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Beginnings of the Nervous System

Marcus Jacobson†*

BEGINNINGS OF THE NERVOUS SYSTEM

> *There is, it would seem, in the dimensional scale of the world, a kind of delicate meeting place between imagination and knowledge, a point, arrived at by diminishing large things and enlarging small ones, that is intrinsically artistic.*
>
> Vladimir Nabokov (1899–1977),
> in *Speak Memory* (1966, revised edition)

A HISTORICAL ORIENTATION

> This was a theory of trial and error—of conjectures and refutations. It made it possible to understand why our attempts to force interpretations upon the world were logically prior to the observation of similarities. Since there were logical reasons behind this procedure, I thought that it would apply in the field of science also; that scientific theories were not the digest of observations, but that they were inventions—conjectures boldly put forward for trial, to be eliminated if they clashed with observations; with observations which were rarely accidental, but as a rule undertaken with the finite intention of testing a theory by obtaining, if possible, a decisive refutation.
>
> Karl R. Popper (1902–), *Conjectures and refutations: The Growth of Scientific Knowledge*, 1962

The Nervous System Is Made of Cells

The history of neuroscience can be viewed as a gradual improvement of techniques with which complex organisms could be analyzed and reduced to their constituent cells and molecules. This can be called the reductionist neuroscience research program. The reductionist program was found on two main assumptions: Firstly, development is the assembly of elementary units in various combinations and configurations, advancing from simple to complex, each stage caused by the conditions of the immediately preceding stage. The second assumption underlying the reductionist program is that one of the main aims of embryology is to deduce the events of development and morphogenesis from the activities of elementary units.

The idea that living organisms are reducible to elementary components, invisible corpuscles and fibers, which were already in the 17th century sometimes termed molecules, was held by Pierre Gassendi (1592–1655) and Robert Boyle (1627–1691) among others (reviewed by Hall, 1979). This idea was one of the essential parts of a research program that culminated in what I have called the mechanization of the brain picture. As Popper states in the epigraph to this section, the idea leads, and the techniques and the results follow, during the construction of a scientific research program. Atomistic and molecular theories of living organisms were proposed in the 17th and 18th centuries, long before the cell theory was advanced in the 19th—reduction of organisms to their constituent parts did not only occur in the logical order from larger to smaller components, from the top down, but also from the atoms and molecules to the macroscopic structures, from the bottom up. Modern theories of cellular structure echo the micro mechanical models of the 17th and 18th century theorists. One of the consequences was that conjectures about molecular mechanisms were made that could not be tested experimentally until centuries later. Many examples (of which Mendel's theory of inheritance is the best) can be given of such premature conjectures that were systematized and generalized to form premature theories (see Jacobson, 1993). Methods to implement the reductionist program would remain unavailable for almost a century, though it was clearly perceived as the desired goal. Koelliker prophetically states in the first English edition of his *Manual of Human Histology* (1852):

> If it be possible that the molecules which constitute cell membranes, muscular fibrils, axile fibres of nerves should be discovered, and the laws … of the origin, growth and activity of the present so-called elementary parts, should be made out, then a new era will commence for histology, and the discoverer of the law of cell genesis, or, of a molecular theory, will be as much or more celebrated than the originator of the doctrine of all animal tissues out of cells.

The cell theory, introduced by Schwann in 1839, defined cells as elementary units whose division, transformation, combination, and permutation to form complex organisms could be observed with the lately improved microscopes and histological methods.

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Because of its inclusivity, the cell theory permitted generalization from one form to all others, so that development of the nervous system was no longer considered to be governed by its own exclusive laws. The cell theory is a paradigm; the neuron theory emerged from it as an important special case. Sherrington says this in the opening sentence of *The Integrative Action of the Nervous System* (1906): "Nowhere in physiology does the cell theory reveal its presence more frequently in the very framework of the argument than at the present time in the study of the nervous reactions."

From 1828 to 1839, the concept of epigenesis was established by VonBaer as a central theory of embryology: "The general before the specific, and so on down to the smallest parts." This theory opened the way for a causal analysis of development of organs and relatively gross structures. Epigenesis was interpreted by VonBaer and most of his contemporaries in terms of homology and the unity of body plan of all animals. The theory of germ layers and the theory of segmentation were results of the interpretation of development. Embryology was still practiced as a branch of morphology not requiring reference to histological structure. That changed after 1830, when the achromatic compound microscope made it possible to see fibers and globular bodies in the central nervous system. In 1837 Purkinje discovered the flask shaped cells in the cerebellar cortex which now bear his name. He concluded that in all animals the nervous system is formed of three components, fluid, fibers, and globules. Such globules were at first thought to develop by precipitation out of a homogeneous fluid substance. This notion was consistent with the theory of epigenesis and it persisted for at least a decade after the introduction of the cell theory.

Beginning in 1839 with the publication of Schwann's book on the cell theory, cells were shown to be the basic units of all multicellular organisms, and the primary roles of cells in heredity and development were very slowly revealed. There was an initial period of confusion, during which cells were still believed to originate by precipitation from a homogeneous "cytoblasteme" (Schwann's term, 1839) in addition to their production by mitosis (Fleming's term, 1860). However, by 1850–1855 Remak could give an account of vertebrate development in terms of cells originating only from other cells, a concept that was finally generalized in the dictum *omnis cellula e cellula* (Virchow, 1858).

Nevertheless, Darwin was able to write *The Origin of the Species* over a period of about 20 years until its publication in 1859 without any reference to the cellular structure. This I find one of the most remarkable facts of the history of science. It shows that scientists do not necessarily work under the influence of the spirit of the times (zeitgeist) or within a "paradigm" as defined by Thomas Kuhn (1962, 1970, 1974).

During the second epoch, particularly as the result of the work of Remak (1850–1855), Koelliker (1852), and Virchow (1858), nerve cells and neuroglial cells were shown to be the basic units of organization of the nervous system, and nerve fibers were recognized as parts of nerve cells. By 1874 Wilhelm His could formulate a purely mechanistic theory of development of the nervous system in terms of cell division, migration, aggregation, differentiation, and changes in cell form and function. This is an indication of the rate of progress that occurred in understanding the behavior of cells following the first glimmerings of the cell theory in the minds of Schleiden (1838) and Schwann (1839). Apart from formulating a general cell theory, these two got almost everything else wrong, so that their original ideas about cells were almost totally overturned before 1870.

Morphogenesis

The history of ideas about morphogenesis has an extensive literature (Russell, 1930; Radl, 1930; Needham, 1931, 1934; Hughes, 1959; Adelmann, 1966; Hall, 1969, to cite only well-known secondary sources). A sketch using broad strokes should be sufficient for the purposes of this historical orientation. Goethe, to whom we owe the term morphology, and his contemporaries and immediate successors, conceived of morphogenesis in terms of plastic transformations of tissues and organs driven by innate formative stimuli and molded by environmental forces. This concept was greatly advanced by Schwann and his successors, who could begin to understand morphology and morphogenesis in terms of cells. Morphogenesis of the nervous system was analyzed in terms of histogenesis, changes of cell shape, cell movements, and migrations (Remak, 1855; Koelliker, 1852; His, 1868, 1887). Wilhelm His (1874, 1894) showed how changes in cell shape are involved in folding of tissues such as the neural plate. The concept of cell migration in the developing nervous system was also worked out by His, first from his observations on the origins of the peripheral nervous system from the neural crest (His, 1868), the migration of cells from the olfactory placode (His, 1889; Koelliker, 1890), and later from the discovery that the neuroblasts migrate individually from the ventricular germinal zone to the overlying mantle layer of the neural tube (His, 1889). The revolutionary discovery of cell migration in the vertebrate central nervous system by His was at first greeted with skepticism, but the universality of this form of cellular behavior soon became apparent. In 1893 Loeb showed migration of pigment cells in *Fundulus*, a teleost, and migration of presumptive skeleton cells and mesenchyme cells in sea urchin embryos was discovered by Herbst (1894) and Driesch (1896). Mechanisms of cell migration were proposed by analogy with the locomotory movements of protozoa (Korschelt and Heider, 1903–1909), but there were no means for making progress along those lines at that time. Other cases of cell migration in the vertebrate central nervous system were also baffling. After discovery of the cerebellar granular layer (Obersteiner, 1883; Herrick, 1891), several attempts were made to follow the migration of granule cells until the problem yielded to Cajal's definitive analysis in the 1890s (Ramón y Cajal, 1911; see Jacobson, 1993).

THE GERMINAL CELL, HISTOGENESIS, AND LINEAGES OF NERVE CELLS

> For all those who are enchanted by the magic of the infinitely small, hidden in the bosom of the living being are millions of palpitating cells whose only demand for the surrender of their

> secret, and with it the halo of fame, is a lucid and tenacious intelligence to contemplate them.
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> The final sentence of Ramon y Cajal's autobiography, *Recuerdos de mi vida: Historia de mi labor cientifica*, Tercera edicion, 1923

Historical Orientation

> A mind historically focused will embody in its idea of what is "modern" and "contemporary" a far larger section of the past than a mind living in the myopia of the moment. "Contemporary civilization" in our sense, therefore, goes deep into the 19th century.
>
> Johann Huizinga (1872–1945), *Homo Ludens*, 1938

From its inception by Wilhelm His in 1887, the concept of subclasses of germinal cells that are the progenitors of corresponding classes of neurons and glial cells has been opposed by the concept of multipotential progenitors, put forward by Vignal (1888), Schaper (1894a, b), and Koelliker (1897). Both concepts have continued to exert powerful heuristic effects for more than a century.

Discovery of the germinal cells of the vertebrate nervous system, by Wilhelm His in 1887, can be fully understood only in historical context—it was only one of many interlocking pieces of research out of which a coherent picture of the behavior of cells during development was rapidly assembled in the second half of the 19th century (O. Hertwig, 1893–1896; E.B. Wilson, 1896). The contributions of His must also be viewed as part of the ongoing program to reconcile comparative anatomy and embryology with the gradual improvements in understanding cell structure and function (Koelliker 1852, 1854, 1896). That program gained momentum throughout the 19th century, and the investigation of the histogenesis of the nervous system was pushed forward rapidly by the advances made by cytologists and embryologists. In many ways it was a period like our own in which powerful new research techniques produced results that challenged the assumptions of time. The cell theory put forward by Schwann in 1838 was radically modified during the next 50 years, most notably by the discovery of cell division and the cell cycle.

Wilhelm His (1831–1904) towers over the field of research on histogenesis of the nervous system in the 19th century. He casts a long shadow into the 20th century through his pupils Franklin P. Mall, who brought the science of human embryology to the United States, and Friedrich Miescher, founder of the chemistry of nucleic acids and nucleoproteins. The new concepts of cell biology were transmitted to neuroembryology by His: He was the first to recognize the significance of cell migration in development of the central and peripheral nervous systems. He discovered the neural crest and showed that cranial and spinal ganglia are formed by cells which migrate from the neural crest. He was among the earliest to give evidence that the nerve fiber is an outgrowth of the nerve cell, the first to try to show when neuronal and glial cell lineages diverge, and he discovered that nerve cells originate by mitosis of stem cells near the ventricle of the neural tube. He showed that neurons originate from specific progenitor cells, which he called germinal cells (*Keimzellen*), recognizable by their mitotic figures lying close to the lumen of the neural tube (His, 1887a, b, 1888a, b, 1889a, 1890a, b).

It should be remembered that Walther Flemming's *Zellsubstanz, Kern und Zelltheilung* (1882) was hot off the press when His discovered the germinal cells in the neural tube of human embryos. Flemming's book provided the first clear demonstration of the transformation of the resting cell nucleus into the mitotic figure, and he showed that the essential event of mitosis is the duplication and division of the chromosomes. Flemming recognize that chromatin (which is the name he gave to the material in the nucleus which he stained with azo dyes) is probably the same as the nucleic acid which Miescher (1871) had purified from the nuclei of leukocytes and had called nuclein (reviewed by Hughes, 1952, 1959). By the mid-1880s, it had become evident that chromatin, the material of the chromosomes (named by Waldeyer in 1888), is the basis of hereditary. With those discoveries the links were forged between cytology, embryology, and evolution.

The great achievements of Wilhelm His are in no way diminished by the fact that he was misled by histological artifacts which were unavoidable at that time. Artifacts led him to conclude that there are two different classes of cells in the neural tube of the early vertebrate embryo: germinal cells that are visible as mitotic figures lining the lumen, which give rise to neurons, and spongioblasts that appear to form a syncytium from which neuroglial cells originate. These observations led His to formulate four separate theories: theory 1 was concerned with the different stem cells for neurons and glial cells; theory 2 was about the syncytium; theory 3 was about the significance of the large extracellular spaces and their contents; theory 4 was about guidance of migrating neuroblasts by radically aligned spongioblasts (see Jacobson, 1993 for a full discussion).

His (1887a, b, 1888a, 1889) deduced that the germinal cell divides repeatedly: one daughter cell remains close to the lumen of the neural tube and reenters the mitotic cycle while the other daughter cell becomes a neuroblast. Then the neuroblast, which is incapable of further division, migrates away from the germinal layer and eventually develops into a neuron. Recognition of the asymmetrical division of the germinal cell was a significant conceptual advance that has become assimilated into modern theory.

The suffix "blast" derives from the Greek word *blastos*, which means a germ or bud, and indicates that a cell is capable of further division. However, neuroblasts in the vertebrates do not incorporate [^{3}H]thymidine, which indicates that they have ceased DNA synthesis and mitosis. Therefore, the cell called neuroblast by His is better referred to as an undifferentiated neuron or young neuron, and the term neuroblast is best reserved for the progenitor of neurons in the invertebrates.

Another theory of neuronal and neuroglial histogenesis, in almost total disagreement with the theory of His, was proposed by Vignal (1888) and Schaper (1894a, b) and supported by Koelliker (1897). They based their theory on essentially the same histological observations as those of His, but they interpreted them in a different way. The observation that mitotic figures occur only in the cells lining the lumen of the early neural tube

had been interpreted by His to mean that the mitotic figures belong to germinal cells (*Keimzellen*), whereas he believes that the other cell nuclei of the neural tube belong to different classes of cells. He identifies some as neuroblasts giving rise to neurons and others as spongioblasts giving rise to neuroglial cells. Alternative interpretations of the histological picture were given by Schaper (1897a, b). He suggested that "*the so-called 'Keimzellen' of His lying near the central cavity of the neural tube, along the membrana limitans interna, are not to be considered as a special type of cell in contrast to the main epithelial cells in process of continuous proliferation*" (Schaper, 1897b). According to Schaper, the so-called germinal cells and spongioblasts are really cells of the same type which move to different levels on the neural tube during different phases of the mitotic cycle. The "*Keimzellen*" of His are merely cells that have rounded up close to the lumen in preparation for mitosis, after which the nuclei of the daughter cells move away from the lumen during interphase and return inward during prophase.

Schaper also showed that the young neuron, with a clear nucleus and abundant cytoplasm, can be distinguished from the neuroglial cell precursors. Some of the glial cell precursors have a small, round, densely chromatic nucleus and very scanty cytoplasm, but others may have different appearances. We would now call these cells glioblasts. Schaper showed that the young neurons and glioblasts migrate away from the lumen and form the mantle layer outside the germinal zone. Some of the cells in the mantle layer undergo mitosis. According to Schaper (1897a, p.100), these are "indifferent cells", which he thought are capable of giving rise either to neurons or to neuroglial cells. These would now be called pluripotential progenitor cells.

Schaper's theory was premature. On the authority of both His and Ramón y Cajal (1909, p.637), Schaper's theory was consigned to oblivion. It was not accepted into a research program until 50 years later when new evidence in its favor was provided by F.C. Sauer (1935a, b). He confirmed that the neural epithelium, until the time of closure of the neural tube, consists of a single type of epithelial cell in various stages of the mitotic cycle. In addition, Sauer showed that the appearance of the cell changes and its nucleus moves to different positions in the cytoplasm during the different phases of the mitotic cycle.

NEUROGLIAL ONTOGENY

> On a perfectly translucent yellow field appear thin, smooth, black filaments, neatly arranged, or else thick and spiny, arising from triangular, stellate or fusiform black bodies! One might say they are like a Chinese ink drawing on transparent Japanese paper. The eye is disconcerted, so accustomed is it to the inextricable network stained with carmine and hematoxylin which always forces the mind to perform feats of critical interpretation. Here everything is simple, clear, without confusion … The technique of dreams is now reality! The metallic impregnation has made such a fine dissection, exceeding all previous hopes. This is the method of Golgi.
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> Ramón y Cajal (1852–1934), *Histologie du systèm nerveux*, Vol. 1 p. 29, 1909

History of Neuroglia

> I have for this young researcher [Ramón y Cajal] the greatest regard, and as I have admired his great activity and initiative, I can appreciate the importance of his original observations. The small differences between his conclusions and my own cannot have an effect on my sentiments, as I am profoundly convinced that such divergence, by which one can push research forward, is always useful to science.
>
> Camillo Golgi (1843–1926), *La rete nervosa diffusa degli organi centrali del sistema nervousa*, 1901

The original concept of neuroglia meaning "nerve glue" was based on Rudolf Virchow's assumption that there must be a mesodermal connective tissue element of the nervous system (Virchow, 1846, 1858, 1867). Even if neuroglial cells did not exist Virchow would have had to invent them as a requirement for his theory—as a bold conjecture thrown out for refutation. But techniques were inadequate to either corroborate or refute that conjecture. The mesodermal origin of neuroglial cells continued to receive corroboration (Andreizen, 1893; Weigart, 1895; W. Robertson, 1897, 1899, 1900a) in the face of strong counter evidence showing that both neurons and glial cells originate from embryonic ectoderm (His, 1889, 1901).

Virchow and his disciples were primarily interested in pathology and thus in neuroglial tumors and in the reaction of the neuroglia to disease and injury. Their theories guided practice in the direction of neuropathology, and away from normal development. Virchow's research program was aimed at showing that the causes of disease can be found in derangements of cells.

It is doubtful whether Virchow saw neuroglial cells in 1846 and there are no convincing pictures of them in Virchow's book, *Die Cellularpathologie* (1858), although he there discusses the theory of a neuroglial tissue. At that time the theory led and the facts followed. Progress was slow because techniques were inadequate in the 1840's and 1850's to provide reliable evidence. Virchow (1885) later described that period: "The great upheavals that microscopy, chemistry, and pathological anatomy had brought about were at first accompanied by the most dismal consequences. People found themselves helpless … filled with exaggerated expectations they seized on any fragment which a bold speculator might cast out." Virchow's bold theory of the neuroglia consisted of two such bold speculations: first, that neuroglial cells form a connective tissue, and second, that neuroglia develop from mesoderm in the embryo. These conjectures were not Virchow's original creations. He derived the concept of tissues from Bichat (1801), who first proposed that tissues are where the functions of life and dysfunctions of disease occur—Virchow extrapolated, saying that cells are where life and disease occur. Virchow obtained the concept of mesoderm from Remak to whom he is also indebted for the idea that all cells originate from other cells.

The problem of the ectodermal or mesodermal origins of the neuroglial cells has a complex history. Wilhelm His (1889) corrected Virchow's misconception that the neuroglia form the connective tissue elements of the nervous system by showing that neuroglia cells as well as neurons originate from neurectoderm.

The problem was resolved in the last decade of the 19th century when numerous stains for connective tissue failed to stain neuroglial cells but only stained blood vessels in the central nervous system: the Unna-Taenzer orcein method (Unna, 1890, 1891) and the Weigert (1898) resorcinol-fuschin method for elastic fibers; the van Geison (1889) acid fuschin-picric acid stain for collagen; and the Mallory (1900) aniline blue stain for connective tissue. That so many different connective tissue stains failed to stain neuroglial cells could not conclusively falsify Virchow's theory because it could be maintained that glial cells are a special form of connective tissue that is not stained by any other method. That was the argument used by Andriezen (1893) and Weigert (1895). Contrariwise, the invention of specific neuroglial stains (Ramón y Cajal, 1913; Rio-Hortego, 1919, 1921a, b, 1932) was not considered to be conclusive refutation of Virchow's theory. Cajal could still maintain in 1920 that the glial cells belonging to his "third element" are of mesodermal origin. The most compelling evidence against it was that neuroglial cells in the embryo originate from the neurectoderm (His, 1889). However, the available evidence did not exclude the possibility of subsequent entry of mesodermal cells into the central nervous system. The gradual resolution of this problem is considered below, in connection with the history of the microglial cells.

Wilhelm His misidentified the progenitors of both the neurons and the neuroglial cells in the neural tube. He conjectured that neurons originate from germinal cells but the glial cells originate from a syncytial tissue named the "*myelospongium*" or "*neurospongium*" formed of spongioblasts. The theory that glial cells remain permanently anastomosed with one another continued to be corroborated by many authorities (Hardesty, 1904; Held, 1909; Streeter, 1912). In 1912, Streeter could confidently invert the truth by pronouncing that "earlier conceptions of neuroglia cells were based on silver precipitation methods (Golgi) which failed to reveal the true wealth of their anastomosing branches, and there thus existed a false impression of neuroglia as consisting of scattered and independent cells." At that time cells were believed to be naked protoplasmic bodies, lacking a membrane, and connected by protoplasmic and fibrous bridges.

The neurospongium theory was based entirely on artifacts and could not be refuted conclusively until the 1950s when the electron microscope showed that all cells in the neurectoderm and neural tube are separated by narrow intercellular clefts from the beginning of development. In the 19th century the counter evidence to the neuroglial syncytium was already quite strong. Golgi staining of the neural tube always showed separate glial cells forming a series of stages of development from the radically aligned spongioblasts to mature astrocytes (Fig. 1) (Koelliker, 1893; Lenhossék, 1893; Ramón y Cajal, 1894). As evidence against the neuroglial syncytium Alzheimer (1910) showed that one neuroglial cells may undergo pathological change while its neighbors remain normal. By the 1920's the neurospongium theory had been abandoned, not because it could be falsified conclusively, but because it failed to predict new observations predicted by the alternative theory that glial cells are always separate (Penfield, 1928, review)

The research program of His split into four separate research programs with different goals. The first dealt with the origins of neurons and glial cells from progenitors on the neural tube—His conjectured that neurons originate from germinal cells near the ventricle, and glial cells originate from spongioblasts which span the full thickness of the neural tube. The second theory dealt with the syncytial connections between cells in the neural tube. The third dealt with the enormous intercellular spaces which appeared as an artifact in sections of neural tube. This space was supposed to contain a "central ground substance" in which the cells were embedded (Boeke, 1942; Bauer, 1953). A fourth theory dealt with the role of migration of neuroblasts in these spaces, guided by the radically aligned spongioblasts

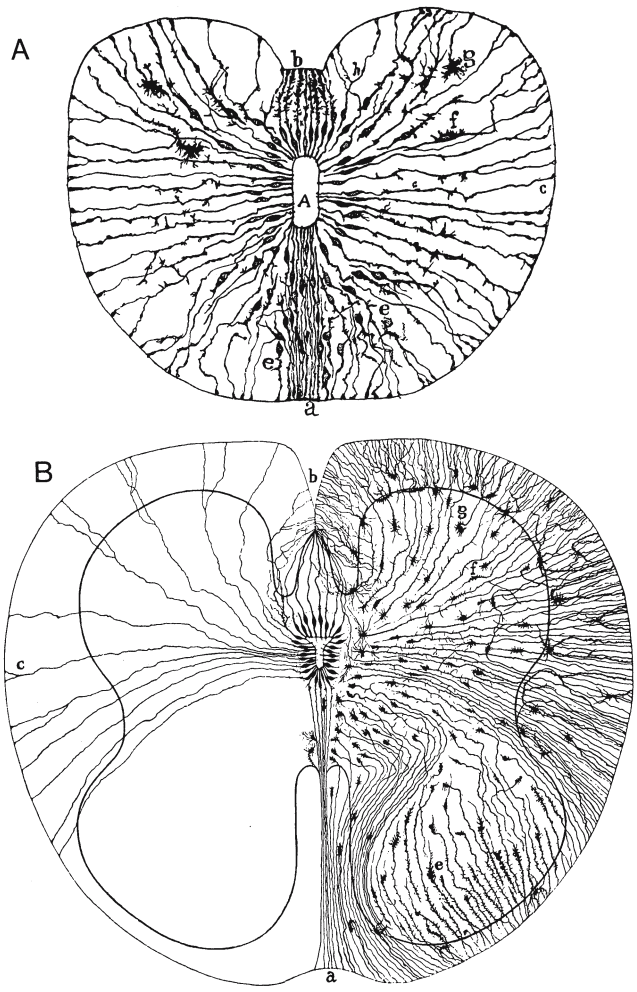

FIGURE 1. Epithelial cells and orgin of the neuroglial cells in the spinal cord of the 9-day chick embryo (A) and 14-cm humand embryo spinal cord (B). Golgi preparations. A = ependymal canal; a, b = epithelial cells of the posterior and anterior median sulci; these two types of cells maintain both peripheral and central attachments; c = ependymal cell; e = displaced epithelial cell migrating in the posterior horn; f = displaced epithelial cell migrating in te anterior horn; these two types of cells have entirely lost their central ends and conserved their peripheral ends terminating by conical boutons at the pia mater; g = epithelial cell about to become a neuroglial corpuscle; there remains no more than an outgrowth going to the pia mater. A from S. Ramón y Cajal, *Les nouvelles idees sur la structure du systeme nerveus*, 1894; B from M. von Lenhossék, Der feinere Bau des Nervensystems, 1893.

(Magini, 1888; His, 1889) (see Jacobson, 1993). These theories illustrate Cajal's dictum that "in biology theories are fragile and ephemeral constructions … while hypotheses pass by, facts remain" (Ramón y Cajal, 1928). He was also well aware that the facts were often indistinguishable from artifacts.

The Golgi research program on neuroglia started in 1875 with Golgi's studies of glial cell tumors. Golgi's earliest papers on neuroglia were published in 1870 and 1871 using hematoxylin and carmine staining which was incapable of showing either neurons or glial cells completely. By 1873 Golgi had developed his potassium dichromate-silver technique, the first of his methods for metallic impregnation which was capable of staining entire neurons and glial cells. In Golgi's 1875 paper on gliomas stained by means of his potassium dichromate-silver technique, he was able to give the first morphological definition of neuroglial cells as a class distinct from neurons. Golgi discovered the glial cell perivascular foot and conjectured that the glial cell "protoplasmic process" mediates transfer of nutrients between the blood and brain (Golgi, 1894). This led ultimately to the modern theory of the blood–brain barrier. This is only one sign of the very progressive character of Golgi's research program, not only in terms of a great technical advance but also because of its theoretical boldness.[1] By showing that the protoplasmic processes of neuroglial cells end on blood vessels and those of nerve cells end blindly, Golgi refuted Gerlach's theory that the protoplasmic processes (later called dendrites by His, 1890) end in a diffuse nerve net by which all nerve cells were believed to be interconnected. Having refuted Gerlach's theory, Golgi conjectured that the nerve net is really between afferent axons and the axon collaterals of efferent neurons. It should be emphasized that it was a mature and progressive theory for its time and became postmature and degenerative only near the very end of the 19th century. Guided by Golgi's conjectures about the relationship between neurological cells and blood vessels and about the active role of neuroglia in mediating exchange with the blood, progressive research programs have continued to the present time (see Chapters 7 and 8).

Golgi published his mercuric chloride method in 1879 but it went unnoticed until after he published his rapid method in 1887. The Golgi techniques were widely used after 1887 and were mainly responsible for the accelerated progress in neurocytology during the 1890's. One of the first advances was recognition of different types of glial cells. Astrocytes were named by Lenhossék (1891) who recognized them as a separate subclass although Golgi had identified them as early as 1873. Koelliker (1893) and Andriezen (1893a) subdivided them into fibrous and protoplasmic types according to the presence or absence of fibers in the cytoplasm. They noted that fibrous astrocytes predominate in white matter and protoplasmic astrocytes in grey matter.

There were two theories of astrocyte histogenesis. Schaper (1897a,b) conjectured that the germinal cells of His, positioned close to the ventricle, give rise to neuroblasts, spongioblasts, and "indifferent cells." He speculated that spongioblasts give rise to glial cells only in the embryo but indifferent cells persist in the postnatal period and give rise to both neurons and glial cells. A different theory was proposed by Koelliker (1890) and supported by Cajal (1909). They believed that spongioblasts persist into the postnatal period and are the only progenitors of all glial cells. Evidence for this was given by Lenhossék (1893), who showed a series of stages of astrocyte histogenesis, starting with detachment of radially aligned spongioblasts from the internal and external limiting membranes, followed by migration into the brain parenchyma where they continue to divide to give rise to astrocytes. Transformation of radial glial cells into astrocytes in the spinal cord was confirmed by Ramón y Cajal (1896) in the chick embryo. Almost a century later those premature discoveries were rediscovered; one might say they matured (Schmechel and Rakic, 1979a, b; Levitt and Rakic, 1980; Choi, 1981; Hajós and Bascó, 1984; Benjelloun-Touini *et al*., 1985; Federoff, 1986; Munoz-Garcia and Ludwin, 1986a, b; M.Hirano and Goldman, 1988). Both Lenhossék and Cajal observed that the radial glial cells are the first glial cells to differentiate and this has also been confirmed with modern techniques (Rakic, 1972, 1981; Choi, 1981). Lenhossék and Cajal found that the peripheral expansions of some of the radial glial cells persist in the spinal cord to form the glia limitans exterior, and that too has recently been confirmed (Liuzzi and Miller, 1987).

Cajal's gold chloride-sublimate method stains astrocytes very well and this enabled him to confirm Lenhossék's theory of the origin of astrocytes from radial glial cells and to refute the theory that astrocytes originate from mesoderm (Cajal, 1913a, 1916). Cajal (1913b) also showed that astrocytes can divide in the normal brain. Mitosis of astrocytes after brain injury was demonstrated by Rio-Hortega and Penfield (1927). When those theories of glial cell origins were originally proposed, they could neither be corroborated nor falsified by means of histological evidence because no techniques for tracing cell lineages existed at that time. Those theories of glial cell lineages were bypassed for a century until more reliable techniques for tracing neuroglial cell lineages recently became available (see Chapter 8).

Oligodendroglial cells were discovered by W. Robertson (1899, 1900a) using his platinum stain. He did not understand their significance in myelination and he conjectured that they originate from mesoderm (he called them mesoglia, not to be confused with the mesoglia of Rio-Hortega, which are brain macrophages). They were rediscovered by Rio-Hortega (1919, 1921), who called them oligodendroglia because their processes are shorter and sparser than those of astrocytes. He made the distinction between perineuronal satellites in the gray matter, most of which he believed to be oligodendroglia, and interfascicular ogliodendroglia situated in rows between the myelinated fibers of

[1] A theory is mature when it is accepted in a research program where it operates to guide techniques to obtain new facts. A progressive theory is defined as one that leads the facts; a degenerative theory is one that falls behind the facts. A progressive theory stimulates research programs aimed at obtaining corroborative evidence or counterevidence. In a progressive research program many theories are proposed and tested. Their refutation and replacement is the basis of progress. A theory becomes degenerative when it fails to predict new facts and when the counterevidence becomes sufficient to falsify it.

the white matter. From their anatomical position, and the fact that they appear only in late embryonic and early postnatal stages during the period of myelination, Rio-Hortega (1921b, 1922) conjectured that oligodendroglia are involved in myelination in the central nervous system. Rio-Hortega (1921b) and Penfield (1924) also conjectured that a common precursor migrates into the white matter and then divides to produce astrocytes or oligodendroglia. This conjecture has only recently been possible to corroborate (see Chapter 7).

Before the introduction of good specific stains for glial cells (Ramón y Cajal, 1913; Rio-Hortega, 1919) it was not possible to differentiate, with any degree of certainty, between processes of neurons and those of neuroglia. Neuroglial cell processes were identified by a process of exclusion. Weigert understood that there was an urgent need for a specific neuroglial stain, and he spent 30 years trying to perfect one. He pioneered the use of hematoxylin for staining nervous tissue, developed a very good stain for myelinated nerve fibers, and introduced aniline dye stains (with the help of his cousin Paul Ehrlich). Weigert (1895) was the first to invent a stain (fluorochrome-methylviolet) that was specific for glial cells, but it only stained glial fibers intensely, the remainder of the glial cell weakly, did not stain neurons, and failed to stain glia in embryonic tissue. It showed glial fibers apparently outside as well as inside the glial cells. Those findings misled Weigert to conclude that glial fibers form a connective tissue in the central nervous system analogous to collagen.

Nineteenth century theories of neuroglial functions in the adult nervous system reviewed by Soury (1899) include their nutritional and supportive functions, formation of myelin, formation of a glial barrier between the nervous system and the blood and cerebrospinal fluid, their role in limiting the spread of nervous activity, their proliferation and other changes in response to degeneration of neurons, and their involvement in conscious experience, learning, and memory. These theories of neuroglial functions were sustained more by clever arguments than by the available evidence; indeed, they continued to flourish because the means to test them experimentally were not available until recent times.

Soury (1899, pp. 1615–1639) gives a masterful critique of the theories of glial function that were being debated at the end of the 19th century. The theories were weakest in dealing with the origins and early development of glial cells and with their functions in the embryo. This is not surprising because the specific methods required for identifying embryonic glial cells were not invented until much later (the glia-specific histological stains of Ramón y Cajal, 1913, and Rio-Hortega, 1919; tissue culture of glial cells in the 1920's; identification of glial cells with the electron microscope after the 1950's; glial cell-specific antibodies after the 1970s). The concept that glial cells help to guide migrating neuroblasts and outgrowing axons, first proposed by His (1887, 1889) and Magini (1888), was not possible to test experimentally, for almost a century (Mugnaini and Forströnen, 1967; Rakic, 1971a, b). Their myelinating functions were suggested by a few but rejected by most authorities in the 19th century. It was thought that myelin in the CNS is produced as a secretion of the axon and in the peripheral nerves as a secretion by either the axon ir the sheath of Schwann.

In 1913 Cajal introduced his gold chloride sublimate method for staining neuroglia. It stained astrocytes well but oligodendroglia were incompletely impregnated. Cajal (1920) mistook the latter for a new type of neuroglial cell lacking dendrites, which he called the "third element." He thought that these *celulas adendriticas* (adendroglia, Andrew and Ashworth, 1945) are responsible for myelination of fibers in the CNS, and that they are of mesodermal origin. Rio-Hortega's ammoniacal silver carbonate method (1919), which clearly stains oligodendroglial and microglial cells,, showed that these are the authentic "third element" and that Cajal's conclusions were based on incompletely stained cells. Cajal opposed this explanation, and although he reluctantly, and with reservations, acknowledged the authenticity of microglial cells, he continued to deny the existence of oligodendrocytes long after they were clearly demonstrated and their role in the central myelination had been revealed (Rio-Hortega, 1919, 1924, 1928, 1932; Penfeild, 1924). However, it is only fair to say that the definitive proof that oligodendrocytes are solely responsible for CNS myelination had to await the ultrastructural evidence (Farquhar and Hartman, 1957; R.L Schultz *et al*., 1957; Mugnaini and Walberg, 1964; R.L. Schultz, 1964).

As Penfield (1924) put it:

> Oligodendroglia has received no confirmation as yet though accepted by several writers. This is probably due to two causes; first, the difficulty of staining this element, and second, the fact that Cajal, repeating the work of his disciple, was unable to stain these cells, and, although he confirmed microglia as a group, he cast considerable doubt upon the validity of del Rio-Hortega's description of the remaining portion of the cells previously termed by Cajal "the third element".

Then comes the critical thrust: "As Cajal, the great master of neurohistology, has himself so often pointed out, it is extremely dangerous to assign value to negative results." Wilder Penfield (1977), who worked with Rio-Hortega in Madrid in 1924, describes how the disagreement resulted in an estrangement between the two great Spanish neurocytologists and may have been a factor that precipitated the older man into a state of depression.

Even Ramón y Cajal did not escape being overtaken and corrected by his intellectual progeny. In historical perspective we see that what Cajal is to the neuron, Rio-Hortega is to the neuroglia (Diaz, 1972; Albarracín, 1982). Rio-Hortega was the first to deduce the origin and functions of oligodendrocytes and microglial cells correctly, the first to show their structural transformations in relation to their functions and to emphasize the dynamic state of these cells in normal and pathological conditions. His artistic talents equaled those of his mentor, but while Cajal's drawings have the nervous vitality and intensity of vision of a Velásquez, Rio-Hortega's figures display the deliberately perfected beauty of a Murillo.

Two types of microglial cells in the mammalian CNS were first described by Rio-Hortega (1920, 1932): ameboid and ramified microglia. Ameboid microglia have shorter processes, appear to be motile and phagocytic, appear prenatally, and increase rapidly in the first few days after birth in the dog, cat,

and rabbit. He concluded that these are macrophages originating from the blood, as Hatai (1902b) had observed earlier. Marinesco (1909) showed that brain macrophages ingest India ink and thus behave like macrophages elsewhere. Rio-Hortega and Asua (1921) showed that microglial cells are morphologically very similar to macrophages in other parts of the body. They conjectured that microglia and macrophages both originate from the reticuloendothelial system, which at that time was being vigorously discussed (Aschoff, 1924). The ameboid microglia appeared to Rio-Hortega (1921a) to originate in what he called "fountains" of ameboid cells at places where the pia matter contacts the white matter: beneath the pia of the cerebral penducles, from the tela choroidea of the third ventricle, and from the dorsal and ventral sulci of the spinal cord. He identified another type, ramified microglia, with long processes, apparently sedentary and nonproliferative. These appear postnatally and persist in the adult. In his 1932 paper Rio-Hortega shows a series of transitional forms between ameboid and ramified microglia and concludes that these represent normal transformations between the two types of microglial cells, thus anticipating recent findings (Perry and Gordon, 1988). In the same paper Rio-Hortega shows that microglia migrate to sites of brain injury, where they proliferate and engulf cellular debris. These are the macrophages of the nervous system, whose roles in defense against infection and injury he was the first to recognize. Confirmation of most of Rio-Hortega's conclusions had to wait until the modern epoch, when the tools were forged that have made it possible to reveal the origins and functions of neuroglial cells.

The debate, started by Rio-Hortega (1932), about whether brain macrophages are derived from the blood or from the brain has continued for 50 years (reviewed by Boya *et al*., 1979, 1986; Adrian and Schelper, 1981; Schelper and Adrian, 1986). The currently available evidence shows that in adults both microglia and blood monocytes can contribute to brain macrophages, depending on whether the blood–brain barrier is intact or not. Present evidence shows that in the embryo the microglia originate from monocytes that enter the brain before development of the blood–brain barrier.

In modern times those who have concluded that brain macrophages are entirely hematogenous in origin include Konigsmark and Sidman (1963), S. Fugita and Kitamura (1975), Ling (1978; 1981), and Del Cerro and Mojun (1979). Those who have concluded that macrophages are derived from microglial cells include Maxwell and Kruger (1965), Mori and Leblond (1969), Vaugh and Pease (1970), Torvik and Skjörten(1971), Torvik (1975), and Boya (1976). The ultimate fate of the brain macrophages after repair of an injury is also controversial: they have been reported to degenerate (Fujita and Kitamura, 1975), return to the blood (Kreutzberg, 1966; McKeever and Balentine, 1978) or transform into microglial cells (Mori, 1972; Blakemore, 1975; Imamoto and Leblond, 1977; Ling, 1981; Kaur *et al*., 1987), but the latter possibility is denied by Schelper and Adrian (1986). The same techniques, in the hands of skillful workers, have led to diametrically opposite conclusions. The brain macrophages may indeed originate from more than one source and have multiple fates, but the neurocytologist tends to select his facts according to prevailing prejudices—in this he is no different from other scientists and nonscientists. The main difference between them is that the scientist, more often than the nonscientist, submits his prejudices for refutation.

THE NEURAL CREST AND ITS DERIVATIVES

> … a physiological system … is not a sum of elements to be distinguished from each other and analyzed discretely, but a pattern, that is to say a form, a structure; the element's existence does not precede the existence of the whole, it comes neither before nor after it, for the parts do not determine the pattern, but the pattern determines the parts: knowledge of the pattern and of its laws, of the set and its structure, could not possibly be derived from discrete knowledge of the elements that compose it.
>
> Georges Perec (1936–1982),
> "*La vie, mode d'emloi*" (1978)

Historical Perspective

> The principle, whereby the germinal discs or organ rudiments are represented in a planar pattern, and conversely, every single point of the germinal disc reappears in an organ, I name the principle of organ-forming germinal.
>
> Wilhelm His (1831–1904),
> *Unsere Körperform*, p. 19, 1874

In 1868 Wilhelm His discovered the neural crest and he traced the origin of spinal and cranial ganglia from the neural crest. These discoveries raised several theoretical problems—the origin and boundaries of the neural crest, the modes of cell migration, and the fates of cells of neural problems—origin, migration, and fate—have been the main conceptual platforms from which research programs on neural crest development have been launched.

The first of those research programs was aimed at finding the origins and morphological boundaries of the neural crest in the brain as well as the spinal cord. Wilhelm His thought that the neural crest originates from the *Zwischenstrang* (meaning "intermediate cord") situated between the cutaneous ectoderm and the neural plate, and he regarded it as distinct from the neural tube and the lateral ectoderm, both anatomically and with respect to the structures to which it gives rise. A priority dispute arose when Balfour (1876, 1878) announced the "discovery" of the neural ridge, part of the neural tube, as the origin of the spinal ganglia in elasmobranch fish. Wilhelm His (1879) then asserted his claim to priority of discovery of the origin of the cranial and spinal ganglia from a distinct organ-forming zone, the neural crest. Wilhelm His (1874) identified the neural crest as one of the organ-forming germinal zones as defined in the epigraph to this section, and he conjectured that it contained subsets of cells with restricted fates. During the following century that conjecture was subjected to numerous weak refutations and partial corroborations,

and it has recently received further corroboration (Maxwell *et al*., 1988; Fontaine-Perus *et al*., 1988; Baroffio *et al*., 1988; Smith-Thomas and Fawcett, 1989).

The problem of migration of neural crest cells was conceived by His as part of the general problem of cell migration and cell assembly during morphogenesis, in purely mechanistic terms of mechanical guidance either of coherent masses of cells or of individual cells. This mechanistic theory was greatly influenced by Carl Ludwig, professor of physiology at Leipzig, to whom His dedicated *Unsere Körperform* (1874), as a kind of mechanistic embryological manifesto. The aim of his manifesto was to refute theories which held that nonmaterial vital forces were at work during development, for example, Ernst Haeckel's "morphogenetic forces" ("*form-bildende Kräfte*") and Justus Liebig's "life forces" ("*lebens Kräfte*"). Mechanistic materialism of the kind upheld by His, Moleschott, Ludwig, and others was unable to refute vitalistic theories because there always remained some phenomena which could not be given a completely mechanistic explanation. Vitalists were unwilling or unable to disclose the conditions for refutation of their theories, and by placing them virtually beyond refutation they also place them outside the empirical sciences.

The history of research on the neural crest can be divided into three overlapping research programs. The first program started with the discovery of the neural crest by Wilhelm His in 1868 and lasted until about 1900. Its primary objectives were to describe development of the neural crest in terms of cellular activities and to fit the observations into comparative morphological and evolutionary theories (summarized in Neumayer, 1906). To achieve those objectives it was necessary to improve microscopic and histological techniques. His made important contributions to those technical advances, such as the invention in 1866 of the first microtome to have a micrometer advance (His, 1870).

This epoch may also be dated from the publication of the first significant treatise on comparative embryology (Balfour, 1881). Twenty-six years later the achievements of this research program were collected in the magisterial compendium edited by O. Hertwig (1906, over 5,000 pages dealing with all the classes of vertebrates, with chapters by such luminaries as O. and R. Hertwig, W. Waldeyer, F. Keibel, K. von Kupffer, H. Braus, E. Gaupp, W. Flemming, and F. Hochstetter, to note only the most famous). There has been no other period of 26 years in the history of embryology in which so much has been done by so few.

Wilhelm His made the distinction between two periods of development in general, and nerve cells in particular: the period of cell proliferation or "numerical growth," as he called it, and the period of "trophic growth," which is characterized by nerve cell differentiation and by outgrowth of nerve fibers (His, 1868, p. 187). He was also the first to give convincing histological evidence that the nerve fibers are outgrowths from the nerve cells, although the concept that nerve fibers are protoplasmic outgrowths of the nerve cell had been proposed earlier by Bidder and Kupffer, Remak, and Koelliker. The observation, first made by His, that nerve fibers grow out only after neural crest cells migrate to the sites at which ganglia are formed, was critical evidence that rendered dubious the theory, then considered to be certain, that all neurons are connected by delicate fibers from the start of the migration.

One of the most significant advances during this epoch was the report by Julia Platt (1893) that she could follow "mesectoderm" from the neural crest to cartilages of the head in *Necturus*. This was the first experimental evidence challenging the dogma of origin of cartilage from mesoderm, and thus a refutation of the germ layer theory. Her findings were confirmed by von Kupffer in cyclostomes (1895), Dohrn (1902) in selachians, and Brauer (1904) in the limbless amphibian *Gymnophiona*. These findings raised fundamental questions about the origins of mesodermal segmentation of the head. The segmental pattern of the head and the relationships of the cranial nerves had been worked out by methods of comparative anatomy (Van Wijhe, 1882; O. Strong, 1895; J.B. Johnston, 1902; C.J. Herrick, 1899). The question arose about how the primary mesodermal segmentation of the head could be related to origin of cartilage cells from the apparently unsegmented cranial neural crest. The answer emerged as a result of the transplantation experiments that were the vogue during the next epoch. It was that the segmental pattern must develop primarily in the mesenchyme of the head that is not of neural crest origin; later the neural crest cells, which are not themselves segmentally determined, migrate into the predetermined metameric pattern of the mesenchyme (Landacre, 1910, 1914, 1921; Stone, 1922, 1929; Celestino da Costa, 1931; Starck, 1937; Ortmann, 1943).

The first research program terminated gradually after about 1900 with the decline of interest in comparative anatomy and with the reciprocal rise of the research program of experimental embryology. These two research programs, which overlapped in time, correspond with what Merz (1904) calls the morphological and genetic concepts of nature. The first tries to explain development in terms of rules that underlie invariant or universal patterns of morphological organization such as segmentation, polarity, and symmetry, or more generally in terms of body plans. It attempts to define homologous forms that show how morphological patterns have been conserved during development and evolution. The second tries to explain the genetic rules by which the patterns are expressed and change during development and evolution. These views are not mutually exclusive, of course, and both views were held by many workers. However, after 1900 the genetic view gained ascendancy.

The second research program can be called the Program of Experimental Embryology. It may be dated from 1888 with publication of a report by Wilhelm Roux showing that destruction of one of the first two blastomeres of the frog embryo results in development of only one-half of the embryo from which partial regeneration of the other half appears to occur. Roux's experimental method of analysis set an example for the entire research program. However, his interpretation that the egg is a self-differentiating mosaic, and even the reproducibility of his results, were later challenged by Oscar Hertwig (1894, Vol. 2 is a critique of Roux). The crucial counterexample to Roux's result was the discovery by Hans Driesch that separation of the first two blastomeres of the sea urchin embryo results in development of

two complete larvae. It has been said that while Roux pointed the way to the methodology of experimental embryology, the fundamental problem of development, namely, embryonic regulation, was raised by Driesch, and that the heir to both traditions was Spemann (Horder and Weindling, 1985). Certainly, with regard to the history of the neural crest, the relative role of self-differentiation and of regulation of crest cells has been an important issue.

This program was defined by Wilhelm Roux in 1894 with his manifesto, the celebrated "*Einleitung*," published in the first issue of his journal *Archival fur Entwickelungsmechanik*. The opening sentence states that "Developmental mechanics or casual morphology of organisms, which this Archiv is dedicated to serve, is the Doctrine of the causes of organic form, consequently the cotrine of the causes of the origin, maintenance, and involution of these forms." The experiment is the method of casual analysis, Roux declared. The comparative embryologists and anatomists have arrived where the new science of *Entwickelungsmechanik* is starting, Roux states, and he refers in a single sentence to the work of Wilhelm His. It is also strange that the name of His does not appear in the long list of those who gave their support to the new *Archiv*.

Manifestos proclaiming new theses always exaggerate their importance and originality, and Roux's was no exception. The three distinctive features of Roux's contribution were all derivative. His performationism was derived from a long tradition and most directly from Weismann and His, albeit with modification. Roux's mechanistic materialism also had a venerable tradition, but for the present purposes it is only necessary to say that Roux's outlook was less rigorously and uncompromisingly materialistic than that of His. Roux's claim that he had introduced a totally new causal-analytic experimental method was true only in that the experimental method became increasingly used in embryology after Roux. Experimental perturbation of development had been used to analyze development long before—for example, Johann Friedrich Blumenbach (1752–1840) made animal chimeras of differently colored hydra to show that the whole animal can be reconstituted by regulation of the different parts and not by regeneration of each part separately (Blumenbach, 1782). Experimental analysis of artificial fertilization of frog eggs, performed by Lazzaro Spallanzani (1784), is another early example of the causal analysis of development prior to the era of *Entwickelungsmechanik*.

Two important experimental techniques were invented at the start of this research program, namely, tissue culture and analysis of chimeras. Tissue culture was invented by Wilhelm Roux and Gustav Born (1895) but was first used for studying neurite outgrowth by Ross Harrison (1910, 1912). Surprisingly fast, a spate of publications appeared in which nervous tissue was studied *in vitro* (Lewis and Lewis, 1911, 1912a, b; Marinesco and Minea, 1912) and the technique has remained indispensable to the present time. The second important technical advance made at that time was the analysis of amphibian chimeras by Gustav Born (1876) and Ross Harrison (1903). The technique of interspecific and intergeneric grafting was very useful in the early studies of neural crest migration in urodeles (Lehman, 1927; Raven, 1931, 1936, 1937; Detwiler, 1937). In frogs, chimeras made up of cells from two species with different cellular markers have also been used quite often since the early experiments of Born and Harrison (G. Wagner, 1948; Thiébaud, 1983; Sadaghiani and Thiébaud, 1987; Krostoski *et al*., 1988). Analysis of neural crest development has been greatly advanced by using the quail/chick chimera introduced by Le Douarin in 1969 (Le Douarin, 1973; Le Douarin and Teillet, 1973).

Another advance made during this period was clarification of the contribution of ectodermal placodes to cranial ganglia. This was first done by Landacre (1910, 1912, 1916) in the fishes *Ameiurus* and *Lepidosteus* and the dogfish *Squalus*. Later he was able to trace the fates of neural crest cells in the head because of their characteristic pigmentation and yolk granules in the urodele *Plethdon* (Landacre, 1921). The placodal contributions to cranial ganglia were further defined by Knouff (1927, 1935) in the frog. The vital staining method of Vogt (1925, 1929) was used to trace the fates of cranial neural crest in urodeles by Detwiler (1937a), Yntema (1943), and Hörstadius and Sellman (1946).

Studies on mammalian neural crest development were limited to descriptive observations, and the advances that were made by the methods of experimental embryology, using amphibian embryos, could not be applied at that time to mammalian embryos. The first description of neural crest in a mammal was given by Chiarugi (1894) in the guinea pig. This was followed by the extensive series of guinea pig embryos studied during the 1920s by Celestino da Costa (1931) and Adelmann's (1925) classical study of cranial neural crest development in more than 200 rat embryos. Those descriptive studies are unlikely to be repeated and are still valuable sources of information.

Perhaps the most significant achievement of the second research program on neural crest development was the demonstration that neural crest cells are pluripotent and that they can give rise to a wide range of cell types in all three classical germ layers (reviewed by Raven, 1931–1933, 1936; Starck, 1937; Hörstadius, 1950). The ablation and grafting experiments of the earlier part of this epoch have been repeated more recently using better techniques and have been extended to include avian and mammalian embryos. That program also remained incomplete, so that the mechanisms of cell migration, inductive cellular interactions, and cell determination remained to be elucidated by molecular biological methods.

In the Developmental Neurobiology Research Program, from the 1960s, the search for molecular mechanisms has dominated the field. The thrust of the new research program was articulated by Joshua Lederberg in the introduction to the first volume of *Current Topics in Developmental Biology* (1966): "The field has had enough fancy; more recently its methodology has been under enormous pressure to accommodate the inspirations of molecular biology and the models of development that can be read into microbial genetic systems. But now, as this volume amply shows, it is responding."

AXONAL DEVELOPMENT

> If there exists any surface or separation at the nexus between neurone and neurone, much of what is characteristic of the

> conduction exhibited by the reflex-arc might be more easily explainable. … The characters distinguishing reflex-arc conduction from nerve-trunk conduction may therefore be largely due to intercellular barriers, delicate transverse membranes, in the former.
>
> In view, therefore, of the probable importance physiologically of this mode of nexus between neurone and neurone, it is convenient to have a term for it. The term introduced has been synapse.
>
> Charles Scott Sherrington (1857–1952), *The Integrative Action of the Nervous System*, 1906

Historical Perspective

> When you are criticizing the philosophy of an epoch, do not chiefly direct your attention to those intellectual positions which its exponents feel it necessary explicitly to defend. There will be some fundamental assumptions which adherents of all the variant systems within the epoch unconsciously presuppose. Such assumptions appear so obvious that people do not know what they are assuming because no other way of putting things has ever occurred to them.
>
> Alfred North Whitehead (1861–1947), *Science and the Modern World*, p. 71, 1925

The history of ideas about the forms and functions of neurons shows that the conditions which permit different scientists to uphold totally opposed hypotheses are, firstly, that the evidence is contradictory and inconclusive and, secondly, that one or both hypotheses are based on erroneous assumptions. One of the major assumptions of the late 19th century was that animal cells lack a cell membrane. The cell surface was believed to be a transition between two phases, without special structure. Therefore, it was assumed that protoplasmic bridges between cells could freely appear and disappear. To recognize the significance of this fundamental assumption is to gain an entirely fresh view of the history of rival theories of formation of nerve connections. Proponents of the *neuron theory* believed that nerve cells only come into close contact and are never in direct protoplasmic continuity, whereas proponents of the *reticular theory* believed that nerve cells are directly connected by protoplasmic bridges or networks. The reticular theory is consistent with the fundamental assumption that cells lack membranes; the neuron theory is in conflict with that assumption.

The concept of the cell without a surface membrane is as old as the cell theory itself. Schwann (1839, p. 177) wrote: "Many cells do not seem to exhibit any appearance of the formation of a cell membrane, but seem to be solid, and all that can be remarked is that the external portion of the layer is somewhat more compact." No special structures or functions were attributed to the cell surface, but the "physical basis of life" that was so much discussed by 19th-century biologists was assumed to reside in minute particles and fibers in "the protoplasm" (reviewed by Hall, 1969). For example, Max Schultze (1861) defined cells as "membraneless little lumps of protoplasm with a nucleus." The concept that the cell lacks a membrane was supported by Carl Gegenbauer in his influential essay on the evolution of the egg, published in 1861. Sedgwick (1895) regarded the embryo as a giant protoplasmic mass in which numerous cell nuclei are embedded. In discussing the structure of nerve cells, Koelliker (1896, Vol. 2, p. 45) believed that the "central cells lack a definite membrane and possess as boundaries only the tissues of the grey substance, which consists in varied proportions of nerve fibers, glial cells and blood vessels." In the 1st edition of E.B. Wilson's very influential book *The Cell in Development and Inheritance* (1896, p. 38), I came across the statement that "the cell-membrane of intercellular substance is of relatively minor importance, since it is not of constant occurrence, belongs to the lifeless products of the cell, and hence plays no direct part in the active cell-life." Wilson maintains the same opinion in the second edition (1902, p. 53), but in the third edition (1925, p. 54) he provides some evidence for the existence of a plasma membrane.

The intellectual climate before about 1920 nurtured the concept of protoplasmic connections between neurons. When the inadequate histological methods of those times failed to resolve membranes between cells, it was quite reasonable to assume that the cells are connected to form a syncytium. This flawed assumption, as much as the histological artifacts, formed the basis for reticular theories of connections between neurons.

All the reticular theories of neuronal connectivity claimed that neurons are in direct protoplasmic continuity, and form various types of networks (Gerlach, 1858, 1872; Golgi, 1882–1883, 1891; Apáthy, 1897). The outlines of these theories have so often been reviewed that they do not require repetition insofar as they narrate the sequences of events and their main ideas (Stieda, 1899; Soury, 1899; Barker, 1901; Ramón y Cajal, 1933; Van der Loos, 1967; Clarke and O'Malley, 1968). However, none of those authors seems to have recognized that the unstated assumption beneath all the variant theories was that cells normally lack a cell membrane. We can now understand more adequately how the minds of the proponents of different theories of neuronal organization were conditioned by the prevalent assumptions about cellular organization, and how far their theoretical speculations exceeded what they could have seen in their histological preparations.

The idea that nerve cells are directly interconnected through a network of fine fibers is usually attributed to Camillo Golgi (1843–1926). However, that notion was originally the brainchild of Joseph von Gerlach (1820–1896). In sections of the spinal cord stained with carmine or gold chloride, Gerlach (1872) saw a fine feltwork of fibers in the gray matter. He interpreted this to be a genuine network formed by anastomosis between branches of the dendrites, which were at that time called protoplasmic processes. Remak (1854) and Deiters (1865) have shown that the branched protoplasmic processes are different from the single, unbranched axis cylinder, but they had not been able to show how they end or form connections in either the central or peripheral nervous systems. Gerlach (1872) depicted the sensory fibers of the dorsal roots originating indirectly by branching from a diffuse nerve network formed by interconnected branches of the protoplasmic processes. Gerlach's concept was accepted by all neuroanatomists at that time because it was consistent with the prevailing belief in protoplasmic bridges connecting cells in general.

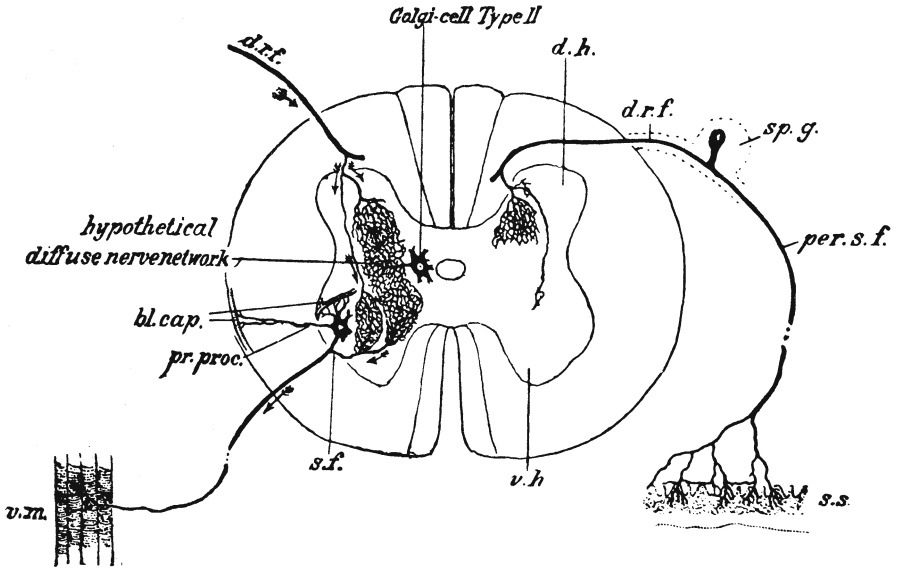

FIGURE 2. Representation of the Golgi's hypothetical diffuse nerve network in the mammalian spinal cord. The netwrok was supposed to be formed by anastomosis between collaterals of incoming dorsal root fibers (d.r.f), the much branched axons of Golgi type II cells, and collaterals (s.f.) of Golgi Type I cells (the motor neuron.) The direction of impulse traffic is shown by the arrows. The cell body and dendrites were supposed to be excluded from the conduction pathways, and the dendrites were supposed to connect with blood capillaries (bl. cap.) and to be purely mitritive in function. From L. Barker, *The Nervous System and its Constituent Neurones* (1898).

Gerlach's construct was eventually demolished by Golgi (1882–1883), using his method of impregnation with potassium bichromate and silver nitrate, which revealed the entire neuron for the first time. Golgi (1882–1883) showed that, contrary to Gerlach's construct, the protoplasmic processes end freely, without any interconnecting network, and appear to make contact only with blood vessels and glial cells (Fig. 2). Therefore, he thought that they must have nutritive functions, and he looked elsewhere for the conducting pathways between nerve cells. He believed that he had found them in the axon collaterals, which he discovered. Golgi then made the distinction between two main types of neurons: type I with a long axon and type II with a short axon. He suggested that the axon collateral of type I neurons are connected directly by a diffuse nerve network *reticola nervosa diffusa* to branches of the axons of type II neurons, thus excluding the dendrites from the conducting pathways. Golgi at first treated this as a speculative hypothesis, and the diffuse nerve network is merely discussed but never shown in any of the numerous figures in his 1882–1883 papers or in his long polemical paper of 1891 which deals specifically with the question of the functional significance of the nerve net. Pictures of the *reticola nervosa diffusa* are shown for the first time in two figures in a communication from Golgi which appears in the *Trattato di disiologia* of Luigi Luciani (1901), and the same figures are reproduced in Golgi's *Opera Omnia* (1903, Figs. 41 and 42) and his Nobel lecture (1907). One figure shows a network formed by axon collaterals of granule cells of the hippocampal dentate gyrus, and the other depicts a network in the granular layer of the cerebellar cortex formed by axonal branches of the basket cells. Neither of these curious illustrations shows the second type of cell participating in the network, as the theory requires. On this point the text also reveals Golgi's reluctance to commit himself to specific details, saying that these figures only *give an idea of the network* (Golgi, 1907, p. 15).

The reticular theory, increasingly destitute of empirical and intellectual support, found its final refuge in structures of incredible subtlety, at the limits of resolution of light microscopy. These are aptly described by Golgi (1901) as "*di organizza-zione di meravigliosa finezza*." These structures took the form of tenuous fibrillar networks that were depicted as direct extensions of intracellular neurofibrils (Apáthy, 1897; Bethe, 1900, 1903, 1904; Held, 1905). Such fancies had to be abandoned when it was shown that neurons are separated at some large synapses by a membrane which is not crossed by neurofibrils and that the neurofibrils do not enter all synaptic terminals (Bartelmez and Hoerr, 1933; Hoerr, 1936; Bodian, 1937, 1947). The neurofibrils that are stained with silver in light microscopic preparations of mammalian central nervous tissue were eventually shown to be clumps of neurofilaments when examined with the electron microscope (Peters, 1959; Gray and Guillery, 1961).

Evidence showing a discontinuity from neuron to neuron came from four quarters: embryology, histology, physiology, and pathological anatomy. The first embryological evidence of the individuality of neurons was reported by Wilhelm His (1886, 1887, 1889). He demonstrated that neuroblasts originate and migrate as separate cells and that nerve fibers grow out of individual neuroblasts and have free endings before they form connections. In 1887 His described with remarkable accuracy the outgrowth of the nerve fiber: "The fibers which grow out from the nerve cells advance by growing into existing interstitial spaces between other tissue elements. *In the spinal* cord and in the brain, the medullary stroma already formed, provides

pathways for expansion and its structure undoubtedly determines the course of the process of extension. … " These observations were later confirmed by Ramón y Cajal (1888, 1890a, b, c, 1907, 1908). This evidence showing free outgrowth of axons did not shake the faith of the reticularists because they could point out that it did not exclude the later development of protoplasmic continuity between neurons after they had made contact (Nissl, 1903; Bethe, 1904; Held, 1905). Of course, the fact that axons and dendrites develop as independent extensions of the neuron did not prove that the entire neuron remains an independent cell, but evidence for that gradually accumulated. Ramón y Cajal (1888, 1890a, b, c, 1907) showed that neurons stained by means of the Golgi technique were always completely isolated from others, and he followed Golgi in assuming that they were revealed in their entirety. This assumption could not be tested before the advent of the electron microscope.

During the 19th century there were three main theories of development of the axon: the cell-chain theory, the plasmodesm theory, and the outgrowth theory. According to the cell-chain theory, originated by Schwann and supported by F.M. Balfour among other excellent embryologists, the axon is formed by fusion of the cells that form the neurilemmal sheath. This theory of development was an extrapolation from interpretations of regeneration of peripheral nerves, in which Schwann cells and fibroblasts were mistaken for the precursors of the regenerating axons. Ramón y Cajal (1928, pp. 7–16) heaps scorn and derision on this theory and on its proponents who are "little acquainted with the severity and rigour of micrographic observation, and with the secrets of histological interpretation," but his rhetoric cannot conceal the fact that histological observations alone, without experimental evidence, were insufficient to disprove the cell-chain theory. It is fair to say that Cajal tended to use such rhetorical flourishes to conceal weaknesses in his arguments. The cell-chain theory was finally refuted by the elegant experiments of Harrison (1904, 1906, 1924a), when he showed that removal of the neural crest, from which the Schwann cells originate, results in the development of normal nerve fibers in the absence of Schwann cells. He also demonstrated that removal of the neural tube, which contains the developing neurons, prevents the formation of nerves, although the Schwann cells are left intact.

The plasmodesm or syncytial theory originated with Viktor Hensen (1835–1924) and was supported by Hans Held (1866–1942). According to this theory, the nerve fiber differentiates from preestablished filaments that connect all the cells of the nervous system. This theory was founded on the fundamental assumption, which has been discussed above, that cells originate as a syncytium in the embryo and retain protoplasmic bridges throughout life. This theory was consistent with much of the evidence available at that time and was widely supported. For example, as late as 1925 in the third edition of *The Cell in Development and Inheritance*, E.B. Wilson was still defining plasmodesms as "the cytoplasmic filaments or bridges by which in many tissues adjoining cells are connected." The plasmodesm theory provided explanation for several observation that were supposed to support the contact theory of the synapse; for example, reflex delay was ascribed to slowing of conduction during passage of the nerve impulse across extremely thin plasmodesms.

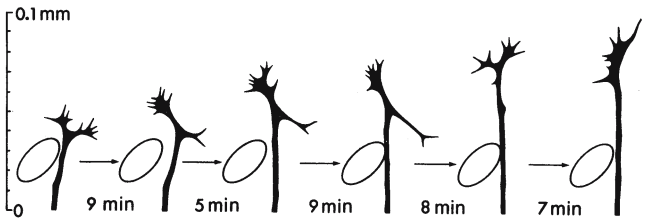

FIGURE 3. Six successive views of the end of a growing nerve fiber, showing its change of shape and rate of growth. The sketches were made with the aid of a camera lucida at the time intervals indicated. The red blood corpuscle, shown in outline marks a fixed point. The average rate of elongation of the nerve was about I μm/minute. The total length of the nerve fiber was 800 μm. The observations were made on a preparation of from embryo ectoderm, isolated in lymph, 4 days after isolation Adapted from R. G. Harrison, *J. Exp. Zool*. 9:787–846 (1910).

The outgrowth of nerve fibers was eventually demonstrated in tissue culture by Ross Harrison (1907b, 1910). Harrison excised pieces of neural tube from early tailbud frog embryos, at a stage before any nerve fibers are present, and explanted the tissue into a drop of frog lymph suspended from a coverslip. Nerve fibers grew out of the explant, in some cases from single isolated cells, for distances up to 1.15 mm at rates ranging from 15.6 to 56 &m/hour (Fig. 3). Harrison's observations were very rapidly confirmed by other reports of outgrowth or regeneration of axons in tissue culture of nervous tissue of amphibians (Hertwig, 1911–1912; Legendre, 1912; Oppel, 1913), chick (Burrows, 1911; Lewis and Lewis, 1911, 1912; Ingebrigsten, 1913a,b) and mammals (Marinesco and Minea, 1912 a–d).

The locomotion and growth of epithelial cells, young neurons, and nerve fibers in tissue culture were shown to occur only when they are in contact with a surface such as fibrin fibers in a fluid medium, or are at the interface between the solid substratum and liquid medium, or are at the liquid-air interface (Loeb, 1902; Harrison, 1910, 1912; W.H. Lewis and Lewis, 1912). This phenomenon was called *stereotropism* by Loeb (1902) and Harrison (1911, 1912), *contact sensibility* by Dustin (1910), and *tactile adhesion* by Ramón y Cajal (1910, 1928). Wilhelm His was the first to recognize the importance of mechanical factors in embryonic development. This and many other important contributions of Wilhelm His to developmental neurobiology are reviewed by Picken (1956). His clearly understood and described cases of axonal guidance by the tissue substratum and his 1894 review of the mechanical basis of animal morphogenesis contain numerous aperçus of the concepts and mechanism of nerve growth later promoted by Ross Harrison and by Paul Weiss.

These results were the final confirmation of the theory of the outgrowth of nerve fibers from young neurons first stated tentatively by Koelliker in 1844: "The fine fibers arise in the ganglia … as simple continuations of the processes of the ganglion-globules. In other words, the processes of the ganglion-globules are the beginnings of these fibers." Only 13 years later Bidder and Kupffer could assert "with the greatest degree of certainty, that … every fiber must … be conceived merely as a

colossal 'outgrowth' of the nerve cell". In 1886 Koelliker could state quite definitely that "*I consider the primitive nerve fibers to be protoplasmic outgrowths of the central nerve cells*" (Koelliker, 1886). This was only one of five theories ultimately united around the end of the 19th century to construct a theory of organization of the nervous system. The others were that the nerve cell and fibers are parts of the same unit; that dendrites are fundamentally different from axons; that nerve cells connect by surface contact and not by cytoplasmic continuity; that the contact regions are the principal sits of functional integration and modifiability; and the nerve cells and their connection are initially formed in excess, and the redundancy is eliminated during later development. Construction of the general theory ("the neuron theory") from these components is considered in detail by Jacobson (1993).

Harrison (1910) observed the outgrowth of the axons from the Rohon–Beard cells, which are the primary sensory cells and which can be seen in the dorsal part of the neural tube just beneath the epidermis in living frog embryos. Beard (1896), in his original description of the development of Rohon–Beard neurons, had accurately depicted the outgrowth of the axon from the cell body, but he failed to draw the general conclusion. Harrison observed that as the axon grows out of the Rohon–Beard cells into the subepidermal tissue, it slowly increases in length and gives rise to many branches. The growth of the axons of Rohon–Beard cells occurs in the same way as the growth of axons in tissue culture. The tip of the initial outgrowth, as well as the end of each branch, consists of an enlargement from which ameboid terminal filaments are constantly emitted and retracted. These were the first observations of the activities of the growth cone during normal development in a living animal. They fully confirmed Cajal's descriptions of growth cones in fixed specimens, and they provided a standard by which to assess whether growth cones in histological preparations are normal or artifactual. The fact that Harrison's observations on the growth of living nerve fibers agreed with descriptions of the growth of axons in histological preparations of the developing nervous system at one stroke established the validity of histological observations of Koelliker (1886), His (1886, 1887, 1889), and Cajal (1888, 1890a–c), which were far more detailed and diverse that any that could be obtained *in vitro* at that time. Harrison's experiments had, in his own estimation, taken the mode of formation of the axon "out of the realm of inference and placed it upon the secure foundation of direct observation."

Harrison coined the term "exploratory fibers" for the nerve fiber that precedes the rest of the development of a fiber pathway. Cajal gives many vivid descriptions of these pathfinders. For instance, during the outgrowth of the dorsal spinal root from the spinal sensory ganglia, "a bundle of precocious bipolar cells strikes with its cones, like battering-rams, on the posterior basal membrane and opens a narrow breach in it. Other sensory fibers, differentiating later, make use of this opening, and assault the interior of the spinal cord along its dorsal portion."

Cajal was the most vigorous advocate of the neuron theory, and it is significant that he never doubted the objective reality of the cell membrane. The sources of his conviction are difficult to trace because he does not discuss the evidence or defend his belief in the existence of the cell membrane. Already in the first edition of *Elementos de Histologia Normal* (1895, p. 303) he defines a "fundamental membrane" which is "a living organ of the cell which is a continuation of the protoplasm." In the fourth edition of his *Manual de Histoligical Normal* (1909, pp. 150, 154) he says: "All the cells of the central nervous system and sensory organs as well as the sympathetic possess a membrane of extreme thinness, a fundamental membrane. … This membrane is not a peculiarity of certain neurons, it is a general property without exceptions." This statement is made *ex cathedra*, without supporting evidence, as if it were a self-evident truth, at a time when most authorities, even some who supported the neuron doctrine, held the opposite opinion, namely, that the cell membrane is either a histological artifact or a lifeless structure without significant function.

I have been unable to determine whether Cajal's belief in the existence of a cell membrane preceded or followed his adoption of the neuron theory. The two beliefs are now seen to be so obviously interdependent that it is not easy to understand how, at that time, it was possible to affirm the one while denying the other. Yet none of the other supporters of the neuron theory shared Cajal's deep conviction, not Koelliker, not Lenhossék, not van Gehuchten. Nor can one find any discussion of the authenticity of the nerve cell membrane the 19 massive volumes of *Biologische Untersuchungen* (1881–1921) of Gustav Retzius, a consistent proponent of the neuron theory. The matter was ignored, either because they did not understand the importance of the cell membrane or because they denied its very existence. For example, Koelliker (1896, Vol. 2, p. 48) states that "with reference to the envelope of the nerve cell, it can be shown with certainty that the latter apparently at all times lacks a cell membrane.*"* Another defender of the neuron theory, Mathias Duval in his *Précis d'Histologie* (1897, p. 774) says of the nerve cell that "formerly one described it as having an envelope, by reason of artifacts produced by coagulating reagents; nowadays it is recognized that it is a naked protoplasmic body." All those who opposed the neuron doctrine were at least logically consistent in also denying the existence of a nerve cell membrane. Thus, after reviewing the evidence, Sterzi (1914, p. 19) concludes that "a cellular membrane does not exist. … The nervous cytoplasm is in direct relationship, through fine reticular fibrils that constitute the interstitial part of the nervous tissue." Cajal's convictions were not dogmatic—he was too shrewd not to be willing to acknowledge that exceptions to the neuron theory may exist. He admits that perineural connective tissue cells but not the neurons are sometimes connected by protoplasmic bridges. He says that "these mesodermic cells form a net with meshes of variable size," and he shows axons "growing through the plasmatic interstices [of the] anastomozed fibroblasts" (1928, p. 183 and Fig. 101D). Cajal treats reports of protoplasmic connections between Schwann cells with skepticism (1928, p. 83) but finally agrees that in degenerating nerves the Schwann cells form a syncytium (1928, p. 130 and Fig. 23).

In his final statement on the evidence for the neuron theory, Cajal (1933) still find it necessary to ask: "Do the terminal nerve arborizations actually touch the nude protoplasm of the

cell or do limiting membranes exist between the two synaptic factors?" Then comes the prescient conclusion: "I definitely favor this latter opinion, although with the reservation that the limiting films are occasionally so extremely thin that their thickness escapes the resolution power of the strongest apochromatic objectives." He then admits that "neuronal discontinuity, extremely evident in innumerable examples, could sustain exception … for example those existing in the glands, vessels and intestines."

The achievements of Cajal may be seen as signals rising far above the intellectual noise. But it is not always possible to see how they were generated in Cajal's mind, or even how he found the empirical stimuli for his creativity. His autobiographical account of his creative processes, *Recuerdos de mi vida* (first ed. 1917, third ed. 1923), deserves to be treated with as much skepticism as respect. His *Reglas y consejos para investigación cientifica* (1923) shows how difficult it must have been for him to discipline his unrepented romanticism (see Jacobson, 1993). In Cajal the genius of the artist and scientist were combined to a unique degree. He had the gift, usually granted only to the artist, of incorporating vague and chaotic elements of experiences into an orderly synthesis. The artist is more or less free to adopt, modify, or invent a language to represent and express his experience. The scientist is not so free and usually lacks the originality and courage that are necessary to liberate himself from the assumptions of his times.

Cajal's methods of drawing from the microscope can be inferred from his own testimony and other evidence. There is a solitary reference to his use of a camera lucida (Ramón y Cajal, 1891, legend to Fig 1), but to suggest that he used such a drawing aid habitually (De Felipe and Jones, 1988) is like saying that a life preserver is needed by a powerful swimmer within reach of the shore (see further discussion in Jacobson, 1993). Cajal describes different models of camera lucida in his *Manual de Histologia Normal*, but he also describes other instruments such as the microspectroscope and the polarizing microscope, which he probably never used. Further evidence against his habitual use of the camera lucida is that the latter is neither mentioned in Cajal's autobiography nor visible in the photographs showing him at his worktable. Cajal's line drawings of Golgi or silver preparations were evidently made with a metal pen or a goose quill, with which the width of the line can be delicately shaped by varying pressure on the point. Penfield (1954) saw Cajal writing with a good quill (but not drawing with one or wiping it on his bed sheets, as stated by De Felipe and Jones, 1988). For halftone figures, he used pencils, crayon, and fine paintbrushes (Penfield, 1977, p. 104). Cajal was well aware also of the special artistic effects obtainable with paper of different grades and textures (cf. Ramón y Cajal, 1905, p. 36). I believe that he could have used the camera lucida for laying out the picture at low magnification, but that the details were drawn freehand, keeping one eye and hand on the microscope while using the other hand and eye for drawing. This was the way in which students were trained to use the monocular microscope for making histological drawings, and it was also the principal method recommended by Cajal. He notes in his *Manual de Histologia Normal* (p. 36, 4th ed., 1905) that this method "requires a facility for copying from nature as well as artistic taste which, alas, does not always coexist in the dedicatees of the natural sciences." Cajal would undoubtedly have found this direct method no less accurate and much less cumbersome than using a camera lucida attached to this microscope, especially when a very strong source of light is required for viewing the image of a Golgi preparation 100 micrometers thick. From his own evidence it is certain that his preferred method of freehand drawing would have been inhibited and frustrated by the used of a camera lucida.

Cajal's unique gift was his ability to grasp in a novel synthesis the relationships between neurons that were seldom if ever seen in a single view through microscope. Justifying this method, he wrote:

> A histological drawing is never an impersonal copy of everything present in the preparation. If that were true our figures would be far too complicated and almost incomprehensible. By virtue of an incontestable right, the scientific artist, for the purpose of clarity and simplicity, omits many useless details. … In order to decrease the number of figures artists are sometimes forced to combine objects which are scattered in two or three successive sections (Ramón y Cajal, 1929a).

Cajal had definite presuppositions regarding the functional significance of the living structure he observed in dead, fixed specimens and was not averse to making bold inferences that went beyond anything that he could have seen (Jacobson, 1993). For example, in his drawings the conspicuous arrows pointing in the assumed direction of flow of nerve activity were meant to show an intrinsic property of the neuron to conduct in one direction only, a "dynamic polarization" of the cell (Fig. 4). His vivid description of activity of the growth cone, which he saw only as a fixed and stained structure, is also typical of the strong inductive vein in his mode of thought. Cajal's procedure was akin to that of the method of Chinese painting called *xie-yi hua*, literally "writing the meaning painting," which I have described as a combination of uninhibited fluency with deep insight (Jacobson, 1985; see also Jacobson, 1993).

The most compelling pathological–anatomical evidence of discontinuity between one neuron and the others comes from the experiments of von Gudden (1869) and Forel (1887). They observed that when axons are cut degeneration is confined to the corresponding neurons. Reactive changes occur in neighboring glial cells (Weigert, 1895; Nissl, 1894), but the neighboring, uninjured neurons remain unaffected. After the discovery of specialized nerve terminals called "endkolben" (terminal knobs) or "endfusse" (endfeet) (Held, 1897; Auerbach, 1898; Ramón y Cajal, 1903; Wolff, 1905), it became apparent that these endings are not in direct continuity with the neurons they contact. This could be deduced from the fact that injury to a neuron results in rapid degeneration of its nerve terminal structures but not of the neurons that they contact (Hoff, 1932; Foerster *et al*., 1933). Conversely, after nerve injury resulting in retrograde degeneration there is not an immediate effect on the nerve endings in contact with the degenerating neurons (Barr, 1940; Schadewald, 1941, 1942). That the acute degenerative changes are confined to

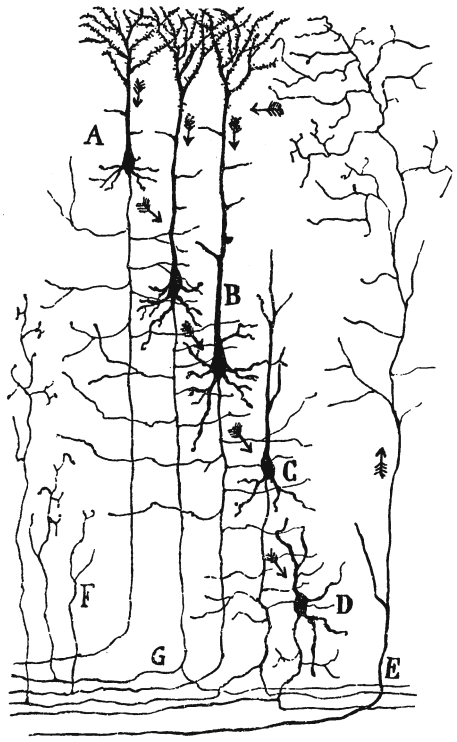

FIGURE 4. Schematic illustation of the probable currents and the nervosoprotoplastmic connections between the cells of the cerebral cortex. A = small pyramidal cell; B = large pyramidal cell; C and D = polymorphic cells; E = fiber terminal coming from other centers; F = collaterals of the white matter; G = axis cylinder bifurcating in the white matter. From S. Ramóny Cajal, Les nouvells ideés sur la structure du système nerveux chez l'homme et chez les vertébrés, Reinwald Paris, 1894.

the injured neuron was much later confirmed with the electron microscope (de Robertis, 1956; reviewed by Gray and Guillery, 1966).

Physiological experiments showed that conduction in nerve fibers is bidirectional whereas conduction in reflex pathways is unidirectional. Sherrington (1900, p. 798) proposed that one-way conduction in the reflex arc is due to a valve-like property of the junction between neurons, which he named the synapse in 1897. Thus the unidirectional conduction in the central nervous system was conceived by Sherrington to be a function of one-way conduction at the synapse and not due to the "dynamic polarization" of the entire neuron from dendrites to axon, as conceived by van Gehuchten (1891) and Ramón y Cajal (1895). Additional physiological evidence supporting the concept of the synapse was provided by measurements of the reflex conduction time, which showed a delay of 1–2 msec more than could be accounted for by the conduction time in the nerve fibers (Sherrington, 1906, p. 22; Jolly, 1911; Hoffmann, 1922; Lorente de Nó, 1935, 1938).

The question of whether transmission of excitation from neuron to muscle is electrical or chemical was first discussed by du Bois-Reymond (1877, p. 700 *et seq*.), who concluded in favor of chemical transmission, largely on the basis of the effects of curare. The accumulation of evidence in favor of chemical transmission at the vagus endings in the heart (Loewi, 1921, 1933), at the neuromuscular junction (Dale, 1935, 1938), and in autonomic ganglia (Feldberg and Gaddum, 1934; Dale, 1935) dealt directly only with transmission at synapses in the peripheral nervous system. Both Adrian (1933) and Eccles (1938) argued that the rapid speed of conduction across central synapses precludes chemical transmission. This objection was removed after it was shown that cholinesterase can act within milliseconds on the amounts of acetylcholine released at the neuromuscular junction and probably also at central synapses (Brzin et al., 1940).

The advent of the electron microscope and of intracellular microelectrode recording finally proved the identity of synapses in both central and peripheral nervous systems. Until the invention of glass knives for cutting ultrathin sections (Latta and Hartmann, 1950), the electron microscope revealed little more than the light microscope, namely, that there was what appeared to be a single membrane separating the neurons at the synapse (reviewed by Robertson, 1987). Later electron microscopic observations of synapses made with tissues fixed in osmium tetroxide, embedded in methacrylate, and sectioned with glass knives showed the presynaptic and postsynaptic and postsynaptic membranes separated by a synaptic cleft about 20 nm wide (Robertson, 1952; Palade and Palay, 1954). Synaptic vesicles, which characterize presynaptic nerve endings (Palade and Palay, 1954; de Robertis and Bennett, 1955), were immediately recognized as possible storage sites of chemical transmitters (del Castillo and Katz, 1956). After the introduction of epoxy embedding (Glauert *et al*., 1956, 1958) and potassium permanganate fixation (Luft, 1956), it became possible to see the structure of the presynaptic and postsynaptic membranes and to obtain more accurate measurements of the width of the synaptic clefts. Even before glutaraldehyde fixation (Sabatini *et al*., 1965) provided reliable pictures of cytoplasmic structure, the first attempts were made to find ultrastructural differences between excitatory and inhibitory synapses (Gray, 1959; Anderson *et al*., 1963; reviewed by Eccles, 1964).

The crucial physiological evidence proving the existence of chemical synaptic transmission was obtained by means of intracellular microelectrode recording at the neuromuscular junction (Fatt and Katz, 1951; Fatt, 1954), in the vertebrate spinal cord (Brock *et al*., 1952), and in the abdominal glanglion of *Aplysia* (Tauc, 1955). Intracellular microelectrode recording showed that the excitatory postsynaptic potential is thousands of times greater than can be accounted for by electrotonic transmission from neuron to neuron, thus making it certain that the observed amplification is mediated by chemical synaptic transmission. This evidence settled the long-standing controversy between electrical and chemical theories of synaptic transmission (reviewed by Eccles, 1959, 1964, who reversed his early position in support of electrical transmission to finally accept the general validity of chemical transmission at central synapses). It is ironic that no sooner had the dispute been resolved in favor of chemical synaptic transmission than the first report appeared of an authentic case of electrical transmission in the crayfish giant fiber to motor fiber synapses (Furshpan and

Potter, 1957). When potassium permanganate fixation allowed membrane structure to be resolved with the electron microscope, it became evident that the presynaptic and postsynaptic membranes are in close apposition at electrically coupled synapses, whereas a synaptic cleft is characteristic of chemical synapses (Robertson, 1963; 1965; Pappas and Bennett, 1966).

In addition to the preconceived ideas about the absence of cell membranes, the other main reason for the slow acceptance of the neuron theory was that the evidence in its favor arrived in bits and pieces from several different disciplines, over a period of more than 50 years. The neuron theory was built up gradually, by a process of conjecture and refutation. Such theoretical constructs can include approximations, need only have sufficient internal consistency to work, and can be continually adjusted to fix the data.

The theory of scientific revolutions, as expounded by Kuhn (1962, 1970, 1974), does not provide a satisfactory explanation of how the neuron theory ultimately replaced the reticular theory, both of which are components of different paradigms. The neuron theory replaced the reticular theory because it was a more consistent predictor of discoveries and ultimately because the electron microscopy evidence falsified the reticular theory. It is said by Kuhn (1970) that scientific paradigms are created under the influence of the *Zeitgeist* (a term invented by Goethe for events that occur "*neither by agreement nor by fiat, but self-determined under the multiplicity of climates of opinion*"). Yet the history of the neuron and reticular theories shows that both existed in the same *Zeitgeist* and people continued to believe in the reticular theory long after the passing of the *Zeitgeist* in which the theory had grown up. There were also some central figures who remained indifferent to either theory, which shows that the climate of opinion did not affect them crucially. As an example of this indifference it is instructive to quote Wilhelm Wundt (1904), the founder of physiological psychology:

> Whether the definition of the neurone in general, and whether in particular the views of the interconnexion of neurones promulgated especially by Ramón y Cajal will be tenable in all cases, cannot now be decided. Even at the present day, the theory does not want for opponents. Fortunately, the settlement of these controversies among the morphologists is not of decisive important for a physiological understanding of nervous functions.

Wundt was wrong but he had enormous influence (see Titchener, 1921; Boring, 1950). Wundt's reactionary view of the neuron theory is consistent with his archaic exposition of neuroanatomy which occupies the first volume of his very influential textbook *Principles of Physiological Psychology* (5th ed., English transp., 1904) in which the important advances in neuroanatomy and neurocytology of the previous decades are completely ignored.

The physiological psychologists, more than any other group of neuroscientists, continued to support the last vestiges of the reticular theory or variants of it. Karl Lashley (1929) was still upholding a theory of equipotentially of different regions of the cerebral cortex 50 years after Golgi had proposed a similar theory. According to Lashley (1929, 1937), learning and memory depend on the quantity of cortex, not on the specific cortical region. After destruction of large regions of cortex, the *engram* (Lashley's term for the form in which memory is stored) moves to another place without losing its essential form, as in the "harmonius equipotential system" proposed by Driesch (1908) to account for embryological regulation in his vitalistic view. Sperry's chemoaffinity theory of formation of specific nerve connections (Sperry, 1963) was a reaction to theories of equipotential or random neural networks and to theories of development of functional specificity from an initially diffuse neural network (see Jacobson, 1993).

The synapse was conceived as a theoretical necessity by Sherrington in 1897, more than 50 years before it was conclusively shown to exist and function as a physical entity. None of the 19th century proponents of the different theories of connections between neurons lived to witness the final solution of their problem. Ironically, neither the intracellular microelectrode technique not electron microscopy owed anything to the rival theories of nerve connections—the stimuli for their invention came from other sources and those techniques were first applied to other problems before being put to use in solving the problem of neuronal connections. The problems were resolved by opportunistic application of any techniques that seemed likely to work, and finally by means of microelectrode recording and electron microscopy, not merely as a result of wrangling about different theories.

This understanding of the history of neuronal connections and of the neuron theory is different from the generally prevalent view. I see the neuron theory reaching its canonical form, as we finally understand it, only gradually as a result of convergence and coalescence of several theoretical positions and research programs rather than as a revolutionary overthrow of one theory by another. The techniques that were favored by the neuronists, especially the Golgi methods, corroborated their position by showing free nerve endings. The reticularists favored techniques, for example the gold technique of Apáthy and neurofibrillar stains, which apparently revealed fine fibrils directly connecting nerve cells. The neuronists conceived of strict localization of function in the nervous system, whereas the reticularists conceived of diffusely distributed functions. These differences were, in general, also related to different values, for example, as reflected in the mechanism-versus- vitalism and in the nature-versus-nurture debates. The importance of values in neuroscience research programs is discussed further in my *Foundations of Neuroscience* (1993).

It is true that the adversarial theoretical positions occupied by the neuronists and the reticularists had a powerful heuristic effect, driving them on to seek evidence to corroborate their own position and to refute that of their adversaries. The research program progressed by means of a dialectical process of conjecture and refutation. Because refutation always lags behind conjecture, and because each side held tenaciously to its own theoretical position for as long as possible in the face of counter-evidence, both theories continued to be contested long after the reticular

theory became impossible to defend. The reticular research program, with its untenable theory, selective techniques, and special world view, became a degenerating program in the sense that it was supported by artifacts and refuted by the facts.

History provides many examples to show that to gain a scientific reputation it is sufficient to apply a new technique to resolve an old problem, but it is neither necessary to be a profound thinker nor to support the correct theory. As Schopenhauer (1851) remarks in a celebrated footnote in *The Art of Controversy*, in order to win a dispute "unquestionably, the safest plan is to be right to begin with; but this in itself is not enough in the existing disposition of mankind, and, on the other hand, with the weakness of the human intellect, it is not altogether necessary."

FORMATION OF DENDRITES AND DEVELOPMENT OF SYNAPTIC CONNECTIONS

> I find the fundamental features of a theory of the central ganglion cells in the observation of Remak, that every cell makes connection exclusively with only one motor nerve cell root, and that this is a fiber chemically and physiologically different from all other central processes … . The body of the cell is continuous, without interruption, with a more or less large number of processes which branch frequently … . These processes which … must not be considered as the source of axis cylinders, or as having a nerve fiber growing from them … will hereafter be called protoplasmic processes.
>
> Otto Friedrich Karl Deiters (1834–1863), *Untersuchungen über Gehirn und Rückenmark des Menschen und der Saugethiere*, 1865

Historical and Theoretical Perspective

> So long as one only substitutes one theory for another, in the absence of direct proof, science gains nothing; one old theory deserves another.
>
> Claude Bernard (1813–1878), *Leçons sur la physiologie er la Pathologie du systèm nerveux*, Vol. 1, p. 4, 1858

Five cardinal theories of the organization of nervous systems originated in the second half of the 19th century and formed the basis of the neuron theory on which modern neuroscience research programs are constructed. The most important advance in our understanding of the historical development of the neuron theory is that it did not originate in the 1880s and 1890s as a single theory but was constructed over a much longer period, starting in the 1840s, by convergence of at least five different cardinal theories and several other auxiliary theories. Those cardinal theories were firstly, that the nerve cell and its fibers are parts of the same unit (Wagner, 1847; Koelliker, 1850; Remak, 1853, 1855); secondly, that nerve fibers are protoplasmic outgrowths of nerve cells (Bidder and Kupffer, 1857; His, 1886); thirdly, that dendrites and axons are fundamentally different types of nerve fibers (Wagner, 1842–1853; Remak, 1854; Deiters, 1865; Golgi, 1873, 1882–1885); fourthly, that nerve conduction occurs in only one direction—from dendrites to axons to dendrites between different nerve cells (van Gehuchten, 1891; Lenhossék, 1893; Ramón y Cajal, 1895); fifthly, that nerve cells are connected by surface contact and not by cytoplasmic continuity (Koelliker, 1887, 1890,; His, 1886; Forel, 1887). In addition to those five cardinal theories, a number of additional auxiliary theories also entered into construction of the neuron theory, especially the theory that the contact regions, named synapses by Sherrington (1897, 1906), are the principal sites of functional integration and modification. One of the consequences of understanding the historical development of the neuron theory in these terms is that priority for its discovery cannot be fairly attributed to a single individual, least of all to Ramón y Cajal, who only appeared on the scene in 1888 after the theory had been largely constructed by others.

A revolutionary advance in understanding the cellular organization of the CNS occurred in the decades before those cardinal theories were promulgated. It was necessary to advance from the elementary level of understanding that the brain is composed of separate globules and fibers suspended in a fluid or ground substance (Purkinje, 1837; Valentin, 1836, 1838, among many others), to the higher level of understanding that the globule and fiber belong to the same cell. This advance was mainly accomplished by Rudolph Wagner (1847), Albert Koelliker (1850), and Robert Remak (1853, 1855). The theory of the unity of nerve cells and fibers was advanced further by the conjecture that nerve fibers grow out of nerve cells (Bidder and Kupffer, 1857; His, 1886).

The discovery of the difference between dendrites and axons (Wagner, 1851; Remak, 1853; Deiters, 1865) intensified efforts to discover how nerve cells are connected together. The earliest theories were based on the assumption that there is a special tissue consisting of fine fibrils forming the link between neurons. Joseph Gerlach (1872) thought this linkage was made by find fibrils connecting dendrites of different neurons. Camillo Golgi (1882–1885) thought that the linkage was formed by a fiber network interposed between afferent axons and collaterals of efferent axons.

Albert Koelliker (1879, 1883, 1886) was the first to conjecture that cells are connected by contact and not by continuity. It is significant that the first good evidence in support of the contact theory was experimental and not merely histological, because histological methods were at that time not capable of resolving the problem—August Forel (1887) showed that, after eye enucleation or lesions of the visual cortex, degeneration was confined to the injured neurons and did not extend to those in contact with them.

It should be emphasized that all these theories, including those which were eventually refuted, were at first progressive, in the sense that they were ahead of the facts (there were few facts on which to base any theory). They were also mature, in the sense that they could be accepted immediately into the construction of research programs and could guide research within the constraints of available techniques. Only in the late 1880s and early 1890s were the facts accumulated to unify and consolidate the five cardinal theories into the neuron theory.

Let us start by considering the level of conceptualization that had been reached in the first half of the 19th century. The concept that the nervous system consists of separate globules and fibers prevailed until the 1840s. It was believed that globules and fibers originate separately and that when they join, which occurs only rarely, it was a secondary and perhaps an impermanent union. The early microscopic observations of nervous globules were probably artifacts created by chromatic aberration. Joseph Lister (1830), who developed the achromatic objective, concluded that virtually all previous microscopic observations of histological structure were invalid because of the gross optical aberrations produced by the available lenses. The poor methods of fixation, sectioning, and staining also set severe limitations on the accuracy of histological observation. Construction of the research program concerning nerve cells and fibers and their connections was closely linked to the progress of microscopic and histological techniques.

The theory that the nerve fiber is an outgrowth of the nerve cell was originally proposed by Bidder and Kupffer (1857, p. 116): "It can be stated with the greatest degree of certainty, that the nerve cell is endowed with the conditions for allowing the fiber to grow as a direct extension out of itself. … every fiber must thereafter until its peripheral termination, regarded morphologically, be conceived merely as a colossal 'outgrowth' of the nerve cell." This theory was based on very flimsy evidence, but it was a mature theory in the sense that it could be accepted into a research program. Its heuristic power was tremendous, and it has continued to guide research until now. It was also a progressive theory in that it was ahead of the facts. It continued to lead the facts for the following 50 years or more, as they were slowly accumulated, culminating in Harrison's (1910) demonstration of nerve fibers growing in tissue culture.

Here we have an interesting exercise in assigning priorities. Priority of discovery of the unity of the nerve cell and fiber could be awarded to Remak for having shown the unity between fibers and sympathetic ganglion cells (1838); to Helmholtz (1842) for showing it in an invertebrate; to Koelliker (1844) for generalizing that concept to all nerve cells. Priority of discovery of outgrowth of fibers from nerve cells could be claimed by Bidder and Kupffer (1857), who first advanced the idea; by His (1886) for histological demonstration of fiber outgrowth from cells in chick and human embryos; by Cajal for discovery of the axonal growth cone, in 1890(c); and by Harrison (1910) for showing nerve fiber outgrowth in tissue culture. Is priority established by the one who pronounced the original theory, or who finally proved the theory? Or should priority be given to those who invented the techniques that made it possible to obtain the facts? No doubt they all did well and all deserve praise, but I think that priority belongs principally to the one who planted the tree of knowledge, less to those who tended it and pruned it, and least to those who marketed the fruit.

Wilhelm His was the first to obtain histological evidence showing that the axon grows out of the nerve cells in the spinal cord of the chick embryo. In 1886 His made this pregnant statement: "As a firm principle I advocate the following law: that every nerve fiber extends as an outgrowth of a single cell. That is its genetic, its nutritive and its functional center; all other connections of the fiber are either merely collaterals or are formed secondarily." From the deduction of Forel that nerve fibers end by contacting other nerve cells in the nerve centers, and those of His that the fiber is an outgrowth of the nerve cell, the neuron theory began to be constructed. His could not see the full extent of outgrowing nerve fibers in his preparations stained with carmine, gold, or hematoxylin, and he did not have the advantage of apochomatic lenses or of the Golgi technique, both of which became generally available after 1886. For example, His was unable to see the growth cones and could not at that time deal explicitly with the question of how one neuron connects with another. It is not sufficiently appreciated that in 1856 His had obtained the earliest evidence that nerve fibers end freely in preparations of cornea stained with silver nitrate and blackened by exposure to light. His suggested that nerve fibers in the center might also end freely but could not obtain evidence with the techniques at this command.

An indirect approach to this problem was taken by August Forel in 1887 which led him to obtain the first experimental evidence showing that nerve cells connect by contact and not by continuity. Forel's work was based on the discovery by Bernard von Gudden (1870, 1874) that removal of the eye of the newborn rabbit results, after a short survival period, in atrophy of the visual centers. He thus provided the first method for tracing pathways in the CNS. He extended this method in 1881 to show that lesions in the cerebral cortex result in degeneration in the corresponding subcortical structures. It was clear that acute degeneration is confined to the injured nerve cells, but von Gudden did not understand the general significance of his results. That was accomplished by Forel, who in 1887 showed that removal of one eye of a rabbit results in degeneration restricted to the optic nerve fibers without extending to nerve cells of the lateral geniculate nucleus, whereas removal of the visual cortex results in loss of the lateral geniculate neurons without apparently affecting the optic nerve fibers. From this Forel made the brilliant deduction that there are two separate neurons linking the retina to the cerebral cortex and that they make contact but do not form direct connections in the lateral geniculate nucleus. In his autobiography (published posthumously in 1935) Forel states:

> I considered the findings of Gudden's atrophy method, and above all the fact that total atrophy is always confined to the processes of the same group of ganglion-cells, and does not extend to the remoter elements merely functionally connected with them … All the data convinced me ever more clearly of simple contact … I decided to write a paper on the subject and risk advancing a new theory … and sent it immediately to the Archiv für Psychiatrie in Berlin. However, this periodical was then appearing at long intervals, so my paper did not appear until January 1887. … Without my knowledge Professor His of Leipzig had arrived at similar results, and had published them in a periodical which was issued more promptly, in October 1886, so that formally speaking the priority was his.

Cajal, in Chapter 5 of his autobiography, deals with the contributions of His and Forel in the following manner: "Two

main hypotheses disputed the battlefield of science: that of the network, defended by nearly all histologists; and that of free endings, which had been timidly suggested by two lone workers, His and Forel, without rousing any echo in the schools. … My work consisted just in providing an objective basis for the brilliant but vague suggestions of His and Forel." Their statements were certainly neither timid nor vague. Perhaps Cajal's perception of the contributions of His and Forel reflects his lack of understanding of their classical or Apollonian style in contrast to his own romantic or Dionysian style (Jacobson, 1993).

Let us now consider the history of concepts of dendritic form and function and the origins of the cardinal theory that dendrites are fundamentally different from axons. In 1851 Rudolph Wagner described the large nerve cells in the electric lobe of the brain of *Torpedo* and noted that usually only one of its several processes is continuous with the nerve fiber (Wagner, 1851, Vol. 3, p. 377). Before that time nerve cells had been described as ganglionic globules, notably by Christian Gottfried Ehrenberg (1833, 1836), Gustav Gabriel Valentin (1836, 1839), and Jan Evangelista Purkinje (1837a, b), but the relationship of globules to nerve fibers was incorrectly understood, and the dendrites had not been identified. For example, Valentin (1836, 1838) thought that the fibers approach the ganglionic globules and even loop around them without making contact. In 1837 Purkinje described the ganglionic globules (they were called cells only after 1839) that now bear his name in the cerebellar cortex and showed the cell body and proximal part of the dendrites without identifying the latter. In the first edition of Koelliker's *Mikroskipische Anatomie* (1850–1852) the dendrites are not identified as such.

Wagner's identification of two different types of nerve cell processes was confirmed in the multipolar nerve cells of the spinal cord of the ox by Robert Remak (1854), who also clearly showed that the axon is in direct continuity with the nerve cell body. Those observations were made by Ramak on tissue section sent to him by Stilling, who was the master of the freehand technique for cutting thin frozen sections. The relationship of the cell body to the two types of processes was described more clearly by Otto Deiters, who dissected single motor neurons from the spinal cord of the ox, after macerating the cord in a weak solution of potassium dichromate. He showed that the dendrites, which he named protoplasmic processes, are different from the axon, and he generously gave priority to Remak for discovery of two different nerve cell processes (see the epigraph to this chapter). Deiters also described a separate system of fine fibers originating from the dendrites, which he believed to run into the ground substance, in which he thought nerve cells are embedded. This was the source of the idea that there are two systems of fibers connecting neurons. Deiters, and later Gerlach, thought that axons connect with one another to form one system but a separate system is formed by the connections between dendrites of different nerve cells. Deiters' observations, which were published posthumously in 1865 by Max Schultze, elevated the difference between axon and dendrites to a general theory of nerve cell morphology. This theory was characterized by Henle (1871, p. 26) in his historical review of the progress of anatomy, as the single most important advance made up to that time in understanding the nervous system. Only the proximal segments of large dendrites could be seen until Golgi, in several works published between 1873 and 1886, showed the complete dendritic trees of neurons in the spinal cord, olfactory bulb, and cerebral and cerebellar cortex. However, it was only in 1890 that they were named dendrites by His. Another important distinction was made by Nissl (1894), who showed that the basophilic granules which now bear his name extend from the cell body into dendrites but never into axons.

By 1860 a major unresolved problem was recognized to be the way in which nerve cells are connected together in the CNS. Koelliker was able to state in 1863 (p. 313): "The case is related undoubtedly to the connections of the nerve cells to one another. Many describe anastomoses and see such where others find nothing definite. I could name many well known researchers who have shown me such variation with which I could not agree." Gerlach (1872, p. 353) claimed to have discovered the necessary link in the form of a very fine feltwork of fibrils between nerve cells in the spinal cord stained with carmine and ammonia or with gold. Gerlach conceived of the feltwork arising from the tips of the fine branches of the protoplasmic processes forming one system of connections between nerve cells and conceived of a separate system of connection between axons. I have been able to find only two reports claiming to corroborate Geralach's theory. One of these is by Boll (1874), and it is of interest because it shows fine fibers linking the protoplasmic processes (dendrites) of Purkinje cells. That might possibly have been a premature discovery of the parallel fibers.

Adequate counterevidence to Gerlach's theory could not be obtained with the existing techniques and was delayed until invention of the Golgi technique and publication of Camillo Golgi's major work. Golgi showed protoplasmic processes (dendrites), fully stained for the first time, in the spinal cord, cerebellar cortex, cerebral cortex, and olfactory bulb (Fig 5). He showed that the protoplasmic processes end blindly, without any connections to one another, and thus totally demolished Gerlach's theoretical construct. We should recognize the significance of Golgi's discoveries in relation to his progress ahead of his predecessors and contemporaries and not only in relation to the later advances made by his followers. Golgi's view of the cellular structure of the CNS was as far in advance of those of his predecessors as the views of Cajal were in advance of those of Golgi. Cajal could see farther not only because he had sharp vision, but because he stood on Golgi's shoulders.

Golgi proposed that dendrites end on or close to blood vessels, and he believed that they have nutritional functions and are not in the main conducting pathways. Golgi rejected the authenticity of Gerlach's fibrillar feltwork, but he proposed that the link between nerve cells is a network of fibrils which form connections between afferent axons and the collaterals of efferent axons. Golgi did not show a picture of his conjectured network or reticulum but in 1886 he described it in very guarded terms:

> Out of all these branchings of the different nerve processes there arises, of course, an extremely complicated texture

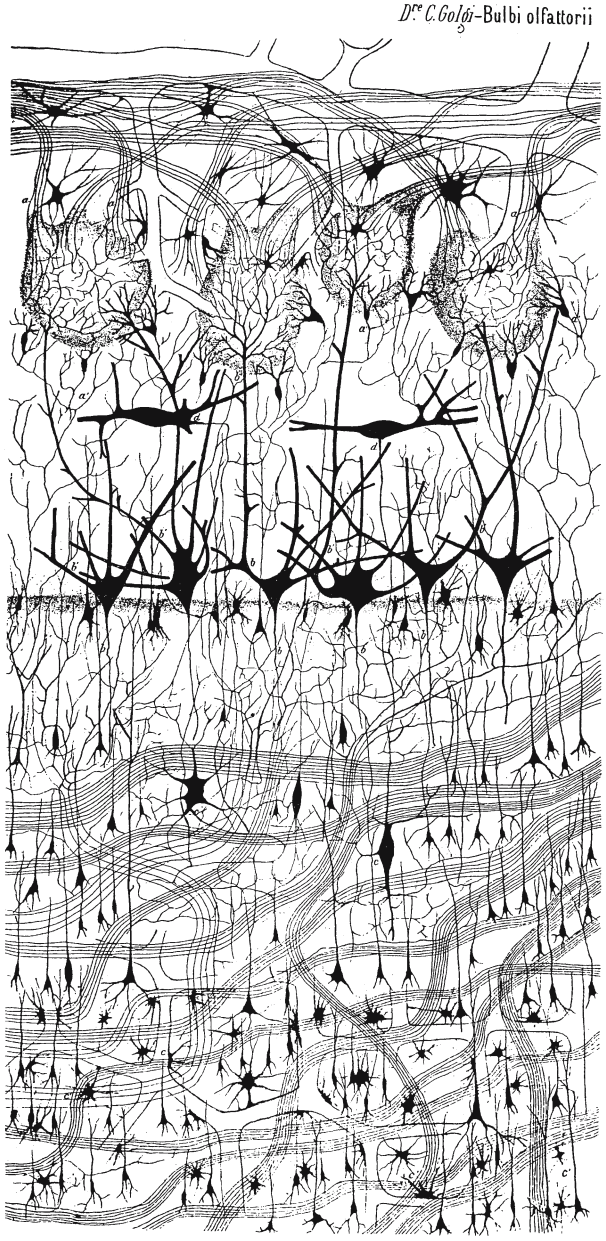

FIGURE 5. Structure of the olfactory bulb of a dog revealed by means of Golgi's newly invented technique of metallic impregnation of nerve cells, using potassium dichromate and silver nitrate. In the original figure the nerve cells and processes are shown in black and blue and the glial cells in red. Golgi states that although this figure is semischematic, it is an accurate depiction made with the aid of a *camera chiara* (same as a *camera lucida*). Golgi discovered the astrocyte perivascular endfeet (seen at the top left). At the time of publication of this figure Golgi was uncertain about the modes of connection of nerve cells, and he shows the axons as well as dendrites ending blindly. The figure was made with an achromatic objective at a total magnification of 250× and was the best that could be obtained unti apochromatic objectives became available in 1886 and enabled others to build on Golgi's work using his staining mehods. From *C. Golgi, Riv. sper. freniat. Reggio-Emilia* 1:405–425 (1875).

> which extends throughout the whole grey substance. It is very probably that out of the innumerable further subdivisions there arises a network, by means of complicated anastomoses, and not merely a feltwork; indeed one would be inclined to believe in it from some of my preparations, but the extraordinary complexity of the texture does not permit this to be stated for certain.

There followed the well-known dispute between the supporters of Golgi's reticular theory (notably Apáthy, Bethe, Held, Nissl) and the supporters of the alternative theory of neuronal connections by contact (Koelliker, 1879, 1883, 1886, 1887; Forel, 1887; His, 1886, 1889; Ramón y Cajal, 1890, 1891; Lenhossék, 1890, 1892; Retzius, 1892; Van Gehuchten, 1892; Vignal, 1889). This polemic is discussed in some detail Jacobson (1993). None of the supporters of the neuron theory actually saw the synaptic contact zone, only the free nerve endings. It should be noted that all these proponents of the theory of neuronal contact at first also admitted that there were some neurons linked by protoplasmic anastomoses. The difference between the opposing factions was that while one side asserted that contact between nerve cells is the rule and anastomoses are the exception, the reverse was asserted by the other side. This is most clearly seen in Koelliker's theoretical position, which shifted progressively from the reticular to the neuron theory. The confrontation between these opposing theories, unpleasant as it often was, had great heuristic value, resulting in efforts to obtain corroborative and refutative evidence.

Evidence showing that dendrites are in the direct conducting pathway accumulated rapidly to refute Golgi's theory that dendrites only have nutritive functions. Firstly, many bipolar neurons had been described, for example, in the spinal ganglia and cranial ganglia of fish (Wagner, 1847) and in the cochlear and vestibular ganglia. In those neurons the dendrites had to be in the conducting pathway. Max Schultze (1870, p. 174) said, "It is obvious that such a ganglion cell is only a nucleated swelling of the axis cylinder." Secondly, many neurons were found in which the axon comes off a dendrite rather than the cell body, for example, in cerebellar granule cells (Ramón y Cajal, 1888, 1890). In the invertebrate nervous system, where the cell body is outside the line of conduction, the beautiful methylene blue and Golgi preparations of Retzius (1890, 1891, 1892a) clearly showed dendrites as a necessary part of the conduction pathways and failed to show any signs of Golgi's conjectured network.

Let us now consider how concepts of the organization of specific regions of the CNS evolved historically. A number of regions were selected by Golgi for study with his metallic impregnation techniques—the spinal cord, cerebellar cortex, hippocampal formation, and cerebral neocortex, and these became the principal battlefields on which different theories of organization of nervous connections were fought (Jacobson, 1993). Briefly, I confine the discussion to the history of concepts of organization of the olfactory bulb.

The direct connection of olfactory nerve fibers to dendrites of mitral cells in the glomeruli of the olfactory bulb was discovered by Owsjannikow (1860) and Walter (1861). Their discovery that the olfactory nerve fibers connect directly with protoplasmic processes (dendrites) was contrary to all concepts at that time. Their findings were disputed by Golgi (1875), who

pointed out that the olfactory nerve fibers often stain when the mitral cell dendrites fail to stain, and *vice versa*, and that he could find no connection between them. Instead, Golgi claimed to have found fiber-to-fiber connections between the olfactory and fine nerve fibers entering the olfactory glomeruli. Golgi's depiction of cellular relationships in the olfactory bulb was correct in many aspects, was a significant advance over earlier concepts, and formed the basis for all subsequent studies. For example, Golgi discovered the astrocytic perivascular endfoot, and he correctly recognized the relationship between astrocytes and brain capillaries. Golgi (1875, 1882) gave the first modern description of the olfactory mitral cells and their relationship to the glomeruli, but he failed to trace the mitral cell axons out of the olfactory bulb. He correctly showed mitral cell dendrites entering the glomeruli, but instead of contacting the olfactory afferents he showed the dendrites contacting blood vessels. Golgi did not fail to see the irregularities on the surface of dendrites but he regarded them as artifacts caused by metallic precipitates. The dendrites are depicted with perfectly smooth surfaces in Golgi's figures of olfactory bulb, spinal cord, cerebellar cortex, and cerebral cortex. He also depicted many mitral cell dendrites ending blindly in the external plexiform layer. Golgi's observations led him to the wrong conclusion that all dendrites end blindly in association with blood vessels and are not nervous conducting elements. Golgi thought that the olfactory nerve fibers connect with fine nerve fibers which enter the glomeruli from the olfactory tract. As we know now, the only fibers from the olfactory tract which end near the glomeruli, but do not enter them, are the centrifugal fibers originating from the nucleus of the horizontal limb of the diagonal band, and it is probably that Golgi erroneously traced those into the glomeruli. He correctly showed some of those fibers ending in the external plexiform layer and also correctly showed other fibers ending in the granule cell layer (these are now known to originate from the anterior olfactory nuclei of both sides). We should remember that he had then only recently invented his method of metallic impregnation of nerve cells, which he gradually improved over the following decade. Also, his observations were made before the invention of apochromatic microscope objectives in 1886. Golgi's advance over his predecessors was at least as great as the further advances that were made by Koelliker, Lenhossék, van Gehuchten, Retzius, and Cajal. To deny Golgi the full credit due to the original discoverer, because he failed to see as far as his successors, is like denying the credit to Columbus for discovering America, because he did not land at New York and failed to explore the entire continent.

Golgi's error regarding the blind ending of the mitral cell dendrites and the fiber-to-fiber connections in the olfactory glomeruli persisted until 1890. The problem could then be resolved with apochromatic objectives, which Golgi did not have when he did his pioneering neurocytological investigations. Ramón y Cajal (1891), van Gehuchten and Martin (1891), Retzius (1892b), and Koelliker (1892), using Golgi's technique, showed that the olfactory nerve fibers end by contacting mitral cell dendrites, and that the mitral cell axons extend into the olfactory tract. Axodendritic contacts were also demonstrated between climbing fibers and cerebellar Purkinje cell dendrites (Ramón y Cajal, 1888, 1890; Retzius, 1892) and between optic nerve terminals and dendrites of neurons in the optic tectum of the chick embryo (Ram Ramón y Cajal, 1891; van Gehuchten, 1892).

The original demonstration of a specialized presynaptic ending at an identified synapse was made by Held (1897a), who showed that during development of the giant presynaptic terminals (the calyces of Held) on the cells of the trapezoid nucleus there is a clear line of demarcation between the axon and the dendrite on which it ends. However, Held incorrectly concluded that the two neurons fuse later in development. The axon terminal expansion were named: "Endfüsse" (endfeet) and "Endkolben" (Held, 1897; Auerbach, 1898). Ramón y Cajal, 1897, immediately understood the significance of these specializations as a means of increasing the contact area, and he was also the first to show that presynaptic terminal expansions occur as a rule and to demonstrate them clearly by means of his reduced silver stain. In 1903 Cajal refers to these axon endings as "mozas," "anillos," "varicosidades," "bulbos," and "botones" (knobs, rings, varicosities, bulbs, and buttons). They were called "boutons terminaux" by van Gehuchten (1904). The terminology was simplified when Sherrington (1897) introduced the term "synapse," and it became conventional to refer to presynaptic and postsynaptic structures and functions.

Confrontation between the reticular theory and neuron theory did not end with these advances—the reticular theory merely changed from a progressive theory, meaning that it was overtaken by the facts and continued to be supported only by histological artifacts. For example, in support of the reticular theory, there were several reports which claimed that fine filaments cross between neurons at the synapse and that those filaments persist even after degeneration of the presynaptic terminals (Tiegs, 1927; Boeke, 1932, Stohre, 1935). In support of the neuron theory it was shown that the end bulbs of degenerating axons swell and disappear completely within 6 days after axotomy, without apparently affecting the cell on which they terminated (Hoff, 1932). This became an effective method of tracing fibers to their terminations, and it generated a vast literature from the 1930s to the 1950s. The reticular theory was by then only a historical relic. It was finally refuted when the synapse could be studied with the electron microscope (Palade and Palay, 1954).

Many examples of axo-somatic junctions were also compelling evidence that a fine network does not form the link between neurons. For example, Ramón y Cajal showed the contacts between cerebellar basket cell axonal terminal and Purkinje cell bodies in 1888, the contacts between centrifugal optic nerve fibers and retinal cells in birds in 1889 and 1892, and the termination of cochlear nerve fibers on cell bodies in the ventral cochlear nucleus of mammals in 1896. The evidence conclusively refuted Golgi's theory, but Golgi continued to adhere to it despite the counter evidence—an outstanding case of tenacity.

A theory of synaptic receptors was first proposed by Langley (1906) from his experiments on the effects of nicotine on neuromuscular transmission in the chicken. Langley (1906) stated that the "receptive substance … Combines with nicotine

and curari [sic] and is not identical with the substance which contracts." This theory was included in a research program that had started with the simultaneous discovery by Claude Bernard and Albert Koelliker, about 1844, that curare blocks transmission at the neuromuscular junction (Bernard, 1878, pp. 237–315), and it culminated in purification of the nicotinic actylcholine receptor, its molecular cloning, and elucidation of its primary structure (reviewed by Changeux *et al*., 1984; Schuetze and Role, 1987).

The theory that dendrites change shape and retract or extend in response to functional demands was widely held at the end of the 19th century. The theory of ameboid movements of the dendrites was proposed by Rabl-Rückhard (1890) and Duval (1895). At first Cajal (1895) supported the theory and added to it the possibility that neuroglial cells penetrate into the space left by retraction of dendrites during sleep or anesthesia. Later Cajal (1909–1911) argued that the theory was unsupported by any evidence showing the required anatomical changes at synapses, but, as we know, lack of evidence is not a good reason for abandoning a theory—for that there must be well-corroborated counterevidence. Some counterevidence was obtained by Sherrington (1906, p. 24), who pointed out that the reflex delay ("latent period") is longer on the second occasion when a reflex is produced in two stages than when a single full-strength reflex is produced: "This argues against an amoeboid movement of the protoplasm of the cell being the step which determines its conductive communication with the next." That was a fine argument at the time, but subsequent research has shown changes in synaptic size and shape as a result of stimulation (Sotelo and Palay, 1971; Baily and Chen, 1983, 1988; Wernig and Herrara, 1986), and the molecular mechanism for rapid changes in size and shape of dendritic spines has been discovered (Coss, 1985; Fifková, 1985a, b).

We should now consider the theory that synapses initially develop in excess and are later eliminated selectively. The concept of competition and selection on the basis of "fitness," "Adaptiveness," and "competitiveness" derives from Charles Darwin. Once the selectionist idea was grasped it could be extrapolated to deal with populations of molecules, cells, nerve fibers, synapses, or any other parts of the organism. The first to do so was Wilhelm Roux in 1881 in his book *Der Kampf der Theile in Organismus* ("Struggle between the Parts of the Organism"). Charles Darwin considered this "the most important book on Evolution which has appeared for some time" and noted that its theme is "that there is a struggle going on within every organism between the organic molecules, the cell and the organs. I think that his basis is, that every cell which best performs its functions is, in consequence, at the same time best nourished and best propagates its kind" (Darwin, 1888, Vol. 3, p. 244). As Roux recognized, competition is keenest between individuals that are similar and will finally result in one type completely displacing the other. In his 1881 book, Roux introduced two other principles of biological modifiability and plasticity: Itrophische Reizung ("trophic stimulation") *and funktionelle Anpassung* ("functional adaptation"). In his autobiography Roux (1923) noted that he had shown that these are "also applicable as a partial elucidation of adaptation during learning in the spinal cord and brain*"* (1881, p. 196; 1883, p. 156; 1895, Vol. 1, pp. 357, 567).

In a single theoretical construct, Roux included competition, trophic interactions, and functional adaptation as causes of plasticity. This was a premature theory in the sense that it was too far in advance of the facts to be of immediate use in constructing research programs. Starting in the 1960s, the technical methods were devised that could be used to test this theory and include it in a research program. The theory of competition and selection was then reinvented in more modern terms. This was done without acknowledging Roux's priority in spite of attention that had been drawn to his contribution in both previous editions of this book. By contrast, the significance of Roux's theoretical construct was well known to his contemporaries, but it was difficult to test the theory with techniques available during the 19th century.

Ramón y Cajal was aware of Roux's theory of cellular competition and selection. Cajal showed that overproduction of axonal and dendritic branches represents a normal phase of development in which excessive components are eliminated. He tells us: "We must therefore acknowledge that during neurogenesis there is a kind of competitive struggle among the outgrowths (and perhaps even among nerve cells) for space and nutrition … However, it is important not to exaggerate, as do certain embryologists, the extent and importance of the cellular competition to the point of likening it to the Darwinian struggle …*"* (Ramón y Cajal, 1929). The last sentence indicates the influence of Roux's theoretical position, which is the origin of so-called neural Darwinism (Edelman, 1988). Cajal (1892, 1910) also adopted Roux's idea of trophic agents in the mechanism of competitive interaction, survival of the fittest, and elimination of the unfit nerve terminals, synapses, and even entire nerve cells. Since then selectionist mechanism have been proposed for development of functionally validated synaptic connections (Hirsch and Jacobson, 1974; Changeux and Danchin, 1976), for development of connections between sets of neurons by various forms of competitive interaction between nerve terminals, and for development of behavior and learning (Jerne, 1967; Changeux *et al*., 1984; Edelman, 1988).

The first evidence of specificity of formation of synaptic connections was obtained by J.N. Langley (1895, 1897), who showed that, after cutting of the preganglionic fibers of the superior cervical ganglion, selective regeneration of presynaptic fibers occurs from different spinal cord levels to the correct postganglionic neurons. Thus, stimulation of spinal nerve T1 dilates the pupil but does not affect blood vessels of the ear whereas the opposite effect is produced by stimulation of T4; T2 and T3 have both effects, but to different degrees. Langley (1895) proposed the theory that preganglionic fibers recognized postganglionic cells by a chemotactic mechanism. Guth and Bernstein (1961) concluded that this selection was made on the basis of competition between the presynaptic terminals.

Experimental tests of competition between cells or cellular elements are very difficult to do. When one structure supplants another during development, the deduction is often made that one has been eliminated as a result of competition. However, there are cases in which one structure is replaced by another

without any competition, for example, the pronephros by the mesonephros and the latter by the metanephros. In that case there is not even a causal relationship between the three kidneys that develop in succession. In general, mere succession is not evidence of causal relationship and is thus not evidence of mechanism, competitive or otherwise (M. Bunge, 1959; Mayr, 1965; Nagel, 1965). An experimental test of neuronal competition was first done by Steindler (1916) by implanting the cut ends of the normal and foreign motor nerves into a denervated muscle. Steindler found no selective advantage of the normal nerve. When two different nerves innervate a muscle, the resulting pattern is a mosaic in which individual muscle fibers are innervated at random by one nerve or the other. Steindler's observations have been repeatedly corroborated (Weiss and Hoag, 1946; Bernstein and Guth, 1961; Miledi and Stefani, 1969). Similarly, when two optic nerves are forced to connect with one optic tectum, their terminals segregate to form strips and patches in the optic tectum of the goldfish (Levine and Jacobson, 1975) and the frog (Constantine-Paton and Law, 1978).

Several theories of the possible mechanisms of competitive exclusion and elimination of synapses have been proposed. The oldest of these is the theory of formation of selective connections between neurons that have correlated activities. This is an extension of the psychological theory of association of ideas. That theory, deriving from the epistemology of John Locke and David Hume, was first given a neurological explanation by David Hartley. In his *Observations on Man* (first published in 1749), Hartley proposed that mental associations form as a result of corresponding vibrations in nerves (an idea that Newton had thrown out in the last paragraph of his *Principia*). The step from a psychological to a neurophysiological theory of association appears to have been made before the mid-19th century, as evidenced by Herbert Spencer's statement: As every student of the nervous system knows, the combination of any set of impressions, or motions, or both, implies a ganglion in which the various nerve-fibres concerned are put into connection" (*Principles of Psychology*, 1855). The hypothesis that synapses form or become altered between neurons whose electrical activities coincide has become widely accepted in approximately the way in which it was formulated by Ariëns Kappers *et al*. (1936): "The relationships which determine connections are synchronic or immediately successive functional activities.

The general idea that learning is predicated by selective strengthening of synapses (Ramón y Cajal, 1895) has been accepted and elaborated in various forms (Hebb, 1949, 1966; J.Z. Young, 1951; Eccles, 1964; Konorski, 1967; Beritoff, 1969; Anokhin, 1968; Stent, 1973). Neurophysiological theories of strengthening of synapses between neurons that have synchronous functional activities imply that linkages initially are extensive but become more restricted, functionally and anatomically, as a result of functional activity. In this view, the final arrangement is the result of cooperative interactions between neurons. This view has been extended to include competitive functional interactions between neurons with equal activities being able to maintain connections with a shared postsynaptic target, while functional imbalance results in the more active neuron excluding the less active neuron from a share of the postsynaptic space (Guillery, 1972a; Sherman *et al*., 1974; Sherman and Wilson, 1975; C. Blakemore *et al*., 1975; Edelman, 1987).

Theories of competitive elimination of synapses are based on the assumption that presynaptic terminals compete with one another for necessary molecules in limited supply such as trophic factors Ramón y Cajal, 1919, 1928; Changeux and Danchin, 1976; M.R. Bennett, 1983); or that synapse elimination occurs as a result of secretion of inhibitory or toxic factors (Marinesco, 1919; Aguilar *et al*., 1973; O'Brien *et al*., 1984; Connold *et al*., 1986). In 1919 Cajal noted that these factors could be produced by and act upon presynaptic or postsynaptic elements, or both, and that neurotrophic factors could also be secreted by glial cells. It was also recognized that the nerve cell body has a trophic influence on the axon and on the peripheral structures with which it connects (Goldscheider, 1898; Parker, 1932). It was also conjectured that a retrograde trophic stimulus travels from peripheral structures to neurons. The observation that dendrites of spinal motor neurons sprout only after their axons have grown into the muscles led to the theory that a neuron's dendritic growth is dependent on its axonal connections (Ramón y Cajal, 1909–1911, p. 611; Barron, 1943, 1946; Hamburger and Keefe, 1944). Related to this is the "modulation theory" of Paul Weiss (1936, 1947, 1952), according to which the motoneuron modulates its central synaptic connections to match the muscle with which its axon connects.

DEVELOPMENT OF NERVE CONNECTIONS WITH MUSCLES AND PERIPHERAL SENSE ORGANS

> The quest of a single neuromuscular unit has in fact had many of the dramatic features associated with the quest for a single atom, and the success achieved by the physiologist is in most respects quite as remarkable as that of the physicist.
>
> John F. Fulton (1899–1960), *Physiology of the Nervous System*, 1st ed., p. 40, 1938

Notes on the History of Ideas about the Connections made by Peripheral Nerves

> If any one offers conjectures about the truth of things from the mere possibility of hypothesis, then I do not see how any certainty can be determined in any science; for it is always possible to contrive hypotheses, one after another, which are found to lead to new difficulties.
>
> Isaac Newton, "Letter to Pardies, 10 June 1672"
> (In *The Correspondence of Isaac Newton* [H.W. Turnbull, ed.], Cambridge University Press, Cambridge, 1959)

We have considered the growth of knowledge about outgrowth of nerve fibers and evolution of ideas about peripheral nerve endings. The history of ideas about the modes of termination of peripheral nerve fibers parallels that of ideas about endings of nerve fibers in the central nervous system (CNS).

Until the 1860s it was generally believed that the peripheral nerves end by anastomosing with one another to form plexuses in the skin and muscles. It was also thought that sensory nerve fibers branch and anastomose in the skin and mucous membranes and then recombine to form fibers that return to the CNS (Beale, 1860, 1862). The concept of anastomosis between the processes of nerve cells in the CNS was supported by the evidence available at that time. Both central and peripheral nervous systems were believed to be organized on the principle of nerve networks. Microscopes could not resolve individual fine unmyelinated nerve fibers in the peripheral nerves. They revealed fascicles which were mistaken for single nerve fibers. Interlacing of such fascicles was construed as true anastomoses between fibers. As we shall see later, this misconception persisted until Ranson (1911) showed that peripheral nerves contain large numbers of unmyelinated fibers and proved that they are sensory (Ranson, 1913, 1914, 1915).

Wilhelm His (1856) was the first to discover free nerve endings in the epithelial later of the cornea stained with silver nitrate, and this was confirmed in 1867 by Julius Cohnheim, using the recently invented method of staining nerve fibers with gold chloride. The use of gold chloride made it possible to see fine peripheral nerve endings in skin, mucous membranes, and smooth muscle. Free termination of nerve fibers in smooth muscle was demonstrated by Löwit in 1875 using gold chloride followed by formic acid, which is the basis of the modern technique of gold staining. When Friedrich Merkel (1875, 1880) described the cutaneous nerve endings that now bear his name, he thought that the *Tastzellen* (touch cells) were ganglion cells from which the nerve fibers originate. That they are modified epithelial cells in contact with disklike expansions of the nerve endings was shown by methylene blue staining of nerve endings at different stages of development in the skin of the pig's snout (Szymonowicz, 1895).

The gold chloride method also led to uncertainty about whether nerve terminals penetrate into the peripheral cells, and even into the cutaneous hairs (Bonnet, 1878). The beautiful Golgi preparations of Retzius (1892, 1894) and van Gehuchten (1892) left no doubt that all the different types of nerve endings end freely in the hair follicles and adjacent skin. Very rapid progress in describing peripheral nerve endings was made after introduction of methylene blue staining (Ehrlich, 1885) and after Golgi published his rapid method in 1886. As an indication of the sudden burst of activity in this field, Kallius (1896) cites 185 papers in his review of the histology of sensory nerve endings. Lenhossék (1892–1893) and Retzius (1892) showed that nerve endings end freely among the cells of the taste bud. Prior to their reports it was believed that the taste cells give off nerve fibers which run to the CNS. The periodic varicosities of autonomic nerve endings in the mucous membranes of the bladder and esophagus were clearly demonstrated by Retzius (1892).

The concept of anastomosis between peripheral nerve fibers was not laid to rest by the evidence that they end blindly and that there are one-to-one relationships between some sensory nerve endings and some peripheral sensor cells and organelles. It seems to have passed unnoticed by historians of neuroscience that the concept of anastomosis between peripheral nerve fibers persisted long after the neuron theory was well established. The reason for this is that until the introduction of the pyridine silver method by Walter Ranson (1911), it was not possible to stain unmyelinated nerve fibers reliably and to count them in peripheral nerves. Before Ranson's work the unmyelinated axons were seen only after dissociation of the nerve fibers by soaking pieces of peripheral nerve in weak acid solutions after fixation in alcohol (Ranvier, 1878). Ranson (1911, 1912a, 1913, 1914) discovered that the majority of small unmyelinating peripheral nerve fibers are sensory, showed that they have their cell bodies in the dorsal root ganglia, and traced their fine central processes into Lissauer's tract of the spinal cord. He also correctly conjectured that they subserve pain (Ranson, 1915). Ranson's evidence that the majority of unmyelinated peripheral axons are afferent was not accepted immediately and continued to be denied for another 20 years, for example, by Bishop *et al*. (1933), and indisputable evidence that they are afferents was finally published only in 1935 (Ranson *et al*., 1935).

The nerves growing into the skin appear to be confronted with a large number of potential targets from which each nerve has to select one target. The situation is complicated by the fact that the density of cutaneous innervation and the number of sensory corpuscles are quite constant in each region of the skin. These aspects of the problem were first fully grasped by Ramón y Cajal, who, in a remarkable paper published in 1919, established the theoretical framework into which all subsequent contributions to the problem have ineluctably had to be fitted. He believed that both selective growth (that is, chemotropism) and selective terminal connection (that is, chemoaffinity) probably play a part in regulating the pattern of cutaneous innervation. He pointed out that the density of innervation of each region of the skin is precisely determined and that "each fiber is destined for an epithelial territory devoid of nerves, and there are no vast aneuritic spaces in some regions nor excessive collections of fibrils in others" (Ramón y Cajal, 1919). He suggested that the nerve fibers are attracted by chemicals in the epidermis, which are either used up or neutralized by the nerves as they grow into the skin, so that "after invasion of the epithelium a state of chemical equilibrium is crested, by virtue of which the innervated territories are incapable of attracting new sprouts."

In addition to the general attractive effect of the epithelium, Cajal proposed a more specific neurotropic effect to account for the specific innervation of different types of sensory organelles and muscles. He pointed out that this specificity is unlikely to be the result of mechanical guidance, because then

> it becomes difficult to understand how, of the large nervous contingent arriving at the mammalian snout, some fibers travel without error to the cutaneous muscle fibers, others toward the hair follicles, others to the epidermis and finally some to the tactile apparatus of the dermis. A similar multiple specificity is found in the tongue, trigeminal fibers innervate the ordinary papillae, and facial (geniculate ganglion) and glossopharyngeal fibers go to the gustatory papillae
>
> (Ramón y Cajal, 1919).

Motor nerves were described as ending freely in both skeletal muscle and smooth muscle, in the form of loops or plexuses on the surface of the muscle fibers. For example, Koelliker (1852) remarks that "with respect to the ultimate termination of the nerves, it may be stated that in all muscles there exist anastomoses of the smaller branches forming the so-termed plexuses." At that time the striated muscle fibers were known to be cells called primitive tubes, containing fibrils, and surrounded by a sarcolemma. Schwann (1847) showed that several mononucleate myoblasts fuse to form a multinucleate myotube. Remak (1844) and Lebert (1850) showed that striated muscle fibers differentiate by elongation of myotubes and that self-multiplication of their nuclei occurs. It should perhaps be noted, because it is not well known, that both Remak (1844) and Lebert (1845) were among the first to apply the cell theory rigorously to development and pathology, and in that respect Lebert's *Physiologie pathologique*, published in 1845, was a forerunner to Virchow's more renowned *Cellularpathologie* published in 1849.

The motor end-plate was discovered and named by Willy Kühne in 1862. He was at first unable to see whether the nerve and muscle are continuous or only contiguous. In 1869 Kühne asked the question: "In what way do nerves terminate in muscle?*"* He came to the wrong conclusion: "We now believe that we are able to perceive the direct continuity of the contactile with the nervous substance." He then expressed some doubts: "Yet it may still happen that, in consequence of further improvements in our means of observation, that which we regard as certain may be shown to be illusory." Sixteen years later, in his Croonian Lecture, Kühne was able to say that "nerves end blindly in the muscles … Contact of the muscle substance with the nonmedullated nerve suffices to allow transfer of the excitation from the latter to the former" (Kühne, 1888). Thus, the concept of transmission of nervous excitation by contact rather than by continuity between nerve and muscle was formed before the concept of contact between neurons in the CNS. Once the concept of nervous transmission by contact between nerve and muscle was accepted, it became easier to generalize it to transmission by contact between nerve and nerve in the CNS. The theory was at that time ahead of the evidence, which was obtained remarkably quickly during the decade at the close of the 19th century.

The anatomical concept of what is now known as the motor unit originated in the late 19th century. However, the modern term was first used in 1925 by Liddell and Sherrington and later defined by Eccles and Sherrington (1930) as "an individual motor nerve fibre together with the bunch of muscle fibres it innervates." Counts of the nerves and muscle fibers were made by Tergast (1873), who showed that the ratio of nerve to muscle fibers ranges from 1 : 80 to 1 : 120 in limp muscles but is only 1 : 3 in the extraocular muscles of the sheep, but he did not know that nearly half of the nerves to muscle are sensory, which was discovered much later (Ranson, 1911). Nevertheless, Tergast (1873) established the principle that muscles which perform fine movements have smaller motor units than those which perform gross movements. This was eventually confirmed by counting muscles and nerve fibers and correlating the counts with the tension developed by each motor unit (Clark, 1931).

The muscle spindle was first identified and named by Willy Kühne (1863). The definitive work on muscle spindles and their sensory and motor nerve endings was accomplished by Ruffini (1892, 1898). After Ruffini there was little that others could add with the methods then available, and Ruffini's account of muscle spindles was not superseded until much later (Denny-Brown, 1929; Boyd, 1960). Sherrington (1894, 1897) proved that muscle spindles are proprioceptors of muscle, and the classical experimental analysis of muscle proprioceptive function was done by Mott and Sherrington (1895), who analyzed the effects of cutting various combinations of dorsal roots supplying the limb in monkeys.

NEURONAL DEATH AND NEUROTROPHIC FACTORS

> According to tradition, the development of the vertebrate nervous system has hitherto seemed to proceed straight on in a gradually ascending path, without turnings, temporary expedients, or regressive changes. As a consequence none were looked for and none were found.
>
> John Beard (1858–1918), *The History of a Transient Nervous Apparatus in Certain Ichthyopsida*, 1896

Prolegomena to a History of Nerve Cell Death during Development

> Men make their own history, but they do not make it just as they please; they do not make it under circumstances chosen by themselves, but under circumstances directly encountered, given and transmitted from the past. The tradition of all the dead generations weighs like a nightmare on the brain of the living.
>
> Karl Marx (1818–1883), *The Eighteenth Brumaire of Louis Bonaparte, 1852*

The tyranny of theory over the evidence is nowhere more glaringly evident than in the history of the delayed discovery of neuronal death during normal development. The tyranny in this case was imposed by the theory that both ontogeny and phylogeny are progressive, from lower and less organized to higher and more organized nervous systems. Evidence of neuronal death during normal development was reported but was ignored because it was in conflict with the idea of progressive development. Reports of neuronal death were buried in the literature, to be unearthed much later as curious historical relics. Such reports come back to haunt us as they haunted previous generations who could not accept evidence that conflicted with their cherished theories.

Neuron death during normal development was discovered and described in considerable detail by John Beard in 1896 in the Rohon–Beard cells of the skate: "This normal degeneration of ganglion-cells and of nerves is now for the first time described and figured for vertebrate animals, in which hitherto such an occurance is without precedent" (Beard, 1896a). Rohon–Beard

cells had been discovered by Balfour (1878), who illustrated them in the spinal cord of elasmobranch embryos, and they were further described by Rohon (1884) in the trout and in other fish embryos by Beard (1889, 1892). Beard traced their origin from "immediately laterad to the medullary place," in other words, from the neural crest. He described their differentiation and outgrowth of their neurites, discovered their degeneration (Beard, 1896a), and tried to build a general theory on that evidence. Beard (1896b) thought that cell death occurs generally during what he called "critical periods" (the first time that term was used in neuroscience). According to Beard, the critical period represents a period of regression and reorganization of embryonic structures and a transition to the definitive structures of the adult. The quotation from Beard that forms an epigraph to this chapter shows that he recognized the prejudice against the idea of normal regressive developmental stages. He noted that his evidence contradicted the biogenetic law of Ernst Haeckel according to which the embryo simply climbs the phylogenetic tree, recapitulating the structures of its ancestors as it ascends to its appropriate level.

After Beard's definitive work on death of Rohon–Beard neurons, the few reports of neuron death during normal development were consigned to obscurity not altogether undeserved in view of their inability to relate the facts to a general theory of neuron death. Cell death during normal development of the nervous system was first reported in the chick embryo neural tube by Collin (1906a, b).

Ernst (1926) was the first to recognize that overproduction of neurons was followed by death of a significant fraction of neurons in many regions of the nervous system of vertebrates. For example, he reported death of a third of the neurons in the dorsal root ganglia. The originality of his findings may be appreciated from the following brief extracts. After discussing the report by Sánchez y Sánchez (1923) of massive cell death during metamorphosis of insects (now undeservedly forgotten, e.g., in the review by Truman and Schwartz, 1982), Ernst says:

> We find ourselves in agreement with Sánchez y Sánchez. He states that he found such extensive cell death in all ganglia of appropriate stages that he at first hesitated to publish descriptions, because he could not believe that such results were not already well known. We too found such massive cell death, above all in the retina, in the trigeminal and facial ganglia, in the upper jaw, and in the anterior horn, that we were at first doubtful whether we were dealing with normal events … We have at the same time the explanation of why ganglia of older embryos always have fewer cells than those of very young stages … The results are in complete agreement in showing that degenerations always occur most strongly in the ganglia from which the nerves grow out to the extremities … There remains only a group of degenerations which are always found, namely in the anterior horns of the spinal cord, the floor of the third and fourth ventricles and at the transition from the thick lateral wall of the brain to the thin roof of the ventricle … Characteristic of all these degenerations is the timepoint of their occurrence: it consists of a striking correspondence between the vascularization of these regions and the occurrence of degenerations … For all these cases we must for the present be satisfied with confirmation of the facts that in the named regions a large number of cells are available for differentiation into nerve cells are available for differentiation into nerve cells but that only a fraction of them are used for that purpose whereas the remainder are destined for disintegration.

Ernst (1926) deserves credit for proposing a general theory of neuron death during normal development and for obtaining a diversity of evidence to support it. The work of Glücksmann (1940, 1951, 1965) merely confirmed the findings of Ernst and others and provided an incomplete but convenient summary in English of some of the literature in other languages. This led to the deplorable practice (e.g., Saunders, 1966, but soon followed by others, e.g., P.G. Clarke, 1985a; Hurle, 1988) of ignoring the work of Ernst and his predecessors and crediting Glücksmann with the concept of three different modes of cell death, when all he did was to give them names. As Ramon y Cajal (1923) noted: "In spite of all the flatteries of self-love, the facts associated at first with the name of a particular man end by being anonymous, lost forever in the ocean of universal science. The monograph permeated with individual human quality becomes incorporated, stripped of sentiments, in the abstract doctrine of the general theories."

Ernst (1926) had provided good evidence of his own and reviewed the previous evidence showing that there are three main types of cell death during normal development: the first occurring during regression of vestigial organs; the second occurring during cavitation, folding, or fusion of organ anlage; the third occurring as part of the process of remodeling of tissues. These were later named phylogenetic, morphogenetic, and histogenetic cell death by Glücksmann (1940, 1951, 1965).

The great neurocytologists of the 19th and early 20th century were in a position to see the death of cells in the developing nervous system but failed to discover it. Why scientists fail to see important things that are staring them in the face is notoriously difficult to understand. Cajal was fond of saying the truth is revealed to the prepared mind, and I should agree that the minds of the great neurocytologists of his time were not prepared for the truth about neuronal death during normal vertebrate development. Another important reason for their failure to see neuronal death was their reliance on Golgi and silver impregnation techniques which do not show cellular debris clearly or obscure it with metallic precipitates. The Nissl stain could have revealed neuronal death to the unprejudiced observer, but the observers were prejudiced by the idea of progressive development. Death of embryonic cells was recognized as a phenomenon of significance only during disintegration of vestigial organs and during metamorphosis, as brilliantly studied by Cajal's student Domingo Sánchez y Sánchez. It is remarkable that the concept of regression of axonal and dendritic structures was easily accepted whereas death of large number of neurons in the vertebrate nervous system was not an acceptable fact. Cajal relished the analog between regression of axonal and dendritic branches and pruning of excessive branches from trees and bushes in a formal garden, but he never conceived of uprooting and destroying large numbers of trees in the process of laying out the garden.

The long delay in accepting the evidence of developmental neuronal death has been regarded as an historical enigma. Here is how the puzzle may now be solved. Nineteenth-century biologists saw that development has an overriding *telos*, a direction and a gradual approach to completion of the embryo, and also saw a terminal regression and final dissolution of the adult; but a fallacy arose when the progression and regression, which coexist from early development, were separated in their minds. Development was conceived in terms of progressive construction, of an epigenetic program—from simple to more complex. For every event in development they attempted to find prior conditions such that, given them, nothing else could happen. The connections and interdependencies of events assure that the outcome is always the same. Such deterministic theories of development made it difficult to conceive of demolition of structures as part of normal development, and it was inconceivable that construction and destruction can occur simultaneously. It became necessary to regard regressive developmental processes as entirely purposeful and determined. For example, elimination of organs that play a role during development but are not required in the adult or regression of vestigial structures such as the tail in humans were viewed as part of the ontogenetic recapitulation of phylogeny. Regression in those cases is determined and is merely one of several fates: cellular determination may be either progressive or regressive. The idea of progress in all spheres, perhaps most of all in the evolution and development of the vertebrate nervous system, has appealed to many thinkers since the 18th century. Such ideas change more slowly than the means of scientific production; thus new facts are made to serve old ideas. That is why the history of ideas, even if it does not exactly repeat itself, does such a good job of imitation.

In the realm of ideas held by neuroscientists, the idea of progressive construction, of hierarchically ordered programs of development, has always been dominant over the idea of a plenitude of possibilities, from which orderly structure develops from disorderly initial conditions by a process of selective attrition. (M. Jacobson, 1970b, 1974b; Changeaux *et al*., 1973; Changeaux and Danchin, 1976; Edelman, 1985). Progressive development implies increasing orderliness gained by the organism, "sucking orderliness from its environment," and by "feeding on negative entropy" (Schrödinger, 1944, p. 74). Schrödinger did not recognize that the organism can lose entropy (that is, gain orderliness) by ridding itself of internal disorder as effectively as by "attracting, as it were, a stream of negative entropy upon itself" (Schrödinger, 1944, p. 74). Cell death may be a quick way for the embryo to reduce its entropy level.

The idea of development of organiztion by means of selective cellular attrition has gained popularity since the 1970s. Before that time, the dominating idea was that matching between different nerve centers is achieved by programs of cell proliferation, migration, and differentiation in which orderly progress always prevails. But this early period of construction is now known to be followed by a period of deconstruction.

Another dominant idea from the beginning of the century until now (e.g., Cowan *et al*., 1984) was that the number of neurons is matched to the size of their targets as a result of reciprocal interactions between nerves and their peripheral innervation fields: neuronal proliferation, migration, and survival were conceived to result from trophic influences coming from the target tissues, and a reciprocal trophic influence of nerves on the target tissues ensured the vitality of muscles and sense organs (reviewed historically by Oppenheim, 1981). For the past 200 years the nutritional functions of nerves have generally been regarded as distinct from their roles in sensation and movement. For example, Procháska (1784) states: "Sylvius, Willis, Glisson and others considered that there were two fluids in the nerves, one thick and albuminous, subservient to nutrition, the other very thin and spiritour, intimately connected with the former, and subservient to sensation and movement …"

A century ago the word "trophic" was on everyone's lips to signify the mysterious life-giving effects of nerves on one another and on the tissues which they supply. In Foster's *Text-Book of Physiology* (7th ed., 1897), trophic action is defined as "the possibility of the nervous system having the power of directly affecting the metabolic actions of the body, apart from any irritable, contractile or secretory manifestations." The first experimental evidence of a trophic action of sensory nerves was the demonstration that taste buds degenerate after denervation and regenerate only if sensory nerves are present (von Vintschgau and Hönigschmied, 1876; von Vintschgau, 1880; Hermann, 1884). Wilhelm Roux (1881) discusses "the trophic action of functional stimuli" under which he has a section "on trophic nerves" (p. 125). There he reviews the trophic effects of nerves on the muscles and other tissues, and he makes the distinction between a direct trophic action of the nerves on these tissues and the indirect effects of lack of stimulation, disease, changes in blood flow, etc. He concludes that nerves have a trophic effect which is not entirely due to excitation. He maintains that not only are the peripheral organs provided with a trophic stimulus independently of the nervous activity, but also "the central nervous substance likewise is influenced in its nourishment by the peripheral organs with which it has formed an excitation-unity" and that "the central nervous tissue should be regarded, so to speak (practically) not as a one-sided provider but at the same time as the nutritive provider by the peripheral tissues." In 1899 L.F. Barker could write, "The more thought one gives to the subject the more he will find in the trophic relations of neurons to make him hesitate before he denies the possibility of conduction of impulses or influences in either direction throughout the neurone." Goldscheider (1898) first conjectured that materials are transported from the nerve cell body to the axon terminals. During the following decades evidence built up to support the theories that trophic factors flow from the nerve cell body to the axonal endings (Olmsted, 1920a,b, 1925; May, 1925) and that nerves release specific trophic factors into the tissues they innervate (Parker, 1932; see M. Jacobson, 1993, for a discussion of the significance of those premature theories.) Perhaps here I should say that those premature conjectures fell on deaf ears and unprepared minds. To arrive on the scene with a message prematurely might be like someone in the position of shouting "fire" in an empty theater.

Experimental analysis of the changes in the developing nervous system resulting from altering peripheral sensory and motor fields was pioneered by Braus (1905) and Shorey (1909) and followed by many others. Removal of limbs or grafting additional limbs was shown to result in hypoplasia or hyperplasia, respectively, and those results were interpreted consistently in terms of regulation of cellular proliferation, as the reader can easily verify from the general textbooks dealing with the subject, such as Samuel Detwiler's *Neuroembryology* (1936) and *Principles of Developmment* by Paul Weiss (1939). The appearance of Glücksmann's 1951 review of cell death during normal development prompted a reconsideration of the effects of limb amputation. Prior to the publication of Glücksmann's review, Hamburger and Levi-Montalcini (1949) concluded that "two basically different mechanisms operate in the control of spinal ganglion development by peripheral factors: (a) the periphery control the proliferation and initial differentiation of undifferentiated cells which have no connections of their own with the periphery; (b) the periphery proivdes the conditions for continued growth and maintenance of neurons following the first outgrowth of neurites" (Hamburger and Levi-Montalcini, 1949). After the discovery that a mouse sarcoma implanted in the chick embryo results in neuronal hyperplasia and hypertrophy (Bueker, 1948) the effect was consistently misinterpreted as a primary action of the factor on neuronal proliferation (Levi-Montcalcini and Hamburger, 1951, 1953).

Victor Hamburger (1958) was able to show that the number of motoneurons in the chick embryo decreases after limb amputation as a result of increased cell death, not because of failure of mitosis or of motoneuron differentiation. However, he did not yet recognize the significance of death during normal development. Arthur Hughes (1961) was the first in recent times to show that a large overproduction of motoneurons occurs during normal development and that motoneuron death is a major factor regulating their final numbers, and Martin Prestige (1965) was the first to demonstrate the same in spinal ganglia. Yet the belief persisted that the periphery controls cell proliferation, even after the discovery of nerve growth factor (NGF), which was at first said to have mitogenic effects (Levi-Montalcini, 1965, 1966; Levi-Montalcini and Angeletti, 1968). The confusion was resolved only after [^{3}H]thymidine autoradiography showed that changes in mitotic activity in the nervous system, following limb grafting or amputation, is confined entirely to glial cells (Carr, 1975, 1976). The path to discovery of the biological effects of NGF and other neurotrophic factors detoured around the difficulties and confusions created by surgical manipulation of limbs. Those were prologues to the biochemical identification of NGF—the rest is history, that ultimate act of imaginative reconstruction.

HISTOGENESIS AND MORPHOGENESIS OF CORTICAL STRUCTURES

> That the cortex of the cerebrum, the undoubted material substratum of our intellectual activity, is not a single organ which enters into action as a whole with every physical function, but consists rather of a multitude of organs, each of which subserves definite intellectual processes, is a view presents itself to us almost with the force of an axiom …. If … definite portions of the cerebral cortex subserve definite intellectual processes, there is a possibility that we may some day attain a complete organology of the brain-surface, a science of the localization of the cerebral functions.
>
> Alexander Ecker (1816–1887),
> *Die Hirnwindung des Menschen*, 1869

Historical Orientation

> In my opinion there are only quantitative differences, not qualitative differences, between the brain of a man and that of a mouse. Accordingly, all cortical regions which are vested with a specific structure and a specific function and are differentiated in humans are also represented—with the corresponding simplification and reduction—in the mammals and probably even in the lower vertebrates.
>
> Ramón y Cajal (1852–1934), Estudios
> sobre la corteza cerebral humana.
> III. Cortez motriz. *Revista Trimestral*
> *Micrográfica* 5: 1–11, 1890

Three important theories of nervous organization, valid for our time, emerged from the cell theory. Firstly, the demonstration that the nerve cell and fiber are parts of the same structure (first claimed by Remak, 1838) was the first step in the formulation of the neuron theory. Secondly, recognition that there are different types of nerve cells, even in the same region, was the beginning of the theory of neuronal typology. Thirdly, realization that there are regionally specific patterns of nerve cells and fibers, especially in the cerebral cortex, was the beginning of a theory of cytoarchitectonics (reviewed by Brodmann, 1909; Lorente de Nó, 1943; Kemper and Galaburda, 1984).

Those extensions of the cell theory were linked to the theory of evolution of the nervous system and, especially as seen from the viewpoint of this chapter, to the theory of evolution of the forebrain. Evolution of the telencephalon was understood as a process which exploited the neural structures—cell groups and their connecting fiber tracts-laid down during earlier stages of evolution. Telencephalization involves selective expansion and elaboration of the front end of the neural tube. This starts phylogenetically with the evolution of the floor plate which becomes the huge basal cell masses of fishes. The later phylogenetic advances may be seen as successive additions of new pallial formations: first the primordial pallium of fishes, next the primary hippocampo-pyriform fallial formation of Amphibia, thereafter the secondary hippocampal and pyriform cortices of reptiles, and finally the neopallium of mammals. Efforts were made to trace the phylogenetic order of emergence of different fields in the neopallium and to relate phylogeny to ontogeny. This research program was constructed, around the end of the 19th and beginning of the 20th centuries, by many workers, notably L. Edinger, C.J. Herrick, Elliot Smith, and Ariëns Kappers.

The two quotations standing at the head of this chapter emphasize the early historical origin of two major concepts of organization of the cerebral cortex: firstly, the concept of parcellation of the cerebral cortex into different areas which subserve specialized functions; secondly, the concept of a common organizational scheme for the entire cortex. Questions arising out of the first concept relate to how the different regional specializations develop. For example, to what extent are the specialized areas preformed from the time of their origin and to what extent do they differentiate epigenetically from a single primordial pattern to a more complex final organization? Karl Ernst von Baer recognized that "each step in development is made possible only by the immediately preceding state of the embryo … From the most general in form-relationships the less general develops, and so on, until finally the most special emerges" (Entwickelungsgeschichte der Thiere, Part 1, pp. 147, 224, 1828). Subsequent studies of brain development were made within the framework of the theory of epigenesis—from simple to more complex stages of ontogeny—and also within the framework of a theory of ontogeny recapitulating phylogeny.

The concept that the mature organization of the cortex develops from a more uniform early state and the final state emerges by addition as well as elimination of components was already well established by the beginning of this century. Korbinian Brodmann (1909, p. 226) summarized that concept of progressive versus regressive differentiation as follows: "Considered genetically it is partially new production of anatomical cortical fields, partially their regression or reversion which are combined here …. Undoubtedly both processes, that is progressive and regressive differentiation, occur concurrently during development of cortical fields." This was a premature theory which could not be substantiated until more than 70 years later.

Several questions emerged regarding the conversation of certain features of cortical organization in different regions in all mammals. For example, how have the six layers and their characteristic cell types, inputs, and outputs been conserved? Are the similarities based on homology, meaning that they share the same evolutionary ancestry, or are they based on analogy, meaning that they evolved under similar functional and adaptive pressures regardless of ancestry?

Franz Joseph Gall (1825) first theorized that different mental faculties are represented in separate regions of the surface of the human brain. Although he claimed to be able to relate the cortical representations to bumps on the cranium, he did not claim to be able to delimit separate cortical areas subserving different faculties. Before the 1860s it was generally believed that the cerebral cortex is the seat of psychic and mental functions while motor functions were believed to be controlled by the brainstem. Those beliefs were established by Jean-Pierre-Marie Flourens (1794–1867) on the basis of his surgical ablation experiments. One of his principal achievements was to demonstrate that the cerebellum functions to coordinate voluntary movements. That was then the strongest refutation of Gall's phrenological theory which localized sexual functions in the cerebellum (Gall, 1835, Vol. 3, pp. 141–239; for a brief history of concepts of cerebellar function see Dow and Moruzzi, 1958, pp. 3–6). Flourens concluded that the cerebrum is the seat of sensation but is not directly involved in control of voluntary movements. Flourens understood that different functions are localized in different parts of the brain, but he concluded that the cerebral cortex functions as a whole, as the organ of sensory perception, intellect, the will, and the soul. (For detailed consideration of Flourens' views, which changed in the two editions of his Recherches, see R.M. Young, 1970.)

There were two opposing schools of thought about cerebral localization—we can call those "lumpers" who saw unity in diversity, and we can call those "splitters" who saw diversity in unity. The prevailing views at different moments of history have tended to oscillate between the extreme lumper and splitter positions. Flourens belonged to the school of lumpers who believed that the cerebral hemispheres function as a whole.

Those beliefs were put in doubt by the observations of Hughlings Jackson (1863) that tumors and other disease processes involving the cerebral cortex sometimes cause seizure movements that progress from distal to proximal limb muscles, often involve the facial muscles, and resemble fragments of purposeful movements. Jackson proposed that the cerebral cortex directly controls body movements is organized in terms of coordinated movements and not of individual muscles, for any muscle could be brought into play in a variety of different movements.

Experimental support for part of Jackson's theory was provided by Fritsch and Hitzig (1870), who evoked coordinated movements of body parts in the dog in response to galvanic stimulation around the cruciate sulcus of the cerebral cortex on the opposite side. Much better evidence of a somatotopic motor representation was obtained by Faradic stimulation of the cerebral cortex of the monkey (Ferrier, 1875, 1876, 1890) and higher apes (Grünbaum and Sherrington, 1902, 1903; Leyton and Sherrington, 1917). The latter also showed that the postrolandic area is inexcitable, contrary to the general belief at that time that the rolandic area is both sensory and motor (Mott, 1894; Bechteres, 1899; see Fulton, 1943, and A. Meyer, 1978, for the history of the concept of sensorimotor cortex and of the efforts to delimit sensory regions of cortex). Cushing (1909) provided the first evidence that stimulation of the postcentral gyrus in humans can result in somatic sensation without movement. It was only after it became possible to record electrical cortical responses evoked by peripheral stimulation that the somatotopic sensory projections to the cortex could be mapped physiologically in cat, dog, and monkey (Adrian, 1941; C.N. Woolsey, 1943).

The area of cerebral cortex from which body movements could be evoked with shortest latency and lowest threshold was defined physiologically as the primary motor cortex. However, it was known that movements can be elicited from widespread cortical areas by using suprathreshold electrical stimuli (Fulton, 1935; Hines, 1947a, b). Mapping those cortical area led to the discovery of the supplementary motor cortical area, which was found first on the mesial surface of the frontal lobe of the human brain (Penfield and Welch, 1951) and later confirmed in experimental animals (C.N. Woolsey, 1951) and later confirmed in experimental animals (C.N. Woolsey, 1952, 1958; G. Goldberg, 1985, review). The areas defined physiologically were correlated

with the anatomical localization of giant pyramidal cells and with the origins of the pyramidal and extrapyramidal pathways (Bechterew, 1899; Brodmann, 1905). The structure–function correlations were strengthened by observation of functional deficits and the extent of nerve fiber degeneration following cortical lesions (Fulton and Kennard, 1934; Fulton, 1935; Hines 1947b).

From those studies the motor cortex appeared to be organized as a mosaic in which each body part is represented in somatotopic order. Whether fundamental units of cortical organization are movements or individual muscles (e.g. H.-T. Chang *et al*., 1947) is an important question that has been reviewed by Kaas (1983) and D.R. Humphrey (1986), but is beyond our scope.

Let us now briefly summarize the evolution of modern concepts regarding the cellular organization of the cerebral cortex (see also M. Jacobson, 1993). The principal concepts regarding cellular organization evolved in parallel with construction of the neuron theory as noted above. There were five crucial conceptual advances made surprisingly rapidly in the final 60 years of the 19th century: recognition that the nervous system is composed of many types of nerve cells and fibers grouped in characteristic morphological patterns; understanding that nerve fibers are outgrowths of nerve cells; making the distinction between axons and dendrites in terms of differences in structure and in the direction of transmission of nervous activity; understanding that nerve cells are linked by contact at synaptic junctions; and conceiving of function in terms of integration of excitatory and inhibitory actions mediated by different synapses. Making allowances for the inevitable overlap between them, it may be useful to consider these concepts evolving in the order given above, and as parts of a research program, advancing to progressively higher levels of understanding.

Koelliker, in the first edition of his *Handbuch der Gewebelehre*, 1852, was already able to classify nerve cells according to shape (pyriform, fusiform, etc.) And according to the number of processes emerging from the cell body (apolar, unipolar, or bipolar). Koelliker's cellular typology was originally based on the appearance of unstained neurons dissociated from fixed brain. The first evidence confirming that similar differences between cell types occurs in a regular histological pattern in section of the cerebral cortex stained with carmine was reported by Berlin (1858). The concept of structural types was linked to that of functional differentiation, termed by A. Milne-Edwards (1857, Vol. 1) the "*physiological division of labour*," one of the dominant concepts of biology in the latter half of the 19th century (see Herbert Spencer, 1866, p. 166; Oscar Hertwig, 1893–1898, Vol. 2, p. 79). In adopting that concept, Cajal (1900) also emphasized that the "principle of division of labour, which holds sway more in the brain than in any other organ, requires that the organs which register sensations are different from those which register memories."

In addition to the principle of functional differentiation, 19th-century studies of the cerebral cortex were guided by two other principles, namely, the principle of functional and structural homology of cortical areas in different mammals, and the principle of divergent differentiation of homologous parts in relation to their use and disuse in different mammals. These three principles are discussed at length by Brodman (1909, Chapter 7), and they continue to influence our current ideas about the development and evolution of the cerebral cortex. For example, evidence that cells with similar functional properties are clustered together anatomically in the cerebral cortex is consistent with the principles of functional differentiation and of functional and structural homology. Examples in the visual cortex are the ocular dominance and orientation columns in the primary visual cortex (Hubel and Wiesel, 1962, 1968) and color clusters in the primary visual area (Livingstone and Hubel, 1984; Tootell *et al*., 1988c) and second visual area (Hubel and livingstone, 1987). Horizontal and corticocortical connections also link clusters or groups of neurons with similar functional specificities (Gilbert and Wiesel, 1989).

The first schemata of cortical architectonics were guided by the principle of regional structural–functional differentiation and were based on differences in sizes and shapes of cell bodies and by their horizontal layering. Those features dominate the histological picture in sections of cerebral cortex stained with carmine, which was the best method of staining then available (Berlin, 1858; Meynert, 1872; Lewis, 1878; Lewis and Clarke, 1878). Despite the limitations of the histological techniques, the architectonics of the cerebral cortex was first worked out in remarkable detail by Theodor Meynert (1867–1868, 1872). Cajal (1911, p. 601) says that Meynert's "study was so exact that, notwithstanding the imperfection of his methods, it is still the best we possess." Meynert (*Bau der Grosshirnrinde*, 1867, p.58) subdivided the cortex into two main types: one with a white surface layer and the other with a gray surface layer. The latter he subdivided into five-layered cortex ("general type" and "claustrum formation") and eight-layered cortex (e.g., calcarine cortex). The white-surface cortex he also called "defective cortex" (including Ammon's horn, uncus, septum pellucidum, and olfactory cortex).

Another guiding principle was that certain cortical regions have been conserved during evolution in all mammals and can be recognized by their functions and structures, especially with respect to layering of certain types of neurons and their afferent and efferent connections. This principle of structural and functional homology generated a terminology in which the homologies are implied. Terminology often reflects the theoretical prejudice of the users. Edinger (1908a, b) coined the term paleoencephalon to mean the phylogenetically most ancient part of the central nervous system (CNS), and the only part in most fishes, as contrasted with what he termed the neoencephalon, of which the neocortex is the most recent culmination. The concept that the CNS of modern amniotes contains a core of ancient structures that are overlaid by layers of structures that evolved at later times was originated by L. Edinger (1908a, b) and Ariëns Kappers (1909). This concept has been extended by MacLean in his theory of the triune brain. According to MacLean (1970, 1972), the brain of higher primates is formed of three systems that originated in reptiles, early mammals, and late mammals. A related concept is that the cerebral cortex enlarges during evolution simply by addition of new areas (Smart and McSherry, 1986a). But evolution does not simply add new levels of organization on top

or by the side of the old levels, so to say, like strata in an archeological site. No, the old adapts to the new and they all continue to evolve. Progression of the old and new occur together. The progression is not A_AB_ABC but A_A′B_A+″B′C and so on. Terms such as paleocortex, neocortex, and archicortex imply a phylogenetic progression which is not well based on evidence, and I use those terms only with certain qualifications. Those terms were coined by Ariëns Kappers (1909) on the basis of comparative studies of lower vertebrates, and their transferral to the mammals, and especially to primates, is a questionable practice. The terms rhinencephalon and pallium were adopted by Koelliker (1896) in his monumental attempt to attach ontogenetic and phylogenetic significance to the different regions of the cerebral cortex.[2] The term "rhinencephalon," for example, was associated with the concept of macrosmatic, and anosmatic brains, that is, with the importance of the sense of smell in the evolution of the species (Broca, 1878; Turner, 1891; Retzuis, 1898). The rhinencephalon was seen as either hypertrophied or atrophied in different species, depending on their use of the sense of smell. For example, the olfactory tubercle, prepyriform area, retrosplenial area, and amygdaloid nucleus were regarded as atrophied in the primates, in which the sense of smell is relatively weak.

Finally, the principle of divergent differentiation embraces the concepts of differentiation of several cortical areas from a protocortex, of progressive adaptation of cortical differentiation as a result of natural selection during evolution and as a result of use and experience of the individual, and also includes the concept of plasticity of the cortex after injury. As Brodmann, (1909, p. 243) clearly understood, all these can occur as a result of progressive or regressive transformations.

We should also remember that a theory prevalent at the end of the 19th century held that nerve cells are all multipotential or even equivalent at early stages of development, and that nerve cell differentiation is controlled by afferent stimulation. Koelliker (1896, Vol. 2, p. 810) summed up the evidence in no uncertain terms: "So I am finally forced to the conclusion that all nerve cells at first possess the same function, and that their differentiation depends solely and entirely on the various external influences or excitations which affect them, and originates from the various possibilities that are available for them to respond to those contingencies." This concept has attained current validity with the evidence that cerebral cortical functions can be specified by afferent nerve fibers. The concept that localization of functions in the cerebral cortex is determined by the input from the periphery and is not autonomously determined within the CNS has endured for more than a century and continues to receive support (e.g., D.M. O'Leary, 1989, review). This theory was held by Golgi and Nissl among anatomists, Flourens and Goltz among physiologists, and S. Exner, Wundt, W. James, and Lashley among psychologists (reviewed by Neuburger, 1897; Soury, 1899; Lashley, 1929; Riese and Hoff, 1950; Walker, 1957; Tizard, 1959). For example, Wundt (1904, p. 150) says, "We know, of course, that the cell territories stand, by virtue of the cell processes, in the most manifold relation. We shall accordingly expect to find that the conduction paths are nowhere strictly isolated from one another. We must suppose, in particular, that under altered functional conditions they may change their relative positions within very wide limits." As Brodmann (1909) says, "All these theories are in fundamental agreement in their concept that the ganglion cells are equivalent forms, unencumbered by their origins, their positions, or their external forms."

The concept of an organology of the cerebral cortex, as expressed by Ecker in the epigraph to this chapter, for example, attained maturity with the cytoarchitectonic and myeloarchitectonic maps, which aimed at showing the structural and presumed functional parcellation of the cortex. This concept can be traced back to the phrenological theory of Gall and Spurzheim, whose *Anatomie et Physiologie due Système Nerveux* (1810–1819), especially in Volumes 1 (1810) and 2 (1812), tried to establish a relationship between the intellectual functions and the shape of the cranium and the underlying convolutions. The phrenological theory, while incorrect in the localization of so-called intellectual and moral functions, was based on much correct anatomical observation, especially that of Gall. Its main significance was to have given an impetus to studies of the relationship between structure and function of the cerebral cortex (see E. Clarke and O'Malley, 1968; R. M. Young, 1970). Out of such studies has come the principle that the magnification of cortical representation is proportional to the functional importance of the peripheral sensory or motor fields and that the primary gyri correspond fairly well, although not precisely, with cytoarchitectonic fields and with functional representation in the cortex (Connolly, 1950, pp. 264–269; Kaas, 1983).

The cortical cytoarchitectonic map of Campbell (1905) is the prototype based on the differences in layering of the cell bodies revealed in Nissl-stained sections. Campbell's structure–function correlations had the virtues of simplicity and reasonableness and were initially communicated to the Royal Society of London by Sherrington in 1903 before publication in book form in 1905. The introduction of the Weigert stain in 1882 resulted in an efflorescence of studies of the fiber tracts of the CNS (Bechterew, 1894; Edinger, 1896) and of the cerebral cortex (Vogt, 1904; Poliak, 1932). Difference in the time of development of myelin in the cerebral cortex was another criterion that was pressed into service to demarcate different regions of the cortex (Flechsig, 1896, 1901, 1927). This direction of research led to the publication of cerebral cortical maps of increasing

[2] The successive editions of *Handbuch der Gewebelehre* by Koelliker (six editions from 1852 to 1896) are invaluable for tracing progress during the second half of the 19th century. A very useful single source of information, in English translation, about the mid-19th century levels of understanding of development and structure of the nervous system is the *Manual of Histology* edited by S. Stricker (English edn, 3 vols, 1870–1873). It contains chapters on research techniques, the cell theory, and embryonic development by Stricker, spinal cord by J. Gerlach, the retina by M. Schultze, and on brains of mammals by T. Meynert. In his autobiography, Cajal refers to Stricker's treatise as "a model … invaluable for the devotee of the laboratory" and notes that he acquired a copy in 1883, before he started his investigations of the histology of the nervous system, and considerably earlier than his initial use of the Golgi technique in 1887–1888.

complexity, in which the relevance to function and development tends to be inversely proportional to the number of cortical regions demarcated (Campbell, 1905, shows 20; Brodmann, 1909, shows 52; Von Economo and Koskinas, 1925, delimit more than 100). We may ask whether the trend shows an increasing departure from reality or a progressive approach to the truth. Neither fits snugly into any theory of history of neuroscience, unless it is a theory of evolution of hypertrophic species which results in the ultimate extinction of the monstrosities. The last in the line of progressively more complicated cortical maps by Von Economo and Koskinas (1925) and the myeloarchitectonic studies of the Vogts (1902, 1904, 1919, review) are probably destined to remain forever enshrined in their gigantic volumes, to be opened by the curious bibliophile, but ignored by the working scientist looking for useful information.

Distrust of the validity of cortical maps was based on what the mappers left out as much as on their excessive zeal to split fields. The reaction to these errors of omission or errors of commission took an extreme, almost nihilistic form in the statement by Lashley and Clark (1946) that in some cases, "architectonic charts of the cortex represent little more than the whim of the individual student." Their skepticism was aroused by the evidence available at that time showing lack of correspondence between electrophysiologically defined cortical areas and anatomically defined architectonic areas. The correspondence has more recently been shown to be quite precise (Kaas, 1983, review). Moreover, quantitative computerized morphometric analysis of cortical cytoarchitectonics has eliminated subjective evaluation and has largely confirmed the validity of classical cytoarchitectonic and myeloarchitectonic maps (Fleischhauer *et al*., 1980; Zilles *et al*., 1980, 1982; Wree *et al*., 1983). The more criteria used to distinguish between different cortical areas, the more subdivisions tend to be revealed: in addition to classical cytoarchitectonics, modern cortical maps take into considera-tion cortical inputs and outputs, intracortical connections, his-tochemical and immunocytochemical markers, electrical activity and functional properties of the constitutent neurons, overall functions, and the effects of lesions (Brak, 1980; Wise and Goschalk, 1987).

Cajal (1922) was alert to the problem of errors of omission resulting from the use of highly selective stains: "There is little value, therefore, in dealing with the differentiation of corticl areas based exclusively on the revelations of the Nissl and Weigert methods, because they show an insignificant portion of the constituent features of the gray matter." In Cajal's hands, the Golgi technique, complemented by staining with methylene blue and reduced silver nitrate, led to the discovery of the dendritic spines and many new types of neurons. The breadth of his observations on the anatomical relationships between different types of neurons in the cerebral cortex and his views on the functional subdivisions of the cortex (for example, he argued that precentral and postcentral gyri are both sensory and motor) can now be appreciated more easily in the English translation by De Felipe and Jones (1988) of a large selection of Cajal's writings on the cerebral cortex.

Koelliker was the pivotal figure connecting the epochs before and after the general use of the Golgi technique. I agree with the assessment by Lorente de Nó (1938a) that "Kölliker's account of the structure of the human cortex (1896) is one of the masterpieces of neuroanatomy and it marks the end of an historical period of research on the cerebral cortex." Golgi occupies a unique position in the earlier epoch as a solitary worker who discovered his marvelous technique prematurely in 1873 and worked with it in virtual isolation unaided by critique, until the significance of his work was first recognized by Koelliker in 1887. Cajal occupies a different position as the person uniquely capable of using the Golgi technique to build on the foundations laid by those in the earlier epoch.

The wealth of information about the cellular organization and functions of the nervous system that had been amassed by the end of the 19th century was codified in the large textbooks published at that time (Koelliker, 1896; Bechterew, 1899; Soury, 1899; Ramón y Cajal, 1899–1904; Sherrington, 1906; Van Gehuchten, 1906). It was finally possible to consider the organization of the CNS in terms of functional assemblies of different types of neurons linked together by synaptic junctions and to analyze their input and output relationships anatomically and physiologically. This synthesis is set forth in Sherrington's lectures on "The Integrative Action of the Nervous System" (1906), in which he gives the evidence showing the importance of reflex inhibition and the concept of the final common path. For a history of the concept of nervous inhibition see Fearing (1930, pp. 187–217) and Fulton (1938, p. 77). We may ask how it was possible for Fearing (1930), in his masterly history of reflex action, to make only two passing references to the work of Cajal.

It is curious that Cajal appears to have failed to grasp the significance of inhibition: he does not refer to it, and he did not accept the challenge of trying to identify the structural substrates of inhibitory functions in the CNS. The question of whether entire neurons or only parts of them had inhibitory functions seems to have eluded him. This may have been because his grasp of anatomical organization was descriptive, albeit at a very high level of insight. His concept of functional organization was dominated by the notion of polarized flow of excitation, which could be altered by changes at the synapses, but he failed to see the significance of inhibition and the final common path as they were worked out by Sherrington (1897, 1904, 1906). By contrast, Sherrington was one of the first to fully understand the significance of Cajal's discoveries.

It has often been said that achievements in neuroscience in recent years are unprecedented. Never before has so much been discovered by so many at such great expense, but I am unable to affirm that we are witnessing a golden age such as that which took place a century ago. It should be remembered that almost all of Ramon y Cajal's important work was done in the four years from the beginning of 1888 to the end of 1891. Other giants of that era, Koelliker, Lenhossek, van Gehuchten and Retzius, were also phenomenally productive in the first decade after 1887, following their use of the Golgi technique. The flow of discoveries in neuroscience has never been stronger than in the 1890's, and it must have been exhilarating to live in a time when the tide came rushing in day after day bearing new gifts. In at least one other respect it resembles our own molecular biological era: priority

for discovery is determined by speed of publication as much as by the moment of discovery. The smart investigator joins the rush to publish every new find, but few can match Cajal, who published 19 of his 28 papers in 1890 and 1891, at the rate of one almost every month, in the *Gaceta Médica Catalana* and other local journals, to establish priority. Later he expanded and republished many of them in journals with international distribution. Not all his contemporaries had the advantage of rapid publication.

By the closing decades of the 19th century less that 100 workers had laid the secure foundations on which neuroscience continues to be upheld. This can be confirmed by perusal of the great textbooks that appeared at the end of that golden age. To go back in search of the golden age is not to deny, but rather to affirm, the values of the present. That more than half my references are to works published since 1980 [i.e., in the decade prior to publication of the third edition in 1991—Ed.] shows that I have chosen to put my money on the future. A final comment—we cannot simply compare the past with the present. Each has to be dealt with on its own terms. We have new problems, and even when we take up their old problems we arrive at new solutions, different from theirs.

DEPENDENCE OF THE DEVELOPING NERVOUS SYSTEM ON NUTRITION AND HORMONES

> He who admits the principle of sexual selection will be led to the remarkable conclusion that the nervous system not only regulates most of the existing functions of the body, but has indirectly influenced the progressive development of various bodily structures and of certain mental qualities. Courage, pugnacity … bright colours and ornamental appendages, have all been indirectly gained by the one sex or the other, through the exertion of choice, the influence of love and jealousy … and these powers of the mind manifestly depend on the development of the brain.
>
> Charles Darwin (1809–1882), *The Descent of Man and Selection in Relation to Sex*, 2nd Ed., 1875

Vulnerability of the Human Brain to Malnutrition

> The destiny of nations depends on the manner of their nutrition.
>
> Jean Anthelme Brillat-Savarin (1755–1826), *The Physiology of Taste, or Meditations on Transcendental Gastronomy*, 1825

A large percentage of children in the "Third World," and a significant number in the rich industrial countries, are unable to obtain food necessary for normal development, and many pregnant women suffer from malnutrition. It is therefore a question of the highest importance whether fetal or childhood malnutrition retards or otherwise alters neurological development. If so, the types of changes, their casual mechanisms, and the permanence or degrees of reversibility of the lesions are of very great moral and social concern. Ethical values are an important component of this research program. It should have attracted great scientific interest and generous public support, yet relatively little money and effort have been expended to answer important questions about causes, prevention, and treatment of physical and functional neurological damage resulting from fetal and neonatal malnutrition. This is evident from the small number of publications in this field in the past decade compared with the efflorescence of publications from other research programs of no greater scientific importance and of lesser human significance. The pregnant phrase of Brillat-Savarin at the beginning of this section should be written on the doorposts of every national legislative assembly.

The effects of maternal malnutrition on the human fetus are not well understood but can result in placental insufficiency causing premature birth, which carries a high risk of mental retardation. Children subjected to chronic malnutrition from birth to 18 months of age, severe enough to result in growth retardation, suffer permanent deficits in emotional, cognitive, and intellectual functions. Acute episodes of neonatal malnutrition also may result in permanent brain damage. The lesions caused by malnutrition in children are not well characterized but are most likely reduction in glial cell numbers and functions, retardation of growth of dendrites and synaptogenesis, and defective myelination. These effects may be totally reversed by nutritional therapy and enriched social conditions provided before age 2 but only partially reversed if therapy is delayed to later ages.

Retardation caused by malnutrition and socioeconomic deprivation interact to produce the syndrome of physical and mental retardation and dysfunction. All the organ systems may be affected. The effects on the nervous system are both direct, due to insufficient nutrients required for growth, as well as indirect, due to lack of trophic and growth factors and hormones required for normal development.

It is necessary to conduct epidemiological studies of the populations at risk in order to discover the causal factors and to design and implement programs to prevent and treat childhood malnutrition. The biological mechanisms can also be analyzed in animal models chosen because they are believed to be relevant to human conditions. However, animals in the wild rarely, if ever, suffer from malnutrition, which is a condition created by the human species on a global scale.

At least half the world's population has suffered a period of nutritional deprivation during childhood, and at present about 300 million children throughout the world are malnourished (World Health Organization, Scientific Publication No. 251, 1972). The majority of these children suffer from chronic lack of proteins and calories punctuated by episodes of acute malnutrition caused by illness and exacerbated by wars and other adverse political and economic malnutrition on the nervous system is difficult because of the other disadvantages of poor children (Pollitt and Thomson, 1977, review).

There is no doubt that economic poverty and malnutrition interact to stunt physical and mental development. For example, in a study of more than 7,000 children in the United States, Edwards and Grossman (1980) found intelligence quotient (IQ)

and scholastic achievement test scores significantly correlated with height and weight, and a history of chronic nutritional deprivation was correlated with subnormal test scores. The fact that sociocultural factors play such an important role in intellectual and scholastic development makes it necessary to analyze the variables, including the contribution of malnutrition to the retardation of impoverished children (Cravioto and DeLicardi, 1972; Herzig *et al*., 1972; Manocha, 1972; Greene and Johnston, 1980; Brozek, 1985). The methodological problems of such a research program are thoroughly discussed by Barrett and Frank (1987).

Prenatal nutritional deprivation of the human fetus may occur in multiple pregnancy as a result of competition between the fetuses for nutrients. Birth weight of twins is lower than that of singletons, and birth weight is significantly correlated with later intelligence (Churchill *et al*., 1966). Birth weights below 2,000 g invariably affect behavioral and intellectual development, whereas birth weights between 2,500 and 4,500 g have variable adverse effects on development of higher nervous functions, depending on other factors such as adequate infant care, disease, nutrition, and sociocultural conditions (Drillien, 1958; Weiner, 1962; Scarr, 1969). Twins average about 7 IQ points below singletons (Stott, 1960; Vandenberg, 1966; Inouye, 1970). The importance of intrauterine competition for nutrients is shown by the fact that identical twins with the same birth weight have the same IQ, but if their birth weights are unequal, the twin with the lower birth weight has the lower IQ in later life (Willerman and Churchill, 1967). The number of variables that enter into human intelligence makes these results difficult to interpret. Thus, in a review of the effect results difficult to interpret. Thus, in a review of the effect of very low birth weight (1,500 g or less) on later intelligence, Francis-Williams and Davies (1974) point out that as many of the harmful factors in the treatment of such infants have been eliminated (excessive use of oxygen, hypothermia, prolonged starvation, infections), there has been progressive improvement of the ultimate IQ attained by children with low birth weight. When babies with low birth weight are cared for under optimal conditions, mental retardation or significant deficits in IQ do not occur (P.A. Davies and Stewart, 1975).

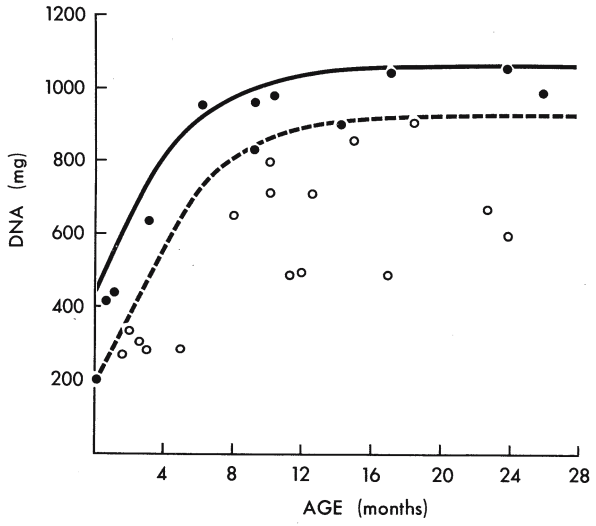

FIGURE 6. Severe childhood malnutrition can result in a reduced DNA content of the brain. The DNA content of the cerebrum of normal children (dark line, black dots) and severely malnorished children (dashed line, circles). From M. Winick, P. Rosso, and J. Waterlow, *Exp. Neurol*. 26:393–400 (1970), copyright Academic Press, Inc.

In humans, it is not known whether twins have a lower brain weight or fewer brain cells than singletons, as would be predicted if animal experimental results, to be described later, can be extrapolated to humans. The deficit is unlikely to be due to failure of nerve cell production because production of nerve cells ceases by 25 weeks of gestation (Dobbing and Sands, 1970), whereas differences in the weight of multiple fetuses compared with a single fetus become apparent only after 26 weeks of gestation (McKeown and Record, 1952). The deficit is more likely to be due to some failure of glial cell production plus retarded neuronal cell growth and differentiation rather than to a reduction in neuronal cell numbers. Nevertheless, there is good evidence that loss of brain cells can occur after severe malnutrition in human infants. Children who die of severe malnutrition in the first 2 years after birth have greatly reduced quantities of DNA in the cerebrum, cerebellum, and brainstem (Fig. 6) compared with well-nourished children of the same age (Winick and Rosso, 1969; Winick, 1970). It is not known whether the deficit is in the number of neurons or glial cells, but it is more likely to be a glial cell deficit which is known to occur in malnourished rats (Robain and Ponsot, 1978; Bhide and Bedi, 1984).

Assessment of the effects of malnutrition on nervous development in children is complicated by at least three main difficulties. First, the effects of malnutrition cannot be entirely separated from the effects of other harmful conditions, such as maternal neglect, environmental impoverishment, and lack of stimulation and incentive. Second, malnourished children show behavioral abnormalities which are variable and are difficult to measure accurately, such as reduced social responsiveness, increased irritability, and emotional disturbances. Third, the effects of malnutrition on the human brain can rarely be assessed directly by postmortem physical and chemical measurements. Instead, less reliable indices, such as physical status, ratio of weight to height, head circumference, and IQ, are generally used. These indices are themselves complex variable, and their interpretation is usually difficult and frequently ends in controversy. For example, general intelligence is a complex product of many variable factors, one of them being the size of the brain. As a result, authorities disagree about the relationship between brain size and intelligence—the relationship can be shown in animals (see Section 2.13) but in humans it tends to be overridden by other factors that vary in different sociocultural contexts. In those cases where IQ is found to be reduced in malnourished children, it is often difficult, if not impossible, to determine whether the reduced IQ is the result of retarded brain development due to malnutrition and associated conditions such as disease, or whether the poor performance is largely or entirely due to social and economic disadvantages. Moreover, a single IQ test has little value, particularly is if is done at an early age: IQ at 1 year of age has no correlation with the IQ at age 17 (B.S. Bloom, 1964).

Beside, the IQ is subject to change during childhood: changing the educational level of children can result in increase or reduction of their IQ by as much as 28 points (Skeels, 1966). Obviously, the rate of development of general intelligence is a more revealing index than a single measurement.

Occipitofrontal circumference of the head is another index that is often used to assess the effects of malnutrition on brain development. However, the circumference of the head also related to the thickness of the skull and scalp, so that it has been claimed that there is a poor correlation between head size and brain size (Eichorn and Bayley, 1962). Intelligence is normal in a small percentage of microcephalic children (H.P. Martin, 1970), and reduced head circumference in infants and young children may not be irremediable (H.P. Martin, 1970; Stoch and Smythe, 1976). Nevertheless, there is a linear relationship between occipitofrontal head circumference over a range of 100–700 g from 25 weeks of gestation to 8 months postnatal (Lemons *et al*., 1981). Head circumference is significantly correlated with brain size in cases of intrauterine growth retardation (Battist *et al*., 1986) and in cases of nutritional deprivation during the first 2 years of life, when most of the growth of the brain occurs (Johnston and Lampl, 1984).

In view of criticisms of the validity of IQ tests, especially that they are racially and culturally biased, it is significant that deficits in IQ of malnourished children have been reported from countries which differ in race, culture, economic, and social conditions: India (Champakam *et al*., 1968), Indonesia (Liang *et al*., 1967), Lebanon (Botha-Antoun *et al*., 1968), Latin America (Pollit and Granoff, 1967; Birch, 1972; Cravioto and DeLicardi, 1975; Klein *et al*., 1977; Barrett and Frank, 1987), Yugoslavia (Cabak and Najdanvic, 1965; Cabak *et al*., 1967) and Africa (Stoch and Smythe, 1963; Fisher *et al*., 1978). In one such study, Stoch and Smythe (1963, 1967) found that severely undernourished South African children from 1 to 8 years of age were 20 points lower in IQ than well-nourished children of similar parentage. When these children were studied 15 and 20 years later, the same severe intellectual deficits were found, and physical abnormalities of the brain were found by computerized tomography scans in some cases, showing that the changes are permanent (Stoch and Smythe, 1976; Handler *et al*., 1981; Stoch *et al*., 1982). Similar conclusions were reached in another study of the effects of severe undernutrition during infancy on subsequent intellectual functions in Yugoslavian children (Cabak and Najdanvic, 1965; Cabak *et al*., 1967). There were marked deficits in IQ in 36 children who had been hospitalized between the ages of 4 and 24 months because of malnutrition but had not suffered from chronic illness thereafter, and who had no significant reduction in body growth. The mean IQ of the malnourished children was 88, in contrast to 101 and 109 for two groups of comparable normal children. Significantly, none of the malnourished children had an IQ above 110 (Fig. 7). No correlation was found between the age of hospitalization and subsequent intellectual impairment. Hospitalization during the first year of life is frequently associated with sensory deprivation and separation of the child from the mother, and these contribute to subsequent emotional disturbances and intellectual impairment (Spitz and Wolf, 1946; Coleman, 1957).

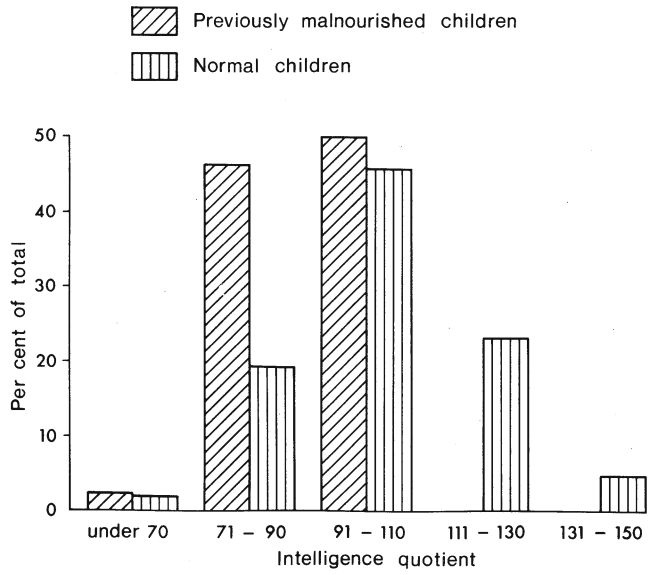

FIGURE 7. Severe malnutrition during infancy may cause intellectual impairment. Distribution of IQs of 36 children aged 7–14 years who had been severely malnourished between the ages of 4 and 24 months, compared with the IQs of normal children of the same ages. Adapted from V. Cabak and R. Najdanvic, *Arch. Dis. Child*. 40:532–534 (1965).

Malnourished children have learning and scholastic difficulties. This is a complex syndrome in which apathy, emotional disturbances, and impaired cognitive and intellectual abilities are synergistic in producing dysfunction (Lester, 1975). Children who are severely malnourished in the first year of life subsequently show behavior disturbances and difficulties with reading, spelling, and arithmetic (Richardson *et al*., 1973; Barrett and Frank, 1987). Reduced ability to integrate inputs from various sensory modalities, for example, auditory–visual integration, has been found in children suffering from malnutrition before 3 years of age (Birch, 1964; Kahn and Birch, 1968; Cravioto and DeLicardie, 1975). Lester *et al*., (1975). studied effects of acute malnutrition in 1-year-old children in Guatemala and found much slower habituation to repeated stimulation. Comparable changes in habituation have been reported in malnourished rats (Bronstein *et al*., 1974; Frankovà and Zemanovà, 1978).

Language development is a significant indication of general intellectual status in children (Cameron *et al*., 1967). Findings which are from different countries, and therefore unlikely to be culturally or racially biased, show retarded language development in malnourished children (Barrera-Moncada, 1963; Monckeberg, 1968; Champakam *et al*., 1968; Chase and Martin, 1970). Retardation of language development has a complex etiology, which includes social deprivation, illness, and maternal deficits as well as malnutrition.

Several studies have provided evidence supporting the concept of a critical period from birth to about 2 years of age during which the nervous system is most vulnerable to malnutrition therapy. The amount of reversibility of impairment following childhood malnutrition is of great practical importance in

planning food supplementation programs and other forms of prevention and treatment. The time and duration of malnutrition, as well as the time at which nutritional therapy and environmental enrichment are started, may be critical.

One study shows that there can be negligible long-term effects of acute starvation—no physical or mental deficits were found in victims of the severe famine in Holland from 1944 to 1945 when they were examined 19 years later (Stein *et al*., 1975). The famine was very severe but of relatively short duration and the amount of intrauterine or neonatal growth retardation is not known. However, it seems that any effects were later reversed by nutrition, education, and good sociocultural conditions. This should be compared with the significant intellectual impairment reported in German school children who were severely undernourished during and after World War I (Blanton, 1919).

Several studies have shown that severely malnourished children can benefit from early nutritional therapy and enhanced sociocultural conditions (Yatkin and McLaren, 1970; McKay *et al*., 1972; Chavez *et al*., 1974; Irwin *et al*., 1979; Monckeberg, 1979; Mora *et al., 1979).* Winick *et al.* (1975) found that IQ and scholastic performance between age 6 and 12 were normal in malnourished Korean children adopted into American families before the age of 2 but were significantly below normal in children adopted after 2 years of age.

Cravioto and Robles (1965) reported that severely malnourished Mexican children admitted to hospital before 6 months of age showed the slowest rate of recovery and greatest loss of intellectual ability compared with malnourished children who entered hospital between age 37 and 42 months, who recovered more rapidly and had least intellectual deficit. McKay *et al*. (1978) found that food supplements given to chronically malnourished Colombian children improved their cognitive, language, and social abilities. The greatest gains were made when treatment was started at age 3, and the gains were progressively less when treatment started at ages 4 and 5. In a study of chronically malnourished pregnant women and children in Louisiana (Hicks *et al*., 1982), a positive relationship was found between the age at which food supplementation was started and subsequent gains of IQ. Mothers received extra food during pregnancy, and their children who continued to receive it from birth to 4 years had higher IQ scores at 6 years of age than their siblings who started receiving extra food after 1 year for 3 years.

Other studies have failed to find any evidence of a critical period although they clearly show that malnutrition and poor socioeconomic conditions result in physical and intellectual impairment. Chase and Martin (1970) reported that children in the United States who were admitted to hospital in a severely malnourished condition before 4 months of age were subsequently less impaired than children who entered hospital after 4 months of age, although both groups later showed significant retardation. This could be interpreted to mean that starting therapy early during the critical period is more effective than starting treatment later. Jamaican children who were hospitalized with severe malnutrition at ages 3–24 months were found to have no correlation between the age of admission to hospital and retardation of IQ at 5–11 years of age (Hertzig *et al*., 1972). Presumably severity of malnutrition and age of onset are only two variables among many, including social and educational deprivation, family instability, and maternal illiteracy, all of which combine to retard child development.

The biological mechanisms of the dysfunction and retardation caused by malnutrition in humans are not well understood, but they are likely to be complex. The nervous system may be affected both directly and indirectly. Nutrients are required for growth as well as to provide materials for synthesis of growth factors, trophic factors, and hormones required for normal development of the nervous system and the other supporting systems. Severe malnutrition directly affects the heart muscle, resulting in cardiac insufficiency and reduced blood supply to the brain. The reduced plasma proteins cause edema in all the tissues including the brain. Anemia, which is often associated with malnutrition, may diminish the oxygen supply to the brain. Neonatal malnutrition results in retarded development of the immune system and consequently in greater risk of infectious disease. Those who survive the insults of malnutrition during infancy and childhood frequently remain with physical and mental disabilities as well as social and economic disadvantages.

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