

Sound Neuroscience: An Undergraduate Neuroscience Journal

Volume 2

Issue 1 *Women in Neuroscience*

Article 1

2015

“Above all, don't fear difficult moments. The best comes from them” (1): The Life and Work of Dr. Rita Levi-Montalcini

Elana Hirsch

University of Puget Sound, ehirsch@pugetsound.edu

Follow this and additional works at: <http://soundideas.pugetsound.edu/soundneuroscience>



Part of the [Neuroscience and Neurobiology Commons](#)

Recommended Citation

Hirsch, Elana (2015) “Above all, don't fear difficult moments. The best comes from them” (1): The Life and Work of Dr. Rita Levi-Montalcini," *Sound Neuroscience: An Undergraduate Neuroscience Journal*: Vol. 2: Iss. 1, Article 1.

Available at: <http://soundideas.pugetsound.edu/soundneuroscience/vol2/iss1/1>

This Article is brought to you for free and open access by the Student Publications at Sound Ideas. It has been accepted for inclusion in Sound Neuroscience: An Undergraduate Neuroscience Journal by an authorized administrator of Sound Ideas. For more information, please contact soundideas@pugetsound.edu.

Elana Hirsch

Neuroscience 201

“Above all, don't fear difficult moments. The best comes from them” (1): The life and work of Dr. Rita Levi-Montalcini

Introduction

In the field of neuroscience, as in many other scientific disciplines, early research was dominated by men and their discoveries. However, many women have greatly impacted neuroscience from the beginning, yet, again and again, their stories go unheard—Dr. Rita Levi-Montalcini is one such woman. Levi-Montalcini is most famous for her work as a neuroembryologist and for her discovery of nerve growth factor with Stanley Cohen. This discovery has proven to be incredibly important to the field of neuroscience; in fact, Levi-Montalcini and Cohen won the Noble Prize in Medicine and Physiology in 1986 for their work.

Nerve growth factors were the first molecules to be discovered in a series of neurotropic proteins that influence the growth and differentiation of sympathetic and sensory ganglia (2), and “provide a regulatory link between targets in the body and the nerve cells that innervate them” (3). In the years after Levi-Montalcini and Cohen’s discovery, it was determined that oncogenes, genes that cause tumors, are actually mutated growth factors, and that nerve growth factors, as neurotrophins, may possibly be used to slow the degeneration of the nervous system in diseases like Alzheimer’s and Parkinson’s (2, 3). Despite the eventual success and acceptance of Levi-Montalcini’s discoveries, she faced both personal and professional challenges along her path to the discovery of nerve growth factor and to winning the Nobel Prize. This paper will explore Rita’s life, as its story is amazing and greatly adds to the history of women in science, as well as explore her scientific work and contributions to the field of neuroscience.

Possessing two X chromosomes: Rita’s early childhood and education

Rita Levi-Montalcini was born on April 22, 1909 in Turin, Italy to a wealthy Jewish intellectual family. She lived very comfortably with her two sisters, her twin Paola and older sister Anna, her older brother Gino, and her two parents. Her father, a domineering and opinionated electrical engineer, expected Victorian obedience from his four children and believed that women should be educated in the ways of true womanhood, not mathematics (3). Because of these Victorian wishes for his daughters, the three girls were sent to finishing school rather than to a secondary school where they could have the chance to go on to university. This

decision would alter the course of Rita's life, and she despised the finishing school because she was unable to study the subjects that truly interested her, like mathematics and science. As Rita points out in her autobiography *In Praise of Imperfection: My Life and Work*, "it was my fate to grow into womanhood in a period when the natural intellectual faculties of an individual possessing an X and Y [chromosome]—that is, a man—were reinforced rather than repressed" (4).

After she finished her education at the girls' high school, Rita was left without a plan for what to do with her life; all she knew for certain was that she did not want to get married and spend the rest of her life acquiescing to the needs of a demanding partner. Then, rather suddenly, when Rita was twenty years old, her beloved childhood nurse fell ill with stomach cancer and died. This event was the catalyst Rita needed to stand up to her father and declare her intent to fulfill her long-life dream of becoming a doctor (3, 4). Although her father did not believe in the higher education of women, he only wanted what was best for his daughter, and agreed to get her a private tutor to pass the medical school examinations. He assured Rita, telling her that, "If this is really what you want, [...] then I won't stand in your way, even if I am very doubtful about your choice" (3). This was exactly what she wanted to do with her life, and in 1930, Rita was admitted to the Turin School of Medicine as a first-year student and began her studies that autumn.

When Rita entered medical school in 1930, she was one of seven female students out of a class of about three hundred. She recalls, "The girls—seven in my day and none of us very attractive—[...] could not avoid the less than gallant remarks about our aesthetic merits" (4). Rita and the other female students were often subjected to blatant sexism inside and outside of the classroom. To combat this, Rita made a point to refuse any male advances, to dress in non-feminine ways, and to only make intellectual rather than social connections. These choices were influenced in part by Rita's lack of intellectual stimulation at the girls' high school, and therefore, she saw medical school as her chance to cultivate her intellect and academic interests, which she did. At the end of her first year of school, Rita earned an internship at the School of Anatomy under the supervision of the famous histologist, Giuseppe Levi. Professor Levi would become Rita's mentor, and eventually they would conduct research together that would influence her understanding and discovery of nerve growth agents. Most importantly, however, Levi taught Rita how to stain embryonic chick neurons with chrome silver; this technique, known as chrome

silver impregnation, is useful because nerve cells have a strong affinity for silver salts and would be vital to Rita's future research.

Bicycling over the countryside, "begging for eggs" (3): Rita's research during WWII

In 1938, two short years after Rita graduated from medical school summa cum laude, Benito Mussolini, the leader of the National Fascist Party of Italy, issued the "Manifesto for the Defense of the Race." This decree, in accordance with other anti-Semitic sentiments gripping Europe at this time, banned Jews from intermarrying with other Italians, from holding academic positions, attending university, and practicing medicine (3). Rita recalls this time period, which also affected her mentor, the famed Dr. Levi, stating that, "I had been dismissed from both my academic position at the Institute of Anatomy and from the Neurology Clinic, and deprived of the right to practice medicine" (4). This meant that in order to continue with her neurological research, Rita was forced to secretly move her laboratory to her bedroom. Her brother, Gino, built her an incubator and she acquired a basic microscope so that she and Dr. Levi, who was acting as her assistant, could replicate a study conducted by Viktor Hamburger which hypothesized that the development of the embryonic nervous system was influenced by some sort of signal from the muscles or organs (3). Hamburger's study intrigued Rita, and she was determined to discover whether some sort of signal did in fact influence the division and differentiation of embryonic neurons.

Hamburger's research indicated that when a limb bud is amputated, the associated motor neurons disappear. Hamburger attributed the atrophy of the ganglia to "the absence of a inductive factor" that was released by the tissue the neurons were intended to innervate; however, Hamburger revealed that he struggled to see the neurons because he was using a light microscope. This is where Rita saw a chance to expand upon his research: Rita and Dr. Levi would repeat Hamburger's experiments, but instead of looking at the embryonic nerves with a light microscope, they would repeat the experiment using the silver-chrome impregnation to visualize the neurons. This proved to be highly successful, and the two found that rather than immediately dying off, the ganglia actually "proliferated, differentiated, and started to grow towards their targets" before they atrophied (5). Rita hypothesized that there was no inductive factor as Hamburger predicted, but rather that there was a factor that was normally released by the budding limb that promoted growth.

Rita and Dr. Levi tried in vain to have their work published in Italy. Most fortuitously, their results were published in Belgian and Swiss journals that also were distributed to American scientists; this is how Viktor Hamburger, who was a professor at Washington University in St. Louis, Missouri, learned of Rita Levi-Montalcini's experiments. In 1946, Hamburger invited Rita to visit him at Washington University so that they could compare and discuss their experimental results. Rita, who had become disinterested in research after the end of WWII, gladly accepted the invitation with renewed fervor and sailed off to America to meet Viktor (3).

Washington University: The “happiest and most productive years” of Rita’s life (3)

When Rita first met Viktor in 1946, she liked him right away and knew that working with him would be pleasant; eventually, the two would become close friends and colleagues (3). The silver-chrome impregnation technique that Dr. Giuseppe Levi had taught her proved to be vital to her work at Washington University with chick embryos. It is worth noting that many neurobiologists favored the chick embryo because it is “particularly suited” as an organism to study because “it’s nerve centers are more clearly segregated and defined and their strong affinity for silver permits a visualization of nerve structures” (6). This means that when the nervous system of the embryo begins to differentiate into cerebral ventricles and the spinal cord, it is easy for researchers to see the migration, differentiation, and degeneration. Chick embryos are also ideal because it only takes three weeks for them to develop and hatch (3, 4, 6).

In late 1947, Rita was examining a set of silver-chrome impregnated embryonic sections, again having used chick embryos, when she saw something strange: spinal cord cells were migrating across the plate into vertical lines, like “large armies on a battlefield,” and the cells were migrating so that certain types of cells were grouped together (4). At the cervical level, Rita also noticed that the membrane was disappearing around the nuclei and there was a decrease in the volume of cell bodies, which indicated that degeneration was occurring. This was an incredibly important discovery because it showed that the nervous system was capable of eliminating unnecessary cells and that its cells could migrate to various places depending on their function or type while managing to innervate completely different tissues (4). These findings changed the way Rita understood the mechanics of the developing nervous system.

In 1948, a graduate student name Elmer Bueker published an article analyzing the regulatory mechanisms governing the development and differentiation of motor and sensory neurons in a chick embryo. To do so, he grafted rapidly growing tissue from a mouse sarcoma

called S180 onto embryos; he found that nerve fibers from sensory ganglia burrowed their way into the tumor cells, creating a neoplastic mass (4, 5). The type of tumor Bueker used seemed to be beneficial to nerve fiber growth and caused precocious differentiation, but it was unclear as to why this occurred.

Rita and Viktor set out to discover the reason for these findings, and to do so, replicated Bueker's experiments. They found that a mass of tumoral cells, yellow in color, was penetrated from all sides by the brown-black nerve fibers. They also saw that the volume of ganglia themselves appeared to have increased with respect to the volume of ganglia that innervated the blood vessels, limbs, and organs on the opposite side of the embryo from the tumor. In 1950, the two discovered that when S180 was grafted onto embryonic tissue, the tumoral masses were completely invaded by sympathetic ganglia and that the tumors seemed to have released a fluid factor that accelerated differentiation, produced an excessive number of nerve fibers, and distributed the ganglia in abnormal ways (4). Rita was convinced that this "humoral factor," as she first called it, was causing the nerve cells to behave in this manner. In fact, Rita and Viktor determined that the tumors were able to effect nerve fiber growth even without direct contact. In other words, this apparent "nerve growth promoting agent" was definitively "humoral in nature" and accelerated the differentiation of nerve cells (4, 7).

Rita still struggled to properly identify the humoral agent, and realized in order to do so she would need to carry out experiments using a technique called *in vitro* culture, a method she was not very familiar with. Rita was still in contact with a good friend from medical school, Hertha Meyer, who had emigrated to Rio de Janeiro during WWII to work at the Institute of Biophysics. Rita acquired a grant to go to Brazil to learn how to grow tissue in culture, and so she flew to Rio de Janeiro with "two little white mice" that each had sarcomas "in the top of [a] small cardboard box that [she] had fitted into [her] overcoat pocket" (4). At first, the tissue cultures Rita grew yielded unsatisfying results. However, once Rita mastered how to culture isolated ganglia, she placed nerve tissue suspended in clotted blood near pieces of the mouse tumors and suddenly saw spectacular results (3). The *in vitro* tissue cultures showed that the transplanted neoplastic cells were completely morphologically different from the non-transplanted cells and had the ability to stimulate the development and precocious differentiation of the sympathetic and sensory ganglia (4). Furthermore, Rita discovered that there were halos of fibrillar fibers growing out of the ganglia "like suns" that were more dense on the side of the

ganglia closest to the tumor, evidence of a neurotropic effect. Rita intuitively knew that these fibrillar halos would prove to be a major clue in the mystery of the identity of this fluid growth agent. Rita was correct, and even today the presence of fibrillar halos indicates that nerve growth factor is present (3, 4, 5). Armed with these data, Rita returned to St. Louis in January of 1953 where Viktor would introduce her to a postdoctoral student named Stanley Cohen; this introduction would mark the beginning of a successful research partnership.

“Together, we are wonderful” (4): Rita, Stanley Cohen, and nerve growth factor

Stanley Cohen joined Rita and Viktor shortly after Rita’s return from South America as the biochemist of the group; his expertise in this area would be essential for uncovering the secrets of the nerve growth agent. As Stanley once told Rita, “you and I are good, but together we are wonderful,” (4) and in the years between 1953 and 1959, Rita and Stanley made amazing discoveries and insights into the nature of the factor that caused the miraculous fibrillar halo growth. Their work during this time period would go on to change molecular biology as well as earn them the Nobel Prize.

Right away, their team set up an *in vitro* culture lab as Rita intended, and within a year, had discovered that transplanted tumoral extracts induced the halo effect around sensory ganglia. They also determined that the tumors themselves contained small amounts of this halo-producing factor. After producing an assay of neoplastic tissue from dozens of embryos, the group identified the tumoral factor as a nucleoprotein, which is a protein complex formed between nucleic acids and other proteins (4). Arthur Kornberg, another biochemist, suggested that Rita and Stanley try purifying the tumoral factor with snake venom because the venom would break down any nucleic acids because of the presence of an enzyme called phosphodiesterase.

Rita and Stanley were amazed by what they found—in the presence of snake venom, halos of unprecedented size and symmetry radiated from sympathetic and sensory ganglia (4). Initially, they suspected that the venom must contain a similar factor that also stimulated nerve growth, and their experiments showed that snake venom contained a “very potent growth-promoting agent,” like that of the mouse sarcomas, that was “3,000-6,000 times as active as crude tumor homogenates in promoting nerve fiber outgrowth in spinal ganglia *in vitro*” (8). This was not the team’s only discovery regarding the effects of snake venom on nerve fiber growth; the two also found that when an anti-snake venom serum was used in culture, the growth of fibrillar halos was significantly reduced. These findings suggested that the active growth factor

was associated with certain protein components of the venom (8) and that, most importantly, snake venom effected embryonic nerve cell growth and differentiation in the same way that tumors S180 and S37 did. This finding would prove to be quite controversial—how could mouse tumors and snake venom both contain this nerve growth promoting factor? Shortly afterwards, Stanley discovered that the submandibular salivary glands of male mice synthesize a toxin, which is injected into competing mice when they fight and bite their opponents, that also contains nerve growth factor that causes fibrillar halo growth. This confounded Rita, as did Stanley's other results that indicated that the antiserum of snake venom also inhibited halo formation around ganglia when it was cultured in the presence of salivary nerve growth factor. Rita recalls that when they examined the sympathetic ganglia, in this case the superior cervical ganglia, which were usually visible with the naked eye, they were barely visible under the microscope. These findings proved that the sympathetic ganglia were almost totally atrophied due to the effects of the antiserum. The two did not fully understand the implications of the results that Stanley reported on July 11, 1959, but Rita remarks in her autobiography that this marks the day that she and Stanley were first able to dramatically show nerve growth factor's role in the differentiation and survival of sympathetic cells (4).

Rita and Barbara Booker, a graduate student, continued with this research even after Stanley left to teach at Vanderbilt University. The two published a paper in 1960 that further strengthened the existence of nerve growth factor in mouse sarcomas, snake venom, and mouse salivary glands. Building off of Stanley's experiments, which found that mouse salivary glands also contained nerve growth factor, Rita and Barbara shifted their investigation to examining what happened when known nerve growth factor-containing sources were cultured with human embryonic cells. When the tissue was cultured with a purified mouse salivary fraction, the two observed the similar “dense halo of nerve fibers” surrounding the ganglia that was seen when mouse sarcomas, snake venom, and mouse salivary extract was used on chick embryos. Ultimately, this report established a relationship between salivary glands and nerve growth agents (9). Rita states that, “we came to the conclusion that the nerve growth promoting protein is a normal constituent of sympathetic cells and is normally present in blood and bodily fluids of birds and mammals” and that it is clear that nerve growth factor plays a vital role in the life and sympathetic nerve cells (6).

At first, the existence of nerve growth factor was treated with some skepticism by other biologists because “it required too great a leap of the imagination to believe in this unlikely soluble factor, which was supposed to diffuse from one tissue and then potently affect specific processes in nerves” (5). But ultimately, the identity and validity of nerve growth factor was vindicated by various discoveries, such as Stanley Cohen’s discovery of epidermal growth factor and the identification of the sequence of its amino acids in 1972 by Bradshaw (3, 5). Nerve growth factor has proven to be essential for understanding how embryonic ganglion differentiation and survive, as well as essential for understanding other complex biological systems like the immune system. Finally, Rita Levi-Montalcini and Stanley Cohen were honored for their discovery of nerve growth factor in 1986, when they jointly won the Nobel Prize in Physiology or Medicine for their discoveries of these all important growth factors.

Improving women’s chances of becoming scientists: Rita’s later life and legacy

Rita’s work in neuroembryology earned her professional respect, despite the fact that her personality was often seen as prickly or challenging. Not only was Rita elected to the National Academy of Sciences in 1968, but she was also the first woman admitted to the Pontifical Academy of Sciences in Vatican City (3). Rita eventually returned to Italy after spending twenty-six years in the United States because she felt homesick for her family, especially her twin sister. Upon her return, she was given her own laboratory space at the CNR, Italy’s largest research association. Rita would continue researching there as well as at her namesake, the European Brain Research Institute-Rita Levi-Montalcini throughout the rest of her life, until she died at 103 in December of 2012. In addition to continuing her research on nerve growth factor and its evolutionary beginnings, Rita was made Senator for Life in the Italian Senate in 2001. While in that position, Rita was often called upon by the government to publicly represent the ideas and opinions of Italian female scientists (3).

Rita also authored twenty-one books, which pleased her greatly as a life-long reader of gothic romances like *Wuthering Heights*. Rita championed many social issues, especially those attempting to improve the state of Italian science education as well as to those supporting women’s education, and encouraging women to enter the sciences. To better these causes, she created the Rita Levi-Montalcini Foundation which supports the education of more than 6,000 African women. Of this project Rita reveals that she hopes that this will “improve [the women’s] chances of becoming scientists” (5). She also, as previously stated, continued to work until the

end of her life, despite becoming hard of hearing and sight, and never went a day without wearing silk, her treasured pearls, and four-inch high-heels, regardless of the weather (3). At the end of her life, Rita simply approached every day as a new chance to contribute and discovery more about the world around her.

Conclusion

Rita Levi-Montalcini was an inspiring woman and amazing scientist. Her discoveries and accomplishments are all the more amazing considering the Victorian climate in which she was raised and the time in which she attended medical school. Rita survived WWII and the Holocaust as a Jewish female scientist, and this alone would be quite an achievement. But Rita did not just survive these events; she overcame them, all the while conducting research and advancing the newly emerging field of neuroscience. Rita's work in discovering the nerve growth factor represents what Thomas Kuhn, a philosopher of science, calls a paradigm shift; in other words, the discovery of nerve growth factor completely changed the way in which biologists understood and thought about the developing nervous system. In fact, the significance of nerve growth factor to modern biology is “huge,” as Dr. William Mobley of UC San Diego says, “and [it] opened up a whole field in understanding how cells talk and listen to each other” (5). These factors make Rita a woman worth knowing about because of her importance to twentieth century biology, and yet when I mention her name to others, including historians of science, they are completely unaware of her importance, even of her existence. I believe that it is important to reach out and learn about the integral women (or other minorities) in various fields of scientific study, especially as scientists ourselves, because it is critical to understand our collective scientific history and our roots so that we can practice science as an informed, educated, and conscientious scientific community.

References

1. “Rita Levi-Montalcini.” *Famous Scientists*. Web. <http://www.famousScientists.org/rita-levi-montalcini/>
2. Binder M.D., Hirokawa N., Windhorst U. (Eds.). “Nerve growth factor (NGF).” *Encyclopedia of Neuroscience*. Berlin, Germany: Springer, 2009. pp. 2544.
3. Bertsch McGrayne, S. *Nobel prize women in science: Their struggles and momentous discoveries*. New York: Carol Publishing Group, 1993.
4. Levi-Montalcini, R. *In praise of perfection: My life and work*. New York: Basic Books, Inc., 1988.
5. Abbott, A. Neuroscience: One hundred years of Rita. *Nature*, 458 (2009): 564-567.
6. Levi-Montalcini, R. “Growth control of nerve cells by a protein factor and its antiserum.” *Science* 143.3602 (1964): 105-110.
7. Cohen, S., Levi-Montalcini, R., Hamburger, V. “A nerve growth-stimulating factor isolated from sarcomas 37 and 180.” *Proceedings of the National Academy of Sciences of the United States of America* 40.10 (1954): 1014-1018.
8. Cohen, S., Levi-Montalcini, R. “A nerve growth-stimulating factor isolated from snake venom.” *Proceedings of the National Academy of Sciences of the United States of America* 42.9 (1956): 571-574.
9. Levi-Montalcini, R., Booker, B. “Excessive growth of the sympathetic ganglia evoked by a protein isolated from mouse salivary glands.” *Proceedings of the National Academy of Sciences of the United States of America* 46.3 (1960): 373-384.