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Rita Levi-Montalcini: the discovery of nerve growth factor and modern neurobiology☆

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The remarkable accomplishments in developmental neurobiology within the past 60 years have depended on two things: (i) a succession of original histochemical and immunohistochemical methodologies for identifying pathways in the nervous system with increasing precision and sensitivity, and (ii) the discovery of growth factors for neurons. Growth factors are naturally occurring, essential biological mediators that promote cell growth, differentiation, survival and function in specific nerve cell populations. The discovery of nerve growth factor (NGF) by Rita Levi-Montalcini in the 1950s represents an important milestone in the processes that led to modern cell biology. NGF was the first growth factor identified, for its action on the morphological differentiation of neural-crest-derived nerve cells. Later, its effect on neuronal cells of the peripheral and central nervous systems, and on several non-neuronal cells was also determined. Thus, Levi-Montalcini's work on NGF represents, as acknowledged by the Nobel Prize Assembly in its press release of 13 October 1986, 'a fascinating example of how a skilled observer can create a concept out of apparent chaos'.

Rita Levi-Montalcini (Figure 1) received her medical degree and specialization in neurology and psychiatry in 1936 from the Faculty of Medicine at the University of Turin, Italy (http://www.unito.it/). She then entered the Institute of Anatomy as a postgraduate student in the laboratory of Giuseppe Levi, a well-known anatomist and tutor of two other future Nobel Prize winners in Physiology or Medicine – Salvatore Luria (who won in 1969) and Renato Dulbecco (who won in 1975). During her early postgraduate studies, Levi-Montalcini investigated the relationship between the developing central nervous system (CNS) and its peripheral targets. The results of these experiments led her to suggest that the failure of neurons to thrive in the absence of peripheral target tissue was because of a degenerative process [1,2] rather than a failure of differentiation, as had previously been hypothesized by Victor Hamburger [3,4], who was a renowned neuroembryologist working at Washington University in St Louis, USA (http://www.wustl.edu/). In his experiments, Hamburger removed the developing limbs of chick embryos to see how such extirpation would affect the subsequent growth and differentiation of nerve cells destined for that region of the embryo. He concluded that the differentiation, or specialization, of nerve cells depends largely on their destination. To resolve their scientific disagreements, Hamburger invited Levi-Montalcini to join his group in 1946. Working together, they obtained direct evidence that many sensory neurons die during normal development, and that limb-bud extirpation causes an increase in the amount of neuronal death [5]. These early collaborative studies led to the hypotheses that developing nerve cells depend on feedback signals that are in limited supply, and that neuronal targets provide a specific signal that is required for neuronal survival. These suggestions confirmed the original hypothesis proposed a few years before by Levi-Montalcini.

Early in her time at Washington University, Levi-Montalcini published findings showing that size differences among ganglia in different segments of the spinal cord could be accounted for by the regulation of neuronal degeneration. Thus, sensory ganglia of the neck are small because they send out more neurites than the periphery can support, whereas brachial ganglia are, ultimately, larger because they innervate the additional mass of the

Figure 1. Rita Levi-Montalcini (1990), Italy.

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limb. Based on these findings, Levi-Montalcini hypothesized that the lack or low availability of specific endogenous biological mediators can cause cell death (now termed 'apoptosis' or 'programmed cell death'). A relevant aspect of these studies is that they prospected the important biological concept that neurons depend on their targets for survival. Later, this concept led to the discovery of NGF and other growth factors [6,7].

The discovery of NGF

The NGF story began in 1949, after Victor Hamburger showed the results obtained by one of his postgraduate students, Elmer Bueker, to Levi-Montalcini. Bueker had observed that, after the implantation of a small fragment of malignant mouse tumor into the body walls of three-day-old chick embryos, sensory fibers invaded the mouse tumor. He was investigating whether homogeneous neoplastic tissue could act as a substitute for a complex limb in supporting the development of the spinal cord and sensory ganglia, and found that the transplanted tumor grew well in chick embryos, that nearby peripheral nerves invaded the tumor mass and that adjacent dorsal root ganglia were markedly enlarged. Bueker hypothesized that this effect was due to the rapidly expanding tumor enabling the sensory fibers to branch in a larger field than the embryonic tissues that had been replaced with the neoplastic cells [8]. This hypothesis did not convince Levi-Montalcini who, with the permission of Bueker, reinvestigated the effects of transplanting two mouse sarcoma tissues into a chick embryo. Working on serial histological sections of chick embryos that were stained using Cajal's silver technique for identifying and charting the connection of many different neuronal circuits, Levi-Montalcini found that the tumor tissues induced hyperinnervation of internal organs, and she hypothesized that the transplanted tissues released a diffusible agent that stimulated the growth and differentiation of the developing nerve cells (Figure 2). She determined that a reliable bioassay to both quantify and demonstrate the biological activity of this, as yet unknown, substance present in the tumor mass was essential to sustain her hypothesis. She carried out these studies during a sabbatical period in Brazil, in the laboratory of Herta Meyer (University of Brazil; http://www.puc-rio.br/), who was an expert in cell culture techniques, and a former student of Giuseppe Levi. There, Levi-Montalcini investigated a method to quantify the stimulating effect of a tumor on neural cells. She conducted tissue culture experiments using sensory and sympathetic ganglia of chick embryos that were exposed to fragments and/or extracts of tumor tissue. She observed that the tumor could release a diffusible factor that promoted neurite outgrowth directly (Figure 3), in addition to nerve cell differentiation [9–11]. Furthermore, this effect was dose dependent, and Levi-Montalcini devised a semi-quantitative method for determining the biological activity of the substance released from the tumor tissue.

The biological characterization of NGF

In the early 1950s, Levi-Montalcini, in collaboration with the young biochemist Stanley Cohen at Washington University in St Louis, USA (who discovered epidermal growth factor and was co-winner of the Nobel Prize in 1986), set out a series of experimental approaches to characterize the biochemical properties of NGF. To determine whether the biologically active molecule was a nucleic acid or a protein, they performed experiments using snake venom (a rich source of phosphodiesterase) to destroy any nucleic acid. They observed that snake venom produced more neural outgrowth than that seen with cultures incubated with tumor extract. The finding that NGF was present in the venom led Cohen to realize that it might be worthwhile to look at the mammalian analog of the snake venom gland, the mouse salivary gland. *In vitro* studies with chick sensory ganglia showed this foresight to be well founded. Indeed, it was discovered that the mouse gland is a rich source of NGF [11,12] (Figure 4) and, in 1960, owing to the high concentration of NGF in this gland,

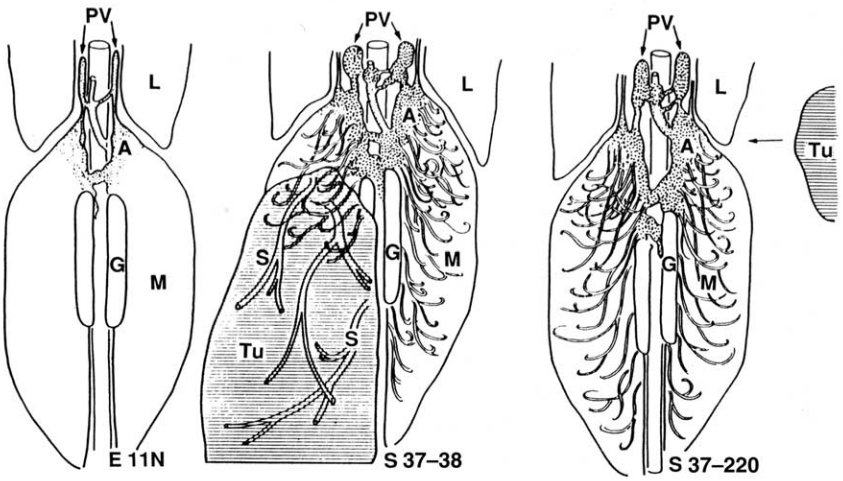

Figure 2. Semi-diagrammatic reconstruction of a normal 11-day-old chick embryo (E 11N), an 11-day-old chick embryo carrying an intra-embryonic transplant of mouse sarcoma (S 37–81), and an 11-day-old chick embryo with a transplant of sarcoma 37 on the chorioallantoid membrane (S 37–220). Note the hyperplastic growth of the prevertebral ganglia in the embryos carrying tumor transplants. Visceral nerve fibers from these ganglia invade the nearby mesonephros. Abbreviations: A, adrenal; G, gonad; L, lung; M, mesonephros; PV, prevertebral ganglia; S, sensory nerves; Tu, tumor. Reprinted, with permission, from Ref. [10]. © (1952) New York Academy of Sciences, U.S.A.

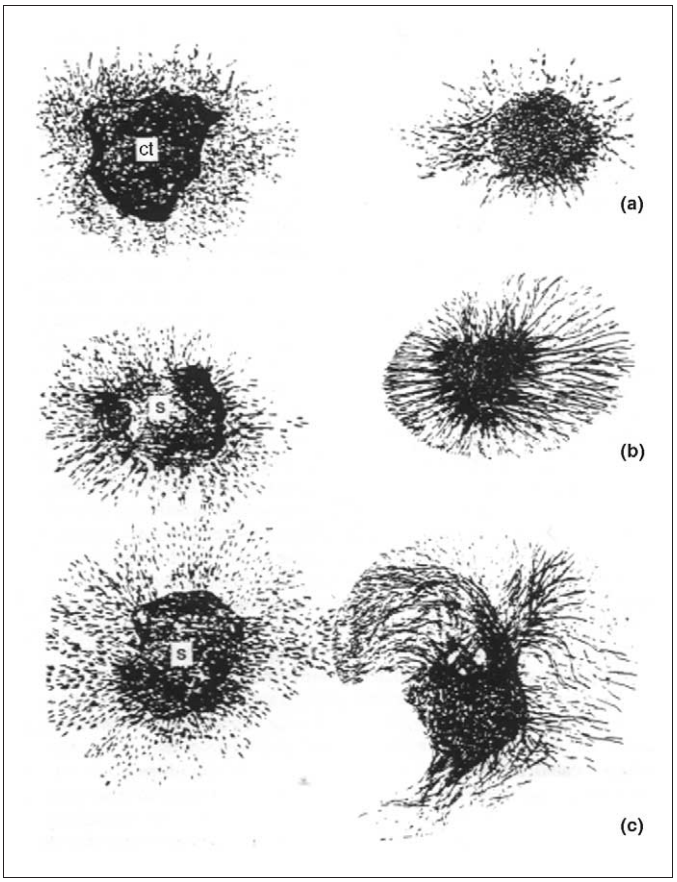

Figure 3. Eight-day-old sensory ganglia from chick embryos. **(a)** The ganglion, which faces a fragment of chick embryonic tissue (ct), shows fibroblasts but few nerve fibers. **(b)** Ganglion cultured in the presence of fragments of mouse sarcoma for 24 h. **(c)** Ganglion cultured in the presence of fragments of mouse sarcoma for 48 h. In (b) and (c), the ganglia, facing fragments of sarcoma (s), show the typical 'halo' effect elicited by the growth factor released from the sarcoma. In (c), note the first evidence of a neurotropic effect of the growth factor. Reproduced, with permission, from Ref. [7]. © (1986) The Nobel Foundation.

Levi-Montalcini and Cohen succeeded in isolating and purifying the molecule and demonstrating that it was a protein [13,14]. With the availability of large amounts of purified mouse salivary NGF, Levi-Montalcini and Cohen could produce large quantities of antibodies against NGF [15,16] and, through the use of these antibodies, they demonstrated the functional significance of NGF in the *in vivo* development of sympathetic and sensory ganglia. In 1970, Ruth Houge Angeletti and Ralph Bradshaw (Washington University in St Louis, USA) demonstrated that the NGF protein is part of a precursor, and that the processor enzyme remains associated to NGF to form a large, multimolecular complex defined as 7S NGF. The 7S complex consists of three different molecular species – the α subunit, the function of which is largely unknown; the γ subunit, which possesses protease activity; and the β subunit, which is the biologically active form of NGF [17]. Subsequent studies have revealed that NGF is a highly conserved molecule with a high interspecies homology, and its biological activity is regulated by two structurally unrelated receptors: a low-affinity receptor known as p75 NGF receptor, and the high-affinity gp140trkA receptor, which is a member of the trk family of tyrosine kinase receptors [18]. Using modern recombinant-DNA technology, the mouse and human genes encoding

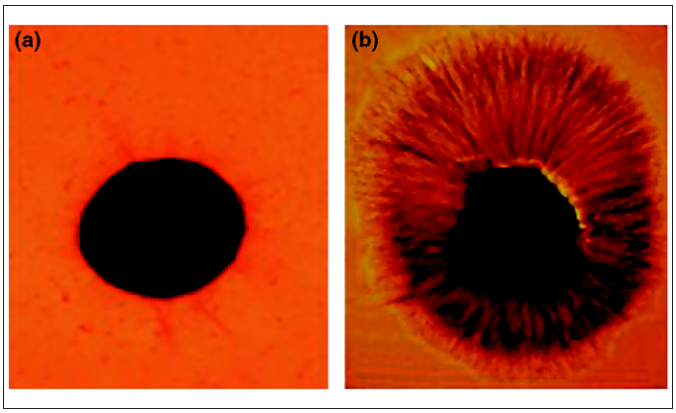

Figure 4. Photomicrographs of sensory ganglia removed from an eight-day-old chick embryo and cultured for 24 h at 37 °C. Ganglia were cultured **(a)** in a medium containing no nerve growth factor (NGF) and **(b)** in a medium containing 10 ng ml^{-1} of NGF. Note that only the ganglion that was exposed to NGF displays a dense halo of nerve fibers. Reprinted, with permission, from Ref. [14]. © (1964) American Association for the Advancement of Science (http://www.sciencemag.org/).

NGF were identified on the proximal short arm of chromosome 1. More recently, murine and human recombinant NGF has been produced and is available for basic and clinical studies [19–21].

Other important scientific contributions from Levi-Montalcini in the early 1970s include the effect of NGF on cell types other than sympathetic and sensory neurons, the influence of NGF on the CNS, and the ability of NGF to act as a chemotropic agent. In 1977, an article by Levi-Montalcini and myself was published that was the first to document evidence that NGF acts on cells of the immune system lineage [21] (Figure 5). Several years later, she published findings that NGF is overexpressed in the bloodstream and the hypothalamus during stressful events, leading to the hypothesis that NGF is involved in homeostatic responses. More recently, NGF has been used successfully for treating human corneal neurotrophic ulcers [22] and pressure ulcers [23]. Currently, NGF and other similar factors are the subjects of intense investigation because of their possible effects on promoting the survival of damaged neurons and on neuroinflammation and neuroimmunopathologies, as discussed in a recent conference about NGF and related molecules in health and disease [24].

Concluding remarks

Since 1950, Rita Levi-Montalcini has been dedicated to investigating the role of NGF in the development of the nervous system. The discovery of NGF is widely regarded by neurobiologists as being the first clear example of the identification of a class of trophic molecules that, although chemically distinct, can serve the same general purpose of providing a regulatory link between targets and the nerve cells that innervate them. NGF and other similar factors are also the subjects of intense current investigations, as mentioned earlier.

Levi-Montalcini's deep knowledge of neuroscience is impressive (she has had more than 200 hundred of her scientific manuscripts published), but equally impressive is her current interest in other human activities, such as

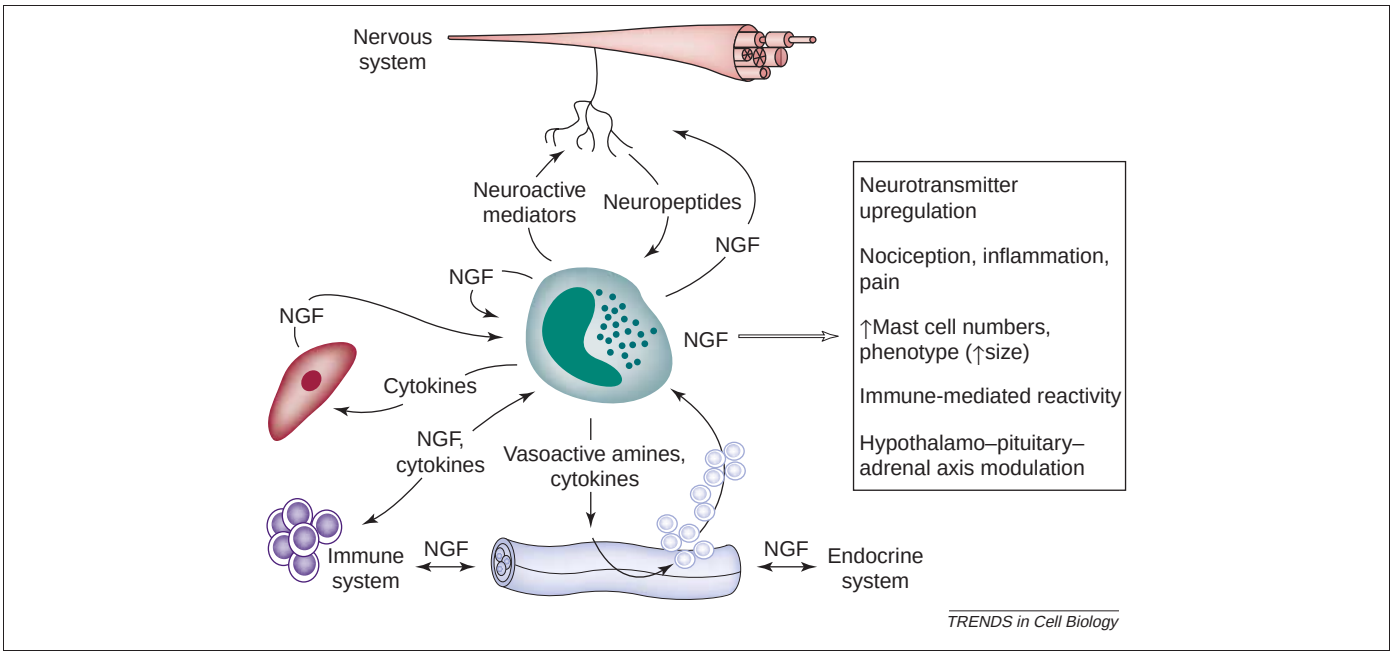

Figure 5. How nerve growth factor (NGF) might modulate nervous–endocrine–immune interaction. NGF released from tissue mast cells as a consequence of inputs from the nervous, immune or endocrine system can, in turn, influence the same system (locally or through the circulation). NGF released from mast cells might also function in an autocrine manner. Reproduced, with permission, from Ref. [21].

social interactions. She is a member of numerous national and international scientific academies, including the American Academy of Sciences, the Italian Academy of Lincei, the Pontifical Academy of Sciences (she was the first woman to be elected to this academy). Levi-Montalcini has also helped to organize a new research institute (the European Brain Research Institute), with headquarters in Rome and, more recently, she set up a foundation that provides mentors, counseling and financial support to African women in higher education.

Levi-Montalcini has written several books. Among them, in 1988, *In Praise of Imperfection* [25] was published, which is an inspirational autobiographical work in which she tells of her successes, but also of her delays and distractions, in addition to recounting the mistakes that typically bedevil research. A second book, *Il Tuo Futuro* [26], is dedicated to young people who often face the need to make vital choices during their early lives. In her third book, *Senza Olio e Contro Vento* [27], she tells ten true stories about well-known and unknown people who are united by courage and honesty in very difficult moments in their lives. In one of her most recent books, *L'asso Nella Manica a Brandelli* [28], she discusses the capacity and creativity of famous elderly people and how to maintain or improve cognitive abilities in later life.

Finally, because of her scientific and social achievements, Rita Levi-Montalcini was nominated as Senator of the Italian Parliament by the Italian Presidente della Repubblica, Carlo Azelio Ciampi.

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I am fortunate to have had the opportunity to work with Rita Levi-Montalcini and be a part of such great science. I thank all of the NGF community in my laboratory, whose exciting and insightful research over the past years has inspired me to prepare this article.

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