Review

Where the thoughts dwell: The physiology of neuronal–glial “diffuse neural net”

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\textbf{ABSTRACT}

The mechanisms underlying the production of thoughts by exceedingly complex cellular networks that construct the human brain constitute the most challenging problem of natural sciences. Our understanding of the brain function is very much shaped by the neuronal doctrine that assumes that neuronal networks represent the only substrate for cognition. These neuronal networks however are embedded into much larger and probably more complex network formed by neuroglia. The latter, although being electrically silent, employ many different mechanisms for intercellular signalling. It appears that astrocytes can control synaptic networks and in such a capacity they may represent an integral component of the computational power of the brain rather than being just brain “connective tissue”. The fundamental question of whether neuroglia is involved in cognition and information processing remains, however, open. Indeed, a remarkable increase in the number of glial cells that distinguishes the human brain can be simply a result of exceedingly high specialisation of the neuronal networks, which delegated all matters of survival and maintenance to the neuroglia. At the same time potential power of analogue processing offered by internally connected glial networks may represent the alternative mechanism involved in cognition.

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1. The neural cells

“Omnis cellula e cellula” this aphorism initially coined by François-Vincent Raspail and subsequently popularised by Rudolf Virchow is an epitome of the biological revolution of 19th century, which begun from the identification of cellular nature of life and ended in theoretical understanding of evolution and genetic code.

The general idea about elementary units of life, from which all tissues and organisms are formed, began to circulate in the early 17th century, being promulgated, for example, by Pierre Gassendi and Robert Boyle. The origins of cellular theory are rooted in the discoveries of the first microscopists (e.g. Robert Hooke, Marcello Malpighi and Nehemiah Grew) made in the 17th century. It all began with the microscopic observations of the plants, and it was Robert Hooke, who, when observing the fine structures of cork, visualised the regular structures that reminded him of the monk’s cells in the monasterial dormitories — and the term “cell” was born (Hooke, 1665).

The first animal cells were discovered, to 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 the sagittal slice of the peripheral nerve (Fig. 1, (Bentivoglio, 1996; Leeuwenhoek, 1673–1696; Leeuwenhoek, 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 an indescribable fineness, running lengthwise to form the nerve” (cited from Bentivoglio, 1996). The fine cylindrical nerve fibres were also described by Felice Gaspar Ferdinand Fontana, who mechanically dissected the nerve and observed his preparations under 700× magnification (Bentivoglio, 1996). More cell types were discovered at the beginning of 19th century, and around 1830 Robert Brown found the nucleus (Ford, 1992). The cellular theory was then formalised by Theodor Schwann and Matthias Jakob Schleiden (Schleiden, 1838; Schwann, 1839; Schwann and Schleiden, 1847) and was rapidly embraced by the scientific community.

The first cells of the nervous system were visualised in 1830-ies. Probably the first observations were done by Christian Gottfried Ehrenberg who was investigating the nervous system of the leech (Ehrenberg, 1836) and by Johann Evangelista Purkinje (Figs. 1B and C) who was studying cerebellum and described the cell named after him (Purkinje, 1837). Purkinje’s pupil, Gustav Gabriel Valentin came with the first published drawing of the neurone (which he called Kugeln — Fig. 1D) where the nucleus and other intracellular structures were visible (Valentin, 1836). Incidentally, Valentin also was the first to suggest that the nervous system contains both active and passive elements.

The passive elements of the brain were soon conceptualised by Rudolf Virchow who introduced connective tissue to the nervous system under the name of neuroglia (Virchow, 1856; Virchow, 1858), for the more detailed account on the history of glia see Kettenmann and Ransom (2005), Kettenmann and Verkhratsky (2008), Verkhratsky (2006b) and Verkhratsky (2009). Virchow did not recognise neuroglia as a specific class of neural cells; the drawings of some round structures, which he reproduced in the Die Cellularpathologie, show, if anything, activated microglia. This did not matter, because for Virchow the neuroglia was a connective tissue of mesodermal origin; the misconception, which was embraced by many histologists (e.g. by Andriezen, Robertson and Weigart) and which was still believed by Cajal as late as in 1920 (Ramón y Cajal, 1920).

In the meantime discoveries of the neural cells continued. In 1838 Robert Remak made the description of nerve fibres and visualised the covering sheath around them (Remak, 1838). In 1851 Heinrich Müller (1851) produced the first images of retinal radial glia (the cells which were subsequently named Müller cells by Rudolf Albert von Kölliker (1852b). Von Kölliker by himself also described nerve elements in the acoustic nerve of the ox (Kölliker, 1852a). In 1858 Max Schulze made a detailed investigation of the Müller cells and produced probably the best possible drawings of them in the pre-staining era of histology. Simultaneously Karl Bergmann (1857) had identified radial glial cells in the cerebellum (the Bergmann glial cells). 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 (that to all probability were astrocytes — Figs. 1E, F and G) were made by young (who untimely deceased at 29) Otto Deiters (1865), and some years later Jacob Henle and...
Friedrich Merkel visualised the glial network in the grey matter (Fig. 1H—(Henle and Merkel, 1869)).

It has to be stressed that all these early cellular images were done mostly on unstained preparations following painstaking cells isolation 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; Golgi, 1903)), which, for the first time, allowed neurohistologists to obtain images of neural cells in their entity (Fig. 2; for a comprehensive and vividly written account of the dissemination of Golgi staining technique through the world see Mazzarello (2010)).

As early as 1871 Golgi recognised glial cells and he was the first to demonstrate that the glia represent cellular population
Fig. 2 – Astroglial cells stained by the silver-chromate technique. A: Protoplasmic astroglial cells in the grey matter stained and drawn by Camillo Golgi; the astrocytes form numerous contacts (the endfeet) with brain capillaries (Golgi, 1883). The image was kindly provided by Prof. Paolo Mozzarello. B. Morphological heterogeneity of human astrocytes. The astrocytes in the brain slices of human foetuses were stained by silver-chromate technique (Retzius, 1894).
distinct from nervous cells, and that the glial cells (which were to all probability the protoplasmic astrocytes) send the processes to the blood vessels where they establish the endfoot structures (Fig. 2A); he was also the first to propose that the glial cells are instrumental in transferring the metabolic substances from the blood to the brain parenchyma (Golgi, 1875; Golgi, 1894), this concept subsequently gained the universal acceptance. The application of Golgi staining led to a further in depth characterisation of glial cells of the grey matter, and in 1891 the term "astrocyte" was introduced by Michael von Lenhossek (Lenhossek, 1891; Lenhossek, 1893); slightly later the astroglia was further subdivided into protoplasmic (Andriezen, 1893) and fibrous (Kölliker, 1893) astrocytes. At the same years 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; His, 1889b).

The end of 1880-ies marked the arrival of the neurone and of Santiago Ramón y Cajal, who was to champion and develop the neuronal doctrine over the next decades. The first papers of Cajal 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 Trimestral de Histología Normal y Patológica, 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). 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 of 1889, Cajal attended the German Anatomical congress where he demonstrated his numerous microscopic preparations (Fig. 3) 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" (Waldeyer von, 1891) and neuronal doctrine begun to conquer the minds of neuroscientists. The neurone was endowed with dendrites (the term was introduced by Wilhelm His in 1889) and very soon it acquired the axon (which was named so by Alfred Kölliker in 1896).

The previous (and the first) grand theory about the brain functional organisation was formalised by Josef von Gerlach (1871), who proposed the diffuse network of neurofilaments and neural cell processes, that are internally connected and act in concert. This "reticular" theory dominated neuroscience for good 20 years and recruited many supporters including Golgi, who was very much convinced in the existence of "diffuse neural net". The history of the neuronism–reticularism conflict was widely popularised, and the curious reader may find all the dramatic details in several comprehensive treatises (Jacobson, 2005; Lopez-Munoz et al., 2006; Mazzarello, 2007; Mazzarello, 2010; Ramón y Cajal, 1933; Shepherd, 1991).

Here we shall continue with a brief outline of further discoveries of glial cells (Fig. 3), which, curiously enough, are very much connected with Cajal’s school and with his pupils, Nicolás Achúcarro and Pío del Río-Hortega. First, Cajal developed gold chloride-sublimate staining method that was specific for both protoplasmic and fibrous astrocytes (García-Marin et al., 2007). 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 (reviewed in Kimelberg, 2004). 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; Ramón y Cajal, 1913b; Ramón y Cajal, 1916). Subsequently 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) and what represented, in fact, the oligodendrocytes and the microglia.

Oligodendrocytes were initially observed and described by William Ford Robertson who developed for this purpose a specific platinum stain technique; Robertson however did not realise the myelinating capacity and role of these cells and thought them external invaders to the brain, hence identifying them as mesoglia (Robertson, 1899; Robertson, 1900). Almost 20 years later Río-Hortega rediscovered these cells and gave them a name of oligodendrocytes (Del Río-Hortega, 1921; Gibson, 1994); he also demonstrated that oligodendrocytes were myelinating cells in the CNS being thus analogous to the Schwann cells in the periphery. It was also Río-Hortega who described the microglial cells, the only glia members of non-neuronal origin (for staining of both oligodendrocytes and microglia Río-Hortega deployed specifically developed ammoniacal silver carbonate method (Del Río-Hortega, 1917)). Initially Río-Hortega called these cells “garbage collectors”; in the literature they also were known as “cells of Hortega” (Gibson, 1993; Gibson, 1994). Del Río-Hortega gave microglia a very detailed and comprehensive characterisation. 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 these cells reacted to brain damage by morphological and functional modification known as microglial activation (Del Río-Hortega, 1919a; Del Río-Hortega, 1919b; Del Río-Hortega, 1920; Del Río-Hortega, 1927; Del Río-Hortega, 1932).

Thus by the 1920-ies the overall understanding of morphological organisation of the brain and the spinal cord has been generally completed, and the following developments were mostly dedicated to uncovering the functional mechanisms by which neural cells communicate and by which they may perform higher brain functions. By then the neuronal doctrine became absolutely dominating, and the synapse, the name of which was introduced by Charles Scott Sherrington (Foster and Sherrington, 1897; Sherrington, 1906) has received a particular attention as the principal place of integration in the nervous system. Rapidly developing electrophysiological experimental techniques allowed monitoring of the functional activity of neurons; and the image of neuronal network where binary signals (action potentials) swiftly convey the information though neuronal membranes and activate synaptic release of neurotransmitters that subsequently trigger changes in postsynaptic potential, which, in turn, provide for the electronic summation thus integrating the incoming signals, was universally accepted.
2. Neuroglia organises and maintains the nervous system

“It may seem strange that, since I have always been opposed to the neurone theory – although acknowledging that its starting-point is to be found in my own work – I have chosen this question of the neurone as the subject of my lecture, and that it comes at a time when this doctrine is generally recognized to be going out of fashion.” (Camillo Golgi starting the Nobel lecture (Golgi, 1906))

The nature of information processing in the human brain and the role of different cell types and their temporal and spatial relations, the role of fast and slow processes and the nature of signalling events that occur between and within the cells as well as comprehension of the relevance of all these immensely complex processes in the generations of thoughts, emotions and creative ideas remain very far from understanding. The neuronal doctrine regards neuronal networks as a sole substance for information processing; the knowledge accumulated in the last 2 decades indicates that the overall picture can be more complex. Here we shall briefly overview the cell biology and physiology of neuroglia from the perspective of the integrated neuronal–glial networks as a substrate of brain function.

2.1. Evolutionary aspect

The primordial glial cells appeared early in evolution (Fig. 4), probably already at the stage of diffuse nervous system when...
they assisted ancestral neurones in their functions (Bacaj et al., 2008; Reichenbach and Pannicke, 2008). Development of neuronal masses organised first in ganglia and then in the functionally segregated centralised nervous system, stimulated the evolution of supporting cells, the proto-astrocytes, which controlled the extracellular homeostasis, provided metabolic support, regulated development and assisted neurones in fulfilling their primary functions. In Caenorhabditis elegans worm the artificial elimination of glia (some of the glial cells are associated with the nematode sensory organ) does not result in wide-spread neuronal death, but dramatically alters function and sensitivity of sensory neurones (Bacaj et al., 2008). Incidentally a number of signalling and enzymatic cascades present in glial cells are exceptionally highly conserved in evolution, being similar in the worms, insects and mammals (Barres, 2008). At the higher evolutionary stages astroglial cells remain an important part of both sensory organs and the CNS, where they assume multiple roles, and much higher significance — elimination of astroglia from the CNS results in neuronal demise. The high degree of specialisation acquired by neurones in the centralised nervous system renders them helpless in the absence of the glial support; this support (which contributes at many levels of brain homeostasis) is the ancestral function of astrocytes that remains throughout the evolution. Another important function that appeared very early in the evolutionary ladder was the isolation of the nervous tissue from the rest of the body by the blood–brain barrier (BBB), which is made solely by astrocytes in crustacean, insects and cephalopods. Interestingly that astroglial blood–brain barrier is also present in the brain of sharks; some of the capillaries are completely surrounded by astroglial process being thus endocellular vessels (Long et al., 1968). At the later stages of phylogenesis the BBB function is shifted to endothelial cells, the latter nonetheless remain under astrocytic control (Abbott, 2005; Abbott and Pichon, 1987).

An increase in the size of living organisms naturally presented an additional survivalist task — an increase of the
speed of communication between the periphery and the central nervous organs. Demand for an increased velocity of electrical propagation through the axons led to an appearance of myelin isolators provided by oligodendrocytes and Schwann cells. The myelin isolation appeared sometimes in Ordovician period (Fields, 2008a; Hartline, 2008; Roots, 2008) in the ancestral invertebrates (e.g. in oligochaetes (earthworms)), in malacostraca (shrimps and prawns), in copepods (small crustaceans) and in gnathostomes (the jawed fishes; although the class includes not only sharks and rays but also tetrapods).

Finally, the isolated position of the nervous tissue required self-defence system and the immune cells began to invade the ganglia in bivalve, crustacean and insects thus forming the ancestral microglia, which is present in brain parenchyma at all subsequent evolutionary stages (Liu et al., 1996; Magazine et al., 1996; Stefano et al., 1996).

Neuroglia attained maximal development in mammals, and moreover an increased complexity of the brain, together with an increased intellectual power was accompanied with remarkable increase in both numbers and complexity of glia (Oberheim et al., 2006; Reichenbach, 1989; Verkrhatsky, 2009; Verkrhatsky and Butt, 2007). Astrocytes became the most numerous cells in the human brain, outnumbering neurones. For example, in layers I and IV of the rat cerebral cortex, neurones/glial cells (astrocytes and oligodendrocytes) ratio is ~2.5 to 1 (Bass et al., 1971). This is inverted in the humans: the glia to neurones ratio in human cortex is ~1.65:1 (Nedergaard et al., 2003; Sherwood et al., 2006). Furthermore, the morphology of human astrocytes is unique when compared to all other mammals (Oberheim et al., 2009); the size of human protoplasmic astrocyte is about 2.5–3 times and fibrous astrocytes ~2.2 times larger than in rodents. The human protoplasmic astrocytes have ~10 times more primary processes, and correspondingly much more complex processes arborisation than rodent astroglia. As a result, human protoplasmic astrocyte contacts and integrates ~2 millions of synapses residing in its territorial domain, whereas rodent astrocytes cover ~20,000–120,000 synaptic contacts.

2.2. Developmental aspect

Neurones and neuroglia both originate from the neuroepithelial cells (Alvarez-Buylla et al., 2001), which phylogenetically correspond to the ancestral neuroepithelium. The latter comprised secretory epithelial cells, which were connected by gap junctions and probably expressed some sets of voltage-gated channels. All in all, the very primordial neural elements were, most likely, physiologically closer to neuroglia, than to the neurones judging on their excitability and syncytia-driven signal propagation.

Be it all as it may, the neuroepithelial cells transform into the radial glia, which act as a universal neural precursor; the asymmetrical division of radial glia produce neuronal precursors that migrate to their destinations using the processes of their progenitor as a scaffolding guide-line. The radial glia also act as progenitors (through several transitional forms) for both astrocytes and oligodendrocytes. Some of the astrocytes, dwelling in the germinal centres of the adult brain, retain the stem cell properties throughout the life span and underlie the adult neuro- and glio-genesis (see Alvarez-Buylla et al. (2001), Doetsch et al. (1999), Gotz and Huttner (2005) and Mori et al. (2005) for a comprehensive review). In addition neuroglial cells are instrumental in promoting neuronal survival at different developmental stages through the release of numerous neurotrophic factors (e.g. epidermal growth factor, EGF, Glial cell-derived neurotrophic factor, GDNF, etc); and by the activation of astrocytic transcriptome-associated genes such as ApoE, ApoJ, MFGE8 and cystatin C (Banker, 1980; Barres, 2008; Wagner et al., 2006).

2.3. Anatomical aspect

In mammalian brain the astroglial cells define the micro-architecture of the parenchyma by dividing the grey matter (through the process known as “tiling” (Bushong et al., 2004)) into relatively independent structural units. The protoplasmic astrocytes occupy their own territory and create the micro-anatomical domains within the limits of their processes (Bushong et al., 2002; Halassa et al., 2007b; Nedergaard et al., 2003; Oberheim et al., 2009; Ogata and Kosaka, 2002; Willemsen et al., 2006). Within the confines of these anatomical domains the membrane of the astrocyte covers synapses and neuronal membranes as well as sends the process to plaster the wall of the neighbouring blood vessel with the endfoot.

The individual astroglial domains are integrated into the superstructure of astroglial syncytia through gap junctions (Giaume and Venance, 1998) localised on the peripheral processes of astroglial cells. These astroglial syncytia are also anatomically segregated being formed within defined anatomical structures, for example in individual barrels of the somatosensory cortex (Giaume et al., 2009).

The second main class of neuroglia, the oligodendroglia, is central in moulding and maintaining the white matter. In the human brain the white matter is much larger as compared to other species; it makes more that 50% of the whole brain, which most likely reflects a high demand for interneuronal connections (see Fields (2008b) for comprehensive review). The oligodendrocytes demonstrate an exceptionally high degree of plasticity and myelination can be rapidly modulated in response to various learning paradigms (Fields, 2008b). The oligodendrocytes are actively communicating with neurones, and this reciprocal signalling, details of which only begin to be uncovered, profoundly contributes to overall brain plasticity and cognitive processes.

2.4. Homeostatic aspect

Astroglia represents the main cellular element of extracellular homeostasis in the brain. Indeed the control over the concentrations of ions, neurotransmitters, neuromodulators, metabolites and other active substances is of a paramount importance for normal operation of neural networks, because even relatively small changes in a number of molecules may result in substantial shifts of their respective concentrations in tiny and often diffusionally isolated extracellular compartments.

2.4.1. Ion and water homeostasis

Astroglia assume the leading role in regulation of extracellular K+. The concentration of the latter may rise up to 10–12 mM...
following frequent action potential firing that may substantially affect the excitability of neuronal networks. Astrocytes remove the excess of extracellular K⁺ via local K⁺ uptake (accomplished by inward rectifier K⁺ channels) and K⁺ spatial buffering (Coles and Orkand, 1983; Kofuji and Newman, 2004; Newman, 1995). The latter mechanism provides for the redistribution of K⁺ from the areas with elevated [K+]o to the regions with low [K+]o, and may occur either in the single glial cells (K⁺ siphoning in retinal Müller cells (Newman et al., 1984)) or in the glial syncytia. The glial syncytia and aquaporine channels expressed in astrocytes also play the most critical role in regulation of brain water homeostasis (Simard and Nedergaard, 2004).

2.4.2. Neurotransmitter homeostasis

The second important homeostatic task accomplished by astroglial cells is the control over extracellular concentration of neurotransmitters, and most importantly, glutamate. The latter, when released in excess or for a long-time, acts as a powerful neurotoxin that triggers neuronal cell death in a multitude of acute and chronic brain lesions (Caudle and Zhang, 2009; Obrenovitch et al., 2000; Schousboe et al., 1997; Westbrook, 1993). Interestingly, the glial function to “chemically split or take up” transmitters was speculated upon by an Italian psychiatrist Ernesto Lugaro in 1907 (Lugaro, 1907).

Astrocytes are responsible for the bulk of glutamate removal from the extracellular space: they accumulate 80% of the glutamate released, whereas the remaining 20% goes to neurones (Swanson, 2005; Verkhratsky and Kirchhoff, 2007a). Astrocytic uptake of glutamate from extracellular space is also an important modulator of the time-course of synaptic neurotransmission (Mennerick and Zorumski, 1994).

There are five types of glutamate transporters operative in the human brain (Gadea and Lopez-Colome, 2001); these are known as EAAT1 to EAAT5 (where EAAT stands for excitatory amino acid transporter). Astroglial cells express almost exclusively the EAAT1 and EAAT2 (known in rodent brain as glutamate/aspartate transporter, GLAST, and glutamate transporter-1, GLT-1) that accomplish the bulk of glutamate uptake (Danbolt, 2001); some expression of GLT-1a has been recently reported at neuronal pre-synaptic terminals (Furness et al., 2008; Melone et al., 2009). The glutamate transporters are co-transporters which utilise the energy saved in the form of transmembrane Na⁺ gradient so that the transport of a single glutamate molecule requires an influx of 3 Na⁺ ions and 1 H⁺ ion coupled with the efflux of 1 K⁺ ion (Kirischuk et al., 2007). The substantial sodium accumulation accompanying glutamate accumulation is counterbalanced by Na⁺ efflux through Na⁺/Ca²⁺ exchanger working in the reverse mode (Kirischuk et al., 1997; Kirischuk et al., 2007); both the Na⁺/Ca²⁺ exchanger and glutamate transporters are co-localised in perisynaptic astroglial processes (Minelli et al., 2009).

Astroglial glutamate transport is crucial for neuronal glutamatergic transmission by operating the glutamate–glutamine shuttle (Verkhratsky and Butt, 2007). Glutamate, accumulated by astrocytes is enzymatically converted into glutamine by the astrocytic-specific glutamine synthetase (Martinez-Hernandez et al., 1977). Glutamine can be safely transported to pre-synaptic terminals through the extracellular space; after entering the neuronal compartment glutamine is transformed into glutamate. It is also of importance that astrocytes possess the enzyme pyruvate carboxylase, and thus act as a main source for de novo glutamate synthesis (Hertz et al., 1999).

2.4.3. Control of local blood flow and metabolic support

The astroglial cells are the central elements of the neurovascular units that integrate neural circuitry with local blood flow and metabolic support. The astrocyte, which determines the confines of the neurovascular unit, bridges the brain parenchyma and local vasculature by virtue of perivascular process and the endfeet. Increased activity of neurones integrated into the neurovascular unit triggers Ca²⁺ signals in the astrocyte, which, in turn, stimulate release of vasoactive agents that regulate the local blood flow (Metea and Newman, 2006; Mulligan and MacVicar, 2004; Zonta et al., 2003). Similarly, astrocytes are also responsible for local metabolic support of active neurones though the postulated glucose–lactate shuttle; the latter being under the control of cytosolic Na⁺ concentration (Magistretti, 2006; Magistretti, 2009).

2.5. Physiological aspect: neuroglia and information processing in the brain

The glia is electrically non-excitable, and therefore is unable to use the plasmalemmal ion conductances to rapidly convey the information. Nonetheless, glial cells are capable of expressing the excitable molecules: the voltage-gated ion channels, the neurotransmitter receptors, and the intracellular signalling chains. Furthermore, the neuroglia is capable of developing propagating signals either through the gap junctions or through the release of gliotransmitters. Conceptually, glial cells can perform all physiological processes as neurones do (recognise the incoming information, generate propagating signals, and secrete the transmitters that form the informational output), albeit the neuroglia executes these physiological processes in a specific and distinct way.

2.5.1. Glial receptors

Neuroglial cells are capable to express virtually every receptor to neurotransmitters, neurohormones and neuromodulators. This fundamentally important property was discovered in the multitude of early experiments on cultured glial cells that demonstrated the sensitivity of glia to a variety of stimulating agents (Condorelli et al., 1999; Cornell Bell et al., 1990; Dave et al., 1991; Fraser et al., 1995; Gallo and Ghiani, 2000; Verkhratsky et al., 1998; Verkhratsky and Steinhauser, 2000). This promiscuity of cultured glia towards neuroactive ligands is generally regarded as an obstacle, and indeed the cultured neuroglial cells are very poor models of the glia in vivo, both morphologically and functionally. Nonetheless, these early experiments were specifically important because they showed, beyond any doubt, that the neuroglia is endowed with molecular machinery to participate in chemical transmission in neural networks.

The receptor patterns expressed by glial cells in situ are very different, as the complement of receptors is quite restrictive depending on the brain region, and is, most likely, controlled by the local neurotransmitter environment. Usually the modality of neurotransmitter receptors expressed by astroglia...
is similar to that expressed in their neuronal neighbours (Verkhratsky and Kettenmann, 1996; Verkhratsky et al., 1998). The most abundant neurotransmitter receptors expressed by neuroglia are glutamate receptors and purinoceptors, although the expression of particular subtypes varies between brain regions. Most of astrocytes and oligodendrocytes express metabotropic glutamate receptors of mGluR3 and mGluR5 variety; the latter receptors are instrumental for generation of glial Ca\(^{2+}\) signals (Hamilton et al., 2008; Kirischuk et al., 1999; Tamaru et al., 2001; Verkrhatsky and Kirchhoff, 2007a). Astro- and oligodendroglia in hippocampus, corpus callosum, optic nerve and cerebellum show relatively high levels of expression of ionotropic AMPA receptors, quite often of high-Ca\(^{2+}\)-permeable form (Garcia-Barcina and Matute, 1998; Matute and Miledi, 1993; Muller et al., 1992; Seifert and Steinhauser, 2001; Steinhäuser and Gallo, 1996; Verkhratsky and Kirchhoff, 2007a). Cortical astrocytes and oligodendrocytes in the corpus callosum, cerebellum and the optic nerve were found in cortical astroglia (Lalo et al., 2008), yet P2X receptors are abundantly expressed in microglia; these receptors arguably, inherited the sensitivity to purines from their epithelial ancestors; be it as it may, all glial cells have sensitivity to purines mediated through several types of P2 (ATP/nucleotide) and/or P1 (adenosine) receptors. Activation of purinoceptors controls many vital functions of glial cells being instrumental for glial signalling, release of neurotransmitters, initiation of astrogliosis and activation of microglia (Verkhratsky et al., 2009). The actual mapping of purinoceptors in various types of glia has not been accomplished; nonetheless majority of astrocytes and oligodendrocytes possess metabotropic A1,2A,2B,3 adenosine receptors and P2Y1,2,4,6, nucleotide receptors coupled to the InsP3-signalling chain and Ca\(^{2+}\) signalling (Abbracchio and Burnstock, 1998; Boison et al., in press; Kirischuk et al., 1995a; Kirischuk et al., 1995b; Verkrhatsky et al., 2009). Glial expression of ionotropic P2X receptors is much less clear; the P2X4,5 mediated currents were found in cortical astroglia (Lalo et al., 2008), yet P2X currents were detected neither in hippocampal astrocytes (Jabs et al., 2007) nor in Bergmann glial cells (Kirischuk et al., 1995a). There is some evidence of functional expression of P2X receptors in oligodendrocytes, although they may operate mostly in pathological conditions (Domerq et al., 2010; Matute, 2008). Finally, multiple P2Y and P2X receptors are abundantly expressed in microglia; these receptors control activation, motility, release of cytokines and exert numerous trophic effects upon microglia (Farber and Kettenmann, 2006b; Haas et al., 1996; Hanisch and Kettenmann, 2007; Inoue, 2008; Inoue and Tsuda, 2009; Inoue et al., 2005; Moller et al., 2000).

The two main receptor systems, glutamatergic and purinergic, expressed in glial cells are complemented by almost limitless combinations of other receptors that allow glia to accurately receive all types of chemical transmission that occurs in the brain. In addition, the morphological proximity of astroglial membranes and synaptic structures (which in fact make the central synapses tripartite — i.e. comprising presynaptic and postsynaptic compartments as well as astroglial perisynaptic process (Araque, 2008; Araque et al., 1999; Halassa et al., 2007a)) almost certainly indicates the continuous involvement of glia in the ongoing synaptic transmission.

2.5.2. Synaptic transmission onto glia

Astrocytes are able to receive inputs from synaptic transmission during the spill-over of a neurotransmitter for which they express appropriate receptors. Dani et al. (1992), using cultured organotypic hippocampal slices, have demonstrated that neuronal activity at mossy fibre—CA3 synapses can trigger [Ca\(^{2+}\)], increases and intercellular waves in an astrocytic network mediated by glutamate released from synaptic terminals. Similarly, astrocytes can respond to neurotransmitter release in acute slices. Porter and McCarthy (1996), using acutely isolated hippocampal slices, have observed that electrical stimulation of Schaffer collaterals caused increases in [Ca\(^{2+}\)]; in astrocytes located in the stratum radiatum of the CA1 region. At the lower level of synaptic activity this effect was mediated by mGluRs, while the higher levels of neuronal activity recruited both mGluRs and AMPA/kainotopic GluRs on astrocytes. Glutamate is not the only mediator of this neurone–astrocyte signalling via spill-over. For example synaptically released acetylcholine also evokes [Ca\(^{2+}\)], elevations in astrocytes in hippocampal slices through the activation of metabotropic acetylcholine receptors (Araque et al., 2002).

Besides glial cells sensing spill-over of synaptically released neurotransmitters, they can receive direct synapse-like (synaptoid) or even classical synaptic connections. Stimulation of pituitary stalk can depolarize stellate astrocyte-like glia, pitiucytes, in situ, an effect mediated by \(\gamma\)-aminobutyric acid (GABA) and dopamine receptors on this glial cell type (Mudrick-Donnon et al., 1993) and occurring via direct inputs from neurones through synaptoid contacts, where axonal projections end on pitiucytes (Buijs et al., 1987; van Leeuwen et al., 1983; Wittkowski and Brinkmann, 1974). Similarly, norepinephrine terminals make synaptoid contacts onto septohippocampal astrocytes (Milner et al., 1995). Direct neuronal glutamatergic and GABAergic synapses made on oligodendrocyte precursor cells were observed in the hippocampus (Bergles et al., 2000; Lin and Bergles, 2004).

2.5.3. Signalling in glial syncytia

The extended complement of plasmalemmal receptors, expressed by glial cells is coupled to intracellular signalling cascades, which provide glia with specific excitability mechanisms. The glial excitability, as we can perceive it today, is based on the excitability of the endomembrane (which forms the endoplasmic reticulum, the ER) endowed with Ca\(^{2+}\) release channels, represented by InsP3 receptors and ryanodine receptors (Berridge et al., 2003; Kostyuk and Verkhratsky, 1994; Verkhratsky, 2005). Stimulation of the
these can be important for the plasticity and information processing, but we know very little about these alternative pathways, and yet... (Rouach et al., 2008), and maybe some other molecules. We know that various second messengers, metabolic substrates (e.g., ATP) release (Anderson et al., 2004; Arcuino et al., 2002; Guthrie et al., 1999; Haas et al., 2006; Scemes and Giaume, 2006; Verkhratsky and Toescu, 2006). The actual mechanisms of generation and maintenance of intercellular Ca\(^{2+}\) waves are complex and involve InsP\(_3\) diffusion through gap junctions as well as gliotransmitter (most notably ATP release (Anderson et al., 2004; Arcuino et al., 2002; Guthrie et al., 1999; Haas et al., 2006; Scemes and Giaume, 2006; Suadicani et al., 2004; Verkhratsky, 2006a)).

The gap junctional route can also form the pathways for alternative signalling in the astroglial syncytia, which may involve various second messengers, metabolic substrates (Rouach et al., 2008), and maybe some other molecules. We know very little about these alternative pathways, and yet these can be important for the plasticity and information processing by neuroglia. Furthermore not only Ca\(^{2+}\) but also other ions may serve as glial signallers. This, for example, may be an important function of Na\(^+\) ions. The concentration of Na\(^+\) is often increased in glia following activation of transporter systems and ionotropic receptors (Deitmer and Rose, 2010; Kirischuk et al., 2007; Langer and Rose, 2009). Sodium ions are capable of activating enzymatic systems. An increase in astroglial [Na\(^+\)], activates Na\(^+\)-K\(^-\) ATPase, which, in turn, stimulates phosphoglycerate kinase thus initiating production of lactate and lactate shuttling to neurones.

The glial networks established by the plethora of gap junctions formed between glial cells can also connect them to neuronal networks. Although gap junctions between glial cells and neurones are not common, their existence has been demonstrated in crayfish (Peracchia, 1981). Additionally, direct heterocellular coupling of mammalian neurones and glia has been demonstrated in neuronal-glial co-cultures (Nedergaard, 1994; Froes et al., 1999), in acute slices from the locus coeruleus (Alvarez-Maubecin et al., 2000) and in the cerebellum (Pakhotin and Verkhratsky, 2005).

2.5.4. Gliotransmission and exocytotic release

Astrocytes and other glial cells can release a variety of transmitters into the extracellular space. The criteria for a chemical released from glia to be classified as a gliotransmitter have been defined (Do et al., 1997; Martin et al., 2007; Volterra and Meldolesi, 2005). Gliotransmitters are classified based on a working set of criteria: (i) synthesis by and/or storage in glia; (ii) regulated release triggered by physiological and/or pathological stimuli; (iii) activation of rapid (milliseconds to seconds) responses in neighbouring cells; and (iv) a role in (patho) physiological processes (Parpura and Zorec, 2010).

Since the first demonstration of the release of GABA from glial cells in superior cervical ganglia (Bowery et al., 1976), a quest for understanding the mechanisms and conditions that underlie transmitter release is underway. Several different mechanisms have been implicated in release of a gliotransmitter: (i) through channels like anion channel opening, induced by cell swelling (Pasantes Morales and Schousboe, 1988), release through functional unpaired “hemichannels” (connexins/pannexins) located at the plasma membrane (Cotrina et al., 1998; Iglesias et al., 2009) and P2X- ionotropic purinergic receptors (Duan et al., 2003); (ii) through transporters, e.g. by reversal of uptake by plasma membrane excitatory amino acid transporters (Szatkowski et al., 1990), exchange via the cystine-glutamate antiporter (Warr et al., 1999) or organic anion transporters (Rosenberg et al., 1994); and (iii) through Ca\(^{2+}\)-dependent exocytosis (Parpura et al., 1994). Gliotransmitters can be divided into three general groups: (i) amino acids and their derivatives, such as glutamate and β-serine; (ii) nucleotides, like ATP, and (iii) peptides (recently reviewed in Parpura and Zorec, 2010). At present, Ca\(^{2+}\)-dependent vesicular release of glutamate and ATP from astrocytes can readily occur under physiological conditions, while there are questions raised as to whether other mechanisms might solely operate during pathophysiological circumstances.

The first documented description of general secretion from glial cells came from the work of a French neuroanatomist-physician Jean Nageotte who proposed that these cells may release substances into the blood, acting like an endocrine gland (Nageotte, 1910). He observed various secretory granules in glial cells of grey matter (astrocytes) using the Altmann method of fucsin labelling (Fig. 5).

He reported: “The facts that I have just observed seems 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 intensely 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 into secretion grains.” (Translated from French; Nageotte, 1910).

Only recently, however, has there been accumulating evidence of biochemical and morphological correlates of the exocytotic process in astrocytes (reviewed in Montana et al., 2006) as briefly below.

Astrocytes express proteins of the core SNARE complex: synaptobrevin 2, syntaxin 1, synaptosome-associated protein of 23 kDa, as well as proteins important for sequestering glutamate/ATP into vesicles: the vacuolar type of proton ATPase (V-ATPase), which drives protons into the vesicular lumen creating the proton concentration gradient necessary for glutamate/ATP transport into vesicles, and the three known isoforms of vesicular glutamate transporters (VGLUTs) 1, 2 and 3 and vesicular nucleotide transporter (VNUT) (reviewed in Parpura and Zorec, 2010). Immunoelectronic microscopy studies demonstrated that VGLUTs 1 or 2 in astrocytes in situ associate with small clear vesicles with a mean diameter of ~30 nm (Bezzi et al., 2004). Astrocytes also display large dense core granules with diameters of ~115 nm, containing the secretory peptide secretogranin II (Calegari et al., 1999) and ATP (Coco et al., 2003). These two populations of vesicles appear to be biochemically and morphologically distinct.

The consequences of Ca²⁺-dependent exocytotic glutamate release from astrocytes can be seen in neurones as: (i) an elevation of neuronal [Ca²⁺], (Parpura et al., 1994); (ii) a slow inward current (Araque et al., 1998a; Araque et al., 1998b); (iii) an increase of excitability (Hassinger et al., 1995); (iv) modulation of spontaneous and action potential evoked synaptic transmission (Araque et al., 1998a; Araque et al., 1998b); (v) synchronization of synaptic events (Fellin et al., 2004), induction of long-term potentiation, LTP (Perea and Araque, 2007) or various combinations of the above (reviewed in Ni et al., 2007). Such effects of the glutamate-mediated astrocyte-neurone signalling pathway have been observed in neurones of different brain regions, including visual cortex, hippocampus, thalamus and nucleus accumbens.

Besides glutamate there are several other vesicularly released mediators of astrocyte-neurone signalling, most notably D-serine and ATP. Astrocytes can exocytotically release D-serine (Mothe et al., 2000) an endogenous NMDA receptor agonist (Schell et al., 1995). Indeed, release of D-serine from glia can contribute to the physiological activation of NMDA receptors expressed on retinal ganglion cells (Stevens et al., 2003), as well as to the hippocampal LTP (Yang et al., 2003). Similarly, ATP can be released from astrocytes and it serves as the major humoral factor in support of astroglial Ca²⁺ wave propagation (Arcuino et al., 2002; Cotrina et al., 2000; Guthrie et al., 1999; Stout et al., 2002). ATP can also contribute to astrocyte-microglia (Verderio and Matteoli, 2001), astrocyte-endothelial cell (Braet et al., 2001), and astrocyte-neurone signalling (Newman, 2003). For example, in the case of astrocyte-neurone signalling, stimulated astrocytes in the whole mount retina can release ATP into extracellular space, where it is rapidly converted by ectoenzymes to adenosine. Subsequent activation of adenosine receptors located on retinal ganglion cells induces hyperpolarization of these cells thus reducing spontaneous firing rate of neurones exhibiting spontaneous spike activity. Similarly, ATP released from astrocytes can cause tonic and activity-dependent suppression of excitatory synaptic transmission in acute slices (Zhang et al., 2003). Taken together, glia-neurone signalling pathway might be a wide-spread phenomenon throughout the brain with glia/astrocytes having the means (by releasing various gliotransmitters) to participate in many information processing functions of the CNS.

2.5.5. Neuroglia controls connectivity in neural networks

The operational connectivity between the elements of the nervous system are of a paramount importance for its function; by and by the maintenance and plastic remodelling of the connectivity status is the raison d’être of the nervous tissue. The connections in the brain are represented by many structures that include chemical and electrical synapses, the neuronal–glial and glial–glial contacts. The synapse appearance, maintenance survival and death — these all are controlled by the neuroglia. Indeed, astrocytes secrete numerous factors indispensable for synaptogenesis (Liuw et al., 2008; Nieweg et al., 2009), and without astrocytes formation of synapses is greatly depressed (Pfrieger, 2009; Pfrieger and Barnes, 1996). The astroglia in the tripartite synapse (which is the most common in the CNS) maintains the efficacy of synaptic transmission through homeostatic control over the synaptic cleft contents and through metabolic support. Removal of astroglial coverage results in synaptic shutdown and synapse elimination (Nagele et al., 2004). The second level of CNS connectivity accomplished by the white matter is almost exclusively maintained by the oligodendrocytes, and oligodendroglial failure severely alters the brain function.

2.6. Pathophysiological aspect

The pathological potential of neuroglia was recognised very early, and several prominent neurologists of 19th-beginning of 20th centuries have stressed the role of glial cells in the
progression of various neurological diseases (Alzheimer, 1910; Del Rio-Hortega, 1919a; Frommann, 1878; Nissl, 1899). Our knowledge of the pathophysiological importance of neuroglia remains fragmentary, because of a long-lasting prevalence of neurocentric views in neurology and neuropathology. Despite substantial gaps in our knowledge we may safely conclude that neuroglia takes the leading role in initiation, progression and outcome of neurological diseases; furthermore we can regard neurological diseases primarily as the gliopathologies (for the state of the art reviews and comprehensive literature see Fields (2008b), Giaume et al. (2007), Halassa et al. (2007a), Heneka et al. (2010), Matute (2010), Nedergaard and Dirmagl (2005), Nedergaard et al. (2010), Rodriguez et al. (2009), Rossi and Volterra (2009) and Seifert et al. (2010)). Indeed, the ultimate specialisation of neurones, which underlie the sophistication of neuronal network in the human brain, robbed the former from the house-holding abilities. Indeed in the higher mammals, the neuroglia is fully responsible for brain homeostasis, for the brain metabolic abilities. Indeed in the higher mammals, the neuroglia is fully responsible for brain homeostasis, for the brain metabolic support and for the brain defence, and every type of lesion to the nervous system tests the ability of neuroglia to withstand and repair the damage. The neuroglial cells are involved in all types of brain pathology from acute lesions (trauma or stroke) to chronic neurodegenerative processes (such as Alzheimer’s disease, Parkinson’s disease, multiple sclerosis and many others) and psychiatric diseases. The pathologically relevant neuroglial processes are many, and they include various programmes of activation, which are essential for limiting the areas of damage, producing neuro-immune responses and for the post-insult remodelling and recovery of neural function (Hanisch and Kettenmann, 2007; Pekny and Nilsson, 2005; Rolls et al., 2009). Recent studies also emphasised the role of astroglial degeneration and atrophy in the early stages of various neurodegenerative disorders, which may be important for cognitive impairments (see Heneka et al., 2010; Olabarria et al., 2010; Rodriguez et al., 2009; Rossi and Volterra, 2009). Similarly, microglia being the innate CNS defence system is intimately involved in all types of brain pathology including the neurodegenerative diseases (Heneka et al., 2010; Rodriguez et al., 2010).

3. Conclusion: the neuronal–glial networks form the substrate of brain function

The human brain is unique in its cognitive power that results from many levels of integration within complex cellular networks. The brain is organised by combining the clearly delineated functional modules (responsible for sensory inputs and executive outputs) with the diffuse information processing and analysis that involves many different structures. In that, when performing the complex functions related to self-consciousness, generation of thoughts and creativity the brain very much acts like a “diffuse neural net” contemplated by Camillo Golgi.

Our understanding of the brain function is very much shaped by neuronal doctrine that assumes that the neuronal networks represent the sole substrate for cognition. These neuronal networks are, however, embedded into much larger and probably more complex networks made by neuroglia. The latter, although being electrically silent, employ many different mechanisms for inter- and intracellular signalling. It appears that astrocytes can control synaptic networks and in such a capacity they may represent an integral component of the computational power of the brain rather than being just a brain “connective tissue”. Consequently, it is not surprising that in an ascending phylogeny, the glia (astrocytes):neurones ratio increases and peaks within the human brain, which has both relatively and absolutely the greatest number of glia. Interestingly, Albert Einstein’s brain contained a significantly higher ratio of glia:neurons in his left Brodmann’s area 39 (Diamond et al., 1985). This makes us wonder.

The fundamental question of whether neuroglia is involved in cognition and information processing remains, however, open. Indeed, the “glial explosion” that distinguishes the human brain can be simply a result of exceedingly high specialisation of the neuronal networks that delegated all matters of survival and maintenance to the neuroglia; to do so the latter obviously is in need of sensing the neuronal activity and communicating with fellow glia. At the same time potential power of analogue processing offered by internally connected glial networks may represent the alternative mechanism of cognitive amplification. Where is the truth? This is the future, which holds the answer.

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