The Golgi staining technique, also called the black reaction after the stain's color, was developed in the 1870s and 1880s in Italy to make brain cells (neurons) visible under the microscope. Camillo Golgi developed the technique while working with nervous tissue, which required Golgi to examine cell structure under the microscope. Golgi improved upon existing methods of staining, enabling scientists to view entire neurons for the first time and changing the way people discussed the development and composition of the brain's cells. Into the twenty-first century, Golgi's staining method continued to inform research on the nervous system, particularly regarding embryonic development.

Golgi began publishing research about the nervous system and its cellular structure between 1870 and 1872, while working in the laboratory of Giulio Bizzozero at the Institute of General Pathology at the University of Pavia in Pavia, Italy. Afterward, Golgi became chief doctor at the Pio Luogo degli Incurabili (Hospital for the Chronically Sick) in Abbiategrasso, Italy. He built a laboratory in the kitchen of the hospital, where he developed a new technique to stain neural tissues. The staining method of his time involved staining an organic tissue with red carmine or black hematoxylin and exposing it to a reagent, like potassium dichromate or chromic acid, that hardened the individual neurons. Scientists then isolated individual cells by separating them under a microscope with needles. Because neurons have a different shape from other cells of the body, scientists often broke the projections off of the cell when trying to separate them. Those projections included web-like dendrites that receive neural impulses and relay them to the cell body, and the long axon on the other side of the cell body that relays those impulses.

Golgi's early attempts to improve staining involved infusing cells with various metals to view whole neurons and other kinds of tissues in greater detail. After experimenting with exposing cells to gold and mercury, Golgi used silver nitrate. The silver nitrate stain enabled him to see the neurons in their entirety, turning them black against a pale yellow background. The black color of the stained neurons led him to name his technique la reazione nera (the black reaction). The staining process dyed five to ten percent of cells per sample, enabling Golgi to see individual cell structures amid tangled masses of nervous tissue.

The mechanisms of the Golgi staining technique were similar to those of his contemporaries. First, to harden the soft neural tissue, Golgi submersed samples for up to forty-five days in a 2.5 percent potassium dichromate solution. Next, he soaked the samples in a 0.5 to 1 percent silver nitrate solution for different amounts of time to dye some of the cells black. Golgi then dehydrated the samples with alcohol and sliced them at 100 micron intervals, the equivalent of the thickness of a piece of paper, wide enough to keep one neuron intact. Golgi then rinsed the tissue in turpentine and attached it to a slide with clear gum damar to preserve it. At that point, he viewed a sample under a microscope.

Golgi published the results of his staining procedure in a 1873 paper titled "Sulla Struttura
della Sostanza Grigia del Cervello” (On the Structure of the Gray Brain Matter). In Germany, Franz Boll published notes of Golgi’s work in German journals, which spread news of Golgi’s work. Golgi continued to use his staining technique to examine neural tissues, and later accumulated his finding in Sulla Fina Anatomia degli Organi Centrali del Sistema Nervoso (On the Fine Anatomy of the Central Organs of the Nervous System) published in 1885. That year, Eugen Bleuler, a psychiatrist in Switzerland, used Golgi’s staining method and presented specimens of rabbit [5]’s cerebral cortex to the Medical Society of Zürich.

In the 1880s, Albert von Kölliker [6], a physiologist in Switzerland, studied the development of the brain, neuro-sensory pathways, and internal organ development in mammals using the Golgi staining technique. He learned the technique under the instruction of Golgi in 1887, and he published “Ueber Golgi’s Untersuchungen, den Feineren Bau des Centralen Nervensystems Betreffend” (About Golgi’s Studies Concerning the Finer Structure of the Central Nervous System) later that year. Kölliker lauded Golgi’s staining technique, leading more scientists to adopt it.

In 1887, Santiago Ramón y Cajal [7] in Spain altered the technique by introducing silver nitrate to the tissue in two shorter soaks, rather than one long soak. That alteration improved the color and detail of the stain. Researchers like Golgi and Ramón y Cajal used results from such staining studies to help develop the neuron [4] doctrine, which stated that neurons were individual cells that transmitted electrical impulses to and from each other. That theory transformed theories of the nervous system’s structure and functionality. Scientists confirmed the theory by using the Golgi staining technique to show the gaps, or synapses, between neurons. Golgi and Ramón y Cajal shared the Nobel Prize in Physiology or Medicine [8] to in 1906.

In 1917, Pío del Río Hortega further adapted Golgi’s method by fixing tissues with formalin, chloral hydrate, and potassium chromate, which decreased the time necessary to complete the staining. Into the twenty-first century, researchers used del Río Hortega’s method to dye non-neuronal brain cells like neuroglia and cells of the cerebellum [9]. After a lull in popularity between the World Wars, the Golgi staining technique regained popularity in the late 1930s with the introduction of the electron microscope [10]. Scientists used Golgi’s staining technique to better explain the formation of neurons, the brain, and sensory pathways. Golgi staining also helped scientists classify organelles within cells, including muscle cell organelles which store and transport calcium, called the sarcoplasmic reticulum, and the protein-modifying organelles in cells called the Golgi apparatus. The Golgi staining technique remained a primary method of staining into the twenty-first century.

Sources


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