# The Neurotropic Theory of Santiago Ramón y Cajal

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*The following article is based on a paper, 'The chemotactic hypothesis of Cajal: a century behind', published by Constantino Sotelo in* Progress in Brain Research*, 2002, 136: 11-20.*

Each of us, interested in the study of the development of the nervous system, has been often amazed to see that migrating neurons follow always the same stereotype pathways and that outgrowing axons are precisely guided from their neurons of origin to their target neurons. How can migrating neurons and growing axons be able to recognize their routes and navigate throughout the meanders of the developing nervous neuropil? The beginning of the answer began in 1890, with the discovery of the growth cone.

**The growth cone**

Cajal (1890) started his studies on Developmental Neuroscience by analysing the development of dorsal commissural neurons of the embryonic chick spinal cord. He was, following Wilhem His, trying to find the ontogenic proof in favor of the independence of the neuroblasts and immature neurons, when he found a new and important structure, the growth cone. Thus, he had not only discovered that the growing axons of immature neurons emerge from the cell bodies and have free endings, but one of the most sophisticated machines imaginable for steering growing neural processes towards their terminal domains, a perfect homing head for axonal navigation. Later on, in his *magnum opus Histologie du Système Nerveux de l'Homme et des Vertébrés* (1909, Vol. I, p. 598), Cajal illustrated his early discovery of the growth cones in the spinal cord of E4 chick embryos (Figure 1). He insisted on the diversity of shapes exhibited by these structures, varying from relatively simple forms in growth cones travelling in the white matter, to much more complex forms in cones within the ventral commissure or in cones passing through the gray matter. In his precise and detailed analysis of the growth cone diversity, he had probably foreseen what we know today about the sensitivity of growth cones to cues in their micro-environment: the cones have simple shapes in straight paths, whereas in decision regions they adopt much more complex morphologies.

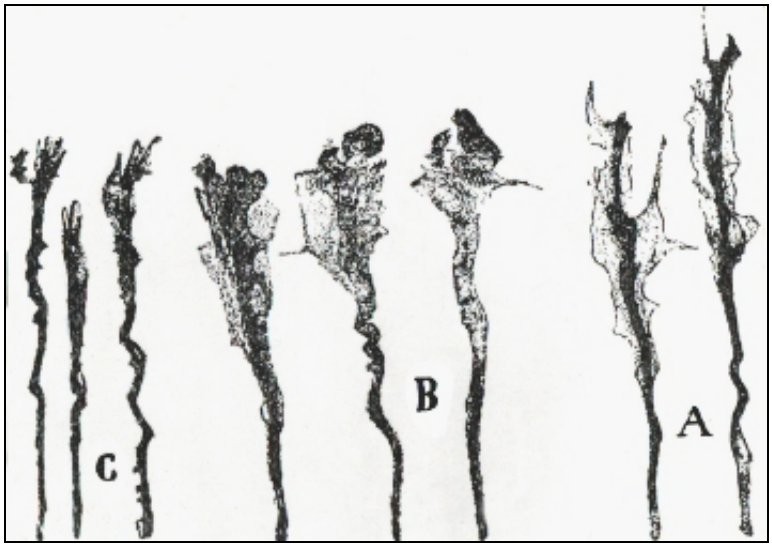


**Figure 1:** This drawing of Cajal illustrates axonal growth cones in the spinal cord of the E4 chick embryos. Note that Cajal represents a diversity of shapes corresponding to growth cones in different situations. Those with the simplest forms 'C' belong to axons traveling through the prospective white matter. In contrast, growth cones with more complex forms are within the ventral commissure 'B' or passing through the gray matter 'A'. It is of interest to note that this diversity corresponds to what we know today as the sensitivity of growth cones to cues in their micro-environment: in straight paths, the cones have simple forms, whereas in decision points the exhibit much more complex morphologies. Copyright Herederos de Santiago Ramón y Cajal.

Cajal (1892) was impressed by the behavior of growing axons and their precise orientation during their growth (Figure 2). He tried to find a plausible explanation for this "intelligent force", as he called this guiding mechanism. He proposed that target cells were able to secrete inducing or attracting substances, and that growth cones are provided with chemotactic sensitivity or chemically elicited ameboidism, formulating this way the "Neurotropic theory" (Cajal, 1892). He ended the presentation of his ideas by saying: "Besides, as weak as might be a scientific hypothesis, it is always better than the complete absence of explanation." He was right because, as we will see, the "Neurotropic theory" is no longer a theory, but one of the more expanding fields in the developmental neurosciences.

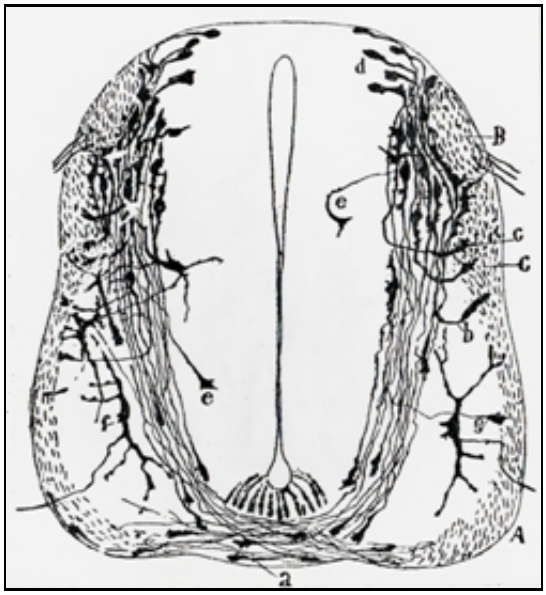


**Figure 2:** This drawing of Cajal corresponds to a modification of the Fig. 1 of his early paper published in 1890 in the Anatomischer Anzeiger. It represents the spinal cord of an E4 chick embryo. Note that axons of commissural neurons 'd' have crossed the midline and form the ventral

commissure 'a'. The motneurons 'f-g' have their axons leaving the cord through the ventral roots. Thus, axons of the different neuronal populations follow stereotyped pathways. Note that there are very few errors, for instance, 'e' marks a growth cone lost in the ventricular zone. Copyright Herederos de Santiago Ramón y Cajal.

**The announcement of the Neurotropic Theory**

Important discoveries in bacteriology, particularly work carried out by the Pasteur school in Paris during the decade 1882-92 concerning the "chemotactic ameboidism" of leukocytes (see Elie Metchnikoff, 1892), were at the bases of Cajal's speculations. For him, similarly to the leukocytes that are guided toward bacteria by diffusion gradients of bacterial toxins, growth cones are also oriented toward their target elements (muscle, neurons) by stimulating substances produced by the targets (neurotropic substances). The end product of this oriented, forward movement is the formation of specific and stable connections.

In his publication of 1892, Cajal envisaged that chemotaxic mechanisms could also be involved in the process of displacement or translocation of neuronal cell bodies during migration, particularly those of the cerebellar granule cells and sensory ganglion cells. He admitted that either a "positive chemotaxis"' (attraction) oriented towards the terminal domains of the migrating neurons, or a "negative chemotaxis" (repulsion) for substances produced by their own axons might be possible in this case of cell body translocation. Indeed, 108 years later, we published the first evidence that netrin-1 (see below) exerts a repulsive action on the glial guided migration of granule cells in the postnatal cerebellum (Alcantara et al., 2000). However, this functional dichotomy is forgotten very soon, and in the *Textura* (Vol. 1, 1899) only attractive chemotropic influences were proposed as mechanisms on axon guidance. He specifically wrote, years later: "nothing indicated the intervention of negative neurotropic substances …" (Cajal, 1913). Another important point is that the chemotactic forces are secreted or produced by the ultimate targets: other neurons in the CNS and, in addition, muscle and peripheral target cells in the PNS (Cajal, 1909). Thus, although chemotropic molecules were supposed to be diffusible, the gradients envisaged by Cajal were much more extensive than those accepted today by modern embryology. In other terms, the current concept of "intermediate target" was missing in Cajal's considerations.

**The search of evidences in favor of the Neurotropic Theory**

The problem with hypotheses, even the most appealing ones, is that they require to be validated by experimental data. Cajal pursued his observations on axons of mislaid neurons in the embryonic medulla oblongata. He followed them, re-entering the nervous parenchyma and eventually reaching their correct destinations. But he soon abandoned the idea of getting the required evidences working with embryonic tissue.

Cajal, although with some reluctance, started to work on the degeneration and regeneration of the nervous system. His aim was mainly to challenge the results obtained in sectioned nerves by Bethe, who claimed that the trophic bases for the neuron doctrine were obsolete because the distal stumps separated from their parent cell bodies were able to regenerate. Thus, Cajal worked hard to innovate a new silver staining allowing him to visualize neurofibrils (the reduced silver method), and at the beginning of the 20th century, started a new direction of his research. The results were first published between 1905 and 1913, and thereafter assembled in his book *Degeneration and Regeneration of the Nervous System* (Cajal, 1913, 1914).

When Cajal started this work, the problem of the regeneration of peripheral nerves was already a very hot topic, and many researchers were involved in these kinds of studies. One of them was J. Forssman, who from 1898 had not only endorsed but also obtained some experimental support to the 'Neurotropic theory' (see Forssman, 1900). This Swedish investigator was, therefore, the first to obtain direct evidence in favor of the neurotropism by putting obstacles to the regenerative process, hindering distal stumps of sectioned sciatic nerves in collodium tubes, or by using peripheral nerve allografts or xenografts. Cajal soon corroborated and extended these results. In his book *Degeneration and Regeneration of the Nervous System* (Cajal, 1913, 1914), he gathered a large number of experiments to demonstrate that regenerating peripheral axons emerging from

the proximal stumps will always enter the distal stumps, whatever the obstacles raised against their growth, as if they were strongly attracted. Moreover, the major contribution of Cajal was to show that the sources of neurotropic substances released by distal stumps were neither contained in remnants of the myelin nor in degenerated axons, as advanced by Forssman, but in Schwann cells. This important result was obtained by transplanting a piece of peripheral nerve untreated (living stage) or treated with chloroform to kill all the Schwann cells. The growth of regenerating axons was oriented towards the grafted nerve only when living Schwann cells were present (Figure 3). These experiments clearly illustrate that the guidance of regenerative growth is not the result of mechanical or physical interactions. It is solely conditioned by the release of substances by living cells in the distal amputated or transplanted aneural nervous stumps, which are particularly rich in Schwann cells.

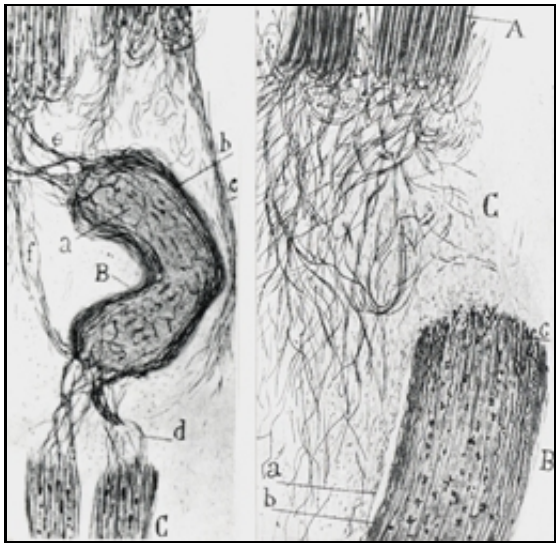


**Figure 3:** Illustrations of the peripheral nerve grafts done by Cajal to demonstrate the neurotropic influences of alive Schwann cells in attracting regenerating nerve fibers from the proximal (up) to the distal stumps. In the left drawing, a portion of a fresh peripheral nerve was grafted in the wound of the sciatic nerve of an adult rabbit. 17 days after the graft, fibers exiting from the proximal stump 'e' cross the graft and enter the distal stump 'C'. In contrast, the right drawing illustrates a case in which the grafting was done with a piece of peripheral nerve killed by chloroform. Young cat killed 12 days after the operation. Note that the regenerating fibers leaving the proximal stump 'C' do not enter the death graft. Copyright Herederos de Santiago Ramón y Cajal.

**The decline and revival of the Neurotropic Theory**

Scientific disciplines are extremely influenced by fashion, and Neuroscience is not an exception. The lack of direct evidence fully to support the growth cone chemotaxis hypothesis, and the use of new tools – in this case in vitro studies, particularly by Ross Harrison (1910) – with new results and interpretations have marked the waning of Cajal's hypothesis. The latter was substituted by the idea that, since the growth cone cannot grow in a liquid medium but attached to specific substrates, what is important in axon guidance is the local environment of the growing axons, a hypothesis that years later was named "contact guidance" (Paul Weiss, 1941). For almost 40 years the word "neurotropism" was only used to designate an affinity for neural tissue (for instances neurotropic viruses), and it was almost forbidden to mention it in the context of the growth cone chemotactic hypothesis.

The importance of Cajal's proposals on the role of neurotropic substances for guiding growing and regenerating axons would have to wait almost a century before its general acceptance. It was only in the eighties that appeared the seminal paper by Andrew Lumsden and Alun Davies (1983) reporting, with a new methodological approach (collagen gel co-cultures), that trigeminal sensory axons were attracted by their final peripheral targets, the whisker pad epithelium. These results provided the first experimental evidence for natural chemoattractant activities during embryonic

development. A few years later, Marc Tessier-Lavigne and collaborators (1988) showed, with the same in vitro approach, that when the dorsal region of the embryonic spinal cord (the favorite material of Cajal) was faced against a floor plate explant, commissural cell axons were strongly attracted. This important experiment demonstrated that not only target tissue but also intermediate targets, in this case the floor plate, can be sources of chemotropic activity. Another milestone was the discovery by Adrian Pini (1993), also with collagen gel co-cultures – in this case of embryonic olfactory bulb and septal neurons (considered here as intermediate target) – that chemotropic influences were not only attractive but that they could be also inhibitory, meaning repulsive. We identified, a few years later, Slit as the repulsive molecule in this system (Nguyen Ba-Charvet et al., 1999). Thus, it became clear that navigating growth cones during their pathfinding are guided, in addition to 'local contact influences' or short-range cues, by long-range guidance cues, some attractive while others are repulsive.

Marc Tessier-Lavigne succeeded in identifying the first molecules with chemotropic action in mammalian embryos. This was a major advance in favor of neurotropic axon guidance at a distance. These chemotactic molecules were named 'netrins' and were mainly produced by intermediate targets (Serafini et al., 1994). Other families of chemotropic molecules have been identified, such as secreted semaphorins, hepatocyte-growth factor/scatter factor, and Slits, that together with neurotrophins constitute the bulk of chemotactic molecules used by growth cones in their oriented elongation. Modern cellular and molecular studies have, thus, elegantly revived in these last 10 years the neurotropic hypothesis of Cajal.

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**Favourite sentences**

*First description of growth cones by Cajal:*

"Each fiber of this commissural bundle ends at variable distances, on account of their state of development, by a conic swelling covered by highly irregular spinous expansions. This terminal swelling, that we shall name growth cone evidently proclaims the distal tip of all growing nerve fibers … Sometimes, the growth cone owns longer, triangular, lamellar or ramified processes, which seem to insert themselves between the nervous elements, opening up a path through the interstitial cement" (Cajal, S. Ramón y, A quelle epoque apparaissent les expansions des cellules nerveuses de la moëlle épinière du poulet? *Anatomischer Anzeiger*, 1890; 21: 606-11, and 22: 631-9).

*On the behavior of growing axons:*

"[They] will adopt pre-determined directions and establish connections with defined neural or extra neural elements ... without deviations or errors, as if guided by an intelligent force."

*The need of axon regeneration studies to consolidate the "Neurotropic hypothesis":*

"Since we formulated in 1892 the hypothesis of chemotactism, that is, of amoeboidism of young axons brought about by an orienting stimulus from attracting or neurotropic substances many experiments have been undertaken with a view to supplying an objective basis for this conception … these experiments have dealt with nervous regeneration … (since) embryonic neurogenesis is too difficult and delicate for experimental proof of the type wanted." (Cajal, S. Ramón y, *Estudios sobre la Degeneración y Regeneración del Sistema Nervioso*, 2 Vols. Moya: Madrid; 1913 and 1914).

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