1
History of Neuroscience and the Dawn of Research in Neuroglia

1.1 The miraculous human brain: localising the brain functions

'Many things seem miraculous until you understand them and some are so marvellous you could call them miracles.'

Merlin to young Arthur (Crossley-Holland, 2009)

Human brain and human intellect – these are still miraculous for us. The scientific endeavours driven by human curiosity have deciphered many miracles of nature. Yet our understanding of how we think, and where lies the fundamental mechanism that distinguishes a man from a beast, remains obscure and hazy.

The general concept that brain functions are produced by immensely complex structures localised in the brain parenchyma evolved slowly over history. In the most

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ancient times, the place for sprit, thoughts and cognition was believed to be associated with the heart, and this was considered to be the hegemonic organ by the Hebrews, the Mesopotamians, the Indians, the Egyptians and possibly the Chinese (Gross, 1995). The 'cardiocentric' doctrine was contemplated by ancient Greeks, who were the first to apply logic, scepticism and experimentation to understand the forces that drive the world and life. Possibly, it all began in about the 7th century BC, when Thales of Miletus made the fundamental discovery that our world is mostly made of water, a statement which, at least as far as life is concerned, remains undisputable. Slightly later, Empedocles broadened the list of basic elements of nature to earth, air, fire and water, and Democritus (460–370 BC) introduced the atomic theory, in which all differences between substances was determined by their atoms and inter-atomic relations. More or less at the same time, the idea of a special substance composed of air and vapours, the *thymós* or *pneuma*, which represents the substance of life, came into existence.

The concept of pneuma as the material substance of life, which acts as a vehicle driving all reactions of the body, was formalised by Aristotle (384–322 BC). The pneuma was a sort of 'air' substance that was diffusely present in living organisms; the mind was pneuma and had no specific localisation. According to Aristotle, the pneuma originated from the heart, and the heart was considered to be the primary organ controlling production of pneuma and also the central seat for sensory integration and initiation of movements. The heart was connected to the periphery by vessels and nerves (between which Aristotle made no distinction). The brain, which Aristotle almost certainly dissected, was of a secondary importance. The brain was a cold and bloodless organ; senseless, indifferent to touch, or even to cutting, and disconnected from the body. Most importantly, a brain was absent in many organisms that were able to move and react to the environment. The primary brain function, according to Aristotle, was to cool the pneuma emerging form the heart and thus temper the passions (Aristotle, 1992; Clarke, 1963).

An alternative concept which identified the brain as an organ of cognition was developed in parallel, being initially suggested by Alcmaeon of Croton (6th century BC), who practised dissections; he described the optic nerves and considered them as light guides connecting the eyes with the brain. Democritus suggested the first mechanism of signalling in the body. He thought about the psyche (the substance of soul and mind) as being made from the lightest atoms, which concentrated in the brain and conveyed messages to the periphery. Heavier atoms concentrated in the heart, making it the organ of emotions, and the heaviest in the liver, which therefore was the organ of appetite, gluttony and lust (Gross, 1995).

Plato was very much influenced by the ideas of Democritus and similarly considered the brain as a cognitive organ. The Hippocratic corpus (the assembly of approximately 60 texts on various aspects of medicine likely written by the members of Hippocrates' school in the 5th and 4th centuries BC) contains the treatise *On the Sacred Disease*, which directly identifies the brain as an organ of cognition: '*It ought to be generally known that the source of our pleasure,*

merriment, laughter, and amusement, as of our grief, pain, anxiety, and tears, is none other than the brain. It is specially the organ which enables us to think, see, and hear, and to distinguish the ugly and the beautiful, the bad and the good, pleasant and unpleasant . . . ' (Hippocrates, 1950).

Systematic studies of the brain developed in the first research institute known to humanity – the Museum at Alexandria, organised and funded by Ptolemaeus I Soter (who in this enterprise consulted Aristotle), and further developed under the reign of the Soter's son Ptolemaeus Philadelphus. The Museum employed, on a tenure basis, about 100 professors, who were provided with laboratories for anatomy and dissection, with an astronomical observatory, zoological and botanical gardens and, above all, with a grand library containing hundreds of thousands of manuscripts.

Two leading neuroanatomists of the Museum were Herophilus (335–280 BC) and Erasistratus (304–250 BC) (Von Staden, 1989; Wills, 1999), who performed numerous dissections of the brains of animals and humans, including vivisections on live human subjects – criminals supplied by royal prisons. Herophilus and Erasistratus were the first to describe macroanatomy of the brain and to discover the brain ventricles. Importantly, Herophilus made a distinction (previously unknown) between nerves and blood vessels and classified the nerves as sensory and motor (Longrigg, 1993). Herophilus and Erasistratus were most likely the first to combine Aristotle pneuma with new anatomical findings, and they proposed the cephalocentric ventricular-pneumatic doctrine (although their works did not survive, and we can judge their ideas only after later texts referring to them). For many other aspects of the history of neuroscience and our understanding of the brain, the reader may consult several comprehensive essays (Clarke and O'Malley, 1996; Longrigg, 1993; Manzoni, 1998; Swanson, 2007).

The ventricular-pneumatic doctrine became widespread and was further developed by Claudius Galen of Pergamon (129–200 AD). According to Galen, the substance of intellect and sensations was the 'psychic pneuma', an extremely light (lighter than the air) substance, which acted as a producer and conveyer of thoughts, afferent and efferent signals. The pneuma was not a gas, however, but rather a fluid which filled the ventricles and hollow nerves. In this scenario, the brain acted as a pneuma producer and as a pump maintaining movement of pneuma through the motor nerves and aspiration of pneuma from sensory nerves. At the same time, the nerves, being rigid, provided for a very rapid signal transduction, much as the pulse wave in the blood vessels. The signal transfer between sensory organs and nerves and nerves and effector organs was made possible by the virtue of microscopic pores that allow free exchange of pneuma between the nerves and peripheral tissues (Galen, 1821–1833; Manzoni, 1998). All these flows of pneuma, according to Galen, had specific anatomic routes; for example, the sensory information were delivered to the anterior ventricles, whereas the afferent signals to the muscles originated from the posterior ventricle.

Thus, the psychic pneuma was assigned the central role in neural processes, from sensation to cognition and memory. The process of pneuma formation was,

according to Galen, complex; it went through several stages that involved a specific processing which transferred the inhaled air into the vital spirit. This vital spirit then entered the choroids plexus, through which it eventually reached the ventricles, where the final refinement took place. The brain parenchyma therefore had a purely supportive role, being involved in the production of pneuma, whereas the latter was the true origin of thoughts, sensations, emotions and voluntary movements. These conclusions were experimentally corroborated in experiments on live animals, in which Galen ligated the nerves and selectively compressed different parts of the brain (he believed that by doing so, he affected only the ventricles). The ligation of

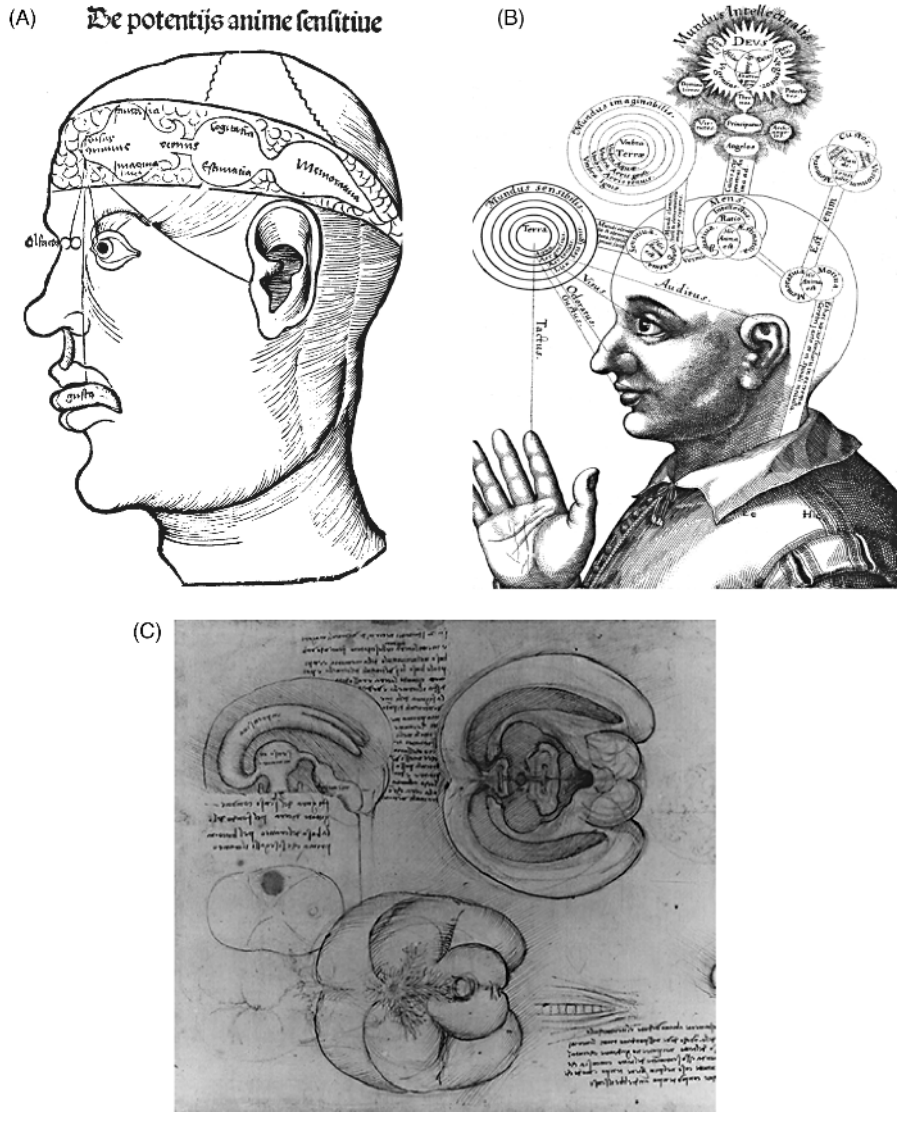


Figure 1.1 (*Continued on next page*)

the nerve, as Galen discovered, led to muscle paralysis; moreover, this process was reversible and removal of the ligature restored muscle contraction. These data were perfectly compatible with an idea of fluid which needed to propagate through the nerve to initiate contraction.

In his experiments on the brain, Galen further found that compression of anterior ventricle caused blindness, whereas compression of the posterior ventricle resulted in paralysis (*De anatomics administrationibus* – cited from Manzoni, 1998). Moreover, he discovered that surgical lesions of the *pia mater* or brain parenchyma did not cause immediate effects unless the ventricle was opened. The damage to the ventricles resulted either in serious sensory deficits (anterior ventricle) or in collapse and death (middle and posterior ventricles). According to Galen, the mechanism concerned was simple – opening of the ventricles led to the escape of psychic pneuma that rendered the brain incapable of performing its functions.

The ventricular-pneumatic doctrine became generally accepted and, with many modifications accumulated during centuries (for a comprehensive account see Manzoni, 1998), it dominated brain physiology through Middle Ages and the Renaissance (Figure 1.1). The main modifications of Galenic neurophysiology were represented by further attempts to localise brain function. In the Middle ages, Arabic (e.g. Avicenna and Averroes), and European (e.g. Albertus Magnus, Tomas Aquinas and Roger Bacon) anatomists and medics associated different faculties of the nervous system with distinct ventricles. Most often, the anterior ventricle was described as a place for sensory inputs and the middle ventricle provided for creative

Figure 1.1 Localisation of brain functions in the framework of pneumatic-ventricular doctrine.

A–C. The three-cell concept that divided localisation of functions between different ventricles and assumed sequential information processing from the first ventricle, which receives the sensory input, to the third, which commands the behaviour.

A. The conceptual scheme of Albertus Magnus in the later version from the 16th century, made by Gregorius Reisch, who was the Prior of House of Carthusians at Freiburg and confessor to the Emperor Maximilian, and who published a concise encyclopaedia of knowledge in 1503 (Reisch, 1503).

B. The much-elaborated scheme from another encyclopaedic book (from the chapter 'The Art of Memory') by English Paracelcian physician Robert Fludd (Fludd, 1617–1621).

C. Cerebral ventricles (ox brain), as seen by Leonardo da Vinci in about 1508. In the small drawing on the right, the syringe can be seen inserted into the floor of the third ventricle, which has expanded somewhat with the pressure. The foramen of Monro, linking the lateral ventricles to the third ventricles, can be seen, as can the aqueduct of Sylvius and the two lateral and the fourth ventricles. In the upper left figure, Leonardo expands the drawing and adds the words '*imprensiva*' in the lateral ventricles, '*senso comune*' in the third ventricle, and '*memoria*' in the fourth. The upper right drawing shows the ventricles from below and the lower left drawing shows the base of the brain, demonstrating the arterial network called the '*rete mirabile*'. (da Vinci, 1978–1980).

imagination, cognition and intellect, whereas the posterior ventricle was a seat for memory (see a comprehensive account written by Manzoni, 1998).

A multitude of scholars throughout Europe (e.g. Petrus Montagnana, Lodovico Dolce, Ghiradelli of Bologna and Theodor Gull of Antwerp) produced their own mapping of brain functions within the ventricles. Leonardo da Vinci, for example, believed in the central role of the middle ventricle, where both soul and judgement dwell (Figure 1.1): '*The soul seems to reside in the judgment, and the judgment would seem to be seated in that part where all the senses meet; and this is called the* senso commune.' (cited from Pevsner, 2002).

Leonardo placed the memory into the posterior ventricle, and the anterior ventricles were responsible for interfacing the sensory inputs with *senso commune*, the function defined as '*imprensiva*' (Pevsner, 2002). Leonardo was the first to make an accurate image of the brain ventricles (Figure 1.1), by filling them with melted wax, thus obtaining their precise cast (da Vinci, 1978–1980): '*Make two vent-holes in the horns of the greater ventricles, and insert melted wax with a syringe, making a hole in the ventricle of memory; and through such a hole fill the three ventricles of the brain. Then when the wax has set, take apart the brain, and you will see the shape of the ventricles exactly*.' (cited from Pevsner, 2002; see also Del Maestro, 1998; Woolam, 1952).

Andreas Vesalius placed the *senso commune* in the anterior ventricle, whereas middle and posterior ventricles were respectively associated with intellect and memory (Vesalius 1543). It was Vesalius who made the most detailed drawings of the peripheral nervous system (Figure 1.2).

Final tuning of the ventricular-pneumatic doctrine was made by René Descartes (1596–1650), who regarded the body as a machine and proposed a mechanical theory of nerve propagation, according to which peripheral stimulation triggered mechanical displacement of nerves that almost immediately caused the central end of the nerve to twitch, resulting in the release of 'animal spirit' or 'a very fine flame' (Descartes, 1664). He also introduced the concept of automatic reflexes, which highlighted the rapidity of signal propagation through the nervous system.

Probably the first neuroanatomists who realised that brain functions are associated with the organ parenchyma, and even more specifically with the grey matter, were Marcello Malpighi (*Epist. de cerebro et cort. Cereb. ad Fracassatum* – Malpighi, 1687) and Thomas Willis (Willis, 1672). This conceptual change induced further interest in localising the brain functions. Starting from the 1780s, the works of Georg Prochaska (Prochaska, 1784), followed by prolific writings of Franz Joseph Gall, Johann Gaspard Spurzeim and George Combe (Combe, 1847; Gall, 1835; Gall and Spurzheim, 1810–1819; Spurzheim, 1826), gave birth to phrenology (literally the 'science of mind'). The term 'phrenology' was introduced by Thomas Ignatius Forster; initially this theory was called 'organology' and was also known as 'craniology' or 'physiognomy' – Macalister, 1911).

Phrenology developed rapidly and gained amazing popularity, especially in America, because of the efforts of the Fowler brothers and Samuel Wells

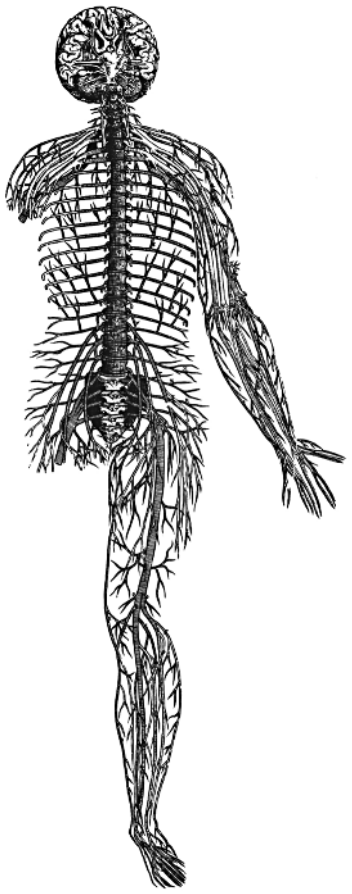


Figure 1.2 The adult human nervous system as seen from the front, with the brain tilted upward to expose the cranial nerve roots emerging from the base, drawn by Andreas Vesalius in the mid-16th century (Vesalius, 1543).

(Fowler & Fowler, 1875; Wells, 1894). Phrenology assigned a multitude of functions to various regions of the brain, which (it was assumed) were mirrored by the surface of the skull (Figure 1.3). This assignment, however, was based on purely empirical observations of the behaviour of different people. Nonetheless, phrenology introduced a fundamental notion that specific functions may be associated with specific regions of the brain, which initiated a further quest for anatomical correlates of these different functions. Ideas of morphological and functional segregations of the brain regions were developed by Luigui Rolando, who was the first to make direct electrical stimulation of brain structures in search for primary motor areas (Caputi *et al.*, 1995).

The idea of functional sub-divisions of the brain was not generally accepted at the time and much opposition was mounted by the most respected neurophysiologists,

Franz Joseph Gall (1758 - 1828)

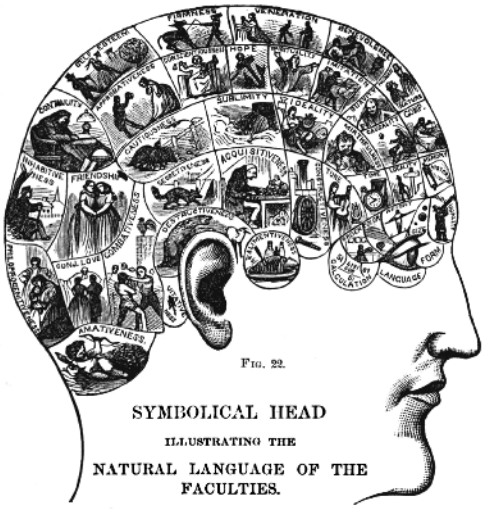


Figure 1.3 Cortical localization of functions according to phrenology.
Portrait of Franz Joseph Gall (1758–1828) and phrenological chart according to Samuel Wells (Wells, 1894).

such as Pierre Frourens, François Magendie and Johannes Müller (Frourens, 1846; Müller, 1838–1842), who all believed that the brain functions as a single organ. Even if they were prepared to give some allowances for motor centres (as Johannes Müller did), they believed that the mind and will and thoughts were the product of the entire organ. The heated discussions on the topic of cortical localization were instrumental in inspiring Paul Broca to search for functionally distinct brain areas. This led to the discovery of the Broca area in the posterior-inferior part of the frontal cortex of the dominant hemisphere – the area that controls the exclusive human function of articulate speech (Broca, 1861).

Nine years later, the first electrophysiological mapping of the motor cortex of the dog was performed by Gustav Theodor Fritsch and Edward Hitzig (Fritsch & Hitzig, 1870), who demonstrated that stimulation of certain areas produced specific motor reactions; in all, they found five distinct motor centres. These first experiments were followed by the truly systematic and comprehensive research of David Ferrier, who developed the first advanced map of functional speciality of various brain regions, including motor and sensory (vision, hearing and taste) areas (Ferrier, 1875, 1876, 1878, 1890). Ferrier and many of his contemporaries, including Charles Sherrington, interpreted these findings as a basis for 'scientific phrenology'.

Incidentally, Ferrier's experiments on primates almost led him to jail, when the Victoria Street Society for the Protection of Animals from Vivisection brought charges for 'frightful and shocking' experiments, using as a legal pretext "The

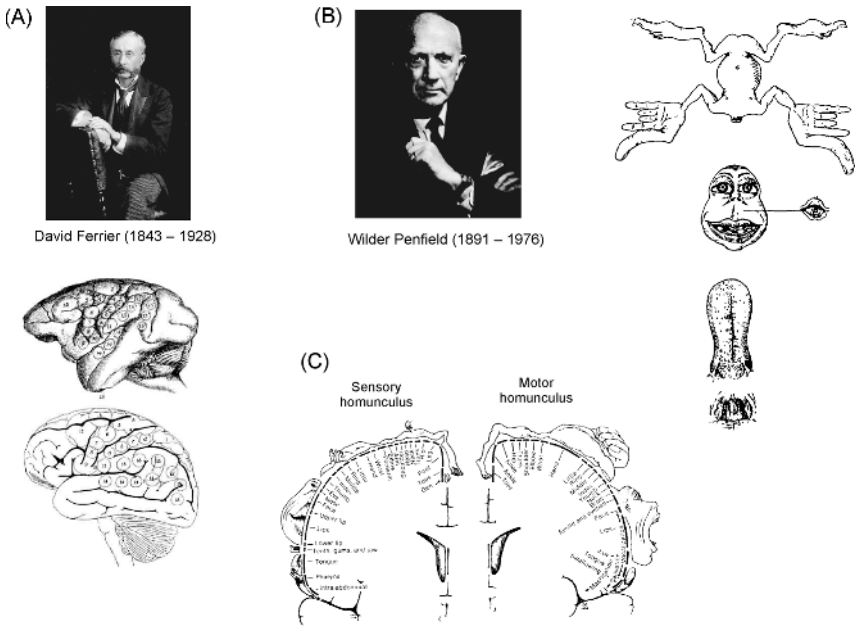


Figure 1.4 The sensory-motor mapping of the brain.

A. Cortical mapping of the monkey made by David Ferrier.

B. The original homunculus, as drawn by Wielder Penfield and Edwin Bouldrey (Penfield & Bouldrey, 1937). In the figure legend, they wrote:

> *'Fig. 28. Sensory and motor homunculus. This was prepared as a visualization of the order and comparative size of the parts of the body as they appear from above down upon the Rolandic cortex. The larynx represents vocalisation, the pharynx swallowing. The comparatively large size of thumb, lips and tongue indicate that these members occupy comparatively long vertical segments of the Rolandic cortex, as shown by measurements in individual cases. Sensation in genitalia and rectum lie above and posterior to the lower extremity but are not figured.'*

C. The modern view of sensory-motor homunculus as represented in textbooks.

Cruelty to Animals Act of 1876" (Fishman, 1995). The medical community mounted a passionate defence[1] and finally the charges were dropped.

The mapping of the brain continued until Wilder G. Penfield accomplished the task of identifying the sensory and motor cortical representations and introduced the widely accepted 'homunculus' to visualise them graphically (Figure 1.4; Penfield & Bouldrey, 1937; Penfield, 1986).

[1] Summons under the Vivisection Act: *British Medical Journal* 1881; **2**, 752. The antivivisection prosecution. *British Medical Journal* 1881; **2**, 785. Dr. Ferrier's localisations; for whose advantage? *British Medical Journal* 1881; **2**, 822–824. Correspondence. Proposed subscription to Dr. Ferrier. *British Medical Journal* 1881; **2**, 834. The Charge against Professor Ferrier under the Vivisection Act: Dismissal of the Summons. *British Medical Journal* 1881; **2**, 836–842.

The contemporary developments of *in vivo* imaging techniques will, without doubt, result in a 'new scientific phrenology', and it is exceedingly interesting to compare the brain maps constructed with Positron Emission Tomography (PET), Computerized Axial Tomography (CAT) or Nuclear Magnetic Resonance (NMR) with the original functional distribution proposed by Gall, Spurzheim, Combe and Fowlers.

1.2 Cellular organisation of the brain

'Omnis cellula e cellula'

This aphorism, attributed by some to François-Vincent Raspail[2], by many to Rudolf Virchow, and by others to Robert Remak (Baker, 1953), is an epitome of the biological revolution of the 19th century, which begun with the identification of the cellular nature of life, and brings us to a theoretical understanding of evolution and the genetic code.

The concept postulating the existence of the elementary units of life, from which all tissues and organisms are formed, appeared in the early 17th century in writings of several philosophers, most notably Pierre Gassendi and Robert Boyle. The origins of cellular theory are rooted in the discoveries of the first microscopists. The very first microscope is believed to be created by Zacharias Janssen in about 1595 (it is likely that his father, Hans Janssen, was involved, too). According to the general view, Janssen invented both single-lens and compound microscopes.

Microscopes were initially used for microscopic observations of plants, and it was Robert Hooke who, when examining the fine structure of cork, visualised the regular structures that reminded him of the monk's cells in the monastery dormitories, and thus the term 'cell' was born (Hooke, 1665). The first animal cells were discovered, in all likelihood, by Antonius van Leeuwenhoek who, in his many letters to the Royal Society, described bacteria (and named them *animalcules* or little animals) and erythrocytes, observed single muscle fibres, followed the movements of live spermatozoids and was the first to see the regular structure (representing single axons) in sagittal slices of peripheral nerves (Figure 1.5A; Bentivoglio, 1996; Leeuwenhoek, 1673–1696, 1798). Leeuwenhoek reflected on the latter observation made in 1717: '*Often and not without pleasure, I have observed the structure of the nerves to be composed of very slender vessels of*

[2] This seems to have become a popular general belief; and is stated in numerous papers, e.g. Tan SY & Brown J. (2006). Rudolph Virchow (1821–1902): 'pope of pathology'. *Singapore Medical Journal* **47**(7), 567–568; Wright NA & Poulsom R. (2012). Omnis cellula e cellula revisited: cell biology as the foundation of pathology. *Journal of Pathology* **226**(2), 145–147; and even in Wikipedia (http://en.wikipedia.org/wiki/François-Vincent_Raspail). The reference to original Raspail writing given in these sources (a paper of Raspail on the development of starch in the grains of wheat – Raspail FV. (1825). Developement de la fecule dans les organes de la fructification des cereales. *Annales Des Sciences Naturelles* **6**, 224) does not contain the sentence in question; we failed to find any original text written by Raspail which contains the phrase.

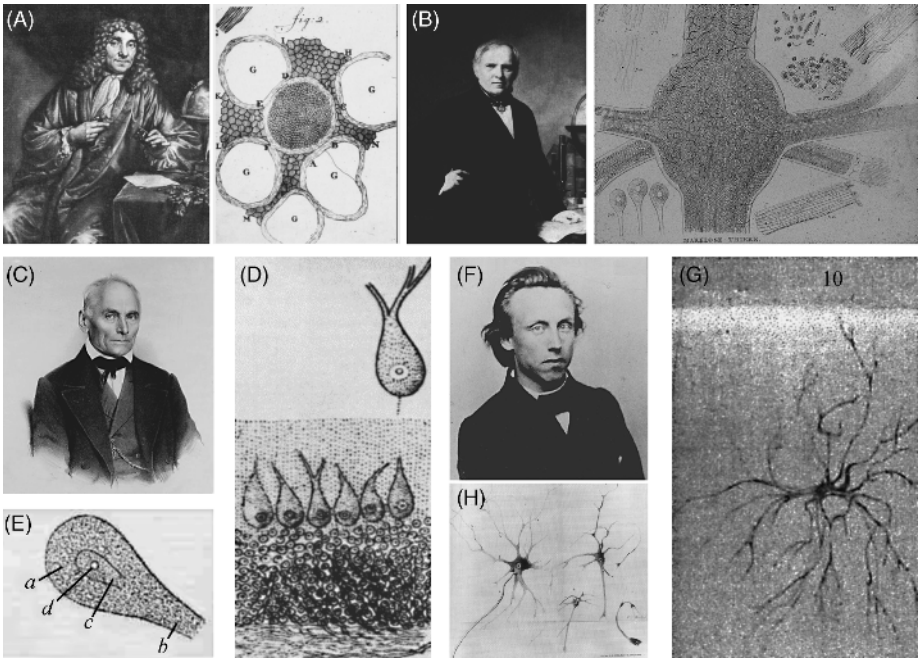


Figure 1.5 First images of neural cells.

A. Antonius van Leeuwenhoek (1632–1723) and his drawing of a '*small Nerve (BCDEF)*', composed by many 'vessels' in which '*the lines or strokes denote the cavities or orifices of those vessels. This Nerve is surrounded, in part, by five other Nerves (GGGGG)*' in which only 'external coats' are represented. The image was kindly provided by Prof. Marina Bentivoglio, University of Verona.

B. Christian Gottfried Ehrenberg (1795–1876) and his image of the nerve cell of the leech (Ehrenberg, 1836) (kindly provided by Professor Helmut Kettenmann, Max Delbruck Center for Molecular Medicine, Berlin).

C, D. Johann Evangelista Purkinje (1787–1869) and the first drawings of the Purkinje neurone made by him for the Congress of Physicians and Scientists Conference in Prague, in 1837. The image was kindly provided by Prof. Helmut Kettenmann.

E. The first published drawing of a neurone, made by Gustav Gabriel Valentin (1810–1883) (Valentin, 1836).

F, G, H. Otto Friedrich Karl Deiters (1834–1863) and his drawings (Deiters, 1865) of motoneurones and 'connective tissue cells' (astrocytes). The images were kindly provided by Prof. Helmut Kettenmann.

an indescribable fineness, running lengthwise to form the nerve.' (cited from Bentivoglio, 1996).

Slightly later, Felice Gaspar Ferdinand Fontana also observed the fine cylindrical nerve fibres that were mechanically dissected from a nerve and observed at 700× magnification (Bentivoglio, 1996). In 1824, Henri Milne-Edwards identified

the basic life unit as 'globule' and, at the same time, Henri Dutrochet made a statement that cells are morphological and functional units of life, and that 'everything is ultimately derived from the cell' (Harris, 1999). Around 1830, Robert Brown defined the nucleus (Ford, 1992), although the first description of the nucleus was made by Franz Bauer, in 1802. Cell division was discovered (in plants) by Barthelemy Dumortier in 1832, and the cellular theory was formalised by Theodor Schleiden and Matthias Jakob Schleiden (Schleiden, 1838; Schwann, 1839; Schwann and Schleiden, 1847).

Early observations of nerve cells were made in the 1830s. Probably the very first descriptions were made by Christian Gottfried Ehrenberg (Figure 1.5B), who was investigating the nervous system of the leech (Ehrenberg, 1836), and by Johann Evangelista Purkyňe (or Purkinje in English transcription) (Figure 1.5C, D), who was studying the cerebellum and described the cell named after him (Purkinje, 1837). Purkinje's pupil, Gustav Gabriel Valentin (1810–1883), made the first published drawing of the neurone (Figure 1.5E), where the nucleus and other intracellular structures were visible (Valentin, 1836).

Purkinje and Valentin named the cells they observed *kugeln* or globules and, in 1845, Robert Todd called them cells: '*The essential elements of the grey nervous matter are "vesicles" or cells, containing nuclei and nucleoli. They have also been called nerve or ganglion "globules"*.' (Todd, 1845, p. 64). In 1838, Robert Remak made the description of nerve fibres and visualised the covering sheath around them (Remak, 1838).

Several types of neuroglial cells (see below) were described by Heinrich Müller, Max Schulze and Karl Bergmann. In 1862, the neuro-muscular junction was described by Wilhelm Friedrich Kühne, who named it the '*endplate*' (Kühne, 1862). Slightly later, the very detailed images of both neurones and stellate glial cells (probably astrocytes – Figure 1.5F, G, H) were made by Otto Deiters (Deiters, 1865). Deiters tragically died very young at 29 from typhoid fever.

It has to be kept in mind that these early cellular images were done mostly on unstained preparations, following painstaking isolation of cells by microsurgery. The histological revolution occurred in 1873, when Camillo Golgi developed the silver-chromate staining technique (the famous '*reazione nera*' or black staining – Golgi, 1873, 1903) which, for the first time, allowed neurohistologists to obtain images of neural cells in their entirety (Figure 1.6; for a comprehensive and vividly written account of Golgi's life and research, see Mazzarello, 2010).

The end of the 1880s marked the arrival of the neuronal doctrine, very much driven by the efforts of Santiago Ramón y Cajal. Cajal's first papers dedicated to the fine structure of the nervous system begun to appear in 1888, about one year after he learned the black staining technique. With characteristic determination and originality, Cajal made a special journal for his papers, the *Revista Truimestral de Histologia Normal y Patologica*, of which he naturally became the editor-in-chief, and the first issues of this journal were almost entirely occupied by his papers (Mazzarello, 2010).

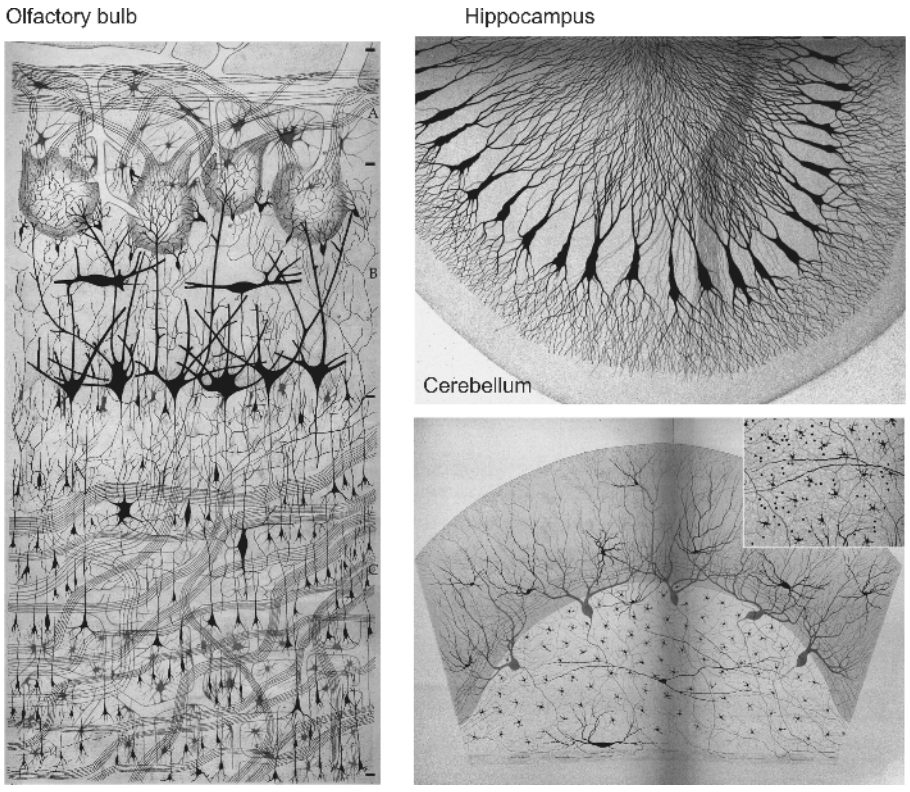


Figure 1.6 Neural cells stained by Golgi's *reazione nera* or black stain reaction. Reproduced from Golgi, 1875, 1903. Images were kindly provided by professor Paolo Mazzarello, University of Pavia. (Full colour version in plate section.)

In the first paper published in the first issue of his journal, Cajal made the seminal statement that '*each nerve cell is a totally autonomous physiological canton*' (Ramón y Cajal, 1888). One year later, in October, 1889, Cajal attended the German Anatomical Congress, where he demonstrated his numerous microscopic preparations to the delegates; this instantly made his international reputation.

In 1891, Heinrich Wilhelm von Waldeyer, a great admirer and follower of Cajal, coined the term '*neurone*' (von Waldeyer, 1891) and the neuronal doctrine began to conquer the minds of neuroscientists. The neuronal processes received the name of '*dendrites*', introduced by Wilhelm His in 1889, and the principal process was named '*axon*' by Alfred Kölliker in 1896.

The first general theory about the brain's functional organisation was introduced by Josef von Gerlach (Gerlach, 1871), who proposed that neurofilaments and neural cell processes are internally connected through anastomoses and form a diffuse network that represents the substrate for brain function. This '*reticular*' theory dominated neuroscience for a good 20 years and recruited many supporters, including Camillo Golgi, who was very much convinced of the existence of a '*diffuse neural net*'. Other

proponents of the reticular theory included prominent figures such as Albert von Kölliker, Max Schulze, Istvan Apáthy, Hans Held, Sigmund Freud and many others. The history of the neuronism-reticularism conflict was widely popularised, and the curious reader may find all the dramatic details and read about the many participants of the struggle in several comprehensive treatises (Jacobson, 2005; Lopez-Munoz *et al.*, 2006; Mazzarello, 2010; Ramón y Cajal, 1933; Shepherd, 1991).

1.3 Mechanisms of communications in neural networks

'Vengo attaccato da due sette opposte – gli scienziati e gli ignoranti. Entrambi ridono di me, chiamandomi "il maestro di danza delle rane". Eppure io so di avere scoperto una delle piùgrandi forze della natura.'

Luigi Galvani (Galvani, 1841)[3]

The notion that there is some substance(s) involved in signal propagation through nerves and between nerves and peripheral tissues, as well as within the brain, has its roots in the ventricular-pneumatic doctrine. Indeed, both Galenic and Cartesian writings describe the 'pneuma' diffusing through the nerves and then being released, either from the peripheral nerve endings to drive muscle contractions, or aspirated from sensory nerves into the ventricles to mediate sensations and drive higher brain functions.

René Descartes introduced a sophisticated mechanistic theory of this 'neurotransmission', contemplating the flow of minuscule particles in the ventricles, from which they diffuse through the multiple pores in the internal surface of the brain and then leave towards the periphery through the nerves. The nerves, in turn, are endowed with a system of valves which allow release of the said particles onto muscles, where they are picked up by a congruent valve system localised in the muscular fibres, thus regulating contraction (Descartes, 1664). The substances for neuronal excitability and communication in the nervous system were found much later and, indeed, they are represented by small molecules – ions and neurotransmitters.

1.3.1 Electrical/ionic nature of excitability

The first experimental preparation for use in physiology (and which was to be the most widely used) was introduced in the 1660s, when Dutch microscopist and natural scientist Jan Swammerdam (Cobb, 2002) developed a neuro-muscular preparation (Figure 1.7). Swammerdam used the frog leg, from which '*one of*

[3] *'I am attacked by two very opposite sects – the scientists and the know-nothings. Both laugh at me – calling me "the frogs' dancing-master." Yet I know that I have discovered one of the greatest forces in nature'*. English translation Atkinson WW. 1907. The secret of mental magic: A course of seven lessons. Chicago: Authors edition. 1–441 p., p. 20.

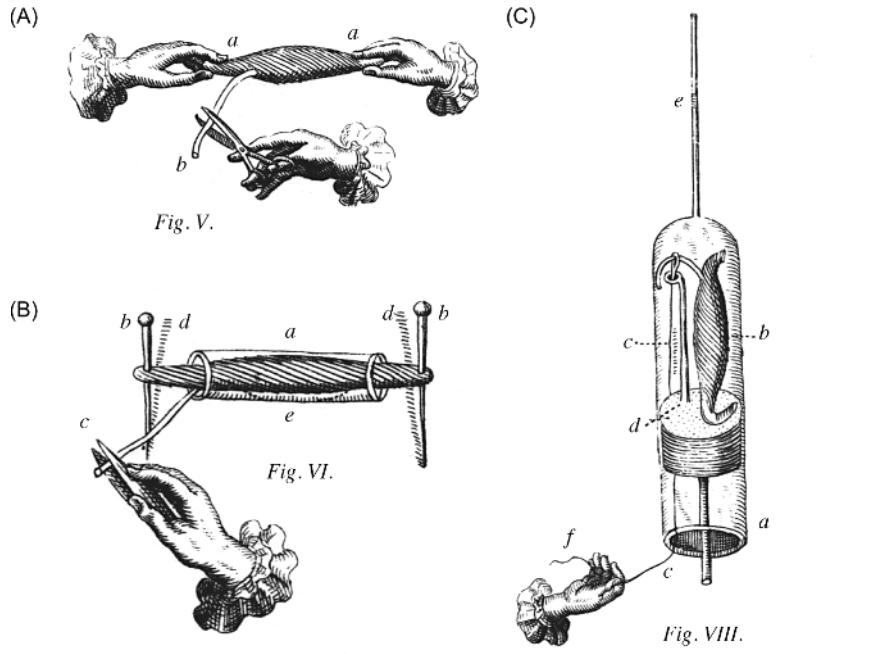


Figure 1.7 Neuro-muscular preparations of Jan Swammerdam, with his original descriptions (Swammerdam, 1758).

A. '*If* [. . .] *you take hold, aa, of each tendon with your hand, and then irritate b the propending nerve with scissors, or any other instrument, the muscle will recover its former motion, which it had lost. You will see that it is immediately contracted, and draws together, as it were, both the hands, which hold the tendons*.'

B. '*If we have a mind to observe, very exactly, in what degree the muscle thickens in its contraction, and how far its tendons approach towards each other, we must put the muscle into a glass tube, a, and run two fine needles bb through its tendons, where they had been before held by the fingers; and then fix the points of those needles, neither too loose nor too firmly, in a piece of cork. If afterwards you irritate, c, the nerves, you will see the muscle drawing dd the heads of the needles together out of the paces; and that the belly of the muscle itself becomes considerably thicker e in the cavity of the glass tube, and stops up the whole tube, after expelling the air. This continues till the contraction ceases, and the needles then move back into their former places*.'

C. The stimulation of neuro-muscular preparation by silver wire: '*a) The glass tube, or siphon. b) The muscle. c) A silver wire with a ring in it, through which the nerve passes. d) A bras wire . . . through which the silver wire passes. e) A drop of water in glass tube. f) The hand that irritates the nerve, in consequence of which irritation the drop on the muscle, contracting itself, descends a little*.'

Images and quotations were kindly provided by Dr. Mathew Cobb, University of Manchester. see also Cobb, 2002.

the largest muscles be separated from the thigh of a Frog, and, together with its adherent nerve, prepared in such a manner as to remain unhurt' (Figure 1.7A; Cobb, 2002; Swammerdam, 1758). Stimulation (which Swammerdam called '*irritation*') of the nerve triggered muscle contraction. Subsequently, he further perfected the preparation, by inserting the muscle into a glass tube and attaching needles to each of the muscle ends (Figure 1.7B). The contraction, initiated by nerve stimulation, could therefore be monitored via the movements of needles and, in principle, these needles could be used for contraction recording (e.g. on charcoaled paper – although we do not know whether such recordings were ever made). Moreover, in one of his experiments, the nerve was fixed by a brass ring and the 'irritation' was done by a silver wire (Figure 1C) – an arrangement that could cause true electrical stimulation (Cobb, 2002; Stillings, 1975).

Swammerdam came close to understanding the nature of signal propagation between nerves and muscles, but it was Isaac Newton who first contemplated the electrical nature of nerve signals. Newton introduced the idea that: '*electric bodies operate to greater distances . . . and all sensation is excited, and the members of animal bodies move at the command of the will, namely, by the vibrations of this spirit, mutually propagated along the solid filaments of the nerves, from the outward organs of sense to the brain, and from the brain into the muscles. But these are things that cannot be explained in few words, nor are we furnished with that sufficiency of experiments which is required to an accurate determination and demonstration of the laws by which this electric and elastic spirit operates*.' (Newton, 1713). Several other scientists contributed to the elevation of neuro-electrical theories in the 18th century; most prominent was Tommaso Laghi, who surmised the flow of some '*electrified*' substances through the nerve, these latter substances also initiating muscle contraction (Bresadola, 1998).

Experimental support for the electric nature of nerve impulses was furnished in Bologna, and the story of electrophysiology, ion channels and ionic nature of excitability began in 1791, when Luigi Galvani published his fundamental work, *De Viribus Electricitatis in Motu Musculari Commentarius* (Galvani, 1791), on animal electricity. This was the result of 10 years of experimentation on isolated frog nerve-muscle preparations, in which Galvani was assisted by his wife, Lucia Galeazzi and his nephew, Giovanni Aldini.

Initially, Galvani used his version of the nerve muscle preparation (Figure 1.8), which consisted of the inferior limbs with the crural nerves, connecting the spinal cord with the limbs, fully exposed, and a metal wire was inserted across the vertebral canal (Galvani, 1791, 1841; Piccolino, 1997, 1998). Using this preparation, Galvani identified electrical excitation of the nerve-muscle preparation, found the relationship between stimulus intensity and muscle contraction (the latter showed saturation, i.e. increasing the intensity of stimulation above a certain strength did not result in an increased magnitude of contraction), and described the refractory phenomenon by showing that repeated stimulation leads to disappearance of contractions, which can be restored after a period of rest.

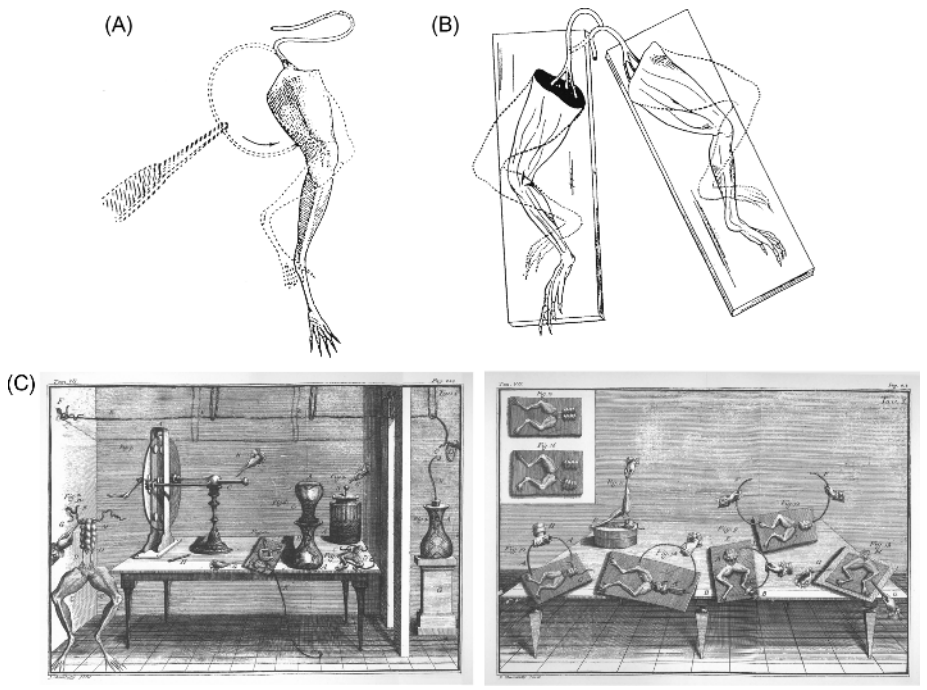


Figure 1.8 Galvani experiments of muscle contraction without metals (Galvani 1841).

A. The 1794 experiment. When the surface of a section of nerve touches the muscle, the leg contracts.

B. The 1797 experiment. When the surface of a section of the right sciatic nerve touches the intact surface of the left sciatic nerve, both legs contract.

C. Plate I of the *Commentarius* shows the frog preparation and the electric machine; Plate III of the *Commentarius* shows the experiments with metallic arcs (Galvani, 1791).

Images were kindly provided by Professor Marco Piccolino, University of Ferrara.

The crucial experiments, however, were performed in 1794–1797 (Galvani, 1841), when Galvani used two frog legs with long sciatic nerves attached (Figure 1.8). When the nerve of the first preparation was in contact with the nerve or muscle of the second, contraction occurred in both preparations. This was the first demonstration of a propagating action potential. Based on his experimental achievements, Galvani developed the theory of electrical excitation. First, he realized that biological tissues exist in a state of '*disequilibrium*' i.e. at rest the tissue is ready to respond to external stimuli by generating electrical signals. Even more importantly, Galvani postulated that '*animal electricity*' results from accumulation of positive and negative charges on external and internal surfaces of the muscle or nerve fibre, which he compared to the internal and external plates of the Leyden jar (Galvani, 1794; Piccolino, 1997).

The electrical current flow that occurs during excitation required a specific pathway, and Galvani contemplated the existence of water-filled channels which penetrate the surface of the fibres and allow electrical excitability. Again comparing the biological tissue to a Leyden jar, he wrote: ' . . . *let one plaster then this conductor with some insulating substance, as wax . . . let one make small holes in some part of the plastering that concerns the conductor. Then let one moist with water or with some other conductive fluid all the plastering, having care that the fluid penetrate in the above mentioned holes, and come in contact with the conductor itself. Certainly, in this case, there is communication through this fluid between the internal and the external surface of the jar*.' (Galvani, 1794, quoted from Piccolino, 1997). This was a very clear model of an aqueous channel penetrating the membranous structure.

Galvani's findings resonated rapidly throughout the world. First, they inspired a fierce fight with Alessandro Volta, who vehemently opposed the concept of animal electricity (Volta, 1918). Volta's experiments, although proven wrong as far as biology was concerned, resulted in fundamental discoveries in the general theory of electricity and the invention of the electric battery in the 19th century. More importantly, however, the idea of galvanism became a cultural phenomenon and spread throughout Europe with lightning speed.

Particularly illustrious were the demonstrations of Giovanni Aldini, who, after the untimely death of Galvani in 1798, continued investigations of animal electricity. In 1803–1804, Aldini published important books, which combined the ideas of Galvani and Volta and made a coherent theory of electrical excitation of biological tissues (Aldini, 1803, 1804). He also made the most exciting electrical stimulations of body parts of freshly executed criminals, which made a huge impact on the general public. So invigorating was the theory of galvanism that, in 1817, it inspired Mary Shelley to write her novel *Frankenstein, or the Modern Prometheus*, which for the first time addressed the problem of the responsibility of the scientist for the products of his mind and hands.

Besides these demonstrations, Aldini made many other fundamental observations. In particular, he was the first to apply electrical currents to mammalian brains and he found that stimulation of the corpus callosum and cerebellum triggered pronounced motor responses (the experiments were done on the ox brain *in situ*, with the skull opened and all brain-spinal cord connections remaining intact (Aldini, 1803; 1804).

The first instrumental recording of animal electricity (using the frog neuromuscular preparation), was made by Leopoldo Nobili in 1828, with the aid of an electromagnetic galvanometer (Nobili, 1828), although Nobili interpreted this recording in strictly physical terms, suggesting that he was measuring a thermoelectrical current resulting from unequal cooling of the two ends of the preparation. Several years later, in 1842, Carlo Matteucci repeated this experiment and demonstrated that the galvanometer reading was the exclusive consequence of currents generated by the living tissue (Matteucci, 1842). Furthermore, he succeeded in measuring the resting current between the intact and cut surfaces of the muscle.

The next step was made by Emile du Bois-Reymond, who was able to measure electrical events accompanying the excitation of nerve and muscle, and who realized that excitation greatly decreases the potential difference between the intact surface and the cut portion of the tissue; hence, he called the excitatory electrical response the '*negative Schwankung*' (negative fluctuation) (du Bois-Reymond, 1884).

In 1850–1852, another fundamental discovery was made by Hermann von Helmholtz, who, using the nerve-muscle preparation, determined the speed of nerve impulse propagation by measuring the delay between the application of an electrical stimulus and the muscle contraction (Helmholtz, 1850). Furthermore, Helmholtz, for the first time, used a smoked drum to record muscle contractions (Helmholtz, 1852). To measure the velocity of nerve impulse propagation, he used a technique developed by Claude Pouillet, who found that galvanometer excursions induced by brief pulses of current were proportional to the pulse duration (Piccolino, 2003) (incidentally, this technique was successfully used in military practice for determining the speed of cannon balls). By using this method, Helmholtz was able to determine the delay between electrical stimulation of the nerve and muscle contraction, a delay, which he rather poetically defined as '*le temps perdu*' (the lost time) (Piccolino, 2003).

The speed of nerve impulse propagation measured by Helmholtz caused some confusion: the values of the propagation velocity were in the range of 25–40 m/s, which was much slower than the propagation of electric current. It was somehow difficult to correlate the Helmholtz data with the excitatory currents of Dubois-Reimond, as the time resolution of the contemporary techniques did not allow measurement of the kinetics of the activity-associated electrical events with any relevant precision. This problem was brilliantly solved by Julius Bernstein, who introduced a truly remarkable piece of scientific machinery – the 'differential rheotome', which allowed adequate recordings of very fast electrical processes. The sampling rate of Bernstein's rheotome was approximately several tens of microseconds (a detailed account of Bernstein's techniques was made by Bernd Nilius (2003).

Using the rheotome, Bernstein made the first true recordings of resting and action potentials (Figure 1.9). He estimated that, at rest, the nerve interior is about 60 mV more negative than the surface, and he showed the kinetics of the action potential (still called '*negative Schwankung*'). The action potential measured by Bernstein had a rise time of about 0.3 ms and a duration of ≈0.8–0.9 ms but, most importantly, the potential deflection actually crossed the 'zero potential' line, causing a 'sign reversal' which clearly reflected the action potential overshoot (Bernstein, 1868). Bernstein also estimated the conduction velocity of the nerve, which was very similar (≈25–30 m/s) to the data obtained by Helmholtz.

Bernstein developed several theories of electrical excitability and, in 1896, being prompted by his student Vassily Tschagovetz, he employed the electrolytic theory of Walther Nernst to biological systems and came up with the hypothesis that K^+ selectivity of the excitable membrane is responsible for the generation of the resting membrane potential (Bernstein, 1902, 1912). This theory was further developed by Charles Ernst Overton, who demonstrated that Na^+ ions are required for producing

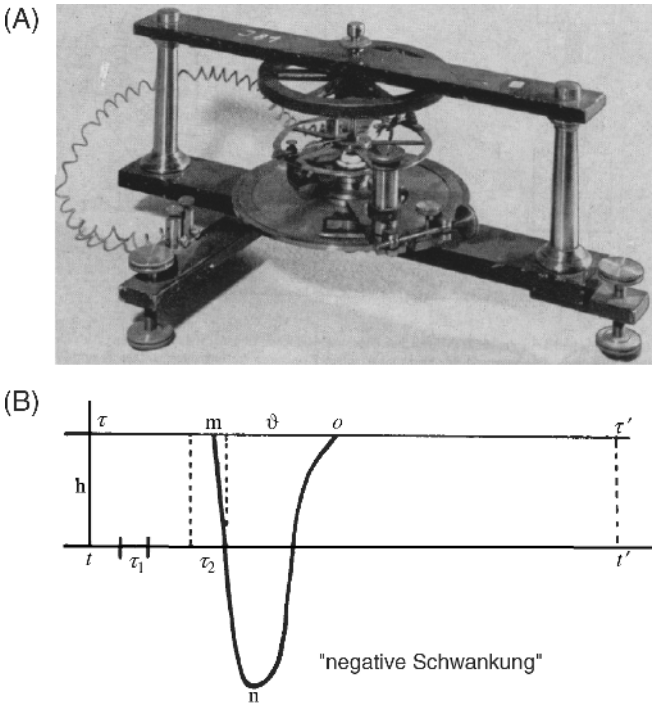


Figure 1.9 First recording of action potential from the nerve made by Julius Bernstein (Bernstein, 1868).

A. The Bernstein rheotome.

B. The recording of an action potential. The t_1 and t_2 indicate 'sampling' intervals of the rheotome; the duration *m – o* is the duration of action potential ('*negative Schwankung*'; and *n* is the *'sign reversal'* (overshoot).

Images were kindly provided by Professor Bernd Nilius, University of Leuven.

the '*negative Schwankung*', and suggested that the excitation process results from the exchange of Na^+ and K^+ (Overton, 1902).

Incidentally it was also Overton who, in 1899, proposed a '*lipoidal membrane*' model of the plasmalemma, after discovering that lipid-soluble dyes enter cells substantially easier than the water-soluble ones (Overton, 1899). The bilayer structure of the cellular membranes was confirmed in 1925 by Gorter and Grendel, who found that the amount of lipids extracted from 'chromocytes' (red blood cells) was sufficient to cover the surface of these cells twice (the surface area was determined from microscopic observations of blood cells), which led them to propose the lipid bilayer structure (Gorter & Grendel, 1925). This theory was further developed by Danielli and Davson (Danielli & Davson, 1935), who introduced the concept of the bilayer lipid membrane, which is associated with numerous proteins and is penetrated by narrow water-filled pores that allow the passage of lipid-insoluble molecules, including ions.

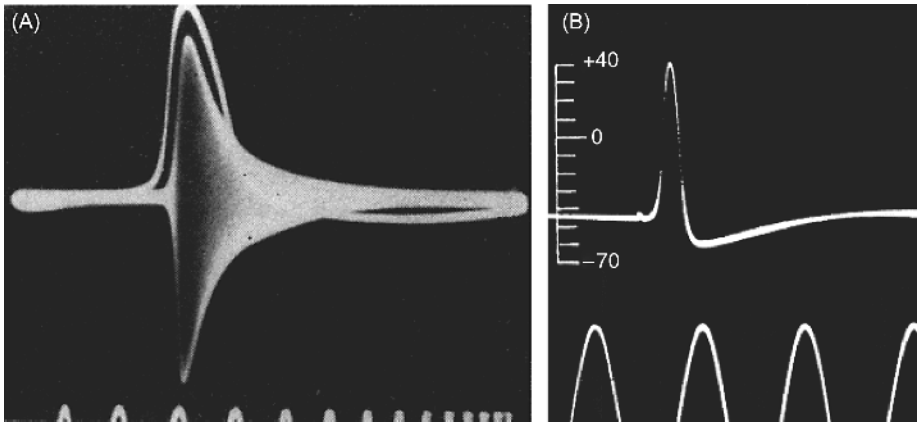


Figure 1.10 First electrophysiological recordings from squid axons.

A. The increase in conductance of the squid axon during the action potential as seen by Cole and Curtis (Cole & Curtis, 1939). Upper trace: action potential; white-dark band: measure of the membrane impedance obtained with the Wheatstone bridge method by applying a high frequency (20 KHz) sinusoidal signal to two electrodes placed on the opposite site of a giant axon. The time marks at the bottom are 1 millisecond apart.

B. The first published intracellular recording of the action potential in the squid axon. Time mark, 500 Hz (Hodgkin & Huxley, 1939).

Reproduced from Hodgkin and Huxley 1939, Journal of Physiology.

All in all, by the mid-1930s, the structure of the cell membrane was known and the prototypes of ion channels suggested. However, direct physiological data were needed to confirm the electrical theory of excitation. These direct electrophysiological experiments became possible after John Z. Young introduced the squid axon into physiological practice (Young, 1936). In 1939, Kenneth Cole and Howard Curtis performed impedance measurements using extracellular electrodes on axons isolated from the '*Atlantic squid,* Loligo pealii . . . *From early May until late June excellent animals were available, but later they were smaller, not so numerous, and did not live long in the aquarium. Slender animals were preferred because the axons were of nearly uniform diameter over their usable length.*' (Cole & Curtis, 1939).

These experiments directly demonstrated the rapid fall in membrane resistance during the development of the action potential (Figure 1.10A). Slightly later, both Cole and Curtis (Curtis & Cole, 1940) and Alan Hodgkin and Andrew Huxley (Hodgkin & Huxley, 1939) developed intracellular electrodes which could be inserted into the squid axon, and performed the first direct recordings of action potentials (Figure 1.10B). These recordings demonstrated a very clear action potential overshoot and determined the resting potential at approximately –50 mV.

In 1949, the voltage-clamp technique was designed by Cole (Cole, 1949) and Marmont (Marmont, 1949), and it was almost immediately employed by Hodgkin, Huxley and Katz (Figure 1.11) to produce the ionic theory of membrane excitation (Hodgkin & Huxley, 1952). Most importantly, Hodgkin and Huxley clearly

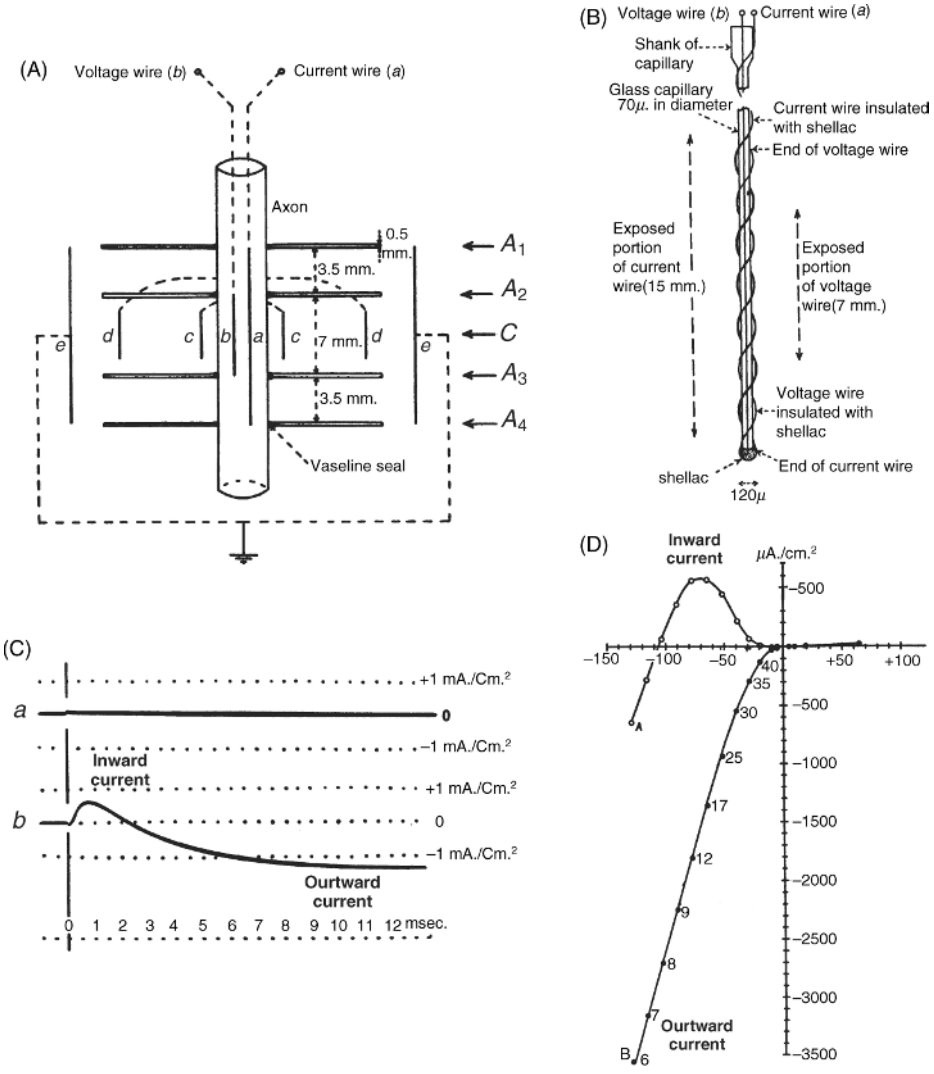


Figure 1.11 First recordings of ion currents.

A. Diagram illustrating arrangement of internal and external electrodes. A_1, A_2, A_3, and A_4 and C are Perspex partitions; a, b, c, d and e are electrodes. Insulated wires are shown by dotted lines.

B. Diagram of internal electrode (not to scale). The pitch of each spiral was 0.5 mm. The exposed portions of the wires are shown by heavy lines.

C. Records of membrane current under a voltage clamp. At zero time, the membrane potential was increased by 65 mV (record A) or decreased by 65 mV (record B); this level was then maintained constant throughout the record. Inward current is shown as an upward deflexion. Axon 41; diameter 585 μm. Temperature 3.8° C.

D. The current-voltage relations for inward and outward ion currents.

Reproduced from Hodgkin *et al.* 1952, Journal of Physiology.

demonstrated that membrane excitability is determined by passive ion fluxes according to their electro-chemical gradients, which implied the existence of transmembrane aqueous pathways. Although the ion channels were not directly incorporated into the theory, their existence was suggested. The quest for ion channels occupied the next 30 years.

From the early days of this quest, several technical obstacles had to be sorted. First, further development of the ionic theory of excitation required recordings not only from axons of non-vertebrates but also from mammalian cells, which are generally rather small and difficult to access because of tissue barriers. Second, precise separation of ion currents and dissection of the mechanisms of their regulation required control over both the extra- and intracellular environments. Third, monitoring of single ion channels' currents ultimately required low noise recordings from exceedingly small areas of cellular membranes (i.e. small enough to contain only a few channel molecules, or better still a single ion channel). In fact, the very first evidence for discrete ion currents were obtained in experiments with artificial lipid membranes, introduced by Paul Müller and Donald Rudin (Mueller & Rudin, 1963). When these membranes were exposed to certain antibiotics (e.g. gramicidin A) or to certain proteins, an ionic conductance was induced, which could be recorded as step-like, discrete events of transmembrane currents (Bean *et al.*, 1969).

The problem of connecting recording instruments to single cells was solved with the development of microelectrodes pulled from glass pipettes. These microelectrodes for low-traumatising penetrations of cells were introduced in 1949 by Gilbert Ling and Ralf Gerard (Ling & Gerard, 1949). The microelectrode technique was rapidly adopted by electrophysiological laboratories.

Glass microelectrodes, filled with ion-containing solution, were first employed for extracellular recordings in 1919 by Frederick Pratt and John Eisenberger (Pratt & Eisenberger, 1919), who manufactured a fine-pointed capillary pore electrode with outer diameter $\approx$4–8 μm and employed these electrodes for focal stimulation of single skeletal muscle fibres. The experiments directly demonstrated that skeletal muscle excitation followed the 'all-or-none' principle.

The first extracellular recordings from cellular membranes of muscle cells were performed by Alfred Strickholm in the early 1960s (Strickholm, 1961, 1962). He used a '*smooth tipped, liquid-filled micropipette (several microns tip diameter) . . . placed against a muscle in such a way that the cell surface under it was electrically isolated except for a leakage resistance path between tip and cell*'. (Strickholm, 1961). Using these pipettes, Strickholm was able to measure the impedance of frog muscles and obtain recordings of currents flowing through the small membrane patch under the tip of this extracellular pipette. Several years later, Karl Frank and Ladislav Tauc revealed a heterogeneous distribution of Na^+ channels in molluscan neurones by voltage-clamping relatively small patches of the plasma membrane with the help of an extracellular glass micropipette (Frank & Tauc, 1963).

In 1969, Erwin Neher and Hans Dieter Lux developed a conceptually similar technique to monitor membrane currents from the somatic membrane of sub-oesophageal

ganglion neurones of *Helix pomatia* snails (Neher & Lux, 1969). They pulled micropipettes from asymmetrical double-barrelled capillaries to obtain an opening of about 100–150 μm in diameter; the tip of the pipette was subsequently fire-polished. Importantly, gentle suction (2–10 mm Hg) was applied to the pipette interior, which helped approaching the membrane of the neurone located within the ganglia (normally covered by glial cells) and improved the shunt resistance between the pipette wall and the cell membrane.

The problem of controlling intracellular ion concentrations was solved in parallel. The first experiments with complete or partial replacement of the cytoplasm with artificial salt solution were performed on squid axons in 1961 by Peter Baker, Alan Hodgkin and Trevor Shaw (Baker *et al*., 1962). About a decade later, the cytoplasm replacement approach was adopted for single cells. The initial version of intracellular perfusion was built around plastic film, which separated two chambers, filled with extra- and intracellular solutions (Kryshtal & Pidoplichko, 1975). A tiny pore, several millimetres in diameter, was made in the film, and the cell soma was placed on top of the pore; a small negative pressure applied to the 'intracellular' chamber helped the cell to invade the pore. After the cell firmly occluded the pore, the membrane facing the intracellular compartment was disrupted and electrical and physical access to the cell interior was gained.

This initial set-up was soon modified and the planar film was replaced by either plastic or glass pipettes (Kostyuk *et al*., 1981; Lee *et al*., 1980), which allowed easy hunting for cells and permitted further modification of the method. These modifications included, for example, double perfusion, where the cell was fixed between two pipettes (Kostyuk *et al*., 1981; Lee *et al*., 1980), which provided for a very good spatial voltage-clamp and fast and effective exchange of the intracellular milieu. The plastic pipettes were also used for extracellular recordings with the aim of measuring single channel currents (Kryshtal & Pidoplichko, 1977). All of these techniques suffered from a relatively low shunt resistance between the membrane surface and the wall of the recording pipette, which prevented low-noise recordings.

Indeed, in intact cells, the main difficulty is to detect single channel currents in the presence of background electrical noise. A background noise associated with the usage of glass intracellular microelectrodes usually exceeds 100 pA, whereas the current flowing through a single channel is as small as several pA, being therefore only a relatively tiny fraction of this background noise. To overcome this problem, Ervin Neher and Bert Sakmann (Neher & Sakmann, 1976) used an extracellular glass micropipette, the tip of which was pressed against the surface of an isolated skeletal muscle fibre. In this configuration, a patch of a membrane was electrically isolated (Figure 1.12). Intrinsic noise decreases with the area of membrane under voltage-clamp, so when a small area (1–10 μm^2) is isolated, the extraneous noise levels can be made so low that the pico-ampere currents flowing through single ion channels can be recorded (Figure 1.12).

These first recordings (which measured currents through single nicotinic acetylcholine receptors) were still far from ideal, because the resistance between recording pipette and cell membrane remained relatively low (in a range of tens

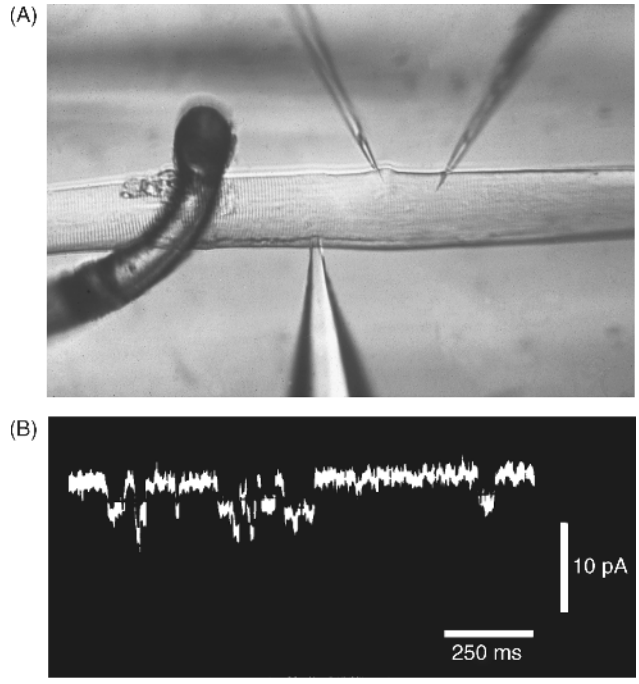


Figure 1.12 First recordings of acetylcholine receptor single channel currents from denervated frog *(Rana pipiens)* cutaneous pectoris muscle.

A. The micro-photograph of the preparation.

B. Current recordings. The pipette contained 0.2 mM suberyldicholine, an analogue of acetylcholine, which induces long-lived channel openings. Membrane potential –120 mV; temperature 8°C.

of MΩ). The technique was substantially improved in 1980, when the ultra-high resistance (giga-ohm) seal between highly cleaned and very smooth micropipette tips and smooth surface cell membranes (the so-called giga-seal) was achieved (Hamill *et al.*, 1981). This was the patch-clamp technique that revolutionised electrophysiology, allowing entirely new types of experiments to be designed. The astonishing stability and tightness of the giga-seal interaction between micropipette and cell membrane permitted not only electrical isolation but also complete mechanical isolation of a patch of cell membrane in either the inside-out or outside-out configurations. Furthermore, the patch-clamp technique can be used for intracellular perfusion and it can be applied to virtually every cell type in the body, in isolation (cell culture) or in tissues (acutely isolated slices) or, indeed, *in vivo*. The patch-clamp technique was instrumental in the detailed characterisation of ion channels underlying electrical excitability, being one of the very few techniques allowing direct recordings of the functional activity of single protein molecules.

1.3.2 Chemical signalling between neural cells

'Of known natural processes that might pass on excitation, only two are, in my opinion, worth talking about. Either there exists at the boundary of the contractile substance a stimulative secretion in the form of a thin layer of ammonia, lactic acid, or some other powerful stimulatory substance, or the phenomenon is electrical in nature'. These prophetic words of Emil Heinrich du Bois-Reymond (du Bois-Reymond, 1877) signalled the emergence of the modern theory of molecular mechanisms of information transfer between cells in living organisms. Indeed, all intercellular communications are mediated either by release and reception of transmitter substances, or by direct movement of molecules from one cell to another through intercellular junctions. This latter mechanism provides for direct intercellular electrical and/or chemical signalling by means of intercellular diffusion of ions and/or other compounds, respectively.

As has been already mentioned, the notion that cells communicate between each other with some substances that can, for example, be released from the nerves and act upon other cells, has been circulated for a long time. The modern theory of neurotransmission, however, developed entirely in the last 120 years.

The morphological basis for intercellular communications in the central nervous system (CNS) was defined in 1897, when Michael Foster and Charles Scott Sherrington introduced the concept of the synapse (the word contemplated by classic scholar Arthur Woolgar Verrall from Greek roots *syn*, συν meaning together and *haptein* απτειν meaning clasp). The theory of chemical neurotransmission was formulated in 1904 by John Newport Langley and Thomas Renton Elliott, who also suggested adrenaline (epinephrine) as the neurotransmitter in the sympathetic nervous system (Elliott, 1904). A year later, Langley postulated the existence of specific neurotransmitter receptors (Langley, 1905), which he called the '*receptive substances . . . capable of receiving and transmitting stimuli of target cells*' (Langley, 1906).

Acetylcholine became the first neurotransmitter experimentally discovered by Otto Loewi and Henry Dale (Dale, 1914; Loewi, 1921), and noradrenaline (norepinephrine) followed in 1946 (Von Euler, 1946). Many more neurotransmitters and neuromodulators were discovered in the ensuing decades, including adenosine 5'-triphosphate (ATP), dopamine, serotonin, glutamate, neuropeptides, etc. The theory of chemical transmission in the CNS became fully acknowledged in the mid 1950s, following famous acceptance of this theory by John Carew Eccles, and definition of its cornerstone principles.

The main criteria that allowed a substance to be accepted as a neurotransmitter were formulated by Eccles (Eccles, 1964) as:

1. The substance and the enzymes necessary for its formation must be present in the neurone.
2. The substance must be released from the terminal axon when the nerve is activated.

3. The effect of the transmitter released on nerve stimulation must be mimicked by the exogenous application of the substance to the effector.
4. A mechanism for inactivation of the substance must be present, whether it involves enzyme action or uptake or both.
5. Drugs which reduce or potentiate nerve mediated responses should similarly affect the responses to the exogenously applied substance (quoted from Burnstock, 1972).

Another criterion of neurotransmission, the 'Dale principle', was also defined by Eccles (Eccles, 1976). This principle postulates that each neurone produces and releases only one type of neurotransmitter. Based on Eccles interpretation of Henry Dale's Nothamgal lecture in 1934, the principle became a subject of long-standing discussions to date.

Further developments in the field lead to the realisation that these fundamental principles have to be redefined somewhat. Indeed, some neurotransmitters (most notably glutamate) can not be synthesised *de novo* in neurones releasing them; they have to be transported in the form of precursors and then converted into the active substance. Second, it appears that most (if not all) neurones secrete more than one transmitter (the concept of co-transmission was originally formulated by Geoffrey Burnstock (Burnstock, 1976), which greatly adds to the complexity of chemical transmission in the CNS. Third, it became clear that neurotransmitters are released not only by neurones but also by neuroglia.

1.4 The concept of neuroglia

> *'Ich habe bis jetzt, meine Herren, bei der Betrachtung des Nervenapparatus immer nur der eigentlich nervösen Theile gedacht. Wenn man aber das Nervensystem in seinem wirklichen Verhalten im Körper studiren will, so ist es ausserordentlich wichtig, auch diejenige Masse zu kennen, welche zwischen den eigentlichen Nerventheilen vorhanden ist, welche sie zusammenhäund dem Ganzen mehr oder weniger seine Form gibt.'*
>
> Rudolf Virchow, *Die Cellularpathologie*, p. 246 (Virchow, 1858)[4]

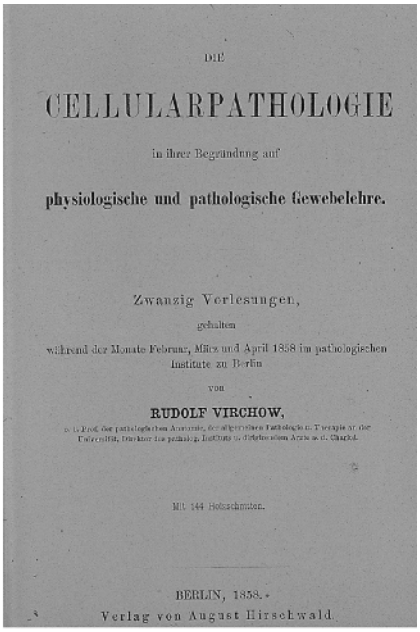
The idea of the coexistence of active (excitable) and passive (non-excitable) elements in the brain was suggested in 1836 by Gabriel Gustav Valentin, who had just been appointed to the Physiology chair in Bern University, in his book *Über den Verlauf und die letzten Enden der Nerven*. The concept and term 'neuroglia' was coined in 1856 by Rudolf Ludwig Karl Virchow (1821–1902; Figure 1.13) in his

[4] *'Hitherto, gentlemen, in considering the nervous system, I have only spoken of the really nervous parts of it. But if we would study the nervous system in its real relations in the body, it is extremely important to have a knowledge of that substance also which lies between the proper nervous parts, holds them together and gives the whole its form in a greater or lesser degree.'*

(A)

Rudolf Virchow (1821-1902)

(B)



(C)

Dreizehnte Vorlesung.

3. April 1858.

Rückenmark und Gehirn.

Das Rückenmark. Weisse und graue Substanz. Centralkanal. Gangliöse Gruppen. Weisse Stränge und Commissuren.
Die Medulla oblongata und das Gehirn. Körner- und Stäbchenschicht desselben.
Das Rückenmark des Petromyzon und die marklosen Fasern desselben.
Die Zwischensubstanz (interstitielles Gewebe). Ependyma ventriculorum. Neuroglia. Corpora amylacea.

Figure 1.13 (*Continued on next page*)

own commentary to the earlier paper 'Über das granulierte Ansehen der Wandungen der Gehirnventrikel' (published in 1846 in the journal *Allgemeine Zeitshrift fur Psychiatrie* vol. 3, pp. 242–250). This commentary (Virchow, 1856, p. 890) indicated the existence of ' . . . *connective substance, which forms in the brain, in the spinal cord, and in the higher sensory nerves a sort of Nervenkitt (neuroglia), in which the nervous system elements are embedded*'.

The concept of neuroglia was presented to the public on April 3rd, 1858, when Virchow delivered the 13th of a series of 20 lectures to medical students of Charite Hospital in Berlin. These lectures were stenographed and published almost without any editing in a book, *Die Cellularpathologie in ihrer Begründung auf*

Figure 1.13 Rudolf Virchow and the concept of neuroglia.

A. Portrait of Rudolf Virchow in the 1850s. Rudolf Virchow was born on October 13, 1821, in Schivelbein, which was then under the rule of the Prussian kingdom and now is a city of Swidwin in Poland. He studied medicine in Berlin and worked as a pathologist at the Charité. After the failure of the 1848 revolution, in which he had actively participated, he was forced to leave Berlin and to move to Würzburg, where he became Professor of Pathology. He returned to Berlin in 1856 and occupied the Chair in Pathology for the rest of his life. He had a broad interest in science, ranging from cancer research and neuroscience to anthropology and, as the editor-in-chief of *Virchow's Archiv*, he could oversee it all. Rudolf Virchow was not only a highly influential scientist, but was actively engaged in different aspects of political and cultural life. He initiated laws for meat inspection at slaughterhouses and, as a member of the German parliament, he was instrumental in installing a modern sewage system in the city of Berlin, borne out of the recognition that there is a relationship between infections and hygienic conditions. His interest in anthropology let him to participate in excavations carried out by his friend Heinrich Schliemann in Troy, and it was Virchow who convinced Schliemann to donate the treasures of Priam to the city of Berlin. Virchow assembled a tremendous collection of pathologic specimens and, at the end of his life, he opened a Pathologic Museum, not only for students and medical practitioners, but also for the public. He died in Berlin on September 5, 1902.

B. The frontispiece of *Cellular Pathology*, published in 1858.

C. Lecture 13 ('Spinal cord and the brain') from *Cellular Pathology*, where the name neuroglia was first coined.

Reproduced with permission from Kettenmann & Verkhratsky, 2008.

physiologische und pathologische Gewebelehre (Virchow, 1858), which became one of the most influential treatises in pathophysiology in the 19th century. Virchow derived the term 'glia' from the Greek '$\gamma\lambda\iota\alpha$' for something slimy and of sticky appearance (the root appeared in the form '$\gamma\lambda\omicron\iota\omicron\sigma$' in writings of the ancient Greek poet Semonides where it referred to 'oily sediment' used for taking baths; in works of Herodotus, for whom it meant 'gum'; and in the plays of Aristophanes, who used it in a sense of 'slippery or knavish'. In modern Greek, the root remains in the word '$\gamma\lambda\omicron\iota\omega\eta\varsigma$', which means filthy and morally debased person[5]).

Virchow did not recognise neuroglia as a specific class of neural cells; the drawings of some round structures (Figure 1.14), which he reproduced in the *Die Cellularpathologie*, show, if anything, activated microglia. This did not matter, because for Virchow neuroglia were a connective tissue, the '*Zwichenmasse* – in between tissue' of mesodermal origin. This misconception of the origin of glia was embraced by many histologists, for example by Andriezen, Robertson and Weigart.

In 1851, Heinrich Müller (Müller, 1851) produced the first images of retinal radial glia, the cells that were subsequently named Müller cells by Rudolf Albert von Kölliker (Kölliker, 1852). In 1858, Max Schulze made a detailed investigation

[5] The linguistic analysis was kindly provided by David Langslow, Professor of Classics at Manchester University.

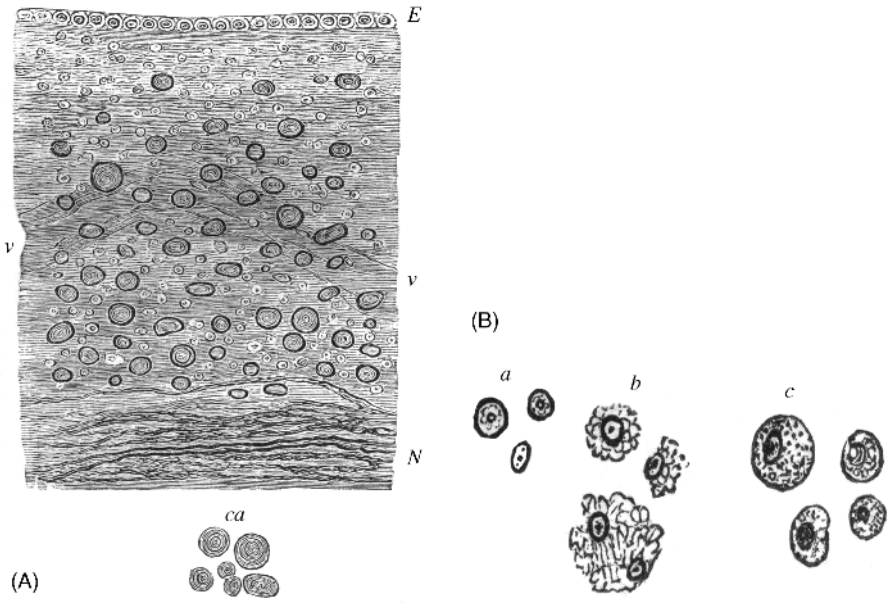


Figure 1.14 Neuroglia as seen by Rudolf Virchow.

A. Ependyma and neuroglia in the floor of the fourth ventricle. Between the ependyma and the nerve fibres is '*the free portion of the neuroglia with numerous connective tissue corpuscles and nuclei*'. Numerous corpora amylacea are also visible, shown enlarged below the main illustration (*ca*). E – ependymal epithelium; N – nerve fibres; v, w – blood vessels.

B. Elements of neuroglia from white matter of the human cerebral hemispheres. a – free nuclei with nucleoli; b – nuclei with partially destroyed cell bodies; c – complete cells.

Reproduced from Virchow, 1858.

of Müller cells and produced probably the best possible drawings of them in the pre-staining era of histology (Figure 1.15). Simultaneously, Karl Bergmann (Bergmann, 1857) had identified radial glial cells in the cerebellum (the cells now referred to as Bergmann glial cells). At the beginning of 1860, Otto Deiters described stellate cells in white and grey matter, closely resembling what we now know as astrocytes (see Figure 1.5H). Curiously enough, these cells were subsequently named as *Spinnenzellen*, or spider cells, by Moritz Jastrowitz (Jastrowitz, 1870). Several years later, Jacob Henle and Friedrich Merkel visualised the glial network in the grey matter (Henle & Merkel, 1869).

Further discoveries in the field of the cellular origin of glial cells resulted from the efforts of many prominent histologists (Figures 1.16, 1.17, 1.18), in particular Camillo Golgi (1843–1926), Gustav Retzius (1842–1919), Santiago Ramon y Cajal (1852–1934), and Pio Del Rio Hortega (1882–1945).

Camillo Golgi was born in Brescia on July 7, 1843. He spent most of his life in Pavia, first as a medical student, then as Extraordinary Professor of Histology, and

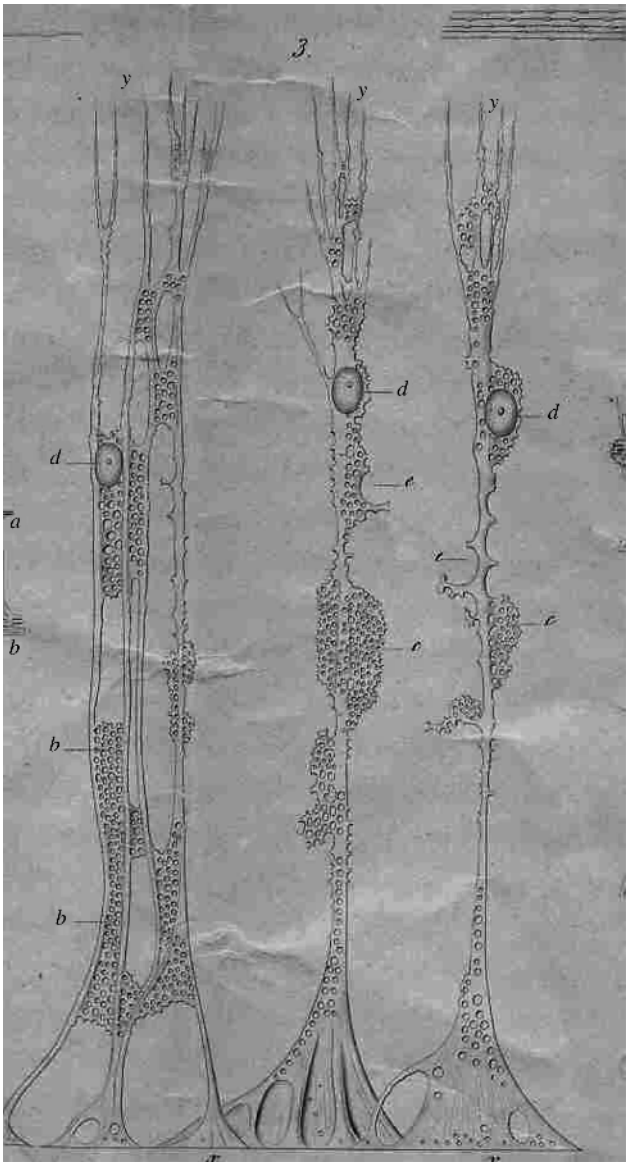


Figure 1.15 Müller fibre of the sheep retina, inspected by Max Schulze with a microscope from Amici.

Yyy: brush-like fibrils extending from the outer Müller fibre in the outer granular layer; xx: internal limiting membrane; a: opening in the limiting membrane; b: very delicate network of fenestrated membranes similar in the ganglion cell layer; cc: network in the so-called molecular layer; ddd: nuclei as part of the Müller fibres; ee: cavity in which the nuclei or the cells of the internal granular layer are located.

From Schulze, 1859. Image kindly provided by Professor Helmut Kettenmann, Max Delbruck Center for Molecular Medicine, Berlin.

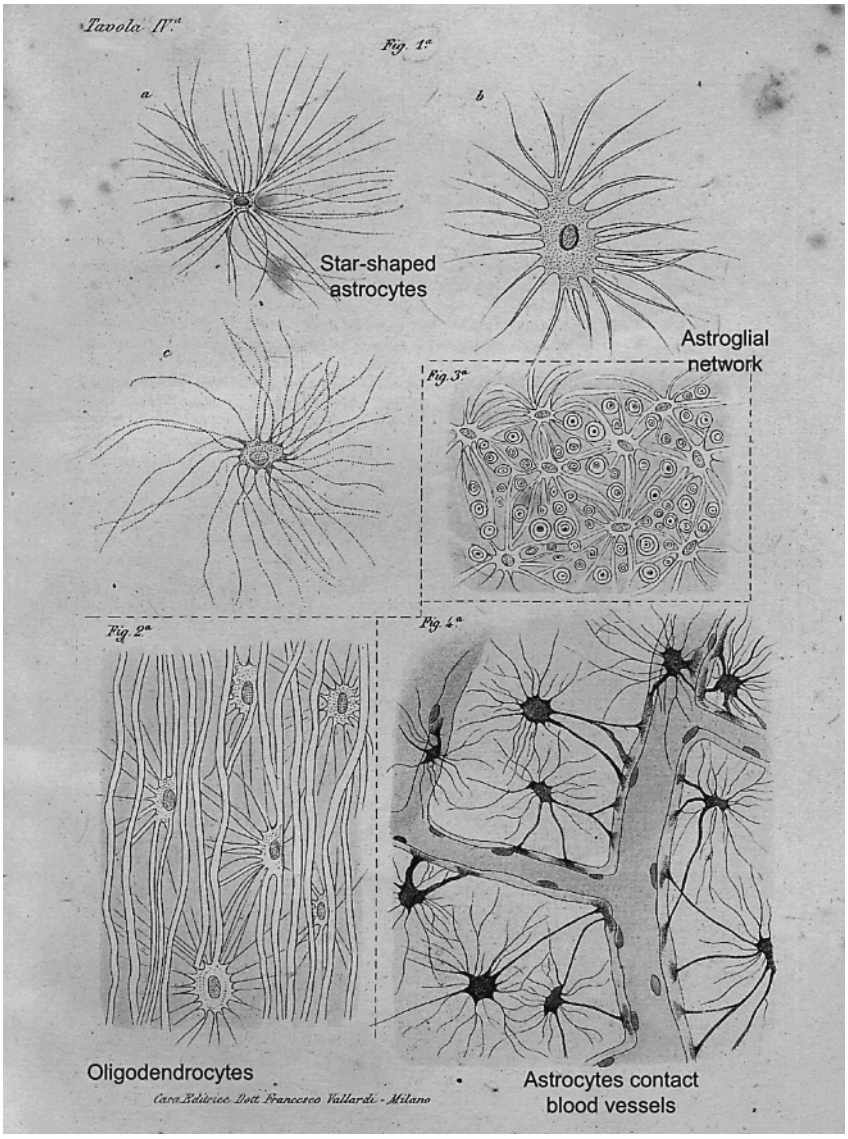


Figure 1.16 Neuroglial cells stained by the silver-chromate technique and drawn by Camillo Golgi (Golgi, 1903).

Top panels show individual star-shaped astrocytes and astroglial networks. At the bottom right, astrocytes forming numerous contacts (the end feet) with brain capillaries are demonstrated. The bottom left panel shows the drawing of white matter with numerous cellular processes oriented parallel to axons, which most likely represent oligodendrocytes.

The image was kindly provided by Prof. Paolo Mozzarello.

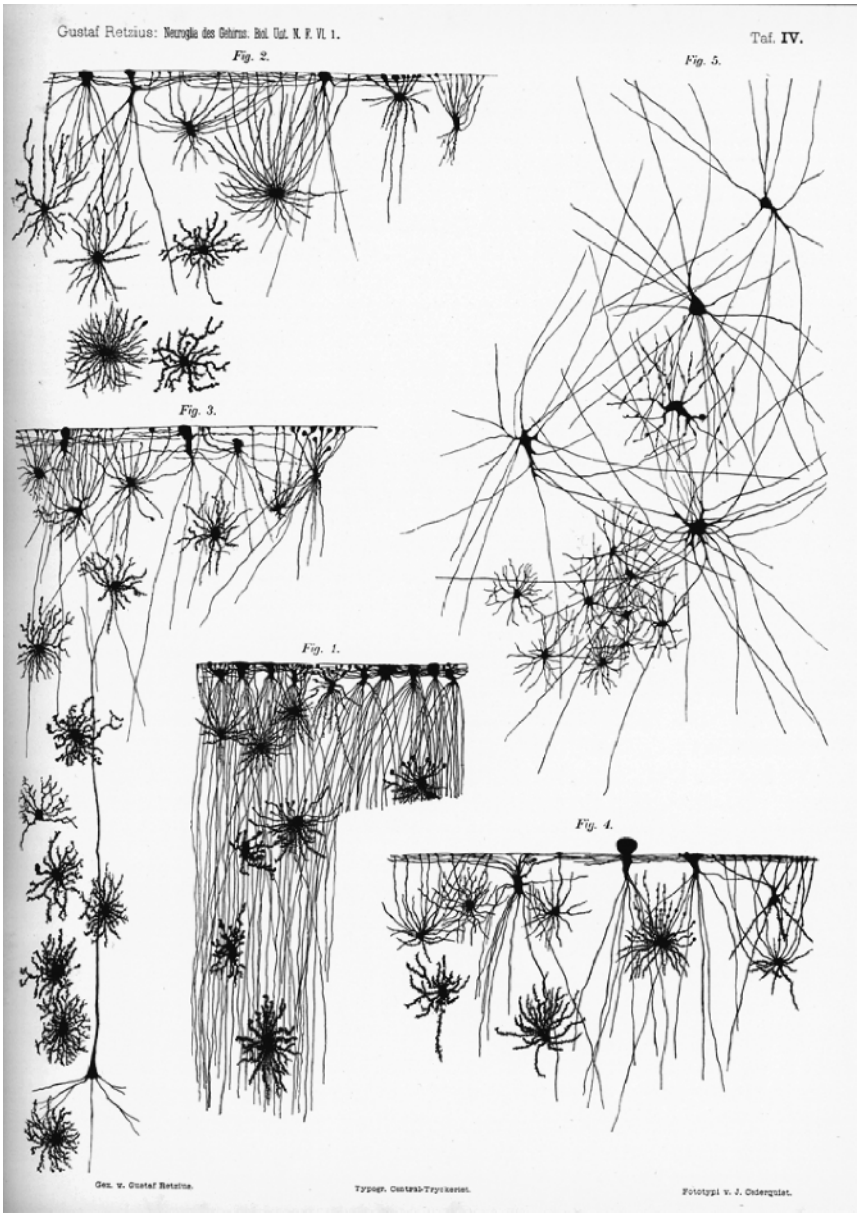


Figure 1.17 Morphological diversity of glial cells in human cortex.

Plate IV: *Fig. 1*. Glial cells from cerebral cortex of 1 year old kid; *Fig. 2*. Glial cells from cerebral cortex of 5.5 years old kid; *Fig. 3*. Glia in *gyrus occipitalis medius* of 17 year old man; Fig. 4. Glia in *gyrus centralis posterior* of 33 year old man; Fig. 5. Glia in *gyrus centralis posterior* of 42 year old woman.

All images are obtained from Golgi stained preparations.

Reproduced from Retzius, 1894, Plate IV.

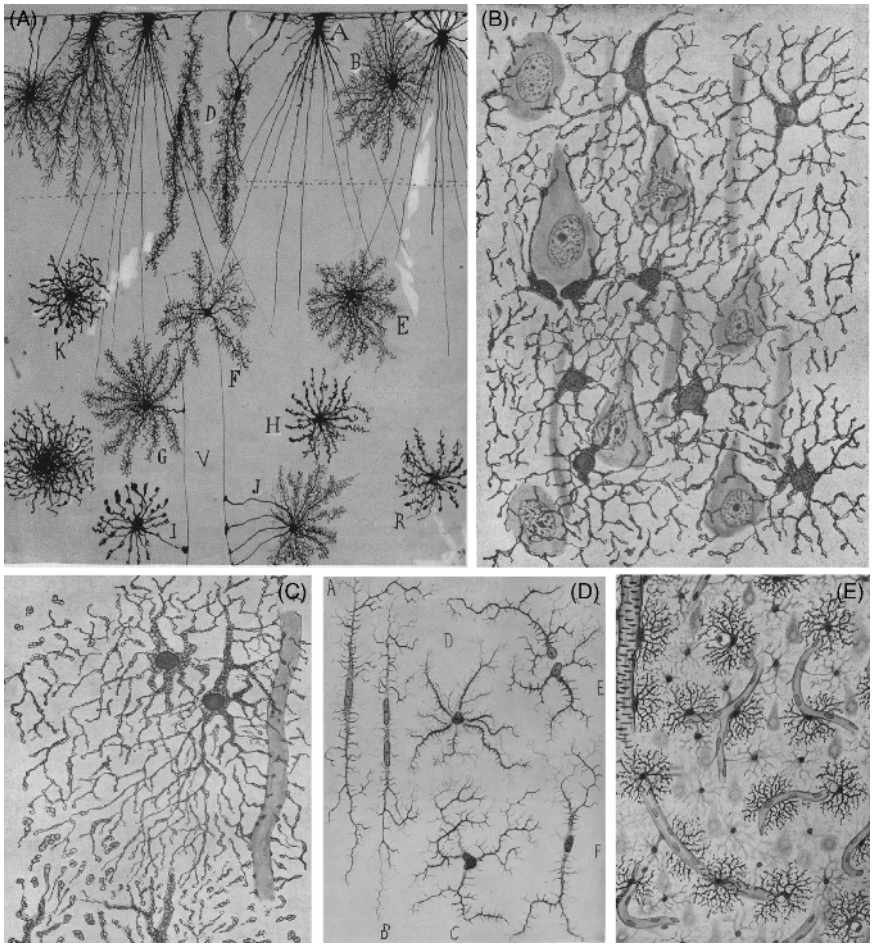


Figure 1.18 Glial cells by the eyes of Santiago Ramón y Cajal and Pío del Río-Hortega.

A. Cajal's drawing of Golgi impregnated glia, showing human cortical neuroglial cells of the plexiform layer (*A-D*), cells of the second and third layers (*E-H* and *K, R*) and perivascular glia (*I, J*).

B, C. Astrocytes in the stratum lucidum of the human CA1 area of the hippocampus, with particular emphasis on the anatomy of perivascular astrocytes in the CA1 stratum radiatum.

D, E. Drawings of Pío del Río-Hortega, showing the different morphological types of microglial cells in the rabbit Ammon's horn and cortical perivascular neuroglia.

Reproduced with permission from Verkhratsky *et al.*, 2011. (Full colour version in plate section.)

from 1881 he occupied the Chair for General Pathology. Camillo Golgi made the first detailed descriptions of glial cells in 1870 (Golgi, 1870), which he observed in thin sections fixed with osmic acid. Already, in this first paper, Golgi identified glia as round cells with many fine processes extended in all directions, many of which

were directed towards blood vessels. Using silver nitrate chromate 'black' staining and microscopic techniques, he discovered a huge diversity of glial cells in the brain, and further characterised the contacts (endfeet) formed between glial cells and blood vessels, as well as describing cells located in closely aligned groups between nerve fibres – the first observation of oligodendrocytes (Figure 1.16). Golgi staining was used by many neuroanatomists, who further characterised morphological diversity amongst neuroglia (Figure 1.17). Golgi was the first to demonstrate that glia represent a cellular population distinct from nervous cells, although he also believed that glial cells and neurones may transform into each other.

Santiago Ramon y Cajal was born on May 1, 1852, in Aragon, Spain. In 1883, he was appointed Professor of Descriptive and General Anatomy at Valencia, in 1887 he assumed a Chair in the University of Barcelona, and in 1892 he became Professor of Histology and Pathological Anatomy in Madrid. Cajal was, and remains, one of the most prominent and influential neurohistologists, who described the fine structure of various parts of the nervous system. He was the most important supporter of the neuronal doctrine of brain structure. He won the Nobel Prize in 1906, together with Camillo Golgi.

Cajal was very much interested in neuroglia throughout his career (Figure 1.18). He developed the gold and mercury chloride-sublimate staining method that was specific for both protoplasmic and fibrous astrocytes (Garcia-Marin *et al*., 2007; Ramón y Cajal, 1913b). We now know that this stain targeted intermediate filaments consisting mainly of glial fibrillary acidic protein (GFAP), a protein used today as an astrocytic marker. Using this technique, Cajal confirmed earlier ideas of the origin of astrocytes from radial glia, and also demonstrated that astrocytes can divide in the adult brain, thus laying the basis for much later discoveries of the stem properties of astroglia (Ramón y Cajal, 1913a, 1916). Cajal contemplated this latter hypothesis about the proliferative capacity of astrocytes after observing pairs of astrocytes joined by their soma; he defined these pairs as twin astrocytes or '*astrocitos gemelos*' (see Ramón y Cajal, 1913a, and a comprehensive historic essay by Garcia-Marin *et al*., 2007).

The term astrocyte (αστρον κψτοσ; *astron, star, and kytos, a hollow vessel,* later *cell*, i.e. star-like cell) was introduced by Michael von Lenhossék (Figure 1.19A) to describe stellate glia, which gained universal acceptance within the next two decades. Lenhossék clearly understood the heterogeneity of neuroglia as he wrote: '*I would suggest that all supporting cells be named spongiocytes. And the most common form in vertebrates be named spider cells or astrocytes, and use the term neuroglia only* cum grano salis *(with a grain of salt), at least until we have a clearer view*.' (Lenhossék, 1895).

More or less at the same time astrocytes were further subdivided into protoplasmic and fibrous astrocytes residing in grey and white matter, respectively (Andriezen, 1893; Kölliker, 1893). William Lloyd Andriezen believed that these two types of cells had different origins, the protoplasmic cells being of mesoblastic origin, while the fibrous cell were ectodermal. Interestingly, he also contemplated

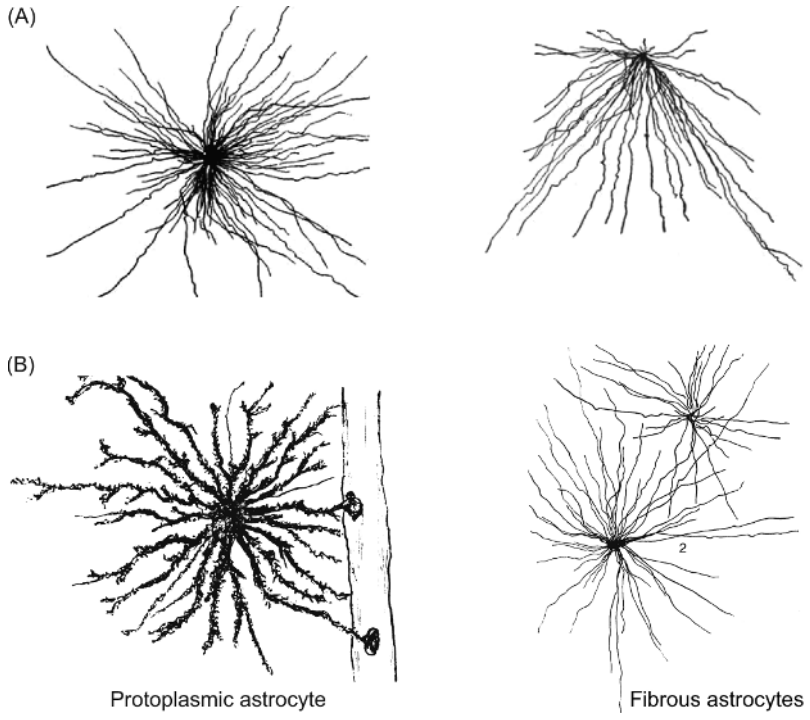


Figure 1.19 Astrocytes.

A. Images of astrocytes drawn by Michael von Lenhossék (Lenhossék, 1895).

B. Protoplasmic and fibrous astrocytes drawn by William Andriezen (Andriezen, 1893). Both preparations were stained with Golgi technique.

the complexity of protoplasmic astrocytes processes, indicating that they have '*the shaggy granular contour, as if a fine moss constituted the protoplasmic processes*'. In the same period, Wilhelm His made a fundamental discovery, when in 1889 he found the neuronal origin of neuroglia and directly demonstrated that both nerve cells and neuroglia derive from the neuroectoderm (His, 1889a, 1889b).

Cajal's pupil (and later his adversary) Pío del Río-Hortega identified two other principal classes of glial cells, initially considered by Cajal as the '*third element*' (a group of adendritic cells) which represented, in fact, the oligodendrocytes and the microglia (Figure 1.18D, E). Oligodendrocytes were initially observed and described by the Scottish neuroanatomist William Ford Robertson, who developed for this purpose a specific platinum stain technique. However, Robertson did not realise the myelinating capacity and role of these cells, and he thought them external invaders to the brain, hence identifying them as mesoglia (Robertson, 1899, 1900a). Almost 20 years later, Río-Hortega rediscovered these cells and gave them the name oligodendrocytes (Del Río-Hortega, 1921). He also demonstrated that oligodendrocytes were myelinating cells in the CNS, being thus analogous to the Schwann

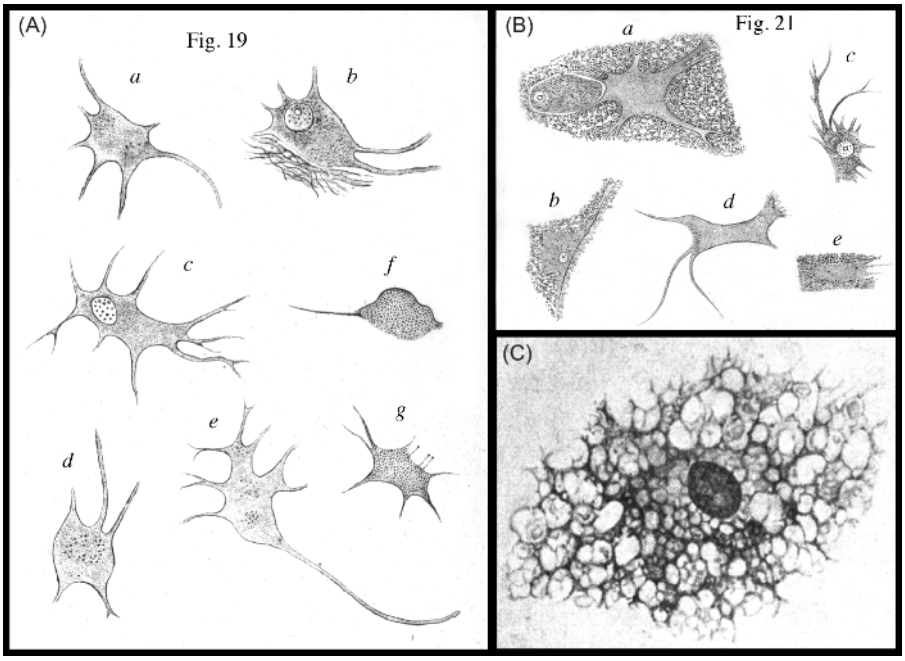


Figure 1.20 Neuroglial cells in pathological context as seen by Carl Frommann and Alois Alzheimer.

A, B. Different types of glial cells found in multiple sclerosis plaques of human cortex.

C. Glial cell close to a 14 days old haemorrhage in human white matter. Axons pass through the network of the cell.

A and B from Frommann, 1878; C from Alzheimer, 1910.

cells in the periphery. It was also Río-Hortega who described microglial cells, the only glial members of non-neuronal origin.

Morphological remodelling of glial cells in neuropathology had already been noted by several neurohistologists by the end of 19th century (Figures 1.20, 1.21). Possibly the first to describe pathology-related remodelling of glia was Carl Frommann, who found, in the brain and in the spinal cord of a patient suffering from multiple sclerosis, glial cells with large somata and small thick processes. He believed these represented their reaction to the disease. Similar pathologically remodelled cells were observed in other pathological contexts, for example *tabes dorsalis* or dementia, by Nissl, Alzheimer and Merzbacher (Alzheimer, 1910; Merzbacher, 1909; Nissl, 1899). These cells received different names, being variously called rod cells (*Stäbchenzellen*), grid cells (*Gitterzellen*) or clearance cells (*Abräumzellen*).

William Ford Robertson introduced the special class of mesoglial cells, which were distinct from parenchymal glia (Figure 1.21A). Robertson found that these

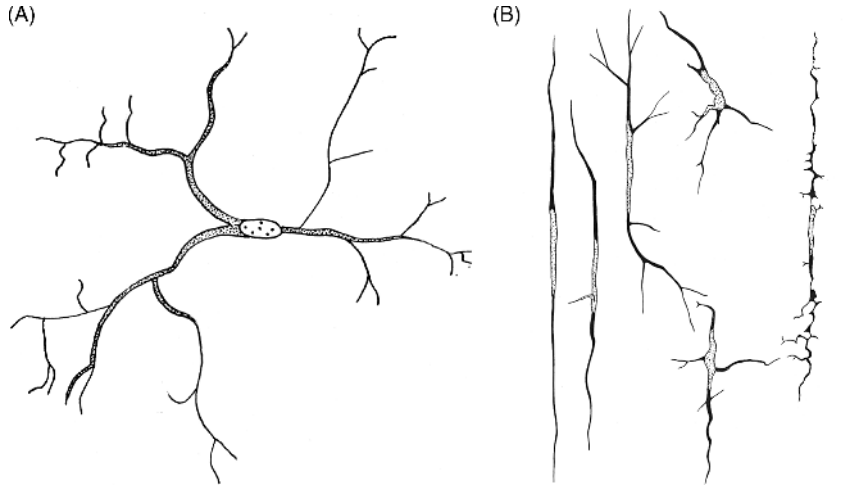


Figure 1.21 Early images of microglia.

A. Mesoglial cells drawn by William Ford Robertson Redrawn from (Robertson, 1900b).

B. The Stabchenzellen or rod cells of Nissl in nerve tissue affected by progressive paralysis. Redrawn from (Nissl, 1899)

cells did not contact blood vessels and withdraw their processes and transform into granule cells in response to injury (Robertson, 1900b)[6]. At the same time, the possibility of cell exchange between blood and the nervous system was also recorded. First, Campobianco and Fragnito (Campobianco & Fragnito, 1898) and Campobianco (Campobianco, 1901) found that at the early embryonic stages a number of mesoblastic cells migrate into the nervous system and are transformed into neuroglia. Some years later, Hatai (Hatai, 1902) described two types of glial cells in the early postnatal brain of mouse and rat. One form he termed the type 'a' cell, which he considered to be the ectodermal derived glial cells. The second type, the type 'b' cell, was morphologically distinct. Based on his morphologic studies, Hatai concluded that these 'b' type cells separated from the vessel wall, became amoeboid and migrated away from the capillary. He concluded that the brain contains two types of glial cells – one ectodermal, the other of mesodermal origin.

Del Rio-Hortega developed a specific silver carbonate impregnation technique to label the microglial cells (Del Río-Hortega, 1917) and made their detailed description (Del Rio-Hortega, 1919a, 1919b, 1920, 1932). Initially, he called these cells '*garbage collectors*', but subsequently he used the term microglia and called an individual cell a

[6] Paul Glees even proposed to call these cells 'Robertson-Hortega cells'. See Glees P. (1955). *Neuroglia morphology and function.* Oxford, Blackwell.

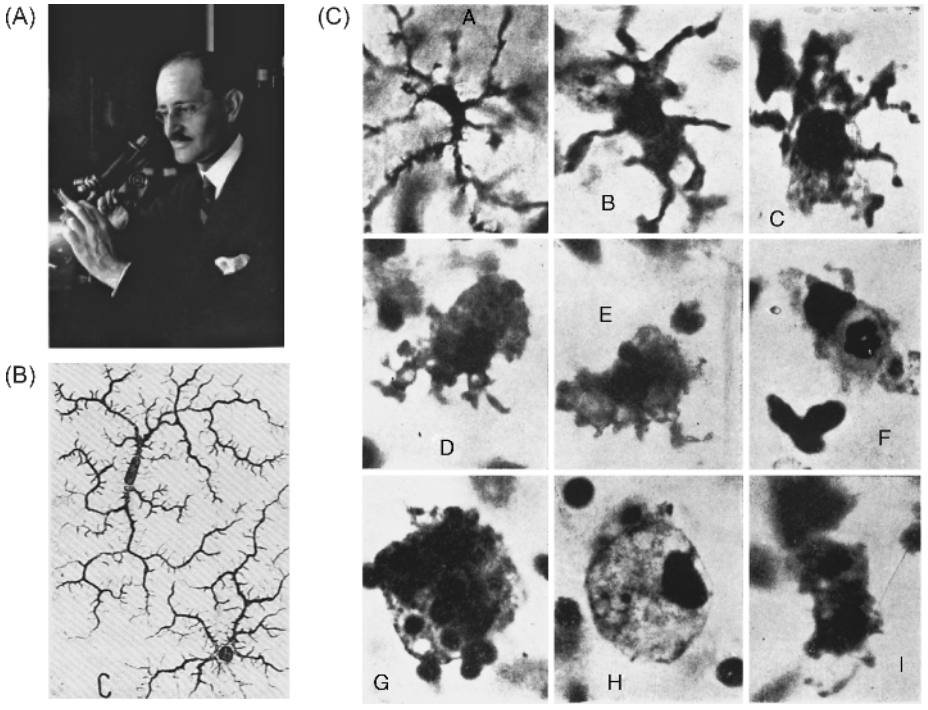


Figure 1.22 Microglial cells discovered by Pio del Rio Hortega.

A. Pio del Rio Hortega (1882–1945).

B. Images of ramified (resting) microglial cells drawn by Hortega (reproduced from Del Rio-Hortega, 1919a).

C. Pathology triggers metamorphosis of microglia. a: cell with thick, rough prolongations; b: cells with short prolongations and enlarged cell body; c: hypertrophic cell with pseudopodia; d, e: amoeboid and pseudopodic forms; f: cell with phagocytosed leukocyte; g: cell with numerous phagocytosed erythrocytes; h: fat-granule cell; i: cell in mitotic division. Photomicrographs: reproduction of Fig. 15 from Del Rio-Hortega, 1932.

'*microgliocyte*'. In some of the contemporary publications, these cells were even referred to as '*Hortega cells*' (Metz, 1924). Del Río-Hortega gave microglia a very detailed and comprehensive characterisation (Figure 1.22), which has changed but little up to now (see Chapter 7). He found that microglial precursors invade the brain shortly after birth, disseminate over the brain parenchyma and develop a distinct phenotype. He also found that that these cells reacted to brain damage by morphological and functional modification known as microglial activation, which eventually turns microglial cells in phagocyting macrophages (Del Rio-Hortega, 1932).

The main peripheral glial element, the Schwann cell, was so called by Louis Antoine Ranvier (1871), following earlier discoveries of Robert Remak, who described the myelin sheath around peripheral nerve fibres (Remak, 1838) and Theodor Schwann, who suggested that the myelin sheath was a product of specialised cells (Schwann,

1839). The word myelin was introduced by Rudolf Virchow and means 'marrow' (Greek μυελοσ), because myelin appearance reminded him of bone marrow.

Remarkably, a further glial cell type in the mammalian CNS was discovered in the 1980s by William Stallcup and colleagues, following their development of an antibody to a novel chondroitin sulphate proteoglycan termed NG2 (Stallcup, 1981; see also Chapter 6). These cells have had numerous names, but in general are called NG2-glia or oligodendrocyte progenitor cells. NG2-glia have unique features amongst glia in that they form synapses, previously considered an exclusive feature of neurones.

The functional role of neuroglial cells puzzled neuroscientists from the very beginning. As already mentioned, Virchow regarded neuroglia as a true connective tissue, which provided the structural support for nerve elements in the brain, in the spinal cord and in the peripheral nerves. To a certain extent, this general concept hindered further enquiries into glial function. As early as 1893, William Lloyd Andriezen complained, '*While in the human brain the nerve elements have been largely and extensively studied both in health and in disease, the neuroglia elements have been comparatively neglected, partly owing to a widely-spread belief in a mere passive rôle they were supposed to play, and partly owing to inadequacy of the methods used . . .* ', although '*the growing importance of these elements is becoming daily obvious . . .* ' (Andriezen, 1893).

Possibly the first general hypothesis of glial cell function was developed by Camillo Golgi, who clearly understood that neuroglia are quite distinct from mere connective tissue. He suggested that glial cells are mainly responsible for feeding neurones, by virtue of their processes contacting both blood vessels and nerve cells. In Chapter 8 (dedicated to neuroglia) of his comprehensive treatise, *Sulla fina anatomia degli organi centrali del sistema nervoso* (Golgi, 1885), Golgi wrote: '*Credo conveniente notare che la parola connettivo, da me viene qualche volta usata per indicare il tessuto interstiziale dei centri e quale sinonimo di nevroglia, senza punto voler assimilare il tessuto medesimo col tessuto connettivo ordinario di origine mesodermica o parablastica. Dichiaro anzi che, dopo tutto, la parola nevroglia adoperata nel senso passato in uso, mi sembra abbia titoli di preferenza, valendo ad indicare un tessuto, che sebbene sia connettivo, perchè connette elementi d'altra natura e alla sua volta serve alla* distribuzione del materiale nutritizio*, pure si differenzia dal connettivo comune per caratteri morfologici, chimici, e quasi certamente, come dirò in seguito . . .* '[7].

Golgi, and many other neurohistologists (e.g. Nissl, Striker, Unger and Bauer (Glees, 1955)), being the proponents of the reticular theory of nerve system

[7] 'It is convenient to note that I usually use the word "connective tissue" to indicate the interstitial tissue as synonymous with neuroglia, without comparing this particular tissue with the usual connective tissue of mesodermal or parablastical origin. I declare that, however, the word neuroglia, used as in the past, indicates a tissue that although is a connective tissue, because connecting elements of another nature and in turn it serves for *distributing the nutritive material*, it is different from the common connective tissue for morphological and chemical features . . . '.

organisation, considered that astroglial cells were connected into the syncytial structure and were making anastomoses with neurones. In contrast, Carl Weigert believed that glial cells were mere structural elements that filled spaces not occupied by neurones. Weigert actually thought that glial fibres exist independently of glial cell somata, and that these fibres provide for structural scaffolding and filling of the interneuronal spaces (Weigert, 1895). Incidentally, this opinion postulating glial fibres existing independently of cell bodies survived well into 1940s and found many supporters (see Glees, 1955).

Both the nutritional theory of Golgi and the space-filling views of Weigert were opposed by Cajal, who, together with his brother Pedro (see Ramon, 1891), suggested that the main function of neuroglia in the grey matter lies in isolation of neuronal contacts in the CNS, thus controlling information flow. '*In short, as a framework with a thousand beams the neuroglia cells act as a material support and protection of the nerve cells and blood vessels. As an insulator their many branches interpose themselves among the dendritic expansions and nerve fibres which for whatever reason must not be in contact*'. The glial processes in their turn '*provide a medium resistant to the passage of nerve waves*' (Ramón y Cajal, 1909–1911).

Cajal's ideas about the role of glia were, however, in a state of continuous flux and modification. As early as in 1895, he proposed astrocytes as core regulators of functional hyperaemia, suggesting that contraction/relaxation of astroglial

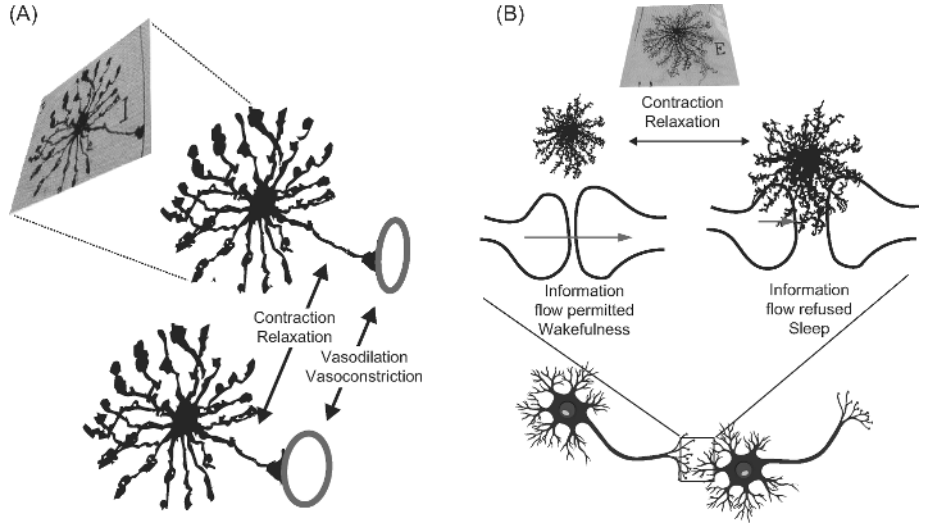


Figure 1.23 Integrative functions of astroglia according to Ramon y Cajal (Ramon y Cajal, 1895, 1925).

A. By contracting and relaxing perivascular processes, astroglia regulate the diameter of brain blood vessels, thus controlling the local blood flow.

B. Astrocytes, again by contracting or relaxing their processes, may interrupt/permit information flow through neuronal networks, thus regulating transition between sleep and wakefulness.

perivascular processes can increase or decrease the diameter of brain capillaries, thus regulating the blood flow (Figure 1.23A; Ramón y Cajal, 1895).

The idea of active neuronal-glial interactions as a substrate for brain function was first voiced in 1894 by Carl Ludwig Schleich (1859–1922) in his book *Schmerzlose Operationen* (Schleich, 1894; and Figure 1.24). Schleich believed that glia and neurones were equal players and that both acted as active cellular elements of the brain. He also thought that glial cells represent the general inhibitory mechanism of the brain. According to Schleich, neuronal excitation is transmitted from neurone to neurone through intercellular gaps (i.e. synapses), and these interneuronal gaps are filled with glial cells, which are the anatomical substrate for controlling network excitation/inhibition. He postulated that the constantly changing volume of glial cells represents the mechanism for control – swollen glial cells inhibit neuronal communication, while impulse propagation is facilitated when glia shrink. This was also the mechanism for general anaesthesia, which, according to Schleich, led to a maximal increase in glial volume, and therefore in complete inhibition of neuronal transmission.

Similar ideas were developed by Cajal, who suggested that perineuronal glia may actively regulate neuronal transmission, and even considered this as being a mechanism of sleep (Figure 1.23B). Cajal suggested that astrocytes act as a switch between active and passive states of the neuronal networks; retraction of astroglial processes allows information flow to promote wakefulness, while extension of astroglial processes would put a halt to interneuronal connectivity, thus inducing sleep (Ramon y Cajal, 1895; 1925).

The notion that neuroglial cells can be actively involved in information processing, in learning and memory and in other higher brain functions, have been considered by several neuroscientists. Probably the first was Fritjoff Nansen, who, as early as 1886, postulated that neuroglia was '*the seat of intelligence, as it increases in size from the lower to the higher forms of animal*' (Nansen, 1886, quoted from Glees, 1955). Fernando De Castro (De Castro, 1951) suggested that neuroglial cells may release neuroactive substances and directly participate in neural transmission. In 1961, Robert Galambos considered neuroglia as a central element for higher brain functions, whereas neurones '*merely execute the instructions glia give them*' (Galambos, 1961).

Neuroglia were also considered to act as a secretory element in the nervous system. Neuroglial secretory activity was proposed by Hans Held (1909) and Jean Nageotte (1910). Using the molybdenum hematoxylin stain, Held discovered darkly stained granular inclusions (granules) in processes of specialised astrocytes, marginal (subpial) glial cells. This stain also allowed him to determine that glial fibres were actually cellular extensions (not an interstitial mass, as per Virchow), forming an elaborate three-dimensional intercellular (astrocytic) network that interacts with vascular endothelium.

These two findings led him to hypothesize that the granular inclusions he observed, referred to as '*gliosomes*' by Alois Alzheimer (1910), was evidence

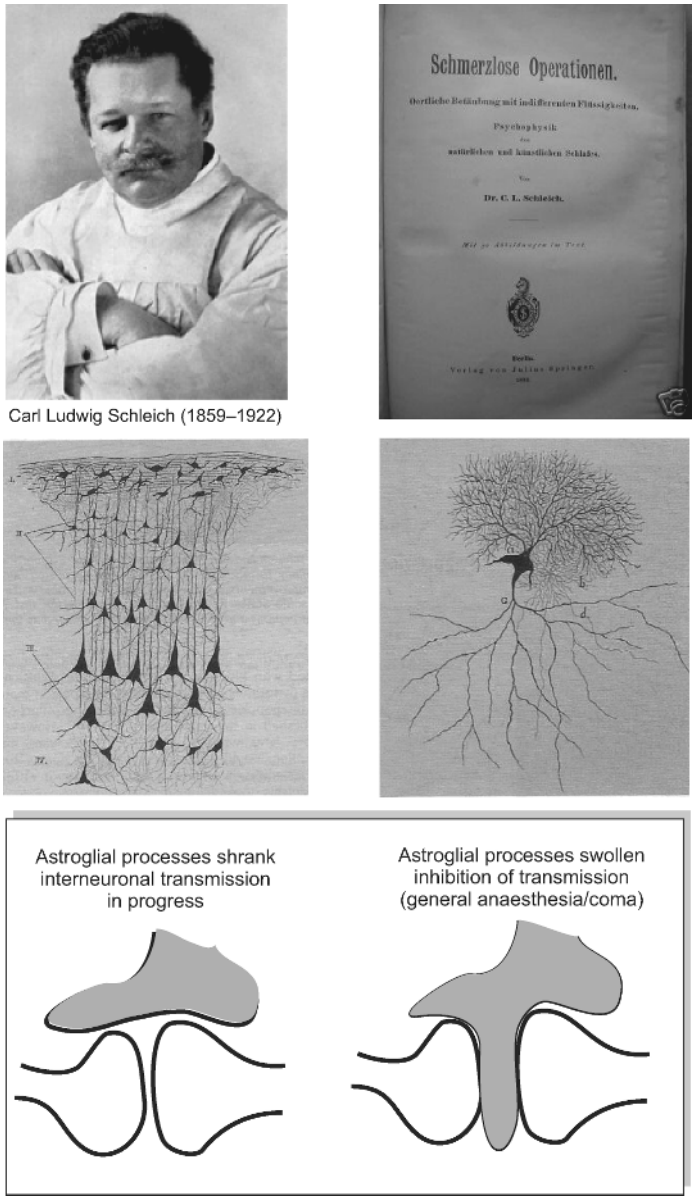


Figure 1.24 Carl Ludwig Schleich and the neuronal-glial interactions hypothesis.

Schleich was a pupil of Virchow and a surgeon who introduced local anaesthesia into clinical practice. In 1894, he published a book, *Schmerzlose Operationen* (Schleich, 1894), the frontispiece of which is shown here on the right upper panel. Apart from describing the principles of local anaesthesia, this book also contained the first detailed essay on interactions in neuronal-glial networks as a substrate for brain function. Mid panels show original drawings from this book, depicting intimate contacts between glial cells and neurones, and the low panel shows, in a schematic manner, Schleich's theory of neuroglia controlling information flow in neuronal networks.

for glial secretion or metabolic provision for neurones, since glial cells reside at the interface between the blood vessels and brain parenchyma. Gliosomes in the cytoplasm of glial cells were, under various terms, also reported by other micro-anatomists of that era – Eisath (1906), Fieandt (1910), Ramón y Cajal (1913a, 1913b), Achucarro (1915) and Hortega (1916) (see Glees, 1955, for appropriate references). As Penfield (1924) improved his staining methods to better disclose oligodendrocytic processes, it became apparent that gliosomes can be seen in astrocytes and oligodendrocytes alike (Figure 1.25).

In 1910, Jean Nageotte observed various secretory granules in grey matter astrocytes using the Altmann method of fucsin labelling (Figure 1.26), and suggested that astroglial cells may release substances into the blood, acting like an endocrine gland. Nargeotte reported: '*The facts that I have just observed seem to me shed new light on the physiology of the neuroglial cells, not only of cells that are associated with neuronal cells, which have been named satellite cells, but also, and particularly, of cells that are in connection with the vasculature walls. Indeed, I was able to present evidence of robust and active secretion phenomenon in the protoplasm of these cells in rabbit and guinea pig. This observation was visible especially within the protoplasmic expansions which cross the empty space created by the retraction of tissues around the vascular walls, on which the neuroglial cells attach using an enlarged foot. In a previous note, I have described the mitochondria that exist in these protoplasmic expansions, and I have shown that many, and maybe all of the granulations located in the grey substance outside the protoplasm of neuronal cells, in reality belong to the neuroglia. Today, I am poised to follow the evolution that occurs within these granulations and to show their progressive transformation into secretion grains. These phenomena are exactly similar as those described by Altmann in the glandular cells; the granulations observed are of three types: 1° round grains excessively small that, by the Altmann method, colour intensively in red; 2° more voluminous grains, with clear centres; 3° grains that do not colour with fucsin. The last ones are slightly smaller than the more voluminous red grains. All intermediates exist between the three types, which represent the successive phases of the transformation of mitochondria in to secretion grains*.' (Translated from French; Nageotte, 1910).

The hypothesis of glial secretion was further investigated by Cajal's pupil Nicolas Achucarro, who postulated that astrocytes may establish a humoral connection between the nervous system and peripheral organs. Achucarro described neuroglial processes as hollow tubules designed to convey various endocrine factors to the blood vessels (Achucarro, 1915). Similar endocrinological roles of neuroglia were considered by another of Cajal's pupils, Fernando De Castro. Several prophetic ideas about neuroglial function were developed by Ernesto Lugaro (a prominent neurologist who, in particular, introduced the concept of neuronal plasticity), who wrote a comprehensive review on neuroglia (Lugaro, 1907). Lugaro suggested close interactions between astroglial processes and synaptic structures and the critical role

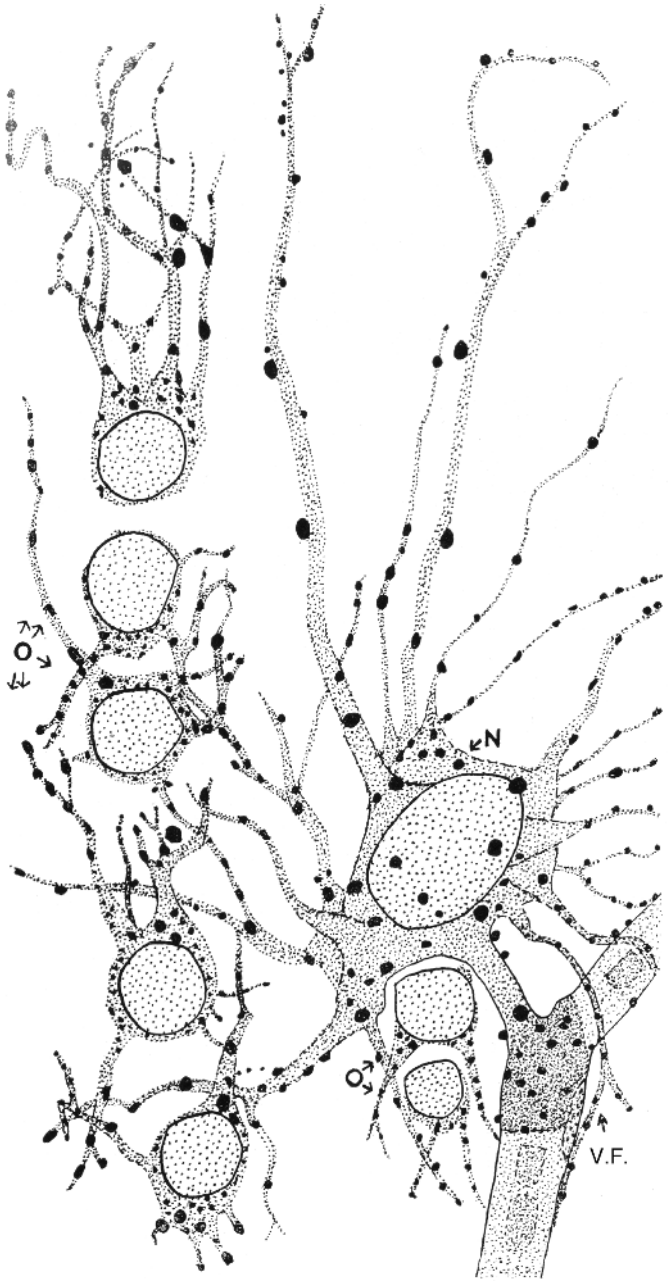


Figure 1.25 Gliosomes.

Oligodendroglia of the white matter and one astrocyte with a vascular endfoot, showing the distribution of darkly staining granules (gliosomes) in their processes.

Drawn from Penfield, 1924.

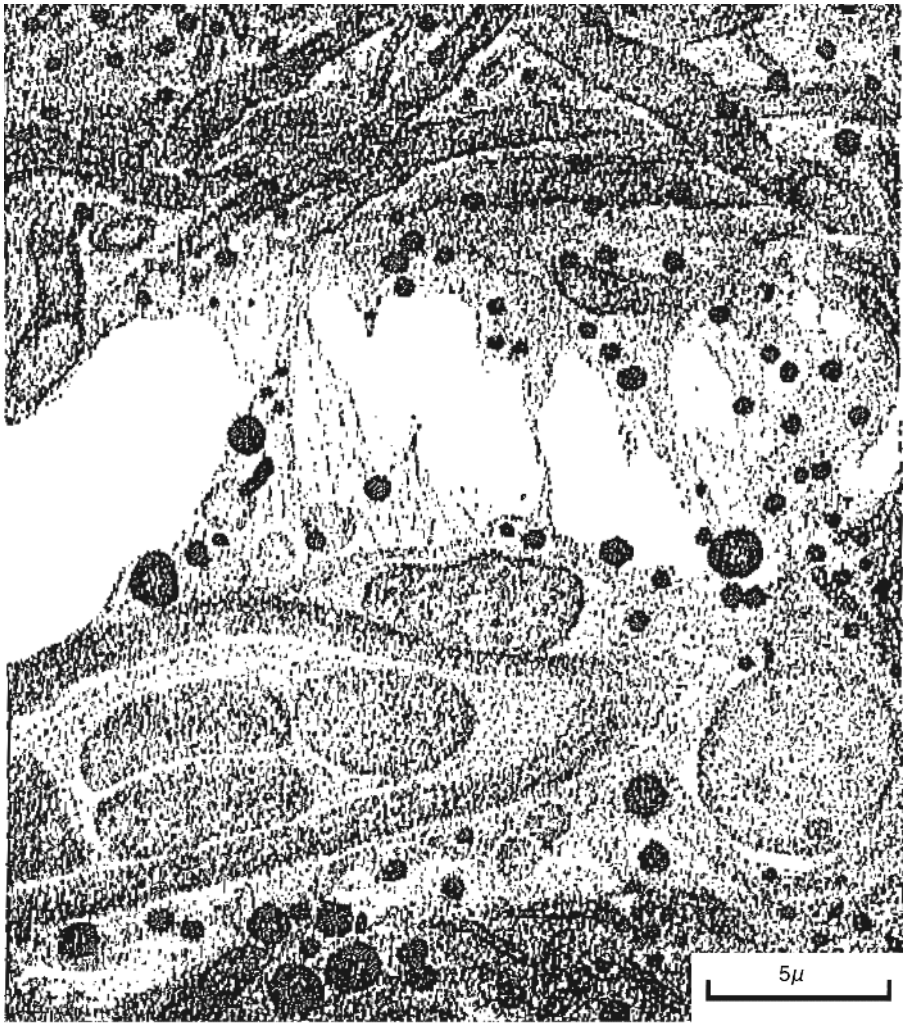


Figure 1.26 Neuroglial cells of grey matter in rabbit medulla, most likely astrocytes, contain various secretory granules.

Scale bar, 5 μm. Reproduced from Nageotte, 1910.

of neuroglia in taking up or degrading transmitters employed for interneuronal communications.

Finally, neuroglial cells were implicated in various pathological processes. Nissl and Alzheimer considered the pathological role of neuroglia in a variety of neurological diseases (Alzheimer, 1910). Astrocytes were considered to '*exhibit a morbid hypertrophy in pathological conditions*' (Andriezen, 1893). The role of astrocytes in scar formation was perceived by Cajal, Hortega and Penfield in the early 1920s. The phagocytic activity of glia, which can be instrumental in removal

of injured or dying neurones form the lesion site, had already been found in 1896 by Gheorghe Marinesco (Marinesco, 1896).

1.5 Beginning of the modern era

The modern era in glial physiology began with two seminal discoveries made in the late 1950s to mid 1960s, when Walter Hild and his colleagues made the first microelectrode recordings from cultured astrocytes (Hild *et al*., 1958) and Steven Kuffler, John Nicolls and Richard Orkand (Kuffler *et al*., 1966) demonstrated electrical coupling between glial cells. Slightly later, Milton Brightman and Tom Reese (Brightman & Reese, 1969) identified structures connecting glial networks, which we know now as gap junctions.

Nonetheless, for the following two decades, glial cells were still regarded as passive elements of the CNS, bearing mostly supportive and nutritional roles. The advent of modern physiological techniques, most notably those of the patch-clamp and fluorescent calcium dyes, dramatically changed this image of glia as 'silent' brain cells. Developments in glial cell physiology were also greatly assisted by the introduction of neuroglial cell cultures (Hild *et al*., 1958; McCarthy & de Vellis, 1980), which allowed the study of these cells in isolation and excluded indirect effects associated with neuronal excitation.

The first breakthrough discovery using these new techniques was made in 1984, when groups led by Helmut Kettenmann and Harold Kimelberg discovered glutamate and GABA receptors in cultured astrocytes and oligodendrocytes (Figure 1.27; Bowman and Kimelberg, 1984; Kettenmann *et al*., 1984).

Several years later, in 1990, Ann Cornell-Bell and her co-workers (Cornell-Bell *et al*., 1990) found that astroglial cells are capable of long-distance communication by means of propagating calcium waves (Figure 1.28). These calcium waves can be initiated by stimulation of various neurotransmitter receptors in the astroglial plasma membrane.

Finally, in 1994, two studies carried out by Maiken Nedergaard, Philip Haydon and Vladimir Parpura demonstrated that astrocytes can trigger calcium increases in neurones when growing together in co-cultures (Nedergaard, 1994; Parpura *et al*., 1994). These discoveries were fundamental in demonstrating that glial cells can mount an active response to brain chemical signalling as well as signal back to neurones, and thus can be involved in information processing in the CNS.

Detailed analysis of the expression of these receptors performed during subsequent decades has demonstrated that glial cells, and especially astrocytes, are capable of expressing virtually every type of neurotransmitter receptor known so far. Moreover, glial cells have been found to possess a multitude of ion channels, which can be activated by various extracellular and intracellular stimuli. Thus, glial cells are endowed with the proper tools to detect the activity of neighbouring neurones. Neurotransmitter receptors and ion channels expressed in glial cells turned out to be truly operational. It has now been shown, in numerous experiments on various regions of the CNS and PNS,

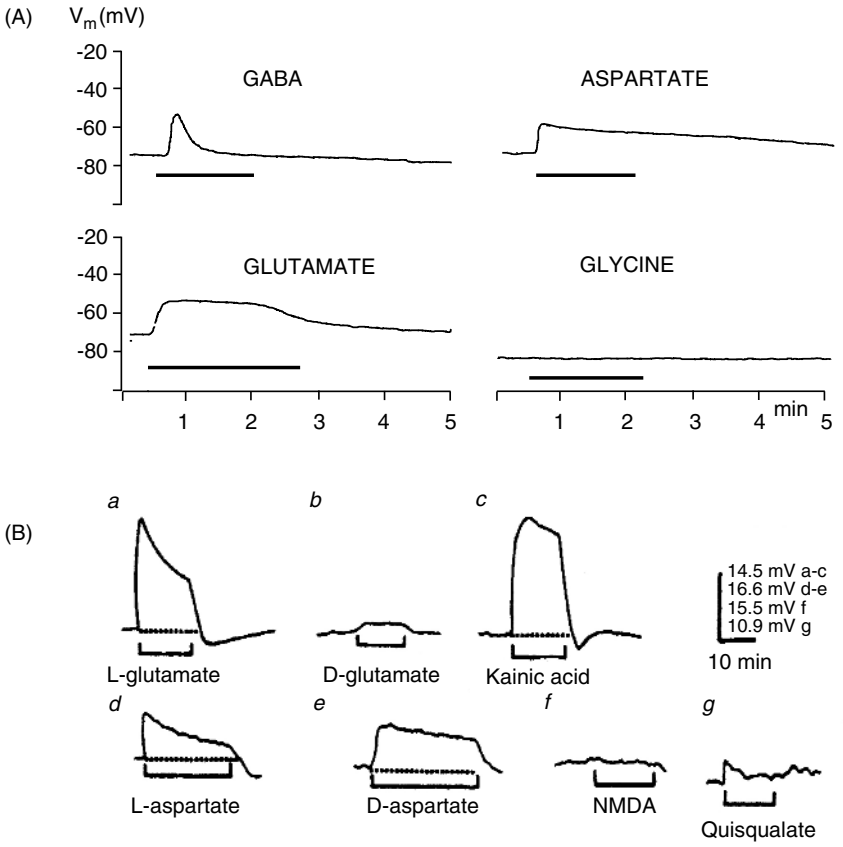


Figure 1.27 Identification of functional neurotransmitter receptors in cultured astrocytes.

A. Microelectrode recordings from cultured astrocytes. The traces show effects of γ-aminobutyric acid (GABA), glutamate, aspartate and glycine all applied at a concentration of 1 mM. Reproduced with permission from Kettenmann, H. et al. (1984) Aspartate, glutamate and y-aminobutyric acid depolarize cultured astrocytes. Neuroscience Letters 52:1–2, pp. 25–29 © Elsevier

B. Effects of excitatory amino acids on membrane potential of cultured astrocytes. Amino acids were applied at a concentration of 10 mM. Membrane potentials of the cells shown before addition of the amino acids were: a, –85 mV; b, –87 mV; c, –78 mV; d, –56 mV; e, –70 mV; f, –86 mV; and g, –64 mV. Reproduced with permission from Bowman & Kimelberg, 1984

that neuronal activity triggers membrane currents and/or cytosolic calcium signals in glial cells closely associated with neuronal synaptic contacts.

Finally, glial cells can also feed signals back to neurones, as they are able to secrete neurotransmitters such as glutamate and ATP. This discovery resulted from the efforts of several research groups, and it has led to the concept of much closer

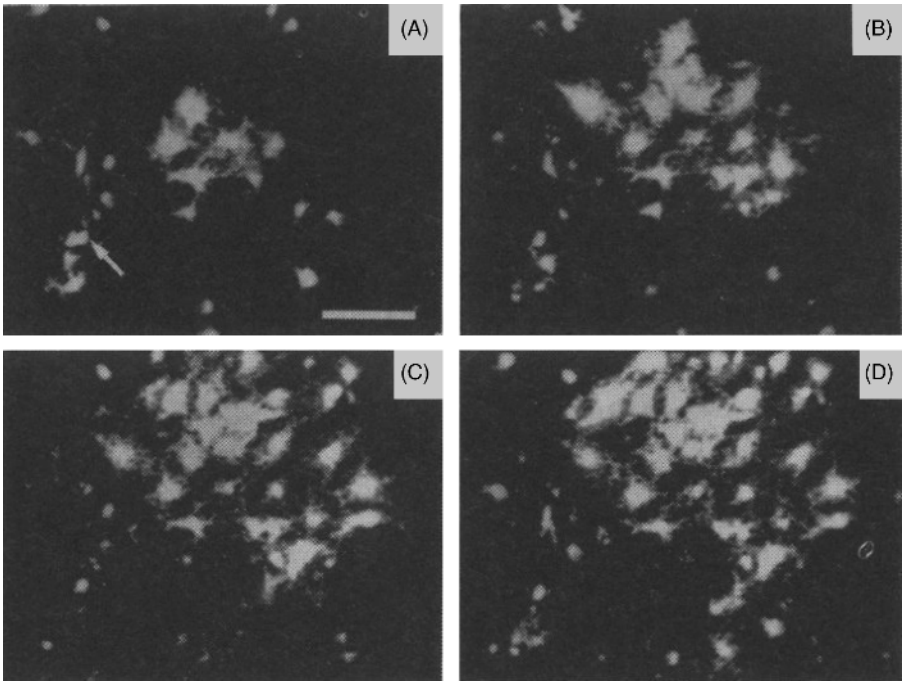


Figure 1.28 First recordings of Ca^{2+} waves propagating through astroglial syncytium in culture after focal stimulation with glutamate.

Reproduced with permission from Cornell-Bell *et al.*, 1990.

interactions between two circuits – neuronal and glial – which communicate via chemical and electrical synapses.

1.6 Concluding remarks

The 150 years of glial research resulted in remarkable changes in concepts and understanding of the role of these cells in the nervous system. Glial cells underwent a long evolutionary history and developed into indispensable homeostatic elements of the brain. Many of the theories of glial cell function originate from the early research of the 19th century, and many of these theories have been forgotten for decades. However, they are now re-emerging, supported by a wealth of newly acquired experimental data.

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