympathetic chain (SY) and spinal ganglia (SP) in the region of the tumor implant are obviously enlarged. In addition, the peripheral nerves are hypertrophied and appear to be directed toward the tumor. Numbers indicate spinal segments. (After Levi-Montalcini and Hamburger, 1951.)*

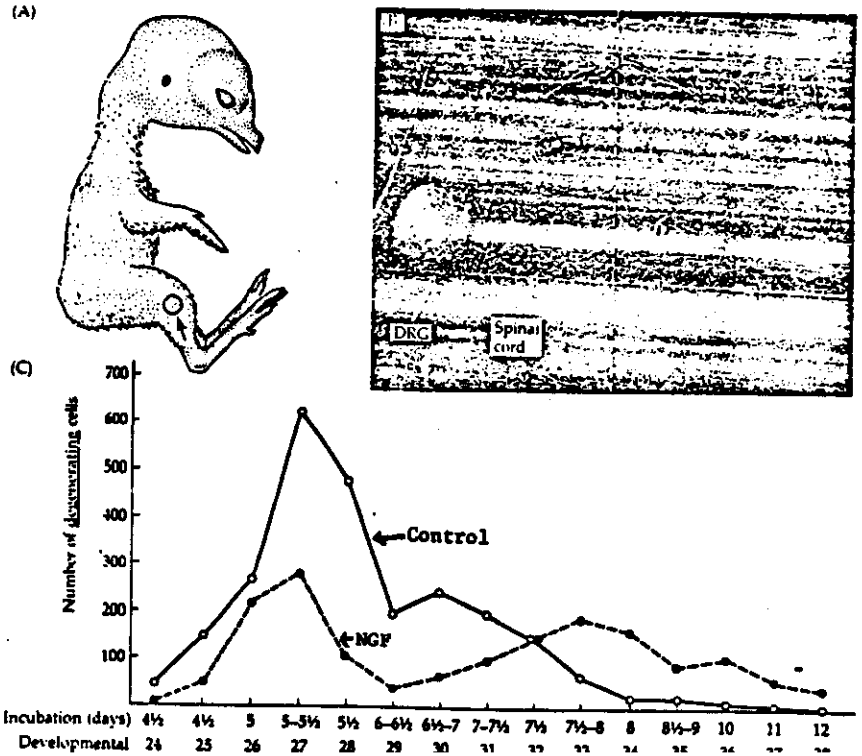
Development of Bioassay: sympathetic or dorsal root ganglion in culture.

Sources of NGF:

Mouse submaxillary gland.

Sympathetic targets - small quantities

Biological actions: Anti NGF causes functional sympathectomy. NGF is taken up and transported in retrograde direction to cell body.



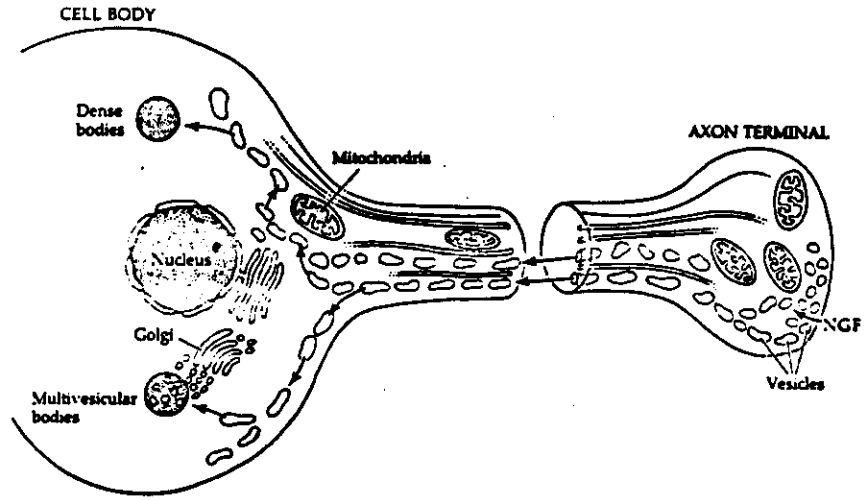


FIGURE 22. *Diagram of the current view of NGF uptake and subcellular distribution in sensitive neurons. NGF binds selectively to membrane receptors at the axon terminals of peripheral sympathetic and sensory neurons, where it is then internalized by endocytosis (arrow). Within the axon, NGF is localized in smooth cisternae and vesicles; after retrograde transort to the cell body, these membrane-bounded sacs apparently fuse with dense bodies and multivesicular bodies. There is no clear evidence for transfer of NGF to the cytosol itself or to the nucleus. (After Thoenen et al., 1979.)*

B. The search for other trophic factors: Continues

Table 1. Derivatives of the neural crest (From Weston, 1982)

A. Cranial crest
  1. Skeletal and connective tissue
    (a) Cartilages
      Visceral arch cartilage
      Chondrocranial cartilage
    (b) Bones
      Upper and lower jaw
      Dental papilla (odontoblasts)
      Palate
      Cranial vault floor
    (c) Mesenchyme
      Corneal endothelium and stromal fibroblasts
      Contribution to adenohypophysis, lingual gland, parathyroid, thymus and thyroid
      Contribution to dermis and subcutaneous adipose of face, jaw and upper neck
    (d) Muscles
      Ciliary muscles (striated)
      Cranial vasculature and dermal smooth muscle
B. Trunk crest or cranial crest
  1. Neural derivatives
    (a) Sensory neurons
      Spinal (dorsal root) ganglia
      Trigeminal (V) ganglion
      Facial (VII) root (geniculate)
      Glossopharyngeal (IX) root (superior)
      Vagal (X) root (jugular)
    (b) Sympathetic (adrenergic) neurons
      Superior cervical
      Paravertebral chain
      Prevertebral complexes (coeliac, mesenteric, adrenal and retroaortic)
    (c) Parasympathetic (cholinergic) neurons
      Ciliary
      Submandibular, ethmoid, otic, lingual, sphenopalatine
      Remak's ganglion
      Meissner's and Auerbach's plexus
      Pelvic plexus
      Visceral intrinsic ganglia
    (d) Neurosecretory
      Carotid body Type I and II cells
      Calcitonin-producing (C-cells) of the thyroid
      Possible contribution to ACTH/MSH producing cells of pituitary
      Adrenal medulla
    (e) Supportive cells of the peripheral nervous system
      Glia
      Schwann sheath cells
      Satellite cells
  2. Pigment cells (melanocytes) of skin, hair and irides