THE MORPHOLOGY OF THE NERVE CELL: A NINETEENTH CENTURY MULTI-NATION SUCCESS STORY[1]

foredrag på jubilumssymposiet Nansen's Doctorate and the Neuron Doctrine – 130th anniversary symposium

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av Ortwin Bock, dr.med., Cape Town

Introduction

At the beginning of the nineteenth century virtually nothing was known of the morphology of the nerve cell, but by the end of the century the basic structure (histology) of the cell, now known as the neuron, had been described. In addition, one of the most important concepts in neuroscience – the Neuron Doctrine – had been formulated and received general acceptance.

Although the advances in knowledge that took place could in many instances be attributed to the new histological techniques that became available during the century, the special attributes of the scientists who made the discoveries cannot be denied. They came from England and all the corners of Europe, but mainly from the German-speaking countries. Not surprisingly they were all men because it was only towards the end of the century that women were allowed to study at a university.[2, 3]

The 'too pulpy' brain – fixing and hardening: two Danes

By 1800 the macroscopic anatomy of the brain and spinal cord was well-known, but efforts to study the finer anatomy had failed because the excised brain tissue putrefied quickly and was too soft to cut into the thin slices

1. The title of this essay was the sub-title of a talk *Fridtjof Nansen and the Neuron* given at Oslo on 3 May 2018 and includes some of the material used that day.
2. A close version of this essay has been published elsewhere: *Research* 2018; 5: 2659
3. Where needed the German into English translations were by the essayist.

needed for microscopic examination; in 1666 Marcello Malpighi (1628–1694) had boiled the excised human brain and then wiped ink over the cut-surface to see what it looked like. [1]

Alcohol ('spirits of wine') came into use as a preservative of organic material during the latter part of the eighteenth century – Robert Boyle (1627 – 1691), the Anglo-Irish polymath and one of the founders of chemistry, had suggested its use in 1666. Although the alcohol also hardened the tissue sufficiently for thin slices to be cut by hand, the extraction of water caused the tissue to lose volume and the consequent compression distorted its in – life architecture. Johann Christian Reil (1759–1813), a Frieslander who became professor of medicine and town physician of Halle in 1788, reported in 1809 that this loss of volume could to a large extent be prevented by adding carbonate of potash or ammonia to the solution. [2] This '… was an important landmark in the history of neuroanatomy'. [3]

The story now moves to Copenhagen. Ludvig Levin Jacobson (1783–1843) was a surgeon who invented an instrument capable of crushing bladder stones. In 1806 he was appointed a lecturer in chemistry at the surgical academy (as a Jew he had been barred from studying at Copenhagen University; he refused to be baptized). A keen traveller he went to Paris to visit Louis Nicolas Vauquelin (1763–1829) who had a special interest in beryllium and chrome. Vauquelin must have mentioned to Jacobson that he thought chrome might prevent putrefaction, because when he returned home Jacobson looked into this and found that potash of chromate had this property. On a visit to Scotland in 1833 he told the Editor of the Edinburgh Philosophical Journal that he had '…laid before the Medical Society of Copenhagen, the results of a series of experiments, relating to the oxides and salts of chrome, (and) that chrome is one of those metals which in a particular manner acts on the nervous system'. An abridged version of the communication was published in German in *Liebigs Annalen der Chemie* the same year. [4]

This affinity of chromic acid for nervous tissue was taken much further by another Dane. Adolph Hannover (1814–1894), the brilliant son of a prominent Copenhagen Jewish family, went to Berlin to study with the legendary professor of anatomy and physiology Johannes Petrus Müller (1801 –1858) after obtaining his medical licence in 1839. There he met Robert Remak (1815–1865), undoubtedly Müller's most brilliant assistant, who suggested that Hannover join him in the study of the microscopic anatomy of the central nervous system. From Berlin Hannover wrote Jacobson a long letter – Die Chromsäure, ein vorzügliches Mittel bei mikroskopischen Untersuchengen – which was reproduced in *Müller's Archive* of 1840 (10 pages). The first paragraph includes these two sentences: 'During my ongoing research I was looking for an agent that would preserve not only the outer appearance of the organ but also its inner structure, in particular that of the retina and the central nervous system', and 'The only fluid I found that preserved the outer form and the inner structure of the organ without making it too hard to cut into thin slices was chromic acid'. [5]

Hannover's work opened the door for other researchers interested in the fine structure of the central nervous system. He had a passion for microscopy and his book on the construction and use of the microscope of 1847 was translated into English and several European languages. He was the author of many books. Despite his distinguished career as a clinician and administrator and the high regard with which he was held by medical societies throughout Europe, Hannover never became a professor at Copenhagen University.

Staining: a German and an Italian

Two stains, carmine and silver, helped to unlock the secrets of the nerve cell during the nineteenth century.

The rationale for staining organic tissue with chemical substances was explained by Salomon Stricker (1834–1898), Professor of Pathology in Vienna, in the first volume of his widely read textbook on human and animal histology (1870): 'The contours (outlines) of the morphological elements not previously visible can often be made so by treating the preparations with certain colouring matters because not all the various constituents of the tissue combine with these agents. The tissue is left in a solution of the colouring agent for some time and then washed'. [6]

Carmine, a red pigment derived from the cochineal insects of Mexico and Peru, was used in 1770 by John Hill (1717–1775) to study the structure of wood, and by Alfonso Corti (1822–1876) when he studied the epithelial lining of the cochlea in 1851. But it was the work of Joseph Gerlach (1820–1896) in the 1850s that promoted the virtues of carmine as a dye for use in neuro-histology. Gerlach was born in Mainz where his father worked as a dyer. In the autumn of 1838 he went to Würzburg to start his medical studies and by the time he returned to Mainz to open a practice in March 1846, he had graduated from Munich (12 August 1841), spent some time with Müller in Berlin and with the famous pathologist Carl Rokitanski (1804–1878) in Vienna, and visited Paris, London and Dublin. In Mainz Gerlach continued his histological research and in 1848 published *Handbuch der allgemeinen und speciellen Gewebelehre des Menschlichen Körpers für Aertze und Studirende*. Of the 500 pages of this book 45 pages are devoted to the histology of the nervous system in general and 46 pages to the histology of the eye, his special interest. [7]

In 1850, when he was 30 years old, Gerlach was offered and accepted the Chair of Anatomy and Physiology at Erlangen. He flourished. In 1858 he completed *Mikroskopische Studien aus dem Gebiete der menschelichen Morphologie*. In his introduction he could hardly wait to extol the virtues of carmine. In 1854 he had started to inject a transparent mixture of carmine, ammonia and gelatine into the capillaries of tissue that had been fixed in potassium dichromate with a concentrated solution of carmine, and had noted that the nuclei took up the dye more avidly than the cytoplasm or intercellular substance. One day, he continued, he emptied the flask of carmine but did not clean it. Pouring water into the flask produced a rose – coloured solution into which he dropped a piece of cerebellum before going home. The next morning the white matter of the convolutions were as before, but the inner layers of the grey matter were stained a deep red, much darker than the colour of the solution in which the tissue had laid overnight. Microscopically the granules were stained dark red, whereas the ground substance and nerve fibres had not taken up the dye. He concluded the chapter by telling the reader exactly how to go about staining brain tissue with carmine; many did. [8]

Although carmine, usually combined with haematoxylin (an extract obtained from the logwood Haematoxylon campechnianum), became the preferred stain for nervous tissue, it had its limitations because the dye picked out only bits and pieces of the cell. Gerlach touched on this issue in his treatise on the spinal cord in the second volume of Stricker's book (1872) where he compared the results obtained with carmine with those obtained with gold chloride. [9] It was clear that the search was still on for somebody to find a chemical substance that would stain the whole nerve cell.

The breakthrough came a year later. In a letter written on 16 February 1873 by Camillo Golgi (1843–1926) to his close friend Nicoló Manfredi (1836–1916), the 30 year old recorded that 'I have regained the energy that for a few months I had completely lost. I spend long hours at the microscope. I am delighted that I have found a new reaction to demonstrate even to the blind the structure of the interstitial stroma of the cerebral cortex. I let silver nitrate react on pieces of brain hardened in potassium dichromate. I have already obtained magnificent results and hope to do even better'. He reported the new method in the *Gazzette Medica Italiana – Lombardia* of 2 August 1873. [10]

Golgi, the son of a doctor, had graduated from Pavia in 1865. After a stint of military service he completed the internship needed to become an assistant physician. But Golgi longed to return to scientific research and by 1869 he was once more studying the effects of a variety of fixing and staining substances on nervous tissue. Because there was no prospect of an early academic appointment at Pavia and because he needed financial security, Golgi applied for and was offered the position of Chief Physician and Surgeon at a hospital that cared for people with long – term physical and mental disabilities at Abbiategrasso near Milan. He started there on 1 June 1872 and soon converted part of the kitchen in his lodgings into a laboratory.

The silver chromate had an affinity for membranes and stained the whole nerve black. But, for an as yet unknown reason, this applied to only 1–5 % of the cells in a microscopic field. This quirk of the stain had the advantage that it made it possible for an observer using thick sections to trace out the entire structure of an individual cell against a contrasting yellow background. The technique of the silver stain, also known as the black reaction or *la reazione nera*, was not easy to learn and the procedure took months to complete. Many scientists who tried it gave up, except for a young Spaniard who improved the stain and set the fledgling neuroscientific world alight with the results he obtained using it (of this more later).

It is of interest that if Golgi could have afforded to buy osmic acid, a better fixing agent for nervous tissue than potassium dichromate, he would not have discovered 'Golgi's stain'.

The microscope gets better: the engineer from Modena and the Jena Trio

At the beginning of the nineteenth century microscopy was no longer an activity confined to scientists working away in dark rooms. In England and elsewhere it had become a social event like playing bridge; the Microscopic Society of London was founded on 20 December 1839. A wide variety of instruments made by firms in England, France and Germany were on the market. They were either simple, a single magnifying glass mounted on a stand, or compound with a lens at one end of a tube made from paper, wood or leather – the eye piece – and another at the other end of the tube – the objective; some instruments had a third lens mounted inside the tube. The column of lenses was attached to a stand and focussing was achieved by moving the object stage up or down.

In skilled hands these microscopes offered a surprisingly high degree of magnification, but to a greater or lesser extent they all exhibited annoying spherical and colour aberrations which blurred the images being looked at. These aberrations were due to the curvature of the spherical lenses which refracted the transmitted light to different focal points. Joseph Jackson Lister (1786–1869), a London wine merchant and opticist, in 1830 offered a solution. On theoretical grounds and after experiments he found that an objective made by cementing a plano – concave lens made from flint glass to a spherical lens made from crown glass considerably lessened the aberrations and brought two of the three primary colours of light, red and blue (the other is green), to the same focus. [11]

This development of the 'achromatic doublet' was the key to the construction and widespread use of the achromatic microscope in the middle of the nineteenth century. To this Giovanni Battista Amici (1786–1863) of Modena made major contributions. Amici was an engineer, teacher of mathematics, astronomer and maker of telescopes and microscopes. In 1827 he travelled to London taking with him his newest achromatic microscope. There he met Lister – now regarded as the father of English microscopic optics – who owned a powerful achromatic instrument which compared favourably with the instrument Amici brought along. [12] Amici's instruments gained legendary status. He was a prolific correspondent and over a period of 50 years made microscopes for many of the scientists working in America, Britain, Europe and Russia. For some of these microscopes and telescopes Amici fashioned the lenses out of glass produced by Michael Faraday (1791–1867), better known as the discoverer of electromagnetic rotation.

In 1886 there came onto the market a finely crafted sturdy microscope supplied with objectives which corrected for the three primary colours of light. The apochromatic microscope was the product of a co-operation between a 70 year old instrument maker, a 46 year old physicist and a 35 year old glassmaker. They lived and worked in Jena. Carl Zeiss (1816–1888) had started to repair scientific instruments and to make simple microscopes in 1846 but in 1857 he decided to concentrate on constructing compound microscopes. His instruments were noted for their fine focus which was achieved by movements of the column that housed the lenses instead of adjustments of the optic stage. He found the trial and error method of selecting and placement of the lenses in the column tiresome and looked for help.

In 1866 he approached Ernst Abbe (1840–1905), a teacher of physics and mathematics at the local university. Zeiss and Abbe soon became partners. Defining the optics needed for microscopic work was not an easy task but by 1873 Abbe had proved that the resolution – equal sharpness of several non-adjacent points in one field – of a microscope is inversely dependent on its aperture and not on the curvature and placement of the lenses; he also determined the conditions that lenses had to fulfil to produce sharp images (Abbe sine condition). What Abbe and Zeiss wanted was glass with a higher refractive index where every batch met these criteria because their suppliers in England, France and Switzerland had been letting them down. They found their glassmaker in Otto Schott (1851–1935), the son of a window glass-maker in Witten (Westphalia).

Schott had studied chemistry, minerology and physics at university with the intention of becoming a glassmaker and experimented with newer ways of making glass. He wrote to Abbe on 27 May 1879 and in January 1882 moved to Jena to set up a glass technical laboratory. There he produced a glass of exceptional qualities that met the theoretical requirements. Together with Abbe, Zeiss and Zeiss' son Roderick (1850–1919), Schott opened *Jenaer Glasswerk Schott und Genossen* on 23 July 1885. The company flourished and the manufacturing methods had to be adapted to meet the increasing demand for Zeiss apochromatic microscopes. Recovering from a stroke Zeiss was able to attend the celebration on the completion of the 10 000th Zeiss microscope on 24 September 1886. He had several more strokes after that and died on 3 December 1888. [13]

The early microscopists had been aware that the images at high magnification were blurred. This was because 'When light passes from a material of one refractive index to material of another, as from glass to air or air to glass, it bends. Light of different wavelengths bends at different angles, so that as objects are magnified the images become less and less distinct. … Placing a drop of oil with the same refractive index of glass between the cover slip and the objective lens eliminates two refractive surfaces, so that magnifications of 1000 x or greater can be achieved while still preserving good resolution'. [14]

In 1840 Amici produced an immersion lens designed to be used with oil (obtained from a variety of plants) which had the same refraction as glass. However these oils were costly and could damage the surface of the lenses if allowed to dry on it. In addition the public were not keen to put oil on their expensive slides. So in 1853 Amici switched to water. Others followed suit, notably Edmund Hartnack (1826–1891), a young German working in the workshop of Georg Oberhäuser (1798–1868) in Paris. In 1859 he exhibited his water immersion objectives. He sold many and for many years his objectives were regarded to be the best on the market. Zeiss produced a water immersion objective in 1871. But, Abbe having found an oily liquid that did not damage the lens, Zeiss from 1877 onwards supplied microscopes with oil immersion objectives – called homogeneous immersion objectives. In 1904 the firm presented Robert Koch (1843–1910) who had discovered the tubercle bacillus in 1882 with the 10 000th homogenous oil immersion objective.

First look at the nerve cell: a star pupil, a Pole in a foreign land, and a German who died young

The three were: Gabriel Gustav Valentin (1810–1883), Robert Remak (1815–1865) and Otto Friedrich Karl Deiters (1834–1863) who died from typhus at the age of 29.

At the Congress of the German Physicians and Naturalists held in the Prague National Museum on 23 September 1837 Jan Evangelista Purkyně (1787–1869), Professor of Physiology at Breslau (now Wroclac in Poland) described the features of large cells in the cerebellum which now carry his name. Although this is generally regarded by medical historians to be the first clear description of a nerve cell, this honour in fact belongs to his star pupil, the son of a Breslau jeweller. Valentin, who qualified in 1832, stunned the world of science in 1836 (the manuscript was dated 9 February) when *Acta Leopoldina* published *Über den Verlauf und die letzten Ende der Nerven.* The text consisted of 199 pages and eight plates with 86 images. In image 54 on plate Vll Valentin picked out four components of the cell – the granular parenchyma, the nucleus, the nucleolus and the axonal cone. Rudolf Albert Kölliker (1817–1905)[4], Professor of Anatomy at Würzburg and doyen of late nineteenth century histologists, in 1850 called the publication 'An epoch-making treatise, the first description of the nerve elements '. Throughout the text Valentin gives credit to his teacher mentioning what Purkyně had taught and showed him. Purkyně had a large achromatic compound microscope made by Simon Plössl (1794–1868) of Vienna which he shared with his pupils. [15]

Valentin had to leave Breslau because his and Purkyně's personalities made it impossible for them to share the same space. Valentin accepted the Chair of Physiology and Pathology at Berne where there was no anti-Jewish

4. Rudolf Albert Kölliker: spelling of his surname. The title page of his autobiography reads: *Erinnerungen aus Meinen Leben* von A. Koelliker Leipzig Wilhelm Engelmann 1899.

sentiment. He started there on 16 June 1836 and stayed until his resignation 45 years later; his research output in biochemistry, embryology, histology and physiology was enormous.

Valentin thought that the cell and the nerve fiber were not connected but were merely closely related to one another. This brought him into conflict with Remak, another Pole, another Jew, another touchy personality. Remak was born in Poznan but went to Berlin in 1833 to study medicine. He was soon attracted to microscopy and the histology of the nervous system and between 1835 and his graduation in 1838 published several papers on the subject. As far as Remak was concerned the nerve fiber was an extension of the nerve cell – the axon cylinder. That a twenty one year old could make such a radical suggestion upset Valentin a lot (there were other scientists who reacted sceptically at first) and it was only in 1842 after an unbecoming exchange of letters and public comments that Valentin conceded that Remak had been correct and that he had made a mistake. In 1859 Berlin University rather belatedly promoted Remak to associate professor. He retained a close relationship with Poland all his life. Complications of diabetes caused his death at the age of 50. [16]

But it was the studies of Deiters who was born and studied and worked at Bonn all his short life (he spent some time with Müller in Berlin as a student) that brought the histology of the nerve cell to light. During the 1860s he identified the soma (nerve cell) and differentiated two kinds of processes, protoplasmic (dendrites) and axis cylinder (axon), and noted that each cell had a variable number of dendrites which frequently branched but only one prominent axon which did not branch. The axon originated from a distinctive part of the cell – later named the axon hillock or cone – whereas the dendrites were extensions of the cell. Deiters had fixed the tissue in weak chromic acid or potassium dichromate solutions and stained them with carmine before cutting the sections; he also teased out from fresh material with a fine needle the individual nerve cells and their branches. It is not known what microscope he used. A 300 page monograph, *Untersuchen über Gehirn und Rückenmark des Menschen und der Säugenthiere*, compiled from his notes by his musicologist brother Hermann (1833–1907) and his professor Max Schultze (1825–1874), was published posthumously in 1865; it contained 16 lithographs of nerve cells that Deiters had drawn. [17]

Contrary to the Cell Doctrine: the Erlangen anatomist

Although Deiters' observations – he saw no connections between adjoining nerve cells – suggested that each nerve cell was an independent unit, the prevailing opinion at the time was that at some point the nerve cells must be joined together. So when Gerlach, one of the most influential anatomists of his day, announced on 4 May 1872 that he had new evidence to support this contention, it was no surprise that this fact was soon accepted: the Reticular Theory was launched.

On that day Gerlach published a two page article in the *Centralblatt für die medizinischen Wissenschaften* with the title: 'On the structure of the grey matter in the human cerebrum. Preliminary communication'. He reported that he had during the winter been studying the central convolutions of the human brain using the gold method and noted that the medullated nerve fibers (axons) were connected to one another to form a network (mesh or reticulum), and that this network was continuous with the protoplasmic projections (dendrites) of the nerve cells. [18] In his chapter on the spinal cord in Stricker's book, which was published more or less at the same time, he surmised: 'Had Deiters advanced one step further, he would have discovered the fine nerve fibers of the grey substance; but as he did not apply carminate of ammonia to his preparations, and was unacquainted with the chloride of gold method, this plexus escaped his observation'. [19] Of the several microscopes Gerlach had at his disposal he might have used the one made by George Oberhäuser labelled 1840/50 No. 895 most often.

Gerlach's study did much to underpin the Reticular Theory which stated that the cells of the central nervous system were joined together to form an enormous mesh of cells. Many scientists, including Camillo Golgi, agreed and joined the band of 'reticularists' whose concept held sway until the 1930s when the amassed studies using the silver stain and apochromatic microscopes proved beyond doubt that Deiters had been correct when he suggested that each nerve cell was an independent unit.

However, a puzzling aspect of the belief by the nineteenth century reticularists that the central nervous system was made up of a network of anastomosed cells, was the fact that by 1872 the Cell Doctrine was firmly established. As enunciated by botanist Mathias Schleiden (1804–1881) and his physiologist friend Theodor Schwann (1810–1882) in 1839, animals, like plants, were made of myriads of individual cells.

Gerlach was familiar with the Cell Doctrine. Why he ignored the doctrine when he drafted the 'Preliminary Communication' of 4 May 1872 is anybody's guess because he did not write an autobiography and so far nobody has produced a biography of him which might offer an explanation. He was succeeded by his son Leo Gerlach (1851–1918) in the chair, which had been established in 1743. In 1904 Leo wrote a long essay detailing his father's life as an anatomist. In it appears the following sentence: 'This view of Gerlach (mesh) found almost universal acceptance; it had to be abandoned, however, when studies with the Golgi chrome method led to the Neuron Theory which was adopted by the majority of neurologists '. The only reference to the Cell Doctrine occurs early in the essay: 'Theodor Schwann's well-known descriptions of animal cells, which had such an impact on histological research, coincided with Gerlach's first years of study'. [20] Gerlach was knighted in 1880 and retired in 1891 at the age of 71. He died on 17 December 1896 from an acute liver ailment. Gerlach never admitted that he had made a mistake when he proposed the concept of the cellular-mesh when he was a 52 year old. It must have pleased him that Emil Fischer (1852 – 1919), who was married to his daughter Agnes, was awarded the Nobel Prize for Chemistry in 1902.

The nerve cells are not joined: the first observations

In 1878 there were two reports that the nerve cells are not joined together that did not receive any attention from the scientific community at that time and only came to light much later.

Edward Schäfer (1850–1935), Assistant Professor of Physiology at University College, London, reported to the Royal Society on 10 January 1878 in a communication read by Professor William Sharpey (1802–1880) that he had the previous August studied the nerve elements in the hoods of jellyfish at the request of his friend George Romanes (1848–1894); he had used gold chloride as stain. He found that each fiber was entirely distinct and nowhere structurally continuous with any other fiber. The published report contains a foot note to a monograph that had just come to his notice. The authors were the Hertwig brothers of Jena. Oskar (1849–1927) and Richard (1850–1937) also studied jellyfish and noted that the two nerve rings were separated by what they thought was a delicate membrane. [21]

Years later – in 1935 – Schäfer, now Sir Edward Sharpey-Schafer of endocrinology fame, wrote in pencil on the envelope enclosing the original 1878 manuscript: 'So far as I know this paper contains the first account of the nervous system being formed of separate nerve units without anatomical continuity. Previously it was universally held that the nervous system was composed of networks of nerve fibres'. [22] If he did know of the Reticular Theory he did not refer to or comment on the network in his paper.

A near dead – heat

In late 1886 – early 1887 there appeared almost simultaneously three studies from different parts of Europe in which the authors, who were unknown to one another and were using different techniques, questioned the veracity of the concept that the nerve cells were all joined together to form a web.

The first was published in the *Bergen Museums Årsberetning* for 1886 in 1887. Fridtjof Nansen (1861–1930), a trainee zoologist, went to Bergen in late 1882 as Curator of the Museum. At the suggestion of Willi Kükenthal (1861–1922), a visiting doctoral student of Jena who was interested in the nervous systems of marine invertebrates, Nansen decided to study the micro-anatomy of the nervous systems of the primitive sea creatures he found in the fjords near Bergen. He tried his hand at the silver stain with the help of Gerhard Armauer Hansen (1841–1912) who had learnt the stain when he worked with Antoine Ranvier (1835–1922) in Paris. Hansen had a Zeiss achromatic microscope. In April 1886 Nansen went to Pavia to learn more about the silver stain. He continued his research at the Stazione Zoologica near Naples for about four months. The director of the research institute was Anton Dohrn (1840–1909) who was a friend of Ernst Abbe and always had a number of the latest Zeiss apochromatic microscopes on hand. On his return to Bergen Nansen completed *The Structure and Combination of the Histological Elements of the Central Nervous System.* On page 146 he wrote: 'If a direct combination is the common mode of combination between the cells as most authors suppose, direct anastomoses (connections) between their processes ought, of course, to be quite common. When one has examined so many preparations … as I have, without finding one anastomosis of indubitable nature, I think one must be entitled to say, that *direct anastomosis between the processes of the ganglion cells does not exist, as a rule'* (his italics). [23]

The second article was written by Wilhelm His Sr. (1831–1904), the Swiss-born Professor of Anatomy at Leipzig. In October 1886 he reported that in the tissues of human embryos which he stained with haematoxilin he had found that in the early stages of development of the foetus the nervous system was a mass of independent cells: 'I believe that one can also arrive at simple concepts regarding the nervous system if one abandons the idea that the nerve fibers, in order to affect a part, must necessarily be in continuity with each other'. [24]

The third paper came from Zurich. In January 1887, August-Henri Forel (1848–1931), Professor of Psychiatry, reported that he had used the technique of 'secondary degeneration' which he had learned while a student of

Bernhard Gudden (1824–1886) in Munich to trace the finer connections in the brain. He found that degeneration was sharply limited to cells whose fibers had been cut and did not spread to adjacent cells. He was of the opinion that '… a nerve network does not exist and each cell is in contact with, but not in continuity with, its neighbour'. [25]

The authors of a review published in 1998 concluded: 'These three, Nansen, His and Forel ... had sown the seeds of doubt concerning the reticular theory. They became the co-founders of the modern view of the nervous system. Nansen's English translation appeared in September 1886, followed by His's paper on the subject in October and Forel's in January 1887'. [26]

Whereas the two professors continued their academic activities until retirement, Nansen changed tack as soon as he had defended his PhD thesis on 28 April 1888. He went on to become a polar explorer extraordinaire, oceanographer, a leading activist for the dissolution of the 1814 Union of Sweden and Norway, and the Nobel Peace Prize Laureate of 1922 for the humanitarian work he did in Eastern Europe after World War I on behalf of the League of Nations.

Enters Santiago Ramón y Cajal (1852–1934)

Working in Spain at the same time was Santiago Ramón y Cajal who became known to the scientific community by his mother's surname, Cajal.

Cajal was born at Petilla in rural Spain where his father was a doctor. He wanted to be an artist but his father persuaded him to study medicine and he qualified at Zaragoza in 1873. A period of military service in Cuba followed. He obtained his doctorate in 1877 and was Director of the Anatomical Museums at Zaragoza before his appointment as Professor of General and Descriptive Anatomy at Valencia in 1883. Four years later he became Professor of Histology and Pathological Anatomy at Barcelona. While there he went to Madrid where he met Luis Simarro (1851–1921), a psychiatrist who had just returned from working with Ranvier in Paris. Simarro showed him slides of nervous tissue stained with the silver method.

Cajal was immediately captivated: 'Realizing that I had discovered a rich field, I proceeded to take advantage of it, dedicating myself to work, no longer merely with earnestness, but with fury. In proportion as new facts appeared in my preparations, ideas boiled up and jostled each other in my mind. A fever for publication devoured me'. [27] He improved on Golgi's recipe for the silver stain ('slow procedure') with his 'double impregnation procedure' and reduced the time for hardening from one to two months to

two to six days by using a mixture of potassium dichromate and osmic acid ('rapid procedure'). 'This mixture ... also produces staining that is even more delicate than the slow procedure'. [28] Cleverly, Cajal chose to study the brains of small immature animals because there was less myelin – the proteinaceous substance that cossets the nerve fibers within the nerve sheath – to impede the impregnation of the silver. He used a Zeiss apochromatic microscope, the whereabouts of which are now unknown.
On 1 May 1888, his 36th birthday, Cajal launched his own journal *Revista trimestral de Histologia normal y patológica.* The first 10-page article in it is now a neuroscience-literature classic. After studying the cells of the cerebellum of birds he concluded that the axons of the Stellate cells did not anastomose with each other and seemed to end freely; the same applied to the axons of the Purkyně cells. [29]

Towards the end of 1888 Cajal decided that for reasons of language and geography he was too isolated from the main stream of neurological research in Europe, and that the time had come for him to meet other scientists interested in the histology of the nervous system. Their publications, he lamented, '… either did not mention me or did so contemptuously … and without attributing any importance to my opinions'. [30] He applied for membership of the German Anatomical Society and in October 1889 went to their meeting in Berlin. This was a masterstroke. Not only did he meet 'the then world celebrities', but the demonstration of his work that he gave led him later to write: 'Finally, the prejudice against the humble Spanish anatomist vanished and warm and sincere congratulations burst forth'. [31]

Cajal continued his studies of the nervous systems of animals and humans when he went to Madrid as Professor of Histology and Pathological Anatomy in 1892 where he worked until his retirement in 1922. He published more than 200 scientific articles and 22 books, and his last book *Neuron Theory or Reticular Theory* published in 1933 '… finally laid to rest the issue whether the nervous system was formed by structurally independent units or was a more or less continuous syncytial network as a vocal generation of his contemporaries had believed'. [32]

Two Master collators: a Würzburger and a Berliner

In 1852 Wilhelm Engelmann of Leipzig published *Handbuch der Gewebelehre des Menschen für Aertze und Studirende.* The author was Zurich-born Albert Koelliker, Professor of Physiology and Microscopic and Comparative Anatomy of Würzburg. Koelliker updated his text book regularly and the sixth and final edition was published in three volumes in 1889, 1896 and 1902 respectively; the latter in co-operation with Victor von Ebner (1842–1925), Professor of Histology in Vienna. These books were regarded as the best of their genre and were translated into several languages, including English.

Koelliker had a special interest in the histology of the nervous system and quickly recognised the importance of the work of Golgi and Cajal. Koelliker and Golgi corresponded regularly before they met and became lifelong friends. It was at the Berlin meeting in October 1889 that Koelliker first met Cajal who wrote: 'The most interested of my hearers was A. Kölliker, the venerable patriarch of German histology '. Koelliker, who was also editor of the *Zeitschrift für wissenschaftliche Zoologie,* was so impressed by Cajal's work that he learnt Spanish so as to spread the Cajal-news. Later Cajal recalled: 'I am very deeply grateful to the distinguished master of Würzburg … it was due to the great authority of Kölliker that my ideas were rapidly disseminated and appreciated by the scientific world'. [33]

The other collator was Wilhelm Waldeyer (Heinrich Wilhelm Gottfried von Waldeyer-Hartz) (1836–1921), since 1883 Director of the Anatomical Institute of Berlin University. He was the editor of *Deutsche medizinische Wochenschrift* and in six articles published between 29 October and 10 December 1891 he reviewed the newer researches into the micro-anatomy of the central nervous system. In 'Summary and physiological remarks' published on 10 December he wrote: 'All these nerve fibers terminate freely ... without a network or anastomotic formation'. He added: 'The nervous system consists of innumerable nerve units (neurons) which are anatomically and genetically independent of each other. Each nerve unit consists of three parts: the nerve cell, the nerve fiber and the fiber aborizations (terminal aborizations)'. In the article Waldeyer coined the word '*neuron'* for the nerve cell, a name which was almost immediately adopted by other scientists. [34]

A contributor to Waldeyer's pool of information was the young Hamburg-born physiologist Wilhelm Friedrich Kühne (1837–1900) who in 1862 had published a short monograph *Über die Peripherischen Endorgane der Motorischen Nerven.* In it he noted that the nerve – ending of each axon consists of several branches arising by division and that these ended freely and were not in continuity with the muscle fibers. Kühne had a distinguished academic career and in 1871 was appointed Professor of Physiology and Director of the Institute of Physiology at Heidelberg. [35]

Cajal was rather dismissive of Waldeyer, writing that although '… histology is indebted (to him) for revelations of the utmost importance in other fields, (he) *did not personally investigate the problem of interneuronal con-*

nections, confining himself to making a popular review of my works in a German weekly and inventing the word neuron, etc!' (his italics). [36] The Neuron Doctrine was born.

Another new term needed: the turn of the English

Charles Scott Sherrington (1857–1952) in 1897 proposed the term 'synapse'. He had been asked by Michael Foster (1836–1907) to write the section on the nervous system for the seventh edition of Foster's Textbook of Physiology. 'I had begun it, and had not got far with it before I felt the need for some name to call the junction between the nerve-cell and nerve-cell (because the place of junction now entered physiology as carrying functional importance). I wrote to him of my difficulty, and my wish to introduce a specific name. I suggested using '*syndesm*'. He consulted his Trinity friend Verrall, the Euripidean scholar, about it, and Verrall suggested *'synapse'* (from clasp) and as that yields a better adjectival form, it was adopted for the book '. [37][5]

A short message from Stockholm: a Spaniard and an Italian

'I was surprised one morning in October, 1906, by a laconic telegram sent from Stockholm and written in German. It said merely: Carolinische Institut verliehen Sie Nobelpreiss' wrote Cajal in his *Recollections in my Life*. [38] On the morning of 26 October 1906 Golgi received a short telegram: 'Flueckwuensche nobelpreis sie und cajal – Holmgren' from Emil Algot Holmgren (1866–1922), Professor of Histology at Stockholm. [39]

Golgi and Cajal met for the first time in Stockholm on 6 December 1906. The shy and reticent 63 year old Golgi and the ebullient and self- promoting 54 year old Cajal did not hit it off and never became friends. To make matters worse Golgi, in his Nobel Prize Lecture on 11 December, *The neuron doctrine – theory and facts,* defended the Reticular Theory despite the mounting evidence that this was most probably wrong and in spite of some of his own drawings which showed free nerve ending dendrites and axon collaterals. It is clear that Golgi had not kept up with the newer developments in the his-

5. Arthur Woollgar Verrall (1851–1912) was the first occupant of the King Edward VII Chair of English at Cambridge University. Sherrington became famous for his book *The Integrative Action of the Nervous System* of 1906, and was awarded the Nobel Prize for Physiology or Medicine for 1932.

tology of the central nervous system. After a brief sojourn at Siena he returned to his alma mater, Pavia, in 1876 as Professor of Histology, and in March 1881 moved to the more senior post as Professor of General Pathology. He devoted his energies to the establishment of the Laboratory of Experimental Pathology, the teaching of students and postgraduates, the study of the lifecycles of the malarial parasites, his duties as a local and national politician (he became a Life Senator for High Scientific Merits in June 1900) and his spells as Rector of the University.

The next day it was Cajal's turn: *The Structure and Connexions of the Neurons.* 'I set forth the most fundamental results of my (25 years) research work, adhering strictly to the facts and to conclusions naturally suggested by them'. This work, he wrote, '… confirms that the nerve elements possess reciprocal relationships *in contiguity* but not *in continuity'* (his italics). 'My lecture was, I believe, to the taste of the public. In any case, it received very kind praises in the local newspapers '. [40]

Final word: a New Zealander

In 1994, a little more than 100 years after Wilhelm Waldeyer's definition of the neuron, Edward G. Jones (1939–2011), New Zealand – born Director of Neuroscience at the University of California Davis and former President of the Society of Neuroscience, wrote: ' As we look back at the material assembled by Waldeyer, and especially if we consider along with it the additional contributions made almost immediately afterwards by those whom he had quoted, we can make a restatement of the neuron doctrine in the following terms: *The neuron is the structural unit, the embryological unit, the functional unit and the trophic unit of the nervous system '* (his italics). [41] In the 1950s electron – microscopic studies had provided the final proof that there was no continuity between neighbouring neurons.

Endnote: from Cape Town

When one has read, and reread, the texts that form the narrative for this essay, one comes away with considerable respect for the nineteenth century scientists who put so much effort into studying the histology of the neuron, particularly if one considers the makeshift laboratories in which they worked; it was only in the 1860s that the universities in Europe started to provide scientists with purpose-built facilities.

Not only is it the quality of their research that impresses the modern reader, it is also the presentation and clarity of their reports; many of their self-drawn illustrations were stunningly beautiful.
These scientists were multi-talented men and it is extraordinary that some, like Purkyně, continued to produce ground-breaking work despite never-ending social, academic and personal setbacks. [15]

The neuron doctrine was a consequence of a synthesis of two histological techniques, the silver stain and the apochromatic microscope, the contributions of scientists of many nationalities, and the enthusiasm of two master collators who passed on new information as it became available.

However, to end on a sad note. In a long article published by the Royal Society during 2005 – ‘ Observations of synaptic structures: origins of the neuron doctrine and its current status ’ – Rainer Walter (Ray) Guillery (1929 – 2017), who for decades had mentored budding neuroscientists at Chicago, Oxford and Wisconsin universities, mentioned that young scientists did not want to be told anything about the history of the Neuron Doctrine! [42]

The essay is dedicated to Karen Blaauw Helle, Emeritus Professor of Physiology, University of Bergen.

Thank you
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