DEVELOPMENTAL NEUROCHEMISTRY

Edited by Richard C. Wiggins, David W. McCandless, and S. J. Enna

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Preface

Research in the neurosciences has been expanding in an exponential fashion during the past two decades, and progress has been especially rapid in the field of neurochemistry. Development is an especially complex application of neurochemistry, offering challenges of enormous magnitude and diversity to clinicians and basic scientists alike. Given the breadth of the neurochemical and developmental sciences, the great clinical relevance, and the rapid flux of scientific thought and effort in these areas, we intend to present a concise overview of essential and interesting topics.

The first three chapters describe various morphological correlates underlying neurochemical development of the brain. The next four chapters review the energy requirements and transitions in intermediary metabolism that characterize fetal and postnatal brain development, including the coupling of energy metabolism to the development of function. Following these, two chapters describe the biochemical development of neurotransmission, including differentiation of receptor sites and transmitter metabolism.

The processes of development, differentiation, and growth involve a series of rapid transitions in cellular and biochemical events in addition to the usual metabolic flux of adult homeostasis. The opening chapter provides a perspective on so-called critical events in development. The developing fetal and postnatal nervous system is dramatically different from the nervous system of the adult with regard to the regulation of dynamic events and the vulnerability of these processes to genetic and environmental perturbation. Accordingly, a concluding chapter discusses the clinical relevance of basic developmental neurochemistry.

We encouraged chapter authors to stimulate interest by addressing the limits as well as the depth of knowledge. We placed rather strict page limits on each chapter to keep the book affordable and to strengthen thematic messages. Aiming at a general audience in the developmental sciences and neurosciences, we intended to summarize current (chap-

ters were submitted during the early part of 1983) knowledge about maturation in the nervous system. Although each chapter was authored independently, we structured the complete volume to provide balance of detail and information through the selection of topics.

Critical Periods in Development: Editorial and Historical Perspectives

Richard C. Wiggins

Introduction

The concept that there are critical periods in development is quite old (Dareste, 1891) and is inseparable from the origins of teratology as an experimental science (Stockard, 1921). The original hypothesis stated that developmental processes proceed at different rates and that during periods when a given rate process is predominant, it is subject to permanent arrest by environmental conditions outside a normal range. Restoring a normal environment would not necessarily restore normal development of the organism. Instead, the opportunity for certain critical events to occur would have passed, causing a distortion in the balance of development. Although Stockard's studies involved abnormalities of morphological development, he believed that the underlying causes were biochemical.

Since Stockard's work, the features of the critical interval hypothesis have been widely applied within the neurosciences, especially in behavior (Scott, 1962; Bronson, 1965; Rodier, 1976). Flexner, Flexner, and Straus (1941) observed that growth and differentiation of the fetal pig sensory-motor cortex proceeds by stages rather than by continuous uniform processes. They described the period of neuroblast proliferation and differentiation as a critical period when cells show morphological change and the activities of such enzymes as cytochrome oxidase and succinic dehydrogenase increase rapidly. Similarly, Kavaler and Kimel (1952) demonstrated that acetylcholinesterase activity rises sharply at the midpoint of gestation, a finding from which they speculated that the biochemical event was precursor to a cellular differentiation. Davison and Dobbing (1966) have described myelination as a critical, or vulnerable, period. These early studies of neurochemical development demonstrated the first appearance of enzyme activities or of specific compounds in the developing brain and demonstrated biochemically that at specific periods in development, certain metabolic processes do occur at predominant rates.

History

Physicians of the early nineteenth century generally believed that birth defects were the result of some pathological lesion intervening to produce deformity. However, Dareste (1891) demonstrated with chick embryos that abnormal development can actually result from an alteration of the normal plan of embryonic development and that an early appearing abnormality of development produced a more grave deformity. From the work of Charles Darwin, Dareste believed that embryonic development recapitulated evolutionary stages in the ascent of animal groups. Thus, vestigial characteristics and atavistic characters, such as supernumerary organs, were readily explained by the shared ancestry of animal species. Dareste also recognized that the clitoris and penis were, respectively, in females and males, homologous structures present in varying degrees of development. The clitoris was a rudimentary or arrested penis; the prostate, a rudimentary uterus. Thus, the general theory of teratology offered by Dareste is that the formation of birth defects generally follows a plan in which development is abnormally arrested at a rudimentary stage.

Stockard (1921) further elaborated the theme that abnormal development, if not of hereditary origin, was caused by developmental arrests. However, Stockard surpassed Dareste by showing through experimentation with fish embryos that there are "indifferent" and "critical" moments of development. He observed that the embryonic organs arose with initial moments of extremely high activity and that "such moments of supremacy for the various organs occurred at different times during development" (p. 139). Such particularly sensitive periods are in Stockard's words "critical moments" (p. 139). Thus, it follows that the earlier the arrest the more numerous will be the types of defects found, and the later the arrest the more limited will be the variety of deformity. The complete interruption of development at a critical stage will prevent those organs or parts having supremacy at the moment from resuming their development at their relatively accelerated rate. In other words, organs developing at the most rapid rate, or having the highest metabolic rates at the time of an arrest, will be affected more than the organs or parts developing at a less rapid rate. When development is only slowed but not stopped, all organs lower their rate more or less relative to one another, and on resuming a normal rate of development, the more rapidly developing parts may be able to maintain their supremacy. Stopping or slowing development has no effect on organs at indifferent moments in their development.

Stockard also recognized that the embryonic formation of an organ was restricted to a limited moment of development. He believed that this time limitation on when an organ could form was a function of growth competition between organs. Interestingly, Stockard explained

that the heightened activity of a group of cells reflected increased metabolic activity, possibly linked to the formation of certain specific and labile compounds. Oxygen or temperature insufficiencies, for example, could at such a moment block or suppress the level of activity normally featured by the driven cellular group. In such a case, their moment for normal growth or differentiation would be lost.

The problem that Stockard posed for the future was to identify the critical moments for the different organs. One would predict (and Stockard partly supports) a hypothesis that a type of defect in one organ can be induced by many types of treatments and that different defects in many organs can be induced by the same treatment. One of Stockard's important conclusions regarding the experimental induction of abnormalities is that the result (of a perturbation) depends on the developmental time during which a treatment acts and not on the properties of the substance used.

Applications

Through various processes, including cell multiplication, cell migration, and the differentiation of cells into unique types, a single fertilized egg gives rise to the multicellular gastrula, germinal layers, and eventually the organs and patterns of growth recognizable in each species. Because the expression of genes is temporarily ordered, development proceeds as a series of ordered events. Development is characterized more as a series of stages rather than as a continuum. When the brain is viewed as a whole organ, several stages of particular rapid growth characterize it (Gottlieb, Keydar, and Epstein, 1977), and at the cellular level, the cytoplasmic architecture of individual neurons matures in stages (Lavelle, 1964). During such critical periods, development is interactive with the environment.

Interestingly, the nervous system is one of the first organ systems to develop from the gastrula and thus plays a major role in organizing the induction of other organs and tissues, yet it is probably the last to reach maturity. Because of its intrinsic complexity and the required long period of development, the nervous system is uniquely vulnerable to nongenetic influences during development and growth.

The next chapter provides a comparatively detailed accounting of cellular development of the nervous system. Biochemical differentiation (treated in various other chapters) is more closely linked with the divergence of unique cell types and consequently, is not as readily subject to useful generalization. However, it is useful to roughly identify stages of brain development as a preface to discussing critical or vulnerable events. Useful stages are (1) proliferation and migration of cells (see the chapter by Bayer), (2) development of specific recognition between cells during outgrowth (Edelman, 1983), (3) arborization of dendrites and de-

velopment of receptors for neurotransmitters (see the chapters by Telang and Enna, and by Jones) and (4) myelination (see the chapter by Gould). These events are neither entirely sequential nor synchronous throughout the brain. However, it is clear that interactions *between* cells and cell contacts are fundamental to all these processes.

A major part of brain development occurs postnatally during a period called the *brain growth spurt*. Thus, during much of its development the brain does not have the protection afforded the fetus by the homeostatic mechanisms of the dam. The postnatal brain growth spurt has been called *the vulnerable period* in development because of these circumstances (Dobbing, 1968; Davison and Dobbing, 1966). The critical period in organ development has usually been described (incorrectly) as the time of most rapid cell division, and as one might suspect, deviations from normality become increasingly subtle the later an insult or perturbation occurs in the organism's approach to final maturity. However, biochemical differentiation is a prerequisite to morphological differentiation; consequently, the critical event leading to an abnormality may occur at a time prior to the first appearance or complete development of the structure.

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The Development of the Central Nervous System

Shirley A. Bayer

Development and Characteristics of the Neuroepithelium

Formation

The nervous system begins its long journey to maturity with the formation of a specialized layer of epithelial cells, the neuroepithelium. Largely because of the pioneering work of the experimental embryologist Hans Spemann during the early part of this century (summarized in Spemann, 1938) it is known that in vertebrate embryos, the initial stimulus to form the neuroepithelium occurs during gastrulation. As cells from the dorsal lip of the blastopore (in amphibian embryos) or from the primitive streak (in avian and mammalian embryos) grow anteriorly to form the dorsal mesoderm, they induce the overlying ectoderm to become the neural ectoderm, whose cells constitute the neuroepithelium. Toivonen et al. (1975) found that the dorsal mesoderm retains its inductive capacity when a millipore filter is sandwiched between it and the overlying ectoderm. Since subsequent electron microscopic examination did not reveal any cytoplasmic processes in the filter pores, these workers concluded that morphogenetic signals for the nervous system are carried by biochemical compounds and that no cytoplasmic contact between the inducer and the responding tissue is required.[1]

Initially, the neural ectoderm is a flat sheet of cuboidal cells. Then, two shape changes take place. First, the cells become columnar, each cell being a cylinder of nearly constant diameter throughout its length. In this stage, the columnar ectodermal tissue is called the neural plate (see *A* of fig. 1). Next, the apical end of each cell becomes constricted. Because the cells are firmly attached to each other (by tight and gap junctions) at their apical ends, a constriction of all the cells just at one end will cause them to curve inward to form the neural groove (*B* and *C*, fig. 1; Burnside, 1973; Karfunkel, 1974; Bancroft and Bellairs, 1975). According to Burnside (1973), end-to-end alignment of microtubules probably accounts for the columnar shape change; colchicine, which interferes with microtubule alignment, will block the columnar shape

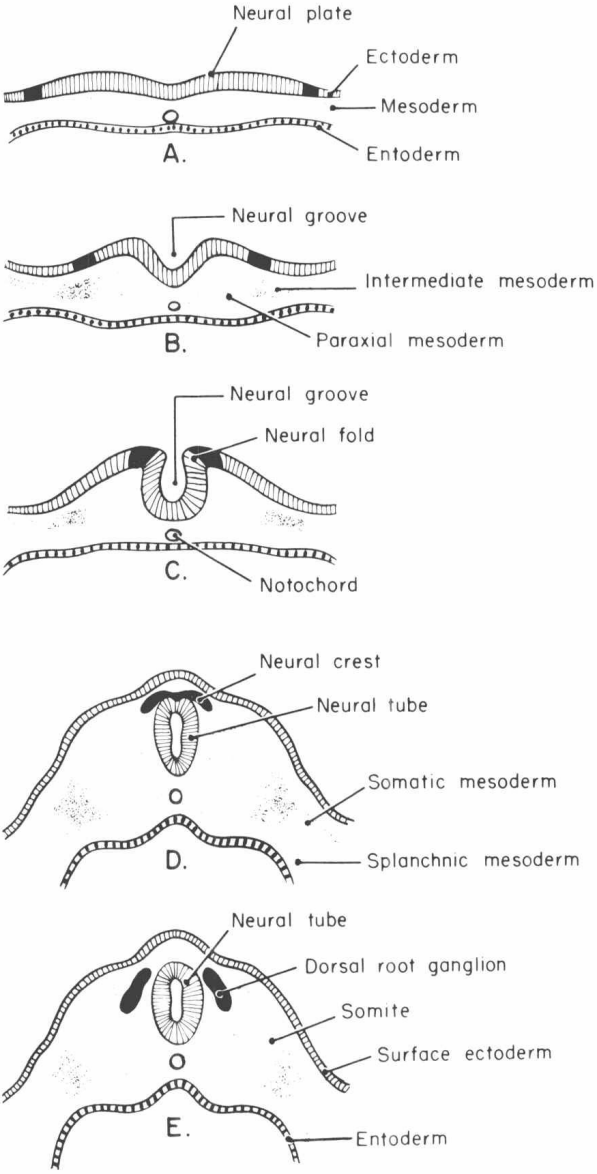

Figure 1. Diagrams of coronal sections (cut is perpendicular to the midline) of mammalian embryos at various stages of early embryonic life to show the initial development of the nervous system. The neural tube differentiates into structures of the central nervous system (see fig. 2), while the neural crest produces all peripheral neurons (sensory neurons in spinal and cranial ganglia and autonomic motor neurons) and glial cells (Schwann cells). (From Truex and Carpenter, 1969; copyright © 1969 by the Williams and Wilkins Co., Baltimore.)

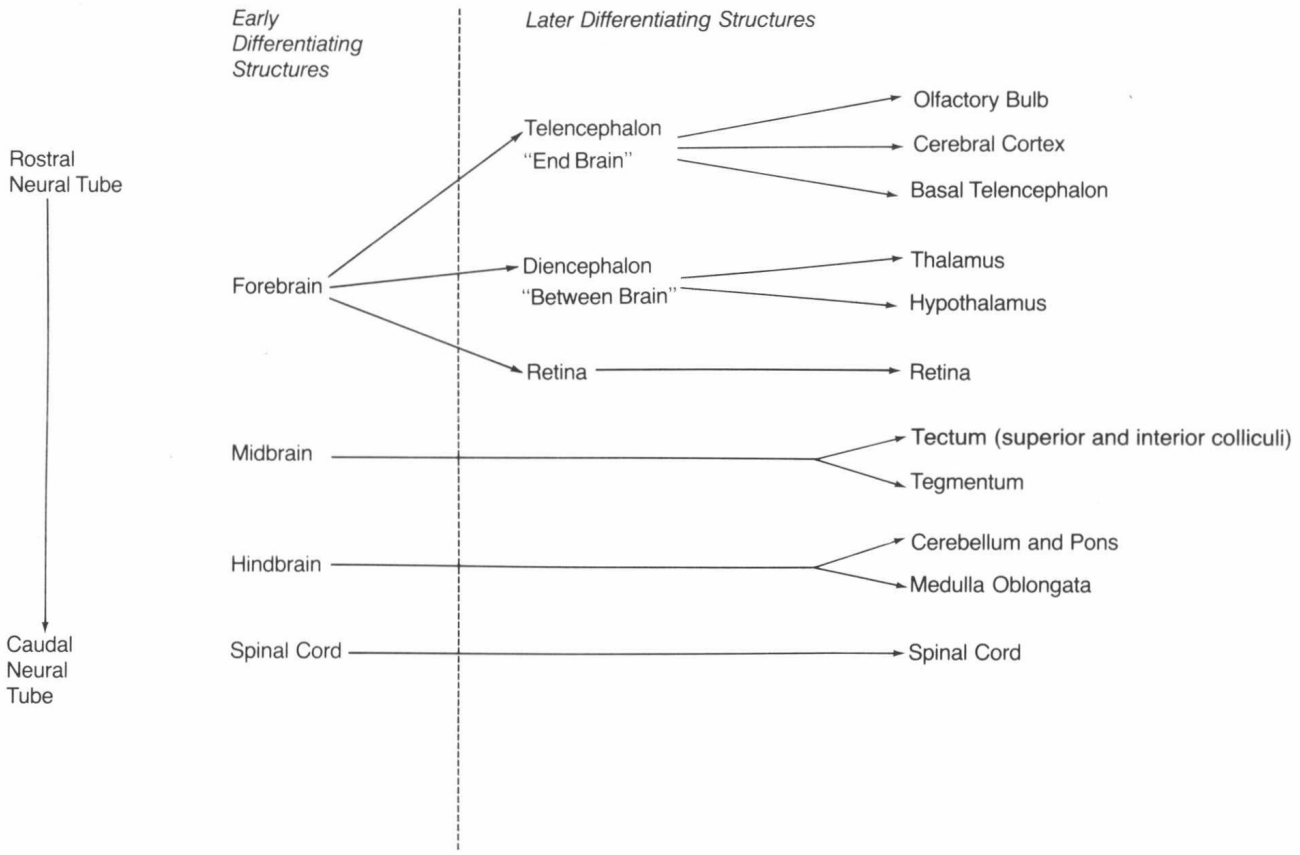

Figure 2. Parts of the nervous system that differentiate from the neural tube.

change. Burnside (1973) also found that microfilaments at the apical end slide past each other to form thick bundles, a formation that decreases the cell diameter much like the contraction of a sphincter muscle. Certain drugs such as cytochalasin B and vinblastine will inhibit apical constriction (Schroeder, 1973).

As the leading edges of the neural groove, called the *neural folds*, approach each other in the midline (*C*, fig. 1), they fuse to form the neural tube (*D* and *E*, fig. 1). During this process, extracellular material (such as carbohydrate residues) accumulate on the apical surfaces of the cells, especially in the zone of future fusion (Moran and Rice, 1975; Lee et al., 1976). If chick embryos are exposed to concanavalin A, a drug that interferes with cell-surface binding of carbohydrate residues, the folds of the neural groove meet but do not fuse (Lee et al., 1976). These results lend support to the idea that the accumulation of extracellular material is important during neural tube formation.

The neural tube is composed of a layer of cells called the *neuroepithelium*. These cells are the source of all structures included in the central nervous system (CNS) (brain and spinal cord). Figure 2 is a diagram showing early and late differentiating structures.

Mosaic Nature of the Neuroepithelium

During formation of the neural tube, the neuroepithelium is mitotically active, and young neurons begin to accumulate along its perimeter. This process continues at a rapid pace throughout embryonic life as the neural tube is formed into the structures of the brain and spinal cord (fig. 2). A given region of neuroepithelium will eventually develop into a highly specialized part of the CNS, each part having distinct structural features. The induction of the presumptive neural ectoderm to form the CNS takes place in a two-step process. In a classical experiment on amphibian embryos, Toivonen and Saxén (1968) removed a portion of the neural plate, which would eventually become the forebrain, along with a portion of trunk mesoderm during early embryonic life. The cells of both explants were separated and then cultured together in various ratios. The results (fig. 3) showed that forebrain neuroepithelium developed into hindbrain and spinal cord structures as the ratio of neural to mesodermal cells became 50:50 or less. During the first stage of induction, neuroepithelial cells are determined to produce neurons, but not those in a particular part of the CNS. Further regional specificity is induced by the proportion of mesodermal cells acting on various zones of neuroepithelium throughout the neural tube. Figure 2 lists the final products of neural tube differentiation throughout its rostral to caudal extent. After the secondary induction, the neuroepithelium is determined to develop into a specific portion of the nervous system. For example, if a "patch" of neural plate cells is stained with a vital dye, the structures these cells begin to produce will retain the stain for several