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Feature Article

Readers will notice a new addition to this issue. The following article by Viktor Hamburger is the first of a series of general interest articles that the Editors plan to include in the Journal pages. Because of the backlog of primary research reports (see Society for Neuroscience Newsletter, Vol. 19, No. 2 (March/April), 1988, pp. 6–7), feature articles will appear only occasionally at first. As the backlog and the resulting publication delays are diminished, however, we plan to make such features a regular part of the Journal. Our intention is to present brief essays on subjects of broad importance to neuroscientists, including historical accounts, tributes to prominent figures, reports of important advances, and other noteworthy issues in our field.

The Editors welcome the response of subscribers to the introduction of this feature section. Further, we are happy to receive specific suggestions from subscribers for future articles.

Dale Purves, Editor-in-Chief

Ontogeny of Neuroembryology

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This essay commemorates the 100th anniversary of the birth of neuroembryology. One cannot, of course, ascribe the beginning of a branch of science to a single year, but the years between 1885 and 1890 saw major publications by the German anatomist Wilhelm His (1831–1904) and the Spanish histologist S. Ramón y Cajal (1852–1934), both of whom laid the foundation to our present understanding of the structure and embryonic origin of the nervous system.

Modern developmental neurobiology emerged from the convergence of two traditions that had their roots in quite different and separate fields of inquiry, and with different conceptual and methodological frames of reference. The one, *the histogenetic tradition,* was descriptive and became sophisticated through refined technology. The other, *experimental neuroembryology,* was causal-analytical and experimental, and was originally a modest side branch of general experimental embryology.

The Histogenetic Tradition

The neurohistologists of the 1860s and 1870s, among them O. Deiters, had already worked out a clear picture of the neuron. It had been obtained by teasing out a motor neuron from the adult spinal cord and clearly shows perikaryon, axon, and dendrites (Fig. 1). Nevertheless, Deiters and his contemporaries subscribed to the *network* or *reticular theory* of the structure of the nervous system. The impulse-conducting elements were pictured not as autonomous units but as part of a network of nerve fibers in which the cell bodies and dendrites were of subordinate importance. Many thought of them as nutritive elements. An earlier version of the reticular theory dated back to Theodor Schwann, one of the founders of the cell theory. He had postulated that the cells bearing his name form cell chains whose protoplasmic connections are transformed into nerve fibers. The more refined versions of the reticular theory of the 1870s and 1880s, associated with the names of Golgi, Hensen, Gerlach, had one important point in common: nerve fibers were supposed to be the product of a preneural protoplasmic network, referred to as *plasmodesms,* which, from the outset, connects the central nervous system with its targets. According to some, the plasmodesms originated by incomplete separation of postmitotic cells; according to others, the plasmodesms were formed secondarily as bridges between cells. The major problem of how the plasmodesms were transformed into nerve fibers remained unresolved.

It is against this background that Wilhelm His's conceptual breakthrough to the neuron theory has to be judged. He was a native Swiss who had become Professor of Anatomy and Physiology at the university of his home town, Basel, at the young age of 26. (At that time the two disciplines were still combined at most universities.) His's title was somewhat deceptive; his institute consisted of two rooms: one his office and laboratory, the other a classroom for his 8 to 12 students that also housed the anatomical collection. In time the department grew rapidly. Later, His, who had a remarkably broad range of interests, became the Director of the Anatomy Department of the prestigious University of Leipzig and one of the leading figures of his generation. Only a very independent mind of his stature could accomplish a complete break with the tradition.

In the early 1880s he began to concentrate on the development of the nervous system. When he looked at the spinal cords of a series of early human embryos he recognized at once that they are not composed of a syncytium but of a layer of individual epithelial cells. He described correctly the neural tube, the precursor of the central nervous system, as a flat epithelium, which became columnar and then loosened up to form what he appropriately called the spongy layer, the *spongiosa.* He identified it as the precursor of the ependymal layer, that is, a glial structure. The mitotic cells at the inner lining of the tube, which he called "*germinal cells,*" had been observed before him, but he

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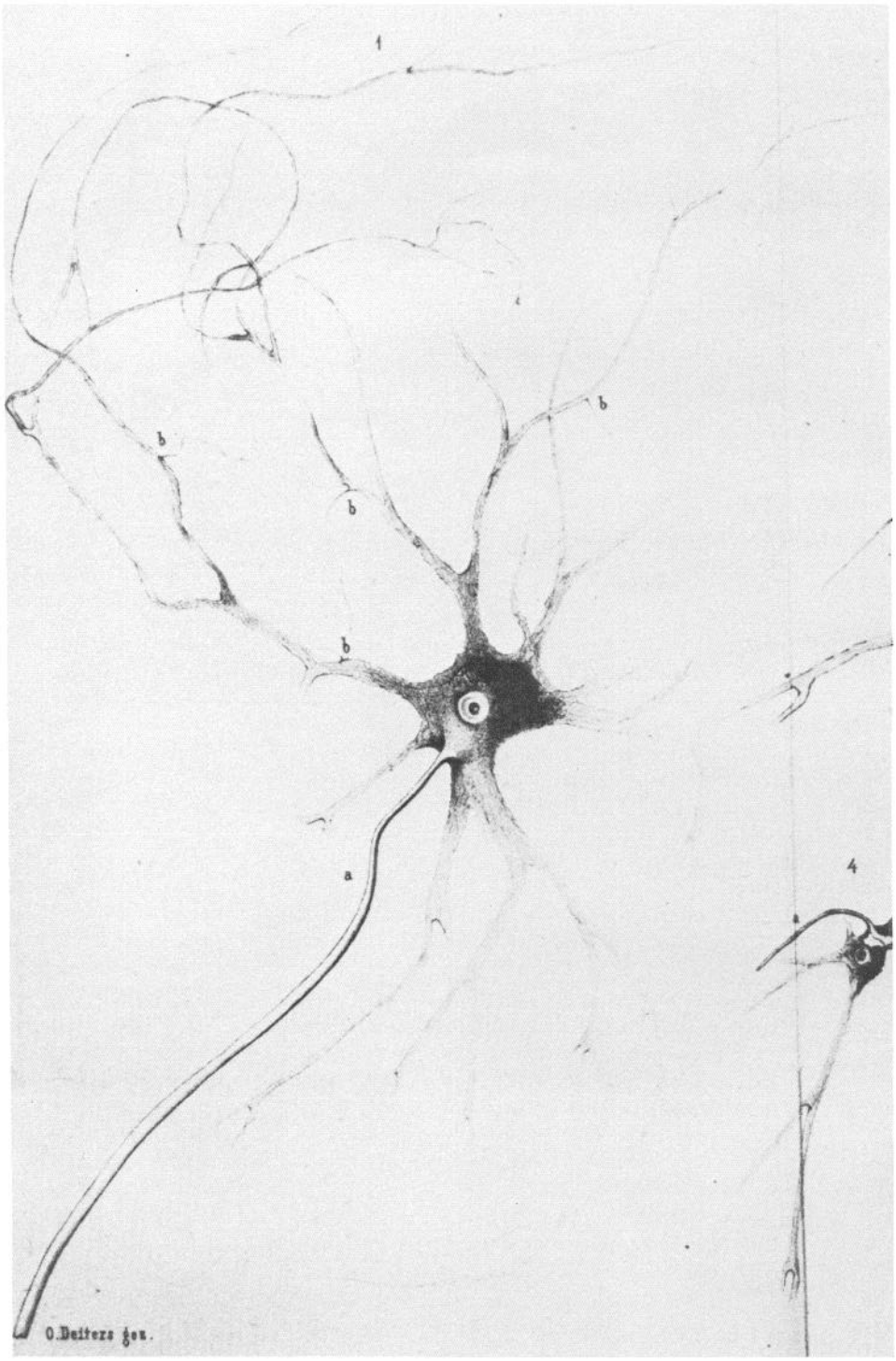

Figure 1. Drawing of a neuron by O. Deiters (1865).

was the first to recognize that they were the precursors of nerve cells. He observed that after their terminal mitosis they became pear-shaped and formed a protoplasmic outgrowth at their distal ends which he identified as the incipient axon. These young *neuroblasts,* as he called them, supposedly migrated across the spongiosa and assembled at the outer margin of the neural tube where they formed what he called the *mantle layer.* In this particular case, the neuroblasts were motor neurons. The tips of their axons pierced through the external limiting membrane, formed a bundle, the motor nerve, and grew toward their target, the somites. They were perhaps supported and guided by the plasmodesms, but were neither nourished nor transformed by them. These observations formed the foundation of the concept of the autonomous neuron on which the *neuron theory* is based. At the end of his classical monograph of 1886 he generalized his findings: "I consider as a definitive principle the theorem that every nerve fiber originates as the outgrowth of a single cell. The latter is its genetic, nutritive and functional center. All other connections are either indirect or they originated secondarily" (1886, p. 513). By genetic he meant embryonic. Among His's other discoveries: the neural crest and the derivation of spinal and sympathetic ganglia from this structure, and the ob-

servation that dendrites (which were named by him) always differentiate later than axons.

Implicit in His's theorem is the idea that there is contiguity but not continuity between nerve cells and other nerve cells or their targets. However, his histological methods did not permit him to demonstrate cell-to-cell contact as a fact. It is at this point that Ramón y Cajal enters the stage. He was a genius in science and an extraordinary human being. One can glean from his delightful autobiography (1937) the magnetism of his personality—a personality that was certainly more colorful than that of Herr Geheimrat His. But his most outstanding trait was his iron will, which he had inherited from his father, and his singleness of purpose. Cajal, who was 20 years younger than His, was born in a small, desolate mountain village in northern Spain where he received very little formal education. Although this lack of education was largely his own fault, he managed to enter professional life. Up to 1887 he had done rather undistinguished work in histology at the University of Valencia, but in 1887 he moved to Barcelona and, according to his own testimony, this year was of decisive importance. On the occasion of a visit to Madrid, a colleague showed him microscope slides of nerve tissue treated with Golgi's silver impregnation method. The method had been available since 1873, and Golgi had made some important discoveries using it, but it was otherwise neglected. The incredible clarity with which the nerve cells and fibers appeared against a faint background made a profound impression on Cajal. It struck him immediately that here was the key to the unraveling of the structure of the nervous system, and the realization of this idea filled the rest of his long life.

His efforts were at first disappointing. The complexity of the central nervous system seemed to be an insurmountable barrier, despite the selectivity of the Golgi method. He then had the ingenious idea to turn to the embryo. "Since the full-grown forest turns out to be impenetrable, why not revert to the study of the young wood in the nursery stage? This was the very simple idea which inspired my repeated trials of the silver method on embryos of birds and mammals. If it is applied before the appearance of the myelin sheaths upon the axons, the nerve cells stand out complete . . . the terminal ramifications of the axis cylinder are depicted with the utmost clearness and perfectly free. The interneuronal articulations appear simple, gradually acquiring intricacy and extension; in sum, the fundamental plan of the histological composition of the gray matter rises before our eyes with admirable clarity and precision" (1937, pp. 324–325).

Cajal looked first at the embryonic cerebellum. He obtained the first convincing evidence for contact—as against fusion—when he observed that the terminal ramifications of the axons of the stellate cells in the molecular layer form basket-like endings around the bodies of the Purkinje cells. In the same preparations he observed the behavior of the climbing fibers. "When they reach the level of the first branches of the dendritic trunks of the Purkinje cells, they break up into twining parallel networks which ascend along the protoplasmic branches, to the contours of which they apply themselves like ivy or lianas to the trunks of trees" (1937, p. 332). It is still incomprehensible to me how he managed within a year or two to unravel the development and structure of the cerebellum in its finest details.

But are we really talking about neuroembryology? Was not the embryo merely recruited by the histologist to provide evidence for the neuron theory, as it had been recruited by the Darwinists to provide evidence for evolution? Does Cajal deserve admission to the guild of neuroembryologists? I would say not, if we consider only his early work on the cerebellum. But the embryonic nervous system captivated his interest in its own right, and he began to study the embryonic spinal cord. At that time, in his seclusion in Barcelona, he was cut off from the mainstream of anatomical research and was not aware of His's investigations. As a result he rediscovered in chick and mammalian embryos the early history of the neuroblast and the outgrowth of the axon. In 1890, His sent him his publication and Cajal acknowledged later the priority of His.

In 1890 Cajal made what he described as one of his most cherished discoveries: the *growth cone.* "In my sections of the 3-day chick embryo, this ending appeared as a concentration of protoplasm of conical form, endowed with ameboid movements. It could be compared to a living battering ram, soft and flexible, which advances, pushing aside mechanically the obstacles which it finds in its way, until it reaches the area of its peripheral distribution. This curious terminal club I christened the growth cone" (1937, p. 369). To this day, the growth cone has remained one of the major challenges to neuroembryologists. Of the many other embryological discoveries of Cajal I mention only one: the mass migration of embryonic neurons. He observed the details of the differentiation of the granule cells in the cerebellum. He saw how the postmitotic cells on the surface became unipolar, then bipolar, how the 2 processes fused and became T-shaped, as in DRG, how the cells migrated to the depth, across the layer of Purkinje cells, and eventually settled down in the granular layer. In the meantime these cells had acquired dendrites. His had already pointed out in 1890 that "the capacity of embryonic nerve cells to migrate seems to be a principle of decisive importance" (1890, p. 115). Indeed, mass migration of embryonic neurons is widespread and is a phenomenon unique in embryonic development.

A penetrating mind of Cajal's stature could not fail to become aware of the central issue in cell migration and axon outgrowth: Which are the forces that give their movements direction? As early as 1892 he opted for *chemotropism,* that is, attraction at a distance by chemical signals emanating from the target. In this speculation he was far ahead of his time, but while he asked the right question, his answer, as described below, turned out to be incorrect.

The way Cajal looked at the growth cone is as interesting as the discovery itself. In the quotation above he reveals one of his prominent traits, his immensely dynamic interpretation of what he saw under the microscope. He "saw" the ameboid movements of the growth cone and the force that pushes aside obstacles, just as he "saw" the climbing fibers climb. As Sherrington (1949) remarked on the occasion of Cajal's Croonian Lecture in London in 1894:

> A trait very noticeable in him was that in describing what the microscope showed he spoke habitually as though it were a living scene. . . . The intense anthropomorphism of his descriptions of what the preparations showed was at first startling to accept. He treated the microscope scene as though it were alive and were inhabited by beings which felt and did and hoped and tried even as we do. A nerve-cell by its emergent fibre "groped to find another"! We must, if we would enter adequately into Cajal's thought in this field, suppose his entrance, through his microscope, into a world populated by tiny beings actuated by motives and strivings and satisfactions not very remotely different from our own. Listening to him I asked myself how far this capacity for anthropomorphizing might not contribute to

his success as an investigator. I never met anyone else in whom it was so marked.

I have dealt with the histogenetic tradition very selectively, focusing on the two leading figures and disregarding the contributions of other important investigators, such as Kölliker, Retzius, van Gehuchten, Lenhossek, and Bielschowsky. However, I should like to add one point. Early in this century, the reticular theory made a remarkable comeback. One of the leading revivalists was Hans Held, who became the successor of His in Leipzig. In 1909 he wrote a weighty tome of almost 400 pages, the gist of which can be summed up in one sentence: What emerges from the embryonic neuroblast is not a protoplasmic outgrowth but a bundle of neurofibrils that become nerve fibers by amalgamating with Hensen's plasmodesm network. This was a futile effort to salvage the reticular theory by combining it with the outgrowth theory. Nevertheless, the reticularist ideas still had adherents in the 1940s. When I attended a conference of neuroembryologists in Chicago in 1949, convened by Paul Weiss, the Dutch histologist Jan Boeke treated us to an animated defense of reticularist ideas. The controversy was finally settled by electron microscopists in the 1950s.

The final victory of the neuron theory was based as much on superior technique and observation as on its rationale. From the physiological perspective, the neuron theory made sense, but the reticular theory was seriously flawed. Cajal points out that the network theory "... takes it for granted that the final axonal branches ... are lost or disappear in the network, in that sort of unfathomable physiological sea into which, on the one hand, were supposed to pour the streams arriving from the sense organs and from which, on the other hand, the motor or centrifugal conductors were supposed to spring like rivers originating in mountain lakes. This was admirably convenient, since it did away with all need for the analytical effort involved in determining in each case the course through the gray matter followed by the nervous impulse.... The reticular hypothesis, by pretending to explain everything easily and simply, explains absolutely nothing'" (1937, pp. 336–337). Like Cajal, His was fully aware that only the neuron theory can account for integrated functional activity which requires specific connections between specific neuronal assemblies.

Experimental Neuroembryology

From the histogenetic tradition, we turn to experimental neuroembryology. It is based on an entirely different tradition, that is, a causal-analytical approach and problem-solving by the analytical experiment. Experimental embryology was conceived and pioneered by the German anatomist Wilhelm Roux in the 1880s, at the same time that His and Cajal started the histogenetic tradition. Roux did his first experiments on frog embryos in 1888. His choice of amphibian embryos was ideally suited for his purpose, so much so that H. Spemann (1869–1941) and R. Harrison (1870–1959), who soon assumed the leadership in the new field, never used any other embryos. Here, then, was another difference which separated the two traditions.

One can consider the organizer experiment of H. Spemann and Hilde Mangold of 1924 as the beginning of experimental neuroembryology because the outcome explains the origin of the neural plate (the precursor of the central and peripheral nervous system) as the result of induction by the organizer. In this experiment, a small piece of the so-called upper lip of the blastopore of a salamander gastrula was transplanted to the flank of another gastrula. The transplant invaginated into the interior and induced in the overlying ectoderm a secondary neural plate and, within a few days, an entire secondary embryo. Embryos of different species differing in the pigmentation of tissues were used, the pigmentation serving as a permanent cell marker. In this way it was established beyond doubt that the secondary neural plate had been induced by the subjacent organizer in tissue that would normally have formed epidermis.

Yet Spemann, like Cajal, can hardly be considered as a neuroembryologist, since his interest in the development of the nervous system ended with the neural tube.

In fact, Ross Harrison was the founder of experimental neuroembryology, although he actually got his start in the histogenetic tradition. He had a Ph.D. from Johns Hopkins University and a German M.D. from the Anatomy Department of the University of Bonn. On the basis of his first investigation of neuron development in the salmon embryo, in 1901, he opted for the axon outgrowth theory as opposed to the reticular theory. While he witnessed the rather acrimonious fight between outgrowth theorists and reticularists, which had flared up again after 1900, he—and apparently he alone—perceived that, in principle, the problem could not be resolved by the histological methods available at that time and that only an analytical experiment could decide the issue. This marked another conceptual breakthrough. He took the bold step of growing embryonic nerve tissue in complete isolation: "The really crucial experiments remained to be performed, and that was to test the power of the nerve centers to form nerve fibers within some foreign medium, which could not by any possibility be suspected of contributing organized protoplasm to them" (1910, p. 790). He succeeded in 1907 in growing pieces of the spinal cord, from early frog embryos, in clotted frog lymph in hanging drop cultures. He was the first to observe axon outgrowth and the formation of growth cones and filaments in the living cell, and he extended these observations over a period of hours and days. He made the important observation that the nerve fibers would not grow out in liquid medium but rather attached to the cover glass, or to fibrin fibers or spider webs which he provided.

One might have expected that this ingenious experiment would have been hailed by the outgrowth theorists and might even have converted some reticularists. Far from it! Nothing can show the gulf between the two traditions better than the cool reception that the tissue culture experiment received in both camps of the histologists. In his last book, which appeared in 1933, Cajal collected once more all the evidence for the neuron theory and against the reticular theory; but he devoted only a few sentences to the tissue culture experiment. From Cajal's vantage point, Harrison had nothing new to say. His opponent, the reticularist Held, in his book of 1909, voiced for the first time a theme which has been repeated ever since: that the behavior of neurons *in vitro* does not necessarily reflect their behavior *in vivo*. He insisted that "the histogenetic investigation of the embryo shows more than the experiment of Harrison. It shows that the intraembryonic nervous system is not formed in the manner of an outgrowth from the neuroblast but that a substance which is present already along its future path and which connects different cells and organ primordia is utilized in the formation of the definitive nerve. For this reason, Harrison's experiment cannot decide according to which principle Nature develops a nervous system in the embryo" (1909, p. 261). Fortunately, posterity has treated Harrison's achievements more kindly.

Harrison was aware, of course, of the problem of how growth cones are guided to their targets. It is interesting to consider the

difference between the approach of the experimental embryologist to this problem and Cajal's speculation on chemotropism, which was not amenable to an experimental test. Harrison made a major methodological contribution by choosing the limb innervation pattern as the testing ground for the analysis. This paradigm has served us well to this day. He transplanted limb buds of frog embryos to the flank and made two observations: that the innervation is provided by the region to which the limb is transplanted, and that the foreign nerves form a normal limb pattern. Harrison concluded from his experiment: "The structures contained in the limb must have a very important directive action upon the developing nerve fibers in that they determine their mode of branching" (1907, p. 276). Of course, he was aware that such a general statement leaves open the problem of the specificity of nerve connections. "One of the most baffling questions . . . is the selectivity of the fibers in establishing their proper terminations—motor neurons with muscle fiber and sensory neurons with the epithelium of the skin or with muscle spindles. . . . It seems necessary to assume some specific reaction between each kind of end organ and its nerve, and Cajal and Tello have pointed out that this could scarcely be other than of a chemical nature" (1935, p. 184). In the meantime, two other mechanisms for guidance had been suggested by others: *stereotropism,* or mechanical guidance, and *galvanotropism,* or orientation in an electrical field. Eventually all theories proposing an action at a distance were discarded and the view was adopted that the growth cone is guided by signals encoded in the structures with which it is in direct contact.

As early as 1904 Harrison opened up the broad field of *trophic relations* between nerves and their target structures. For a long time pathologists had been aware of muscle atrophy resulting from denervation. Harrison inquired whether the initial differentiation of muscles is dependent on nerve supply. He removed the trunk segment of the spinal cord of frog embryos prior to nerve outgrowth and found that the trunk musculature differentiated normally; it showed fiber formation and cross-striation and responded to electrical stimulation; however atrophy and degeneration began soon thereafter. Harrison did not continue the analysis of trophic relations. For reasons of his own, he turned (around 1910) from neuroembryology to other basic problems of development, but his most prominent student, S. Detwiler, continued the tradition.

By chance I became involved in the problem of the trophic role of innervation in the development of target structures. My Ph.D. thesis was supposed to put to the test the rather improbable claim of a German experimental embryologist that eye extirpation in early frog larvae would create a chain reaction of neural deficiencies from eye, to midbrain, to the spinal cord, and to the motor centers that would then result in neurogenic limb abnormalities. The repetition of the experiment gave ambiguous results and I decided to do the crucial experiment: to create nerveless limbs by removing the limb-innervating segment of the spinal cord before nerve outgrowth (1928). I found that limb development was entirely normal. As in the Harrison experiment, the musculature developed normally but atrophied and degenerated later. The pattern of skeletal elements and even joints had been formed normally in these paralyzed limbs. The fact that limb development in amphibians does not require nerve supply is in strange contrast to the dependence of amphibian limb regeneration on innervation. My result was definitive and did not suggest further experiments. I was then prepared to abandon this field and did actually turn to my other interest in developmental genetics.

In the meantime, Detwiler had encountered by chance the one problem that had escaped Harrison's attention but which eventually became one of the most exciting in neurogenesis: the trophic role of the targets in the differentiation of the nerve centers that innervate them. Harrison had suggested to Detwiler the transplantation of forelimb primordia of salamander embryos to different positions on the flank to find out whether limbs innervated by foreign nerves would be capable of motility. Detwiler found that coordinated movements were performed only if at least one limb nerve was derived from the brachial plexus. When he studied his material, he made the seminal discovery that the brachial ganglia, which were deprived of their target, were hypoplastic whereas the thoracic ganglia, which were overloaded, were hyperplastic. Strangely enough, he did not observe changes in the motor centers. The findings were first reported in 1919 and 1920 and were followed by a series of experiments which, however, did not advance the analysis significantly (see Detwiler, 1936).

A decade later fate brought me back into the fold and I landed in Detwiler's territory. In 1932 I joined the laboratory of Dr. Frank Lillie at the University of Chicago as a Rockefeller Fellow. I was supposed to apply Spemann's microsurgery with glass needles on the chick embryo which had been placed on the map by Lillie's classic book on the development of the chick, first published in 1908. By some intuition he had the idea that the limb might have an influence on the development of the nervous system. In 1909 his student, M. Shorey, had destroyed the wing bud by electrocautery and found that, indeed, wing bud removal resulted in the hypoplasia of both spinal ganglia and motor columns. However, there was no follow-up to the experiment and it was almost forgotten. It was only natural that I should start my explorations by repeating this relatively simple experiment. As it happened, my success within a few months in limb extirpation and transplantation shifted the emphasis from amphibian to chick embryos. Their more highly differentiated nervous system was more favorable for in-depth analysis. I could, therefore, add an important point to the findings of Shorey: I established by semi-quantitative methods that the hypoplasia in the motor column was proportional to muscle loss, and that the hypoplasia in the spinal ganglia was roughly proportional to skin loss. In other words, the different centers responded independently of each other. I interpreted this to mean that each center receives a signal from its own target, and I subsequently suggested in 1934 that "the stimuli going from the peripheral fields to their nerve centers are probably transmitted centripetally by the nerve fibers" (1934, p. 491). Thus, I had an inkling of the retrograde axonal transport of a signal from the target. The transplantation of supernumerary limbs resulted in a distinct hyperplasia of the spinal ganglia and a slight increase in the number of cells in the motor column, but otherwise the transplantation experiments shed no further light on the problem of trophic relations (1939).

I come now to a critical issue: How to explain all these findings in terms of a mechanism by which the targets regulate the differentiation of the centers that innervate them. Detwiler and I had two explanations: either the target regulates the proliferation in the nerve centers or it regulates cell numbers in a more complicated fashion. We proposed a highly speculative recruitment hypothesis that involved pioneer fibers which would explore the target area, and a pool of hypothetical uncommitted cells in the nerve centers. The pioneer fibers would send signals back to the centers indicating the size of the target area, and the appropriate number of cells would then be recruited from the pool of un-

differentiated cells. Both explanations had the advantage that they could explain hypo- and hyperplasia by the same mechanism. A disadvantage was that they were wrong.

The correct answer was provided by Rita Levi-Montalcini. She had repeated my limb extirpation experiment in the 1940s, together with her mentor, Guiseppe Levi, who was Professor of Anatomy in Turin and a distinguished neurohistologist. They confirmed my results but provided an entirely different explanation. They had made cell counts in spinal ganglia at different stages and suggested that the so-called hypoplasia comes about, not by interference with proliferation or differentiation, but by the gradual loss of fully differentiated neurons—an entirely novel concept. However, this notion did not explain the hyperplasia resulting from limb transplantation. I suggested to Rita that we collaborate and pursue the matter further. Her arrival in St. Louis in 1947, and the repetition of both limb extirpation and transplantation experiments, turned out to be the start of a new chapter in neuroembryology.

I think that our collaboration profited greatly from our different backgrounds. Rita was more familiar with the intricacies of the nervous system; I was more familiar with the subtle ways of the embryo. The combination of the experimental method with the very powerful silver-impregnation method in which Rita had expertise was indispensable for further progress. The idea that regressive changes could be an integral part of development was not in the conceptual repertory of the experimental embryologist; Rita, however, was not encumbered by this mindset. But I would hesitate to identify Rita with the histogenetic tradition, or, for that matter, with any tradition. I know from my long association with her that her intuition and ingenuity are uniquely her own. Yet, the discoveries of every one of us have roots somewhere in the past. Therefore, one can assert that, in historical perspective, the discovery of NGF by Rita was founded on the confluence of the histogenetic and experimental neuroembryological traditions.

In a broader sense, both the histogenetic-descriptive and the analytical-experimental approaches are now part of history. It is true that the silver-impregnation method and experimentation on embryos are still widely employed tools. And the fundamental questions that were then formulated rather precisely still form one frame of reference for modern developmental neurobiology. Yet, the reductionist turn to the cellular, subcellular, and molecular levels has changed our perspective profoundly. We can now hope for sophisticated solutions of some of these problems, solutions which could not have been anticipated a few decades or even a few years ago. The brilliant successes of the new era have tempted some members of the younger generation to believe that all essential ideas and methods were born in the 1950s. The older generation does well to remind them once in a while that they too stand on the shoulders of their predecessors.

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