

Camillo Golgi's Black Reaction for Staining Neurons

In 1873 Italy, Camillo Golgi created the black reaction technique, which enabled scientists to stain and view the structure of neurons, the specialized cells that compose the nervous system. During the nineteenth century, scientists were studying cells and proposed cell theory, which describes the basic characteristics of cells as fundamental units of life. However, cell theory struggled to explain neurons as they are specialized cells and more complex in structure than cells of other tissues. Prior to Golgi's black reaction, other neuron staining techniques did not enable scientists to clearly and completely view entire neurons without damaging the tissue and obscuring the form. By enabling scientists to study individual neurons and neural tissues, Golgi's black reaction enables researchers to better study the nervous system and how it develops.

Researcher and physician Camillo Golgi developed the black reaction technique in 1873 in a laboratory he constructed in his apartment kitchen at the the Hospital for the Chronically Sick in Abbiategrosso, Italy, where he was the lead physician. Golgi needed a staining technique to reveal neurons in their entire, undamaged form to confirm his reticular theory. Reticular theory stated that the entire nervous system was a continuous network of cells without gaps or synapses in between the cells.

Prior to Golgi's black reaction, researchers used two different staining techniques to study neural tissues. One method involved the cutting and staining of neural tissue using a dark blue or violet stain called hematoxylin. Using that method, researchers could not see neurons very well, as the penetration of the stain into the neurons varied depending on the size of the cell. In smaller neurons, scientists saw only two cellular structures, the nucleus in the center of the cell that contains genetic material, and the cell wall encasing the cell. In larger cells, scientists saw the nucleus, its surrounding cell body, and the dendrites, which are branchlike cellular extremities later shown to receive impulses from other nerve cells. Those variations in visibility between neurons made the hematoxylin staining method unreliable.

The second method of neural tissue staining involved immersing neural tissue in potassium dichromate, a chemical that hardened the cells and enabled scientists to examine their structures. By hardening nervous tissue, scientists could then handle neural tissues with minimal damage to the fragile cells. After the immersion, scientists stained the neuron using carmine, a red dye that enabled scientists to view cellular structures under the microscope. After staining the tissue, scientists separated the individual neurons using needles under a microscope. However, that method was only applicable to larger neurons because the thin cellular extremities of neurons (axons) could tear apart, which distorted the microscopic image of the neuron.

In 1873, Golgi used similar methods to prepare the neurons he studied. He also used the chemical potassium dichromate to harden the tissue. However, after hardening the tissue, Golgi used a different chemical to dye the neurons. Instead of hematoxylin or carmine, Golgi submerged the tissue in a silver nitrate solution, soaking it for one to two days. The silver nitrate solution reacted with the potassium dichromate and formed fragments of silver chromate on the cell membrane. Those fragments were black, hence the name black reaction. Silver chromate primarily clusters around the cell membrane, staining the entire neuron cell black, while earlier methods only stained parts of cells. However, Golgi's method did not stain every neuron in a given sample of hardened tissue. Only 1 to 5 percent of neurons per sample were stained. That selective staining enabled Golgi to analyze individual neurons from end to end, without separating individual neurons under a microscope and risking damage to them.

Golgi used his technique to search for evidence to support reticular theory, the theory that the

nervous system was a continuous network of cells without any gaps between them. Not all researchers agreed with Golgi. Santiago Ramón y Cajal studied the nervous system at the University of Barcelona in Barcelona, Spain, at the same time Golgi was working in Italy. Ramón y Cajal supported the neuron doctrine, a theory that the nervous system was composed of separate, non continuous, individual cells. Both researchers used Golgi's black reaction technique to provide evidence in support of their conflicting theories.

In the late nineteenth century at the University of Barcelona, Ramón y Cajal modified Golgi's staining technique by submerging the tissue sample in the silver nitrate solution for two short soakings instead of submerging the tissue for one to two days. Through the use of the black reaction, later called the Golgi stain, Ramón y Cajal detected spaces between branch-like structures at the end of one neuron (axons) and the branch-like structures at the head of another neuron (dendrites). The presence of those spaces confirmed the neuron doctrine, which Ramón y Cajal supported over the reticular theory.

Researchers used the black reaction to discover many neural structures such as the Golgi apparatus, a structure that functions in transporting newly made proteins within the cell. Golgi discovered that structure in 1897 while working at the University of Pavia in Pavia, Italy. In 1906, both Golgi and Ramón y Cajal received the Nobel Prize in Physiology or Medicine for their work on the structure of the nervous system. The Golgi staining technique continued to be used by scientists into the twentieth century, both on its own and in combination with the electron microscope. Electron microscopes use electrons instead of light to produce high quality images of samples. After applying the Golgi stain to neural tissues, researchers used electron microscopes to produce images of neurons that captured more detail than had earlier microscopes, revealing smaller structures within neurons than were previously visible.

Sources

1. Borrell, Jose, and Juan A. DeCarlos. "A Historical Reflection of the Contributions of Cajal and Golgi to the Foundations of Neuroscience." *Brain Research Reviews* 55 (2007): 8-16.
2. "Camillo Golgi - Biographical." Nobel Prizes and Laureates. http://www.nobelprize.org/nobel_prizes/medicine/bio.html (Accessed April 13, 2016).
3. Costandi, Moheb. "The Golgi Stain, Circa 1873." *The Scientist*, August 1, 2009. <http://www.the-scientist.com/?articles.view/articleNo/27559/title/The-Golgi-Stain--circa-1873/> (Accessed April 11, 2016).
4. Dröscher, Ariane. "Camillo Golgi and the Discovery of the Golgi Apparatus." *Histochemistry and Cell Biology* 109 (1998): 425-30.
5. Golgi, Camillo. "Sulla Struttura della Sostanza Grigia del Cervello." [On the Structure of the Gray Matter of the Brain]. *Gazzetta Medica Italiana. Lombardia* 33 (1873): 244-246.
6. Golgi, Camillo. "Interno alla Struttura delle Cellule Nervose." [Inside the Structure of Nerve Cells]. *Bollettino della Società Medico-Chirurgica di Pavia* 13 (1898): 3-16.
7. Golgi, Camillo. "Intorno alla Distribuzione e Terminazione dei Nervi nei Tendini Dell'uomo e di Altri Vertebrati." [Around the Distribution and Termination of the Nerves in Humans and Other Vertebrates' Tendons]. *Gazzetta Medica Italiana* 38 (1878): 221-224.
8. Golgi, Camillo. "Sull'infezione Malarica." [On Infection of Malaria]. *Gazzetta Medica Italiana* 10 (1886): 109-135.
9. Golgi, Camillo. *Sulla Fina Anatomia degli Organi Centrali del Sistema Nervoso* [On the Fine Anatomy of the Central Organs of the Nervous System]. Reggio Emilia: Tipi di Stefano Calderini e Compagno, 1885. <https://archive.org/details/b21978724> (Accessed April 21, 2016).
10. Martin, Kevan. "A Golgi Stain for Dendritic Function?" *Nature Neuroscience* 3 (2000): 753.
11. Pannase, Ennio. "The Golgi Stain: Invention, Diffusion and Impact on Neurosciences." *Journal of the History of the Neurosciences* 8 (1999): 132-40.
12. Ramón y Cajal, Santiago. *Les Nouvelles Idées sur la Structure du Système Nerveux chez L'homme et chez les Vertébrés* [New Ideas on the structure of the nervous system in humans and in vertebrates]. Paris: C. Reinwald and Cie., 1894. <https://archive.org/stream/lesnouvelle>

sid00ram#page/n7/mode/2up (Accessed April 21, 2016).

13. "Santiago Ramón y Cajal – Biographical." Nobel Prizes and Laureates. http://www.nobelprize.org/nobel_prizes/physiology-medicine/laureates/1906/ramon-y-cajal-bio.html (Accessed April 13, 2016).