THE DISCOVERY OF THE NEURON

For most of the nineteenth century, there was an on-going debate among researchers about the organization of the nervous system. One group of researchers, the so-called reticularists, believed that the nervous system consisted of a large network of tissue, or reticulum, formed by the fused processes of nerve cells. The other group, the neuronists, argued that the nervous system consisted of distinct elements, or cells.

Both groups used the same methods to study nerve cells, but came to different conclusions about the fine structure of the nervous system, which could not yet be seen in detail because of the low magnification and poor resolution of the microscopes available to them at the time. Just as the observable universe increased in size with the development of increasingly powerful telescopes, so did understanding of the organization of the nervous system improve with advances in microscopy.

In 1838 Theodore Schwann and Matthias Schleiden proposed that the cell was the basic functional unit of all living things. The Cell Theory was not, however, believed to apply to the nervous system, and it was not until towards the end of the nineteenth century that it became generally accepted that the brain, too, consisted of cells. The discovery of the neuron was a milestone in brain research, and paved the way for modern neuroscience.

Advances in microscopy and improved histological methods meant that nerve cells could be examined in increasing detail, but at the time that the Cell theory was proposed, the relationship between the cell body, axons and dendrites was still unknown, because each of the components of the neuron had only been seen separately.

The complexity and small size of most neurons made elucidating the structure of the neuron the most formidable task in the field of histology, and the debate about the fine structure of the nervous system spanned most of the nineteenth century. But, because of the work of a number of outstanding individuals, the Neuron Doctrine would eventually prevail over reticular theory as the nineteenth century drew to a close.

The popularity of the reticulum theory peaked during the mid-nineteenth century. The main proponent of this theory was Joseph von Gerlach (1820-1896). Gerlach was interested in methods of staining nerve tissue, and used gold chloride or carmine to stain his tissue samples, popularizing the use of the latter among his contemporaries. Based on his observations using these staining techniques, Gerlach concluded that:

the finest divisions of the protoplasmic processes ultimately take part in the formation of the fine nerve fibre network which I consider to be an essential constituent of the gray matter of the spinal cord. The…divisions… are none other than the beginnings of this nerve fibre net. The cells of the gray matter…are therefore doubly connected…by means of the nerve
process which becomes the axis fibre...and through the finest branches of the protoplasmic processes which become a part of the fine nerve fibre net of the gray matter.

There were disputes among the reticularists about the nature of the nerve net. Whereas Gerlach believed that axons and dendrites fused, other investigators argued that only one or the other of the processes formed anastomoses.

In the 1820s, achromatic lenses were developed, and their use in compound microscopes provided investigators with the clearest images yet of tissue samples. One of the first people to use the new compound microscopes to investigate nervous tissue was Johannes Evangelista Purkinje (1787-1869, left), an anatomist born in Libochovice, Bohemia (in what is now the Czech Republic).

Purkinje studied medicine and philosophy at the University of Prague, graduating in 1819. He then wrote a doctoral dissertation on vision before being appointed as professor of physiology at Prague University. As a professor, Purkinje discovered the phenomenon (now known as the Purkinje effect) whereby, as light intensity decreases, red objects are perceived to fade more quickly than blue ones of the same brightness.

Purkinje obtained an achromatic compound microscope in 1832, and began examining nervous tissue and other biological samples. He was the first person to use a microtome to prepare thin sections of nervous tissue for examination under the microscope.
One of Purkinje’s many observations was that dopaminergic midbrain neurons (which we now know degenerate in Parkinson’s Disease) synthesize melanin:

pigmented spots of various shades of brown and of different distribution…are most noticeable in the substantia nigra of the cerebral peduncles…[where] they have processes which exhibit extravagant shapes.

He also calculated the diameter of the bodies of these cells, or “ganglion granules” as he called them, as being “8-30/800 of a Vienna line,” a Vienna line being 1/11th of an inch.

Purkinje made many important discoveries, including the germinal vesicles, sweat glands in the epidermis, and the fibres in the heart which conduct electrical impulses (now known as Purkinje fibres). He also discovered that fingerprints could be used as a means of identification, and created the world’s first physiology department, at the University of Breslau, in Prussia, in 1839.

Purkinje is, however, most famous for discovering the cerebellar cells which bear his name. Because these cells are among the largest in the vertebrate brain, they were the first neurons to be identified.

The low magnification and poor resolution of the microscope used by Purkinje is evident in the crude (yet beautiful) drawing that he presented to the Congress of Physicians and Scientists in Prague, in 1837 (right). He also gave a very accurate description of the morphology of his cells:

Corpuscles surrounding the yellow substance [the junction between gray and white matter] in large numbers, are seen everywhere in rows in the laminae of the cerebellum. Each of these corpuscles faces the inside [of the organ], with the blunt, roundish endings towards the yellow substance, and it displays distinctly in its body the central nucleus together with its corona; the tail-like ending faces the outside, and, by means of two processes, mostly disappears into the gray matter which extends close to the outer surface which is surrounded by the pia mater.
Purkinje’s speculations on the functions of the entities he had discovered suggest that he contributed more to the Neuron Doctrine than he is generally given credit for:

With reference to the importance of the corpuscles…they are probably central structures…because of their whole organization in three concentric circles [i.e. cytoplasm, nuclear membrane and nucleolus] which may be related to the elementary brain and nerve fibres…as centres of force are related to the conduction pathways of force, or like the ganglia to the nerves of the ganglion, or like the brain substance to the spinal cord and cranial nerves. This means they would be collectors, generators and distributors of the neural organ.

Several decades later, further improvements in microscopy enabled Otto Friedrich Carl Dieters (1834-1863) to produce the most accurate description yet of a nerve cell, complete with axon and dendrites (left). Dieters referred to the axon and dendrites as the ‘axis cylinder’ and ‘protoplasmic processes,’ respectively.

Dieters died from typhoid fever, aged just 29. His description of nerve cells from the spinal cord, completed just before his death in 1863, was completed by Max Schultze, and published posthumously in 1865:

The central ganglion cell is an irregular shaped mass of granular protoplasm… the body of the cell is continuous uninterrupted with a more or less large number of processes which branch frequently [and] have long stretches in between…these ultimately become immeasurably thin and lose themselves in the spongy ground substance…these processes [the dendrites]…will hereafter be called protoplasmic processes. A single process which originates either in the body of the cell or in one of the largest protoplasmic processes, immediately at its origin from the cell, is distinguishable from these at a glance.

From this description, it is clear that Dieters easily differentiated between the dendrites and the axon, but that he did not know if the axon arose from the cell body or from the dendritic tree. Dieters could not see the “immeasurably thin”
terminal branches of the dendrites and, like many others, inferred from his observations that they must fuse to form a continuous network. Dieters believed that dendrites, but not axons, could fuse by anastomosis.

Another significant development, apart from the evolution of the microscope, was the use of new methods to stain nervous tissue for examination. Gerlach, the main proponent of reticular theory, had introduced the use of carmine and gelatin, and then gold chloride, to stain tissues for microscopic examination. Although satisfactory, these methods left much to be desired.

A far more useful staining method was discovered by Camillo Golgi (1843-1926, right). Golgi was born in Corteno, a small mountain village in the Italian province of Brescia. He studied medicine at Pavia University, graduating in 1865 aged just 22. In 1872, Golgi took a post as resident physician at a hospital for the chronically ill, in a small town called Abbiategrasso, near Milan.

Golgi discovered his method while working by candlelight in one of the hospital’s kitchens, which he had transformed into a laboratory. The method, now known as Golgi staining or Golgi impregnation, involves hardening of tissue in potassium bichromate and ammonia, followed by immersion in a silver nitrate solution. The Golgi stain visualizes a small number of cells in a tissue sample; the cells are stained at random and in their entirety, so that silhouettes of the cell body, axon and dendrites are clearly visible. In a letter to a friend, he describes the discovery of *la reazione nevo* (“the black reaction”):

> I spend long hours at the microscope. I am delighted that I have found a new reaction to demonstrate…the structure of the interstitial stroma of the cerebral cortex. I let the silver nitrate react with pieces of brain hardened in potassium dichromate. I have obtained magnificent results and hope to do even better in the future.

Despite discovering a technique which stained neurons in their entirety, Golgi thought that his observations confirmed the hypothesis that the nervous system consisted of a continuous network.
If a method of preparation capable of demonstrating the anastamoses of different cells exists, it must be that of black staining…Thanks to this reagent one notes not only the cell body with its primary extension [the axon] clearly revealed, but, in addition, the most delicate ramifications. The use of the black dye can be limited to a small number of cells or to more extensive groups; occasionally one may even obtain a comprehensive view of an entire area of the central nervous system.

In 1873, Golgi published a paper containing descriptions of hippocampal and cerebellar tissue that he had stained using his new technique. In that paper, Golgi reasserts his belief that the nervous system consists of a reticulum: “I was confronted by the concrete fact; this was the existence of the formation which I have called the diffuse nerve network.”

Golgi argued that, because there were so many connections between the nerve cells he had seen in his samples, a law for transmission between nerve cells could not be formulated. Nervous tissue must, then, be composed of a continuous network rather than discrete units. He also believed that only axons fused by anastomosis, and that delivered nutrients from the blood vessels to neurons.

Because of his adherence to reticular theory, Golgi took a holistic approach to brain function. His belief that the nervous system consisted of a reticulum went against the theory of localization of cerebral function, which by then was gaining ground, due largely to the work of early neurologists such as Paul Broca.

Although it turned out that Golgi was mistaken about reticular theory, he was nevertheless a great scientist who made a number of important discoveries, including the identification of projection neurons, interneurons and tendon organs. He also elucidated the part of the Plasmodium life cycle that took place in red blood cells, and correlated the fever and chills, which are symptoms of the disease, with release of the microbe into the blood. In 1898 he identified what he termed the “internal reticular apparatus”; the identification of this structure, which would later be named the Golgi complex, was a
major breakthrough in cytology. The staining method Golgi discovered was of vital significance; using that method, he laid the foundations of the discipline we now call histology.

It was Santiago Ramón y Cajal (1852-1934) who suggested that the neuron was the anatomical and functional of the nervous system, and it is largely because of his work that the Neuron Doctrine eventually came to accepted. Cajal was an outstanding neuroanatomist who is regarded as the father of modern neuroscience. He made many contributions to our understanding of the organization of the nervous system, many of which were later published in *Histologie du système nerveux de l’homme et des vertébrés* (‘Histology of the Nervous System of Man and Vertebrates’).

Born in a small village in Northern Spain, Cajal studied medicine at the Faculty of Medicine in Zaragoza, where he taught histology upon graduating. Cajal examined nervous tissue from embryos, and noticed that the axon and dendrites grow out of the cell body of the neuron. These embryological studies led Cajal to provide the first description of the growth cone.

In 1887, Cajal started using Golgi’s method of staining nervous tissue, and pioneered an improved method, which involved immersing tissues in fixative and silver nitrate a second time. A second immersion stained the tissues more deeply, allowing Cajal to study them in greater detail. In a make-shift laboratory set up in his kitchen, Cajal systematically examined and described nervous tissue from most regions of the brains of various species. In doing so, he was an early pioneer of comparative neuroanatomy.

As a young boy, Cajal had taken to drawing and painting, at which he became very skillful. Later in his life, he would use his artistic skills to produce highly detailed and very accurate diagrams of his observations of nervous tissue, and started his own journal in which he published his findings.

What beauty is shown in the preparations obtained by the precipitation of silver dichromate deposited exclusively onto the nervous elements! But, on the other hand, what dense forests are revealed, in which it is difficult to discover the terminal
endings of its intricate branching… Given that the adult jungle is impenetrable and indefinable, why not study the young forest, as we would say in its nursery stage.

In the passage below, Cajal describes the basket cells in the molecular layer of the cerebellar cortex:

They are small, globular, and irregular…[and] supplied with numerous protoplasmic prolongations [dendrites]. The special character of these cells is the striking arrangement of their nerve filament [axon], which arises from the cell body but also very often from any thick, protoplasmic expansion [dendrite]. It immediately adopts a horizontal position, runs for a considerable distance through the molecular layer, and is supplied with numerous branches, some ascending and others descending. The ascending ones are delicate, and after various ramifications they end in the molecular layer in a manner as yet unknown, but perhaps by terminating freely since we have been unable to find anastomoses between these fibres and the branches of the cylinders [axons] of more superficial cells.
Because Cajal wrote in Spanish, and was isolated from the scientific community, his work received little attention from others in the field. It was perhaps because of this isolation that Cajal was not influenced by the ideas of other investigators. Eventually, he would have his research findings translated, first into French, and then into German.
In 1888, Cajal described a section of chick cerebellum he had stained using his improved Golgi method (below). He observed that the axons in his samples ended in the gray matter, and that their endings were consistent with the location of dendrites. This led Cajal to formulate the law of dynamic polarization, according to which information flows in one direction through a neuron, from the dendrites, through the cell body, to the axon. More importantly, Cajal found no evidence of a reticulum, noting instead that each “nervous element [neuron] is an absolutely autonomous unit.”

The following year, armed with tissue specimens and his beloved Zeiss microscope, Cajal travelled to Berlin to attend a meeting of the German Anatomical Society. It was at this meeting that Cajal met other investigators, some of whom were so impressed by his work that they abandoned their beliefs in the reticular theory.

During his acceptance speech for the Nobel Prize in Physiology, which he was awarded with Golgi in 1906, Cajal reiterated how he had always believed that the nervous system consisted of discrete elements:

Our observations revealed, in my opinion, the terminal arrangement of the nerve fibres. These fibres, ramifying several times, always proceed towards the neuronal body, or towards the protoplasmic expansions around which arises plexuses or very tightly bound and rich nerve nests…. [the] morphological structures, whose form varies according to the nerve centres being studied, confirm that the nerve elements are possess reciprocal relationships in contiguity but not in continuity. It is
confirmed also that those more or less intimate contacts are always established, not between the nerve arborizations alone, but between these ramifications on the one hand, and the body and protoplasmic processes on the other.

It was only during the last decade of the nineteenth century that the terminology we use today was introduced. The term ‘neuron’ was introduced in 1891. The axis cylinder was then named the ‘axon’ by Rudolph von Kolliker, and the protoplasmic processes were called ‘dendrites’ by Wilhelm His. Also during this decade Sir Charles Sherrington described the junction between nerve and muscle, and named it the ‘synapse’ (from the Greek roots syn, meaning ‘together,’ and haptein, meaning ‘to clasp’) in 1897.

The Neuron Doctrine, then, has four tenets:

- The neuron is the fundamental structural and functional unit of the nervous system.
- Neurons are discrete cells which are not continuous with other cells.
- The neuron is composed of 3 parts – the dendrites, axon and cell body, and
- Information flows along the neuron in one direction (from the dendrites to the axon, via the cell body).

In the twentieth century, the invention of the electron microscope would allow researchers to examine nervous tissue in yet greater detail. The electron micrograph on the left shows a neuromuscular junction; clusters of vesicles can clearly be seen docked at the presynaptic membrane, waiting for an action potential to arrive at the motor neuron nerve terminal so that they can fuse with the membrane and release their cargo of neurotransmitters into the synaptic cleft. On the right, a freeze fracture scanning electron micrograph shows vesicles in the process of fusing with the presynaptic membrane.

This new technique led to a refinement of the Neuron Doctrine. Using electron microscopy, researchers could see that nerve cells use not just chemical synapses but also electrical ones (gap junctions). The technique would also reveal the existence of axo-axonic and dendro-dendritic synapses. Electrophysiological recordings would show that voltage-gated ion channels are present on dendrites, and that action potentials could be back-propagated from the cell body to the dendrites.

Even with such advanced techniques, researchers have focused on those parts of the nervous system that are easily accessible, like the neuromuscular junction. Neurons and synapses in the brain are far more difficult to study than those in the periphery. They are undoubtedly far more complex than their peripheral counterparts too.

Neuroscientists today face different challenges from those faced their predecessors, but those problems are no less perplexing. The brain is yet to yield the vast majority of its secrets, and it is unlikely that too many of them will be
revealed in the near future. Ultimately, the limitations of technology will always hinder the advancement of scientific knowledge.