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Published version:

DOI:10.1016/j.endeavour.2019.100707

(Article begins on next page)

A new season for experimental neuroembryology: The mysterious history of Marian Lydia Shorey

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Introduction

In 1932 Viktor Hamburger was awarded a Rockefeller Fellowship to work in Frank R. Lillie's laboratory in Chicago. Hamburger, commonly known as "Viktor" in the world of developmental biology and neuroscience, came from Hans Spemann's laboratory in Freiburg where he had worked on the "problem of correlation" in embryonic development. Being aware of Viktor's experiments on limb development in amphibians, Lillie suggested replicating the experiments that one of his graduate students, Marian Lydia Shorey (Fig. 1), had conducted 25 years earlier. However, Viktor was to use the less invasive and more "refined microsurgical techniques" (i.e. glass needles and "hair loop" micromanipulation) that he had learned in Spemann's laboratory, and not the electrocauterisation used by Shorey.[1]

In an interview (June 30, 1983) with Dale Purves, chairman of the "Neurobiology Department" at Duke University, Viktor recalled:

> And that was extremely fortunate because Lillie's was the only laboratory in this country that worked with chick embryos, and in 1909, that means twenty-two years before I came here, he had a student who had tried to kill wing buds in the chick embryo to see how the nervous system was reacting. How Lillie ever got that idea I don't know. Then, Miss [M. L.] Shorey, who did it, disappeared from the literature so I couldn't ask her either.[2]

Who was that unknown student of Lillie? What was her career? And, above all, in which field of research did she take her first steps? To answer these questions, it is essential to reconstruct the framework of the scientific environment in which Shorey worked and in particular to report on the multifaceted activities of Lillie, her mentor.

An unknown researcher

The historiography of medicine has very limited information on the biography of Marian Lydia Shorey. Her ancestors had settled in Albion, in Kennebec County, Maine, at the beginning of the nineteenth century. From the city records it has been possible to reconstruct Marian's genealogy, who was born on the February 6, 1873 (but according to other biographical repertoires, in 1872 or 1874) and she had several brothers and sisters. Her father owned a mill and in the *Census of Albion for 1880* her brothers are referred to as "labourers." This information reveals that her family was of modest origins and had probably joined the Freewill Baptist Church. The period at the turn of the nineteenth and twentieth centuries was characterized by profound change in the United States and in

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[1] William Maxwell Cowan, "Viktor Hamburger and Rita Levi–Montalcini: The Path to Discovery of the Nerve Growth Factor," *Annual Review of Neuroscience* 24 (2001): 551–600, on 555.

[2] Dale Purves, "Viktor Hamburger," available: http://beckerexhibits.wustl.edu/oral/interviews/hamburger.html (accessed May 28, 2019).

Fig. 1. Portrait of Marian Lydia Shorey, 1913 (Milwaukee-Downer College People Files, MDC-RG-001, Lawrence University Archives, Appleton, Wisconsin).

particular in Maine, where industrialization and the development of technology attracted large numbers of workers. This process of social and economic development, described by John Dewey in his socio-pedagogical works, also explains Marian's ambition, because despite coming from a humble family she marshalled her intellectual capabilities and perseverance, kept on studying while working, and ultimately secured university admission.

But first Marian attended the College of the State Normal School of Castine, Maine, where future teachers were trained for the various grades of public and private schools; at the same time she taught in a number of schools in the state, probably in order to finance her studies. She enrolled at Brown University in 1900. Here, between 1904 (Bachelor's of Philosophy degree) and 1906 (MA degree), she was "instructor in physiology and household economics."[3] After a transfer to the University of Chicago, she conducted two years of graduate work in biology under the guidance of Lillie. She received her PhD in 1909 with a dissertation discussed at the Department of Zoology, published in the *Journal of Experimental Zoölogy*, which represents her major work. In the same year, she was Professor of Biology at the Milwaukee–Downer College in Wisconsin, and between 1909 and 1914 she repeatedly worked at the Marine Biological Laboratory of Woods Hole, Massachusetts, where she occupied one of its tables.[4] In 1911 a second important article was published in the *Journal of Experimental Zoölogy* devoted to the differentiation of neuroblasts in artificial culture. In 1915 the *Alumnae Bulletin Milwaukee–Downer College* announced the unexpected news that "Marion (sic) Shorey (prof. of Zoology) has resigned." In the same year, a *Bulletin* later noted, "Dr. Shorey, formerly connected with the Faculty of Milwaukee–Downer College, is in Baltimore studying and resting."[5] Just as suddenly, in November 1916, from the *Chicago University Magazine* we learn that: "Marion L. Shorey [. . .] has gone to Cape Colony, South Africa, where she is teaching at the Huguenot College of Wellington."[6]

The Huguenot Seminary in the Cape Colony was founded in 1874 specifically for religious purposes by the Dutch Reformed Church.[7] In 1898 it became the first women's college in South Africa, and from the beginning it increasingly needed to recruit staff for educational purposes from European and American universities. Its courses included laboratories studying Botany, Physics, Chemistry and, after 1908, Zoology, but—for financial reasons—it was difficult to find assistants for these chairs. In 1916 Shorey was appointed lecturer in Zoology. The circumstances of her recruitment remain mysterious, and it is known only that she was very courageous in facing the passage from America. Very little information is available about her course of "Intermediate Zoology" including "Philosophical Zoology" concerning evolution, heredity, and variation, or practical work on dissection. Her "Syllabus" of 1917–1918 is kept in the archives of the college (Fig. 2),[8] and in the "Calendar of the University of the Cape of Good Hope" for 1917–1918 there is the following entry: "Zoology and Geology: Miss Marion Shorer [*sic*], PhD (Chicago)."[9] Despite the mistakes in both her first and family names, it was indeed Marian. She finished her teaching contract in December 1918 and left for the United States via Australia in March 1919. She may have been unhappy with the salary.[10]

The history of the Huguenot College has been reconstructed in several works, just as there is wide literature on the women who worked in that college between 1895 and 1910 when a certain number of young American women went to South Africa to teach and spread evangelical Protestantism.[11] Nevertheless Shorey's name never appears in this research which is of a mostly sociological character. Therefore, from the scarce information available about her time in South Africa, it is not possible to make

[3] Martha Mitchell, *Encyclopedia Brunoniana* (Providence: Brown University Library, 1993, https://brown.edu/Administration/News_Bureau/Databases/Encyclopedia/search.php?serial=D0080). (accessed 19.09.24).

[4] "Marian L. Shorey," *History of the Marine Biological Laboratory: A Website Preserving and Communicating the History of Science at the MBL*, available: https://history.archives.mbl.edu/node/102219 (accessed March 13, 2018). See also "Developments," *The Kodak Literary Magazine* 14, no. 1 (1908): 20; "Twelfth Annual Report of the Marine Biological Laboratory," *Biological Bulletin of the Marine Biological Laboratory* XVIII, (1910).

[5] *Alumnae Bulletin Milwaukee–Downer College* 7, no. 2 (May 1915), p. 5.

[6] "Alumni Affairs," *The University of Chicago Magazine* 9, no. 1 (November 1916), p. 37.

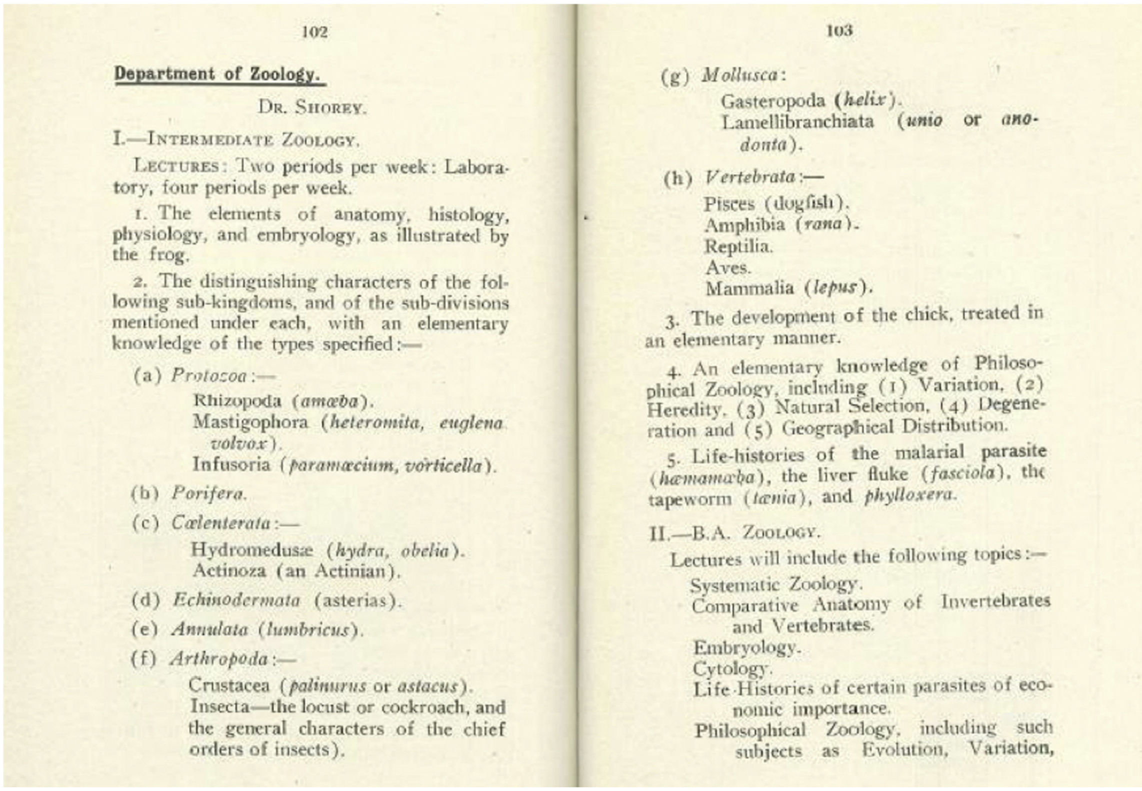
[7] See Dana R. Robert, "Mount Holyoke Women and the Dutch Reformed Missionary Movement, 1874–1904," *Missionalia*, 21 (1993): 103–23; Sarah E. Duff, "'Oh! for a blessing on Africa and America': The Mount Holyoke System and the Huguenot Seminary, 1874–1885," *New Contree* 50 (2005): 21–45; Dana R. Robert, *Changing Childhoods in the Cape Colony: Dutch Reformed Church Evangelicalism and Colonial Childhood, 1860–1895* (London: Palgrave Macmillan, 2015).

[8] A description of Dr. Shorey's Zoology "Syllabus" is given in detail in the Huguenot College Yearbook, 1917/18, Dutch Reformed Church (DRC) Archives, K-DIV733 (personal communication of Isabel Murray, NG Kerk in Suid-Afrika, Stellenbosch).

[9] *University of the Cape of Good Hope, Calendar, 1917-18* (Cape Town: Juta, 1917), p. 355. We thank Cornelis Plug for this reference.

[10] Shorey was classified as lecturer with a salary of $275. But she complained that "she was at the time of her engagement verbally assured that her position would be similar to the other members of the Staff and since others were getting $350 she applied to the Council to make good the difference." Minute of the meeting of June 14, 1918 of the Department of Zoology at Huguenot University College, *Minutes of the Huguenot Council*, p. 372, DRC Archives, K-DIV 672.

[11] See Sarah E. Duff, "Head, Heart, and Hand: The Huguenot Seminary and College and the Construction of Middle Class Afrikaner Femininity, 1873–1910," unpublished MA thesis, University of Stellenbosch, 2006; Sarah E. Duff, "From New Women to College Girls at the Huguenot College, 1895–1910," *Historia* 51 (2006): 1–27.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Department of Zoology.

Dr. Shorey.

I.—Intermediate Zoology.

Lectures: Two periods per week: Laboratory, four periods per week.

1. The elements of anatomy, histology, physiology, and embryology, as illustrated by the frog.

2. The distinguishing characters of the following sub-kingdoms, and of the sub-divisions mentioned under each, with an elementary knowledge of the types specified:—

(a) *Protozoa*:—
Rhizopoda (*amœba*).
Mastigophora (*heteromita, euglena volvox*).
Infusoria (*paramœcium, vorticella*).

(b) *Porifera*.

(c) *Cœlenterata*:—
Hydromedusæ (*hydra, obelia*).
Actinoza (an Actinian).

(d) *Echinodermata* (asterias).

(e) *Annulata* (*lumbricus*).

(f) *Arthropoda*:—
Crustacea (*palinurus* or *astacus*).
Insecta—the locust or cockroach, and the general characters of the chief orders of insects).

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(g) *Mollusca*:
Gasteropoda (*helix*).
Lamellibranchiata (*unio* or *anodonta*).

(h) *Vertebrata*:—
Pisces (dogfish).
Amphibia (*rana*).
Reptilia.
Aves.
Mammalia (*lepus*).

3. The development of the chick, treated in an elementary manner.

4. An elementary knowledge of Philosophical Zoology, including (1) Variation, (2) Heredity, (3) Natural Selection, (4) Degeneration and (5) Geographical Distribution.

5. Life-histories of the malarial parasite (*hæmamœba*), the liver fluke (*fasciola*), the tapeworm (*tænia*), and *phylloxera*.

II.—B.A. Zoology.

Lectures will include the following topics:—
Systematic Zoology.
Comparative Anatomy of Invertebrates and Vertebrates.
Embryology.
Cytology.
Life-Histories of certain parasites of economic importance.
Philosophical Zoology, including such subjects as Evolution, Variation,

Fig. 2. Dr. Shorey's Zoology "Syllabus" in the College Yearbook 1917/18, Huguenot College 1917/18 Calendar [Dutch Reformed Church-Archives K-DIV733]. (By permission of Andrew Kok, Manager Dutch Reformed Church Archives in Stellenbosch, South Africa).

assumptions about the motivations that led her to accept the teaching post in that country—or to leave it.

It is similarly impossible to reconstruct the last years of her life in detail. She seemed to have disappeared into thin air when in two local newspapers the news appeared that she had committed suicide in Waterbury, New Haven, Connecticut on August 26, 1922. Forgotten and disappointed by the world, she left a pathetic letter in which she expressed a wish "to remain as unknown [. . .] as [she had] been alive."[12]

An ante litteram *"Embryo Project"*

In the late nineteenth century an important tradition of experimental embryology emerged in the United States, thanks to the role of the increasing number of marine stations (*e.g.*, Woods Hole and Puget Sound) and to the activity in other laboratories working on the development of non-marine vertebrates. According to the program of the *Entwicklungsmechanik*, or "developmental mechanics," understood as "physiological or causal" embryology, scientists were determined to uncover the mechanisms of morphogenesis and of differentiation, and how external and internal forces act on its development.[13] In the same field in which August Weismann, Wilhelm His, Wilhelm Roux, Hans Driesch, and Oscar and Richard Hertwig had started their embryological research, so also Lillie showed interest in the study of the function and the power of generation of the embryonic organs. In order to discover the role of parts and organs on the normal development of the embryo, researchers destroyed certain parts and transplanted specific tissues within and between embryos. Between 1903 and 1908 Lillie was convinced that chick embryos "were the best choice for almost any type of experimental work on embryological problems."[14] To this aim he prepared an extensive series of sections of the chick embryo at various stages. His method consisted in the destruction, by cauterization, of some identified parts in order to verify whether the wing bud or other parts could survive and develop after extirpation.[15] In fact, according to the concept of *correlative development* expressed in 1903, it was evident that the removal of one part could have an influence on other parts and, in the very first experiments performed on chick embryos, Lillie attempted to destroy amniotic rudiments to show the "power of regulation" on the formation of the tail-fold, head-fold, lateral fold, and so on.[16] In other words, it was imperative to know how an embryonic rudiment is *dependent* on other components of the same organism.[17]

This program reached a new level in the first half of the new century with the introduction of a new branch, to the so-called "physiology of development." This term was coined by Lillie in order to emphasize that all embryological phenomena have a *functional* significance. In his classic book, *The Development of the*

[12] *Waterbury American*, New Haven (August 28, 1922) (source of quote); also, *The Sunday Republican*, New Haven (August 27, 1922).

[13] Jane Maienschein, "Epistemic Styles in German and American Embryology," *Science in Context*, 4 (1991): 407–27.

[14] Karen Wellner, "Frank Rattray Lillie (1870–1947)," Embryo Project Encyclopedia (July 22, 2009), ISSN: 1940–5030, available: http://embryo.asu.edu/handle/10776/1755 (accessed June 6, 2019).

[15] Benjamin H. Willier, "Frank Rattray Lillie (1870–1947)," *National Academy of Sciences Biographical Memoirs* 30 (1957): 179–236, on 213, n. 1.

[16] Frank R. Lillie, "Experimental Studies on the Development of the Organs in the Embryo of the Fowl (*Gallus domesticus*)," *Biological Bulletin* 5 (1903): 92–124, on 92.

[17] *Ibid.*, pp. 105*ff.*

Chick (1908), where he emphasized the role of "physiology of development" (not to be confused with embryology), he established the *principle of correlative differentiation*:

> Physiology of development must proceed from an investigation of the composition and properties of the germ-cells. It must investigate the role of cell-division in development, the factors that determine the location, origin and properties of the primordia of the organs, the laws that determine unequal growth, the conditions that determine the direction of differentiation, the influence of extra-organic conditions of the formation of the embryo, and the effects of the intra-organic environment, i.e. of component parts of the embryo on other parts (correlative differentiation).[18]

In addition to his qualities as a scientist, administrator and research organizer in the Zoology Department of the University of Chicago, Lillie should be also remembered as a great teacher. His PhD students were first trained and later selected according to their value and even his graduate students played a conspicuous role in the advancement of his work, because he used "to assign [them] a research problem for the doctorate which was along the lines of his research program at the time" and leave the development of the topic to them.[19]

When he was writing his book on chick development, Lillie, as was his custom, gave a new topic for investigation to the above-mentioned young lady, Marian Shorey, to verify whether the wing primordium plays any role in the development of the nervous system. In 1909 she tackled this issue in her dissertation at the Department of Zoology to achieve the degree of Doctor of Philosophy (Fig. 3). Based on "work that had begun at the suggestion of the Professor Frank R. Lillie," this dissertation was published in *The Journal of Experimental Zoölogy*.[20] Nevertheless, although Lillie was the supervisor of Shorey's research, it should be emphasized that her scientific horizon was not confined to his approach. While maintaining the research methodology learned by her mentor, Shorey showed she could work autonomously and critically. In fact, right from the *incipit* of her dissertation, she moved with great maturity in the context of the embryological research regarding the problem of the influence of peripheral organs on the development of the nervous system, referring to three main guidelines of research, whose traces we now propose to follow.

Shorey and the birth of neuroembryology

Although as late as 1890 Santiago Ramón y Cajal felt the need to criticise the "*obscurité*" on the emergence of the nerve fibres, resulting from the lack of a suitable method of investigation, it must be acknowledged that in the first decade of the twentieth century there was already a fairly large literature on this topic.[21] In an article published in 1909 in a popular magazine aimed at disseminating scientific knowledge, describing his "theory of individual development," Lillie mentioned an experiment where the bud of a leg of a tadpole, that had as yet no nerves, may be transplanted to any region of the body where it develops as a leg. In this regard, he mentioned the names of the two embryologists who had adopted this experimental model to shed light on "the problems of nerve development," Ross Granville Harrison at that time at the Johns Hopkins University, and the German anatomist Hermann Braus first at Würzburg, then at Heidelberg, who faced the problem of amphibian embryonic development "from the nerve center out to periphery."[22] In particular, the experiments by Braus were the first in which limb transplantations were used to address neuroembryological questions.[23] Lillie reached the conclusion that the bud of the leg of the tadpole receives its innervation from the nerves of the region to which it has been transplanted, and the mode of branching of the nerve is that of the leg nerves. This assumption was proposed as a general rule by observing that any nerve, regardless of its normal mode of branching, may be made "to branch like leg nerves, by bringing a leg bud into its innervation area at the time that the nerve is still growing." Therefore, he reached the conclusion that the constancy of distribution of peripheral nerves "is a function of the intra-organic environment in each generation."[24]

In the context of these research lines, a point of particular relevance was the question of the relationship between the development of the musculature and that of the nerves. Between 1897 and 1904 the anatomist Charles Russell Bardeen of the Johns Hopkins University became interested in the development and variability of the nerves in limbs. In an article devoted to the issue of the development of the musculature of the body-wall in the pig embryo he came to the conclusion that each muscle-cell has its origin in a single myoblast which elongates, and the number of the nuclei increases by direct division. As regards the development of the nervous system in the early stages of the embryonic life he observed that, in the pig embryo, the musculature was well differentiated *before* the nerves establish a connection with it: "The peripheral nerves are shown to develop independently of the myotomes, and to become associated directly with musculature only after the muscles have become differentiated."[25] However, at that time, Bardeen admitted that "on no subject in vertebrate embryology is the literature less complete than that concerning the development of the voluntary apparatus."[26]

In this conceptual frame, the contribution of Shorey emerged as an answer to the questions posed by these three embryologists, Harrison, Braus, and Bardeen, on the "intimate relation [which] exists between the life of a muscle and that of its motor nerves."[27] In particular, the assertion made by Bardeen that "The early development of the nerves is one of a passive independence, without any immediate relations to myotomes" to some extent addressed the investigations of Shorey. This was the starting point, since she was convinced that it was not a conclusive one, due to

[18] Frank R. Lillie, *The Development of the Chick: An Introduction to Embryology* (New York: Holt, 1908), p. 8.

[19] Willier, "Frank Rattray Lillie" (ref. 15), p. 186.

[20] Marian L. Shorey, "The Effect of the Destruction of Peripheral Areas on the Differentiation of the Neuroblasts," *Journal of Experimental Zoölogy* 7 (1909): 25–63, on 28.

[21] Santiago Ramón y Cajal, "Sur l'origine des ramifications des fibres nerveuses de la moelle epinière," *Anatomischer Anzeiger* 5 (1890): 85–95, 111–19, on 85.

[22] Ross Granville Harrison, "Observations on the Living Developing Nerve Fiber," *The Anatomical Record* 1 (1907): 116–18, on 116. Further on Harrison, see Ross Granville Harrison, "Further Experiments on the Development of Peripheral Nerves," *American Journal of Anatomy* 5 (1906): 121–31; Ross Granville Harrison, "Experiments in Transplanting Limbs and their Bearing upon the Problems of the Development of Nerves," *Journal of Experimental Zoölogy* 4 (1907): 239–81; John S. Nicholas, "Ross Granville Harrison (1870–1959)," *National Academy of Sciences Biographical Memoirs* 35 (1961): 132–62; on Braus, see Hermann Braus, "Einige Ergebnisse der Transplantation von Organanlagen bei Bombinatorlarven," *Verhåltnisse der Anatomische Gesellschaft* 18 (1904): 53–65; Hermann Braus, "Vordere Extremität und Operculum bei *Bombinator*–larven. Ein Beitrag zur Kenntnis Morphogener Correlation und Regulation," *Gegenbaurs Morphololologische Jahrbuch* 35 (1906): 509–90.

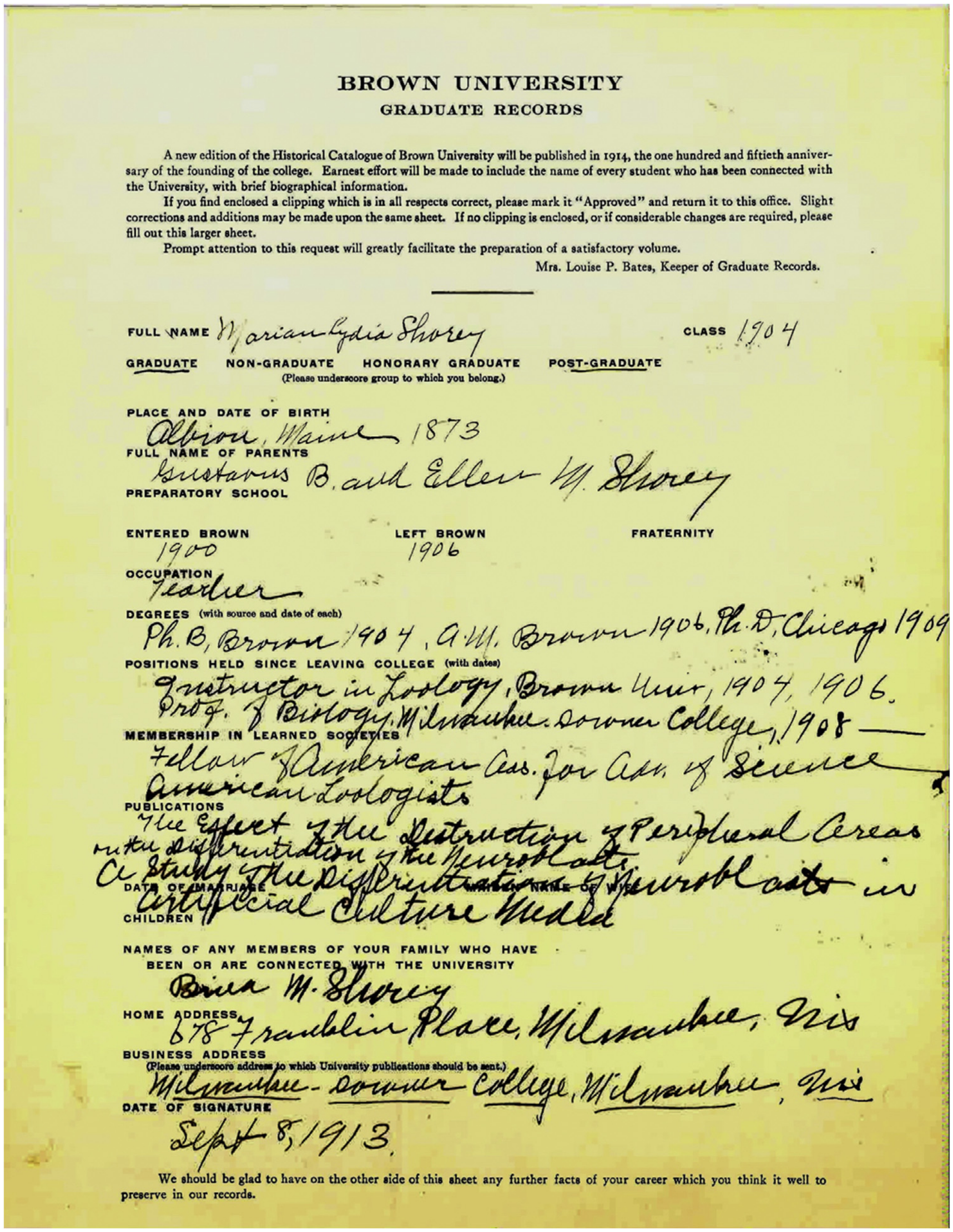
[23] See Viktor Hamburger, "S. Ramón y Cajal, R. G. Harrison, and the Beginnings of Neuroembryology," *Perspectives in Biology and Medicine* 23 (1980): 600–16, on 609.

[24] Frank R. Lillie, "The Theory of Individual Development," *Popular Science Monthly* 75 (1909): 239–52, on 244.

[25] Charles R. Bardeen, "The Development of the Musculature of the Body-Wall of the Pig," *Johns Hopkins Hospital Reports* 9 (1900): 367–400.

[26] Beatrice N. Rafter, *Myogenesis of the Chick*, MA thesis, Boston University, 1936.

[27] Shorey, "The Effect of the Destruction" (ref. 20), p. 26.



BROWN UNIVERSITY

GRADUATE RECORDS

A new edition of the Historical Catalogue of Brown University will be published in 1914, the one hundred and fiftieth anniversary of the founding of the college. Earnest effort will be made to include the name of every student who has been connected with the University, with brief biographical information.

If you find enclosed a clipping which is in all respects correct, please mark it "Approved" and return it to this office. Slight corrections and additions may be made upon the same sheet. If no clipping is enclosed, or if considerable changes are required, please fill out this larger sheet.

Prompt attention to this request will greatly facilitate the preparation of a satisfactory volume.

Mrs. Louise P. Bates, Keeper of Graduate Records.

FULL NAME Marian Lydia Shorey CLASS 1904

GRADUATE NON-GRADUATE HONORARY GRADUATE POST-GRADUATE

(Please underscore group to which you belong.)

PLACE AND DATE OF BIRTH Albion, Maine 1873

FULL NAME OF PARENTS Gustavus B. and Ellen M. Shorey

PREPARATORY SCHOOL

ENTERED BROWN 1900 LEFT BROWN 1906 FRATERNITY

OCCUPATION Teacher

DEGREES (with source and date of each) Ph.B, Brown 1904, A.M. Brown 1906, Ph.D, Chicago 1909

POSITIONS HELD SINCE LEAVING COLLEGE (with dates) Instructor in Zoology, Brown Univ, 1904, 1906. Prof. of Biology, Milwaukee-Downer College, 1908 —

MEMBERSHIP IN LEARNED SOCIETIES Fellow of American Ass. for Adv. of Science; American Zoologists

PUBLICATIONS The Effect of the Destruction of Peripheral Areas on the differentiation of the Neuroblasts. A Study of the Differentiation of Neuroblasts in Artificial Culture Media

DATE OF MARRIAGE NAME OF WIFE

CHILDREN

NAMES OF ANY MEMBERS OF YOUR FAMILY WHO HAVE BEEN OR ARE CONNECTED WITH THE UNIVERSITY Bruce M. Shorey

HOME ADDRESS 678 Franklin Place, Milwaukee, Wis

BUSINESS ADDRESS (Please underscore address to which University publications should be sent.) Milwaukee-Downer College, Milwaukee, Wis

DATE OF SIGNATURE Sept 8, 1913

We should be glad to have on the other side of this sheet any further facts of your career which you think it well to preserve in our records.

Fig. 3. Biographical information on the career of the student Marian L. Shorey contained in the Brown University Graduate Records. (Brown University Archives).

"the fact that two organs are not in direct contact does not exclude the possibility that the one may influence the other."[28] By commenting the view of Bardeen about the relationship between myotomes and nerves, Shorey observed that decisive conclusions on the interdependence of two organs or tissues in development could be obtained by studying the behaviour of one in absence of the all possible effects from the other. As far as the nervous system is concerned, this may be achieved by destroying (or removing) some regions of the developing organism, and to verify the effect of this destruction on the innervated organs. To this aim Shorey mentioned also the experiments of Braus and Harrison, although the two works of these scholars had only recently been published and she was not able to read them during her experimentation.

It is clear that Shorey worked on the line of the experimental paradigm of her mentor Lillie, though her approach was completely original. Accordingly, chick embryo was a favourable model for this kind of experiment and there was no nerve regeneration after the destruction of specific embryo portions, a result "entirely confirmed" by Shorey herself. The experiments consisted in destroying the primordia of some muscles before nerve fibres penetrated into them. In the first experiments the wing bud was removed by electrolysis or scalpels, depending on the stage of development. Lillie had observed that the wing develops from seventeenth, eighteenth, and nineteenth somites and it is innervated by the fourteenth, fifteenth, and sixteenth nerves. Only later in the development do these three nerve trunks form a plexus irradiating to the muscles and to the sensory area, but at this stage of the operation there is no contact with the myotome. The aim of Shorey was to proceed serially: first she looked at the older embryos, killed between five and six days after the operation, then she went on to examine specimens between 1 and 4 days after the surgery.

[28] *Ibid*.

In the experiments on embryos after three days of incubation Shorey removed the wing primordium and she put the egg in the incubator again for over five days. The embryo proved to be normal except for the missing limb. Concerning the motor neurons, she noticed a quantitative loss in the nerve trunks: "wherever a muscle is missing the corresponding nerve is also missing."[29] Even the ganglia on the operated side were smaller, and a loss in the spinal cord was evident as well, and the cells of the ventral horn (antero-lateral part) were numerically inferior. Also in embryos of different incubation periods the distribution of the peripheral nerves turned out to be smaller with less extensive branching in the operated side, and there were abnormalities in the spinal cord. Therefore, well-defined defects were detected in the nervous system after the extirpation of peripheral areas. The logical reason for this fact could be a slower development of the injured part, a degeneration of the nervous elements or, more likely, the dependence of the neuroblasts on the surrounding tissue: when this is missing, the nerve elements do not develop. Shorey could rely on material provided by Lillie for experiments at later stages of development and at a certain point she decided to remove some somites. By destroying the primordium of the muscles of a given somite, she observed that some cells and motor fibres developed in the medullary tube of this portion, but they were fewer and less extensive. In addition, they did not form a well-defined nerve trunk, but rather "an irregular mass."[30] She condensed her observations in a summary of sixteen points, whose essence was that in the early stages of development nerve fibres follow "a definite path leading to a muscle or other end organ" and for this reason it was not a question of degenerating fibres, but of "the failure of the neuroblasts to develop."

The fact that some motor neuroblasts differentiated on the injured part could imply that there were two classes of neuroblasts dependent on or independent of influence from the periphery. But this seemed unlikely, and it was more plausible to think that the neuroblasts that develop normally in the injured side are under the control of the muscles of adjacent somites. In this regard, she appealed to the theory of chemotaxis, "to the chemical substances or physical forces" coming from the medium surrounding the cell, without any "vital force" being postulated, a reflection that could be considered "almost philosophical."

> The cell during its whole embryonic history has been repeating the same cycle of processes, namely, assimilating food in a definite way, increasing in size and dividing, and it is impossible to conceive that any tendency to develop in a certain direction, any adaptation to conditions, or any need of the organism can produce a new chemical substance or inhibit the action of one already present. Differentiation of any cell must therefore occur because of a change in the chemical composition or physical properties of the lymph surrounding it.[31]

Therefore, contrary to the belief that neuroblasts were self-differentiating, Shorey concluded that a more plausible hypothesis was that they could differentiate into motor nerves thanks to the contribution of muscular end organs ("a necessary corollary of this condition"), and in their absence the neuroblasts would have no power of self-differentiation.

It is therefore evident that the presence or absence of muscles in a given somite must influence the character of the medium surrounding the neuroblasts in its immediate neighbourhood, and thus a change in the chemical inter-reactions may be effected. This would give at least a possible explanation of the influence of the muscles on the nerves by which they are normally innervated.[32]

Her observations on the chicken embryos were complemented by others on amphibians, and she proceeded along the Harrison's approach. Although it was an indirect test, the very method of lymph drop in which nerve fibres developed was a test in favour of the influence of metabolism products. In fact, that method was criticized because it was claimed that in the lymph products of the metabolism of other parts of the body of the larva could be present. However, this fact in Shorey's view showed also in a non-crucial way that neuroblasts are self-differentiating, and at the same time that they are dependent on stimulation from end organs or their products. Although it was not possible to obtain crucial results in amphibians, animals in which limbs are repeatedly regenerated, nevertheless the experiments showed "that the process of differentiation of the neuroblasts is essentially the same in the amphibians as it is in the chick." In fact, the experiments in which the limb was repeatedly removed, which involved a considerable decrease in the musculature and consequent defective nerve trunks and ganglia, revealed that *there was never any degeneration*, but that the neuroblasts failed to differentiate regularly. This phenomenon was called *hypoplasia* which means a deficient development. A similar influence was exerted by the presence of the muscles that were located in close contact with the neuroblasts, thus favouring their differentiation in motor nerves.

Further research on neuroblast differentiation

Two years later, in a subsequent paper Shorey went back to the theme of differentiation. She asked whether intrinsic factors were sufficient for the differentiation of the neuroblasts or whether external factors were necessary and, if so, what their nature and source were. Shorey referred to the two "extreme" views that were opposed at that time. On the one hand, Driesch championed a theory which considered the *position* of the blastomeres relevant, "what a cell becomes depends on its position in the embryo":

> The relative position of a blastomere within the whole will probably determine in a general way what shall come from it; if it be situated differently, then it will give rise to something else; or, stated another way: its prospective relation is a function of [its] place."[33]

On the other hand, Roux and Weismann (reformulated by Edmund B. Wilson),[34] interpreted the development as a *mosaic* of self-differentiating cells, according to which each element can interfere on the development of the other parts.[35] Nevertheless, Shorey was convinced that none of these views were sustainable and she reaffirmed the thesis maintained in the previous essay that the development of nerve fibres was possible due to the influence of some substance in the circulating medium. If the interdependence of the tissues was to be explained in the adult, it was necessary to postulate a physiological discontinuity between the cells and the issue of the relationship between muscles and nerves was particularly significant. To carry on its normal metabolic processes the nerve needs its end organ, whose metabolic products act as a stimulus; but even when it begins its differentiation and then it performs its tasks in adult life, the cell must maintain a physiological inter-relationship with other cells. Consequently, Shorey suggested as a working-hypothesis that the development is

29 *Ibid*., p. 33.
30 *Ibid*., pp. 47–48.
31 *Ibid*., p. 53.
32 *Ibid*., pp. 53–54.

33 Hans Driesch, "Entwicklungsmechanische Studien," *Zeitschrift für wissenschaftliche Zoologie* 55 (1892): 1–62, on 39.
34 Edmund B. Wilson, "Mosaic Development in Annelid Egg," *Science* 20 (1904): 748–50.
35 Klaus Sanders, *Landmarks in Developmental Biology, 1883–1924* (Berlin–Heidelberg: Springer, 1997).

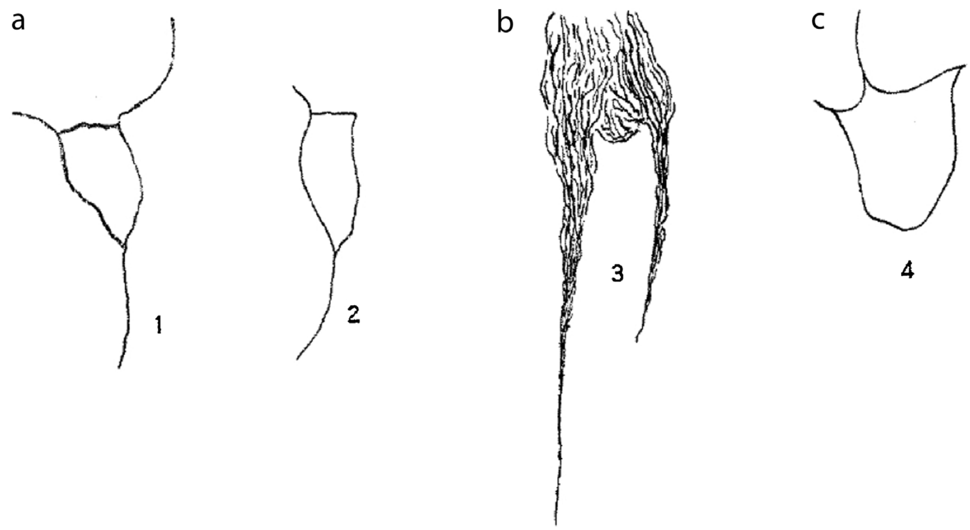


Fig. 4. Development of wholly undifferentiated neuroblasts taken from *Necturus* and placed in a culture medium consisting of gelatine enriched with peptone or beef extract. Insets 1, 2 and 4 show the emergence of fibres from the neuroblasts after 40 to 70 hours. Inset 3 is a bundle of fibres emerging from the medullary canal after 67 hours. Untreated neuroblasts have not been outlined by the author. Usually they have a small spherical shape with tiny protrusions on their surface. (From M.L. Shorey, "Study of the differentiation of neuroblasts in artificial culture media," *Journal of Experimental Zoölogy*, 10, 1911, p. 91).

the result of "a chain of chemical reactions and inter-reactions" between the cytoplasmic substances of the egg and substances forming the organs. Although external forces to the organism can play a role, "*one of the sources of stimulation will always be found to be the metabolic products of other tissues*."[36]

Shorey moved from a famous experiment of Harrison, which consisted in placing a tiny portion of medullary tube in a drop of lymph.[37] Of particular interest were the cases in which the fibres under observation had not yet completed their development: their enlarged ends which extend simple or branched filaments were "very active," and their amoeboid movements consisted in "the drawing out and lengthening of the fibre to which [they are] attached." Harrison hypothesized that it was "a specific characteristic of nervous tissue," particularly accentuated in the nerve cells at this period of development.[38] Starting from the experimental results achieved in 1909, Shorey had instead come to the conclusion that "motor neurons are in some way dependent on the muscles for differentiation."[39] In a new experimental series, she added gelatine solution with peptone or beef extract to culture medium in which neuroblasts were alive but without developing nerve fibres. In particular, experiments on *Necturus* were interesting. She placed isolated neuroblasts in culture media enriched with peptone and beef extract for several days. In all her experiments, she found that the cells maintained the typical spherical shape without appreciable changes. In contrast, when using neuroblasts derived from the central canal of the spinal cord (about 67 h after being placed in this medium) many fibres were emerging and elongated to a considerable distance (Fig. 4). In conclusion, in a number of experiments masses of fibres were emerging from the cells or from small portion of medullary canal.[40] In other experiments, she observed many short fibres or a few longer ones. In several instances the changes were absent. Anyway, when the beef extract was absent, no development of fibres could be observed.

She assumed that probably the variability of these results was due to the conditions of the cells at the time they were placed in the culture medium. The influence and change in metabolism constitute a gradual process and neuroblasts have to reach a certain stage of development in order to be stimulated by the products of adult muscle. The fact that the development of nerves did not occur when the primordium of a muscle had been destroyed was further evidence of the role of the products of the muscular metabolism as a stimulus for the growth. A conclusion drawn from these experiments was that the neuroblasts are not self-differentiating and that some external factor or factors must be present in the extracellular environment where the neuroblasts are physiologically developing.

Epilogue

In an address to the Biological Club of the Chicago University on the occasion of the Darwin anniversary in 1909, Lillie returned to the subject of the organic development and he summarized the role of the extra- as well as intra-organic environment. His hypothesis was that the developing embryo is a living mosaic: "each element of which may conceivably enter into the development of any other in the sense of being a factor in the process. Each part of the embryo, therefore, has an intra-organic environment consisting of all the other parts, some of which constitute relatively immediate environmental factors, others relatively remote ones".[41]

To better illustrate this principle, Lillie took the following example: some nerves arise from some centres in the embryo and grow out as roots in the soil. The muscles arise separately and the nerves grow onto them by making the proper connections. He was asking whether it was an "innate tendency" of the nerves to grow along "particular paths" and to branch according to definite laws, or whether there was a driving stimulus exerted on nerves by the developing muscle tissue. Lillie remarked that in his laboratory

[36] M. L. Shorey, "Study of the Differentiation of Neuroblasts in Artificial Culture Media," *Journal of Experimental Zoölogy* 10 (1911): 85–93, on 87 (emphasis in the original).

[37] Harrison, "Observations on the Living Developing Nerve Fiber" (ref. 22); see Kimberly A. Buettner, "Ross Granville Harrison (1870–1959)," *Embryo Project Encyclopedia* (2007), available: http://embryo.asu/edu/handle/10776/2106 (accessed April 10, 2019).

[38] Harrison, "Observations on the Living Developing Nerve Fiber" (ref. 22), p. 117.

[39] Shorey, "Study of the Differentiation of Neuroblasts" (ref. 36), p. 88.

[40] *Ibid.*, p. 91 (Fig. 3).

[41] Lillie, "The Theory of Individual Development" (ref. 24), p. 244.

"Miss Shorey" demonstrated in the chick that origin and growth of the nerve cells are dependent on muscle development, and for this reason the anatomy of the central nervous system and not only of the peripheral nervous system is dependent on the intra-organic environment.

At this point, the question of whether Shorey conducted any further research and where it led still remains. As explained above, the biographical information about this extraordinary, "secluded" neuroembryologist is practically non-existent. In the rare papers in which her name is mentioned, it is misspelt as "Marion"[42] or "Elizabeth."[43] Fortunately, in their history of American neuroscience, Magoun and Marshall accurately cite her name.[44]

However, at least in the first half of the twentieth century her name was not destined to disappear from the neuroscientific scene. In fact, in the twenties, a student of Ross Harrison's, Samuel Detwiler, performing manipulations of limb removal and transplantation in salamanders (*Ambystoma,* or "Amblystoma" in Hamburger's report), had come to different results than those of Shorey. He found sensory hypoplasia, but few effects on the motor column: "This remarkable discrepancy in the results [demanded] further investigation," since it could be hypothesized that the results obtained by Shorey were an artefact due to the electrocautery technique.[45]

Ten years later, as has been said, Hamburger was working in the Lillie's laboratory, and the latter assigned the continuation of Shorey's research to him. Reconstructing the story of "the rise of experimental neuroembryology" in a Kuffler Lecture, he commented "I knew Detwiler from his visit to Freiburg, but I knew nothing of Ms. Shorey; her name had disappeared from the literature. I found out a few years later that she had been a student of Dr. Frank Lillie of the University of Chicago, whose classic book "The Development of the Chick" had put the chick embryo on the map for research and teaching".[46]

This reinvestigation was not futile and repetitive, and it proved to be crucial regarding the relationship between peripheral structures and the central nervous system. The wing extirpation experiments on the chick embryo showed that "the results obtained by Miss Shorey were fully confirmed."[47] They were confirmed "in every detail and there is not the least deviation in the experimental results": both the brachial dorsal root ganglia (DRG) and the brachial lateral motor column were hypoplastic.[48] Nevertheless, Hamburger added a further important point: he established "by the semiquantitative methods that hypoplasia in the motor column was proportional to muscle loss."[49] In fact, the number of the motoneurons in the lateral motor column appeared to be reduced between 22 % and 60 % proportionally to the muscle tissue loss between 31 % and 96 %.[50] At the same time, he confirmed that "no degenerated neurons were found in the area affected" and also in this regard he agreed with Shorey: "The idea that hypoplasia might be due to cell degeneration did occur to Shorey, but she dismissed it."[51]

In 1934, Viktor arrived at the conclusion that:

> The different peripheral structures while growing are in some direct connection with their appropriate centres in the nervous system. Thus, they are enabled not only to control the growth of their own centres in general but even to regulate this growth in quantitative adaptation to their own progressing increase in size [. . .] [e]very structure within the growing limb, muscle as well as sensory organs, send stimuli to the central nervous system. Each part of the peripheral field controls directly its own nervous centre, i.e., the limb muscles affect the lateral motor centres, the sensory fields control the ganglia, etc.[52]

However, Hamburger was not sparing in his criticism of the interpretation that Shorey had given to the processes that led to a decrease in the number of cells. In fact, according to Shorey the products of the muscular metabolism filter in the lymph and they are carried to the spinal cord, "where they as a stimulus for motoneuron growth and maintenance."[53] Nevertheless, this suggestion did not explain why only certain parts of the spinal cord reacted and others did not, and above all this hypothesis did not allow an explanation of why the nerve centres reacted "in quantitative relation to the growth of their fields proper."[54] A more suggestive idea was that the nerves acted as *mediators* to constitute "strictly specific paths": from limb muscles to lateral motor cells, from sensory fields to spinal ganglia. Hence the conception of the nerves as *pathfinders* would be derived. They were charged with the "double task" of locating the peripheral field and of reporting back centripetally the "information" of this exploration to the central organ. "The pioneer fibres would send signals back to centres indicating the size of the target area, and the appropriate number of cells would then be recruited from the pool of undifferentiated cells."[55]

This long and multifaceted story on the relationship between the neural centres and the periphery in embryogenesis was apparently well accepted by the scientific community. The paper published by Hamburger in 1934 was a kind of reference site on the state of the art on this issue. The results were interpreted on the basis of the induction theory elaborated by Spemann. Accordingly, the wing or limb primordium was responsible for promoting the maturation of the motor and sensory neurons of the spinal cord and setting a proper quantitative relationship between the centre and the periphery.

However, it was only a few years later that an earthquake destabilized the picture. The entire story is told by Rita Levi-Montalcini in her autobiographical record, *In Praise of Imperfection* (1988).[56] She was a young scientist working in the Institute of Human Anatomy of the University of Turin led by Giuseppe Levi. On June 1940 when Levi-Montalcini was reading Hamburger's paper, she envisaged a new way of approaching the issue. In collaboration with Levi she planned to repeat the experiments performed by Hamburger in 1934. Although the experiments were

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[42] Lijin Jiang, "The Effects of Wing Bud Extirpation on the Development of the Central Nervous System in Chick Embryos," (1934), by Viktor Hamburger, Embryo Project Encyclopedia (2010–11–22), available: http://embryo.asu.edu/handle/10776/2315 (accessed March 19, 2014).

[43] Garland E. Allen (2004): "A Pact with The Embryo: Viktor Hamburger, Holistic And Mechanistic Philosophy in the Development of Neuroembryology, 1927–1955," *Journal of History of Biology* 37, no. 3 (2004): 421–75.

[44] Horace W. Magoun and Louise H. Marshall, *American Neuroscience in the Twentieth Century* (Lisse: Swets & Zeitlinger, 2003), p. 190.

[45] Viktor Hamburger, "The Effects of Wing Bud Extirpation on the Development of the Central Nervous System in Chick Embryos," *Journal of Experimental Zoölogy* 68, no. 3 (1934): 449–94.

[46] Viktor Hamburger, "The Rise of Experimental Neuroembryology: A Personal Reassessment," *International Journal of Developmental Neuroscience* 8, no. 2 (1990): 121–31, on pp. 127–31.

[47] *Ibid.*, p. 450.

[48] *Ibid.*, p. 480.

[49] Viktor Hamburger, "Ontogeny of Neuroembryology," *Journal of Neuroscience* 8, no. 10 (1988): 3535–40.

[50] Viktor Hamburger, "The Effects of Wing Bud Extirpation" (ref. 45), p. 491.

[51] Viktor Hamburger and Ronald W. Oppenheim, "Naturally Occurred Neuronal Death in Vertebrates," in Viktor Hamburger, *Neuroembryology: The Selected Papers* (Boston, MA: Birkhäuser, 1990), 126–42, on 127.

[52] Hamburger, "The Effects of Wing Bud Extirpation" (ref. 45), pp. 473 and 470.

[53] Cowan, "Viktor Hamburger and Rita Levi–Montalcini: The Path to Discovery of the Nerve Growth Factor" (ref. 1), p. 558.

[54] Hamburger, "The Effects of Wing Bud Extirpation" (ref. 45), p. 474.

[55] Hamburger, "Ontogeny of Neuroembryology" (ref. 49), pp. 3539–40.

[56] Rita Levi–Montalcini, *In Praise of Imperfection*, trans. by Luigi Attardi (New York: Basic Books, 1988).

done in the most difficult environmental conditions due to the Jewish persecutions and the war, the results signalled the beginning of a new age in neurobiology.[57] By observing the growth of neurons in the chick embryo with the aid of a silver staining technique, they showed that in the absence of the primordium, central neurons were able to reach a high degree of maturation and an axonal elongation toward the periphery despite the absence of the peripheral target. However, at day twelve all neurons started to degenerate, a phenomenon which escaped the attention of Shorey and Hamburger. Therefore, Levi and Levi-Montalcini concluded that the death of differentiated neurons depended "on the impossibility of establishing connections with the peripheral structures, the muscles and the skin" which were necessary for their survival and maintenance.[58]

In St. Louis, where "Rita" was invited by Hamburger in 1947, Rita and Viktor repeated together the experiments of limb extirpation: the results were confirmed, but the new, different explanation ruled out the role of the primordia as inductor of the central neurons development as hypothesized by Hamburger. "The so-called 'hypoplasia' comes about [. . .] by the gradual loss of fully differentiated neurons—an entirely novel concept," and it was "the lack of a factor released from the primordia that [was] simply needed for neuron survival."[59]

To be fair, it must be remembered that Rita and her mentor Levi were aware of Shorey's work and quoted it in their article, which appeared in French in the *Archive de Biologie* (1942).[60] The two Italians observed that Shorey and Hamburger had been the only ones to work on embryos of chickens and not with amphibians, but they had adopted the method of ordinary staining and this had prevented them from distinguishing the differentiated neurons. Moreover, they had limited themselves to observing the embryonic nervous system at a single stage of development. For this series of reasons, they did not pay too much attention to Shorey's research.

In conclusion, it is known that Rita considered the discovery of NGF as "her" child and that, while acknowledging the contribution of Viktor, she strongly defended "her" Nobel. So we should not struggle to see why she conceded so little merit to the neglected Marian. Notwithstanding this, we intend to conclude that the story of the discovery and "the saga" of the NGF began with experiments of Hamburger, who in turn repeated and extended the experiments made by Shorey. If the findings of Ramón y Cajal and Wilhelm His were "the cornerstones of the neuroembryology, and the chapters on NGF are still open-ended," then the beginning of this exciting adventure must be sought in "the pioneering efforts" of Marian Shorey, a scientist too long forgotten.[61]

Acknowledgements

The authors wish to thank Prof. Marco Piccolino, Centre of Neurosciences, University of Ferrara, who is writing a book on the life and science of Marian Lydia Shorey, for sharing and discussing biographical information about Shorey obtained from archives. The authors acknowledge, also on behalf of Prof. Piccolino, assistance from Debra Morehouse, Wilson Museum, Castine, Maine (USA); Isabel Murray, Archives of the Dutch Reformed Church, Stellenbosch (South Africa); Carolyn M. Picciano, Connecticut State Library, Hartford Connecticut (USA); Erin K. Dix, Lawrence University, Appleton, Wisconsin (USA); Cornelis Plug, University of South Africa; Raymond Butti, Brown University, Providence, Rhode Island (USA), and Joseph Doore, Albion Historical Society, Albion, Maine (USA).

[57] Rita Levi–Montalcini and Giuseppe Levi, "Correlazioni nello sviluppo tra varie parti del sistema nervoso. I. Conseguenze della demolizione dell'abbozzo di un arto sui centri nervosi nell'embrione di pollo," *Pontificiae Academiae Scientiarum Commentarii* 8 (1944): 527–75.

[58] Piergiorgio Strata, "Rita Levi–Montalcini and her major contribute to neurobiology," *Rendiconti Lincei. Scienze Fisiche e Naturali* 29 (2018): 737–53, https://doi.org/10.1007/s12210-018-0741-4.

[59] Hamburger, "Ontogeny of Neuroembryology" (ref. 49), p. 3540; Strata, "Rita Levi–Montalcini" (ref. 58), p. 746.

[60] Rita Levi–Montalcini and Giuseppe Levi, "Les conséquences de la destruction d'un territoire d'innervation périphérique sur le développement des centres nerveux correspondants dans l'embryon de Poulet," *Archives de Biologie* 53 (1942): 537–45, on 537.

[61] Viktor Hamburger, "Historical Landmarks in Neurogenesis," *Trends in Neurosciences* 4, no. 7 (1981): 151–55, on 155; Ronald W. Oppenheim, "Introduction. Viktor Hamburger: Pioneer Neuroembryologist, Teacher, Colleague, and Friend," in Hamburger, *Neuroembryology* (ref. 51), pp. ix–xiv, on xi.