

Rita Levi-Montalcini: Neuroscientist par excellence

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The scientific career of Rita Levi-Montalcini spanned more than 75 years, from her training as a graduate assistant to the histologist Giuseppe Levi in Turin; to her research on chicken embryos in her home laboratory, using eggs from local farms; to Nobel-Prize-winning research in Washington University, St Louis; and back to Italy. Along the way she faced and overcame many hurdles, including the prejudice that women were not suitable for higher education and research and the vicious persecution of non-Aryans in Mussolini's Italy.

From the start, Levi-Montalcini's work on chicken embryos addressed a key question in developmental biology: how do nerves growing out from an embryonic nervous system find the limbs or other targets that they will innervate? In 1934 Victor Hamburger at Washington University published the results of a key experiment on developing chicken embryos, in which he showed that the unilateral removal of a wing bud led to a profound reduction in neurons in the spinal cord and spinal ganglia on the operated side (1). Hamburger speculated that one explanation of this result was that the peripheral fields transmitted signals to the appropriate nerve centers "...centripetally by the nerve fibres." This prescient hypothesis was eventually shown to be correct, and Levi-Montalcini's work helped to unlock this mystery.

Working with Levi in Turin and later in her home laboratory, Levi-Montalcini repeated Hamburger's experiment and showed that the reduction of neurons after limb removal was not the result of a failure of the formation of neurons, but rather because of their degeneration when they failed to make contact with a suitable peripheral field (2). In 1947 Hamburger invited her to St Louis, to continue research in his laboratory. Both researchers set out to find the agent from the peripheral field that allowed the survival of innervating neurons. As often happens in scientific research, serendipity played an important role. Elmer Bueker, a former student of Hamburger, showed that the transplantation of small pieces of a mouse sarcoma

tumor could take the place of a limb bud in sustaining neuronal survival (3). Levi-Montalcini repeated these experiments (4) and showed that the survival factor could penetrate through the amniotic membrane surrounding the chicken embryo; the cancerous tissue thus provided the first unequivocal evidence that the tumor cells released a soluble nerve growth-promoting factor.

In the 1950s a brilliant young biochemist, Stanley Cohen, joined Levi-Montalcini and Hamburger in the quest to identify the soluble "nerve growth factor" produced by the mouse sarcoma. The two researchers succeeded in preparing a cell-free extract, which replicated the growth-promoting effects on chicken-embryo neurons in tissue culture (5). When a snake venom was used to further purify the extract, it was surprisingly found to contain its own nerve growth factor (NGF) activity (6). Reasoning that the venom came from the snake's salivary gland, Cohen investigated mammalian salivary glands and found that mouse salivary glands contained high levels of nerve growth factor activity, a crucial step for the purification of the protein.

This research culminated with the publication of a classic trio of papers in the March issue of PNAS in 1960. Cohen described the isolation of a novel protein, NGF, and the preparation of an antiserum (7). In two other papers Levi-Montalcini and her assistant Barbara Booker described the nerve growth-promoting activity of purified NGF on a wide variety of embryonic mammalian ganglia (including human tissue), and the growth-promoting effects of NGF on sympathetic ganglia when administered in vivo newborn, young, and adult mice (8). In a third paper Levi-Montalcini and Booker showed that the administration of an antiserum to NGF to newborn mice, rats, rabbits, and kittens lead to the almost complete destruction of the developing sympathetic nervous system, without damaging any other organs or tissues (9). The dramatic effect of such "immunosympathectomy" was the first example of a highly selective tool able to ablate particu-



Rita Levi-Montalcini in 2009 on the occasion of her 100th birthday. Photo courtesy of Prof. G. Nistico, European Brain Research Institute, Rome.

lar populations of nerve cells. In the 1960s there was a high level of interest in sympathetic nervous system research, and immunosympathectomy offered an important new tool to probe its physiological functions (10). The demonstration of the specific actions of anti-NGF antibodies on the sympathetic nervous system in a wide variety of mammals helped to persuade skeptics, who had questioned the biological importance of NGF derived from such exotic sources as mouse tumors, snake venom, and mouse salivary glands. It was not until 1986, however, that Levi-Montalcini and Stanley Cohen were finally awarded their well-deserved Nobel Prize for Physiology or Medicine.

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The discovery of NGF prompted a search for other nerve growth factors with the first success in the 1980s with the discovery of BDNF by Yves Barde and Hans Thoenen (11), and the subsequent discovery of families of other neurotrophins (12).

The question of how NGF gained access to its target cells in the nervous system remained unanswered for another decade after its discovery. In 1972 Levi-Montalcini and the Angeletti reported that after *in vivo* administration of [¹²⁵I]-labeled NGF, the protein accumulated selectively in sympathetic ganglia (13), but this did not prove whether the protein gained access directly to the ganglion cells or via their axons. The fact that the accumulation of labeled NGF in ganglia was reduced in animals in which sympathetic terminals had been destroyed by prior treatment with 6-hydroxydopamine suggested that the uptake of labeled NGF in sympathetic ganglia might occur "...via the end terminals with subsequent retrograde transport to the cell body." (13). However, it was not until Ian Hendry, a graduate student from my laboratory in Cambridge, joined Hans Thoenen's group in Basel that this could be confirmed experimentally. Injection of radiolabeled NGF into the anterior chamber of the eye led to a preferential accumulation of labeled protein in the sympathetic ganglion on the injected side, reaching a peak after 16 h. Transection of the sympathetic axons or their destruction with colchicine completely abolished the preferential accumulation on the injected side. Autoradiographic studies confirmed that the accumulated labeled protein was located in the cell bodies of the sympathetic neurons (14). The concept that neurons respond to specific chemical signals produced by their target tissues represented a radical step forward in understanding how the nervous system and target tissues are correctly "wired" during development, and the selective retrograde transport of numerous other subsequently discovered neurotrophins has been demonstrated (15, 16).

Levi-Montalcini continued her research for another 50 years after the classic papers

of 1960; she was not one to give up work because of retirement and continued actively in research, with sponsorship from the Italian government at the Research Institute of Neurobiology in Rome. Her review in 1995 "Update of the NGF saga" (17) summarizes some of the findings that emerged concerning the role of NGF, not only during development but also in the adult. In particular, NGF was found to exert a profound modulatory influence on pain mechanisms in the adult, and was associated with a heightened responsiveness to pain in response to tissue inflammation. A broad role of NGF in neuroimmune reactions and tissue inflammation was suggested (17). Franz Hefti and others used such information to plan a novel class of pain-relieving drugs based on antagonism of NGF (18). A number of anti-NGF monoclonal antibodies were developed, with some positive clinical studies, although this has not yielded a new medicine as yet (19).

In her later years Levi-Montalcini was influential in Italian politics and science.

In 2006 she founded the European Brain Research Institute in Rome, with the aim of offering a multidisciplinary research center for young scientists, and she devoted much of her energy to this project in her last 10 years. In 2007, at the age of 98, Levi-Montalcini started a new research project. She had an idea that NGF might play a role in the very early stages of development of the chicken embryo. With her colleague Antonino Cattaneo, anti-NGF antibodies were injected into early chicken embryos. They observed a surprising result and the findings were published in January 2012 in PNAS (20). This was a fitting end to an illustrious career. I remember her as a gracious figure with an unquenchable passion for science. At the height of her quest for NGF, Levi-Montalcini wrote to her parents: "...our work is at its peak and the possibilities for development are such as to make us dizzy. My euphoria is evident to all of the entourage, we are all happy and excited, as if we are about to have a party." (21)

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