Patching the glia reveals the functional organisation of the brain

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Abstract The neuroglia was initially conceived by Rudolf Virchow as a non-cellular connective tissue holding neurones together. In 1894, Carl Ludwig Schleich proposed a hypothesis of fully integrated and interconnected neuronal–glial circuits as a substrate for brain function. This hypothesis received direct experimental support only hundred years later, after several physiological techniques, and most notably the patch-clamp method, were applied to glial cells. These experiments have demonstrated the existence of active and bi-directional neuronal–glial communications, integrating neuronal networks and glial syncytium into one functional circuit. The data accumulated during last 15 years prompt rethinking of the neuronal doctrine towards more inclusive concept, which regards both neurones and glia as equally responsible for information processing in the brain.

Keywords Glia · Neuronal–glial interactions · Ion currents · Neurotransmitter receptors · Calcium signalling · Patch-clamp · History

The birth of glia

The history of glial research is confusing and painful. For a good part of the 20th century, glial cells were very much neglected by neurobiology, and the real advance of glial physiology begun only in the mid-1980s. Yet, the concept of glia appeared almost 50 years before the neuronal doctrine of nervous system organisation was conceived,

and by the end of 19th century, a great variety of glial cells were identified and their morphology was very precisely characterised.

The father of glia, Rudolf Virchow, invented the idea of nervous ‘connective tissue’ in 1846, when he wrote “...this connective substance forms in the brain, in the spinal cord, and in the higher sensory nerves a sort of Nerven Kitt (neuroglia), in which the nervous system elements are embedded” ([89]; quoted from [79]). The word ‘glia’ has a dubious origin, initially appearing in the ancient Greek texts in a form of ‘γλοιος’, which meant ‘oily sediment’ used for taking baths (Semonides, VII century B.C.), ‘gum’ (Herodotus, V century B.C.), or ‘slippery, knavish person’ in Aristophanes (~V century B.C.) tragedies. The latter meaning remained in Modern Greek, where the word ‘γλοιος’ stands for a morally debased and filthy person. Virchow further elaborated the concept of glia in his classical book *Die Cellularpathologie in ihrer Begründung auf physiologische and pathologische Gewebelehre* [90]; for Virchow, neuroglia was a true connective tissue of a non-cellular nature.

The first description of neuroglial cell, the radial glial cell (which is now known as Müller cell) in the retina was made by Heinrich Müller in 1851. Several years later, these cells were also characterised by Max Schultze (for relevant referencing see historical reviews [41, 79]). Around 1865, Otto Deiters described the stellate cells in white and grey matter [26]. These cells closely resembled what we now know as astrocytes and in 1869, Jakob Henle has published the first image of cellular networks formed by stellate cells (i.e. astrocytes) in both grey and white matter of the spinal cord. Further discoveries in the field of the cellular origin of glial cells resulted from the efforts of many prominent histologists, in particular Camillo Golgi (1843–1926), Santiago Ramon y Cajal (1852–1934) and Pio Del Rio

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Fig. 1 First electrophysiological recordings from mammalian glial cells. **a** Passive behaviour of glial cell in the optic nerve of mud puppy, *Necturus*. (**a**) A glial cell was penetrated with two electrodes, with tips located as close as possible; each electrode recorded resting potential of 75 mV. Square pulses of current passed through one electrode displace the membrane potential recorded by the second electrode. The two displacements are symmetrical in the hyperpolarising and depolarising direction. Dashed line indicates zero membrane potential. (**b**) Electrode tips were separated by 50 μ m; current passed as in (**a**), but potentials rise more slowly. When either

the stimulating or recording electrode was withdrawn from the cell, the electrotonic potentials disappeared. Lower traces show current monitored during pulses. **b** Depolarisation of a glial cell produced by nerve impulses in the *Necturus* optic nerve. Glial membrane potential recorded with an intracellular electrode was 86 mV. The isolated nerve was stimulated maximally a few millimeters from the site of microelectrode penetration. Upper trace shows glial depolarisation after a nerve volley. Lower trace shows glial depolarisation in response to three stimuli at 1 s interval; the glial depolarisation sum. Reproduced with permission from [50] (**a**) and [65] (**b**)

Hortega (1882–1945). Using a variety of microscopic techniques, Golgi and Ramon y Cajal discovered a wide diversity of glial cells in the brain, found the contacts formed between glial cells and blood vessels and observed cells located in closely aligned groups in between nerve fibres (this being the first observation of oligodendrocytes). Further advances in morphological characterisation of glia appeared after Golgi developed famous black (silver chromate) reaction, and Ramon y Cajal invented gold-chloride sublimate staining technique, which significantly improved microscopic visualisation of cells (and neuroglial cells in particular) in brain tissues. Closer to the end of 19th century, Michael von Lenhossek proposed the term astrocyte (1893), which within the next two decades gained universal acceptance. The name ‘oligodendrocyte’ appeared slightly later, after Pio Del Rio-Hortega introduced silver carbonate staining technique, which selectively labelled these cells (1921). It was also Del Rio-Hortega who proposed the term ‘microglia’ to characterise the distinct cellular population of mesodermal origin; he was one of the first to understand that these cells can migrate and can act as phagocytes.

The main peripheral glial element, the Schwann cell, was named by Louis Antoine Ranvier (1871), after earlier discoveries of Robert Remak, who described the myelin sheath around peripheral nerve fibres (1838) and Theodor Schwann, who suggested that myelin sheath was a product of specialised cells (1839).

At the end of 19th century, several possible functional roles of glial cells were considered. Camillo Golgi, for

example, believed that glial cells are mainly responsible for feeding neurones, by virtue of their processes contacting both blood vessels and nerve cells. This theory was, however, opposed by Santiago Ramon y Cajal. Another theory (proposed by Carl Weigert) considered glial cells as mere structural elements of the brain, which filled space not occupied by neurones. Finally, Ramon-y-Cajal brother, Pedro, considered astrocytes as insulators, which prevented undesirable spread of neuronal impulses.

For the first time, the idea of active neuronal–glial interactions as a substrate for brain functions was voiced by Carl Ludwig Schleich (1859–1922) in 1894 in his book *Schmerzlose Operationen* [77]. Incidentally, this happened at the same year when the ‘neuronal’ doctrine was introduced by Sigmund Exner [29] and only 3 years after the term ‘neurone’ was coined by Wilhelm Gottfried von Waldeyer [93]. Schleich believed that glia and neurones both act as active cellular elements of the brain. He thought that glial cells represent fundamental inhibitory mechanism in the brain. According to Schleich, neuronal excitation is transmitted from neurone to neurone through intercellular gap; this inter-neuronal gap, surrounded by glial cells is the anatomical substrate for controlling excitation or inhibition in neural networks. The constantly changing volume of glial cell represents the mechanism for this control: swelling of glial cells inhibits neuronal communications, when glia shrinks, the impulse propagation is facilitated. The ideas of Schleich were never fully appreciated by a neuroscience community and from the beginning of 20th century glial cells were firmly believed to represent a

Fig. 2 First patch-clamp recordings of single potassium channels from cultured glial cells (oligodendrocytes). **a** Preparation of isolated membrane patches. **b** Single channel recordings from outside-out patch isolated from cultured oligodendrocyte at different membrane potentials and different time resolution. Channel conductance was 90 pS. Reproduced with permission from [40] (**a**) and [39] (**b**)

Fig. 3 Calcium currents in glial cells. **a** Voltage-sensitive calcium current in type 2 astrocyte in vitro. *Left* Family of calcium currents evoked by voltage steps from a prepulse potential of -100 mV to test steps ranging from -60 to 0 mV. *Right* The I–V curve of calcium current measured in response to ramp stimulation from -100 to 100 mV over 500 ms. **b** Two types of Ca^{2+} currents recorded from cultured oligodendrocyte. The currents shown on the *left* were evoked by test depolarizations to different voltages (indicated near the traces) from the holding potential of -75 mV. On the *right*, the I–V curves for total (LVA+HVA) calcium current is presented. Reproduced with permission from [5] (**a**) and [88] (**b**)

supportive and passive element of the brain, completely devoid of any role in the integration and processing of information.

Beginning of glial electrophysiology

First electrophysiological recordings from glial cells were performed in 1964–1966 in the laboratory of Stefen Kuffler. In 1964, Kuffler and Potter made intracellular microelectrode recordings from leech glial cells [51], and in 1966 Kuffler, Nicholls and Orkand [50, 65] accomplished a detailed analysis of membrane potential in glial cells from the optic nerve of a vertebrate, a mud puppy (Fig. 1). Importantly, they demonstrated for the first time active neuronal–glial interactions by showing that stimulation of the optic nerve triggered depolarisation of the glia. Furthermore, they found that glial cells are electrically connected, and several years later, Milton Brightman and Tom Reese [19] identified gap junctions as structures connecting glial networks.

The turn of the tide-identification of glial excitation

The modern era of glial physiology was triggered by several important technical advances, which arrived almost simultaneously at the end of 1970s to the beginning of 1980s. These were the patch-clamp, developed by Bert Sakmann and Ervin Neher in collaboration with Owen Hamill, Fred Sigworth and Alan Marty [33]; the fluorescent calcium indicators invented by Roger Tsien [83, 84] and the technique of preparation and maintenance of glial cell cultures [57, 58]. These techniques used alone or in combination were instrumental for many fundamental discoveries, which completely change our comprehension of the importance of glial cells in the nervous system.

Electrophysiological recordings from cultured glial cells (Figs. 2, 3 and 4) identified a wide variety of both voltage- and ligand-gated currents. It turned out that glial cells are capable of generating the same types of voltage-gated currents (including, e.g. sodium and calcium currents—Fig. 3) as neurones; main parameters of glial voltage-gated channels were very similar to those expressed in neurones [5–7, 13, 16, 39, 40, 54, 55, 80–88]. In 1984, intracellular microelectrode recordings from cultured astro- and oligodendroglia [18, 31, 37, 38] clearly demonstrated that glial cells can be depolarised by neurotransmitters such as glutamate, GABA and aspartate (Fig. 4); further patch clamp studies characterised functional glutamate and GABA receptors in macroglial cells [17, 81]. Within the next decade, a huge variety of neurotransmitter receptors were discovered in cultured glial cells (see [72, 85–87] for

Fig. 4 Identification of functional neurotransmitter receptors in cultured astrocytes. **a** Microelectrode recordings from cultured astrocytes. The traces show effects of GABA, glutamate, aspartate and glycine all applied at a concentration of 1 mM. **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 [37] (**a**) and modified from [18] (**b**)

Fig. 5 Bergmann glial cell and its neighbour Purkinje neurone bear a similar set of neurotransmitter receptors. *NT* terminals from tuberomammillary nucleus of the posterior hypothalamus which carry histamine innervation of the cerebellar cortex; *LC* terminals from locus

coeruleus which utilize noradrenalin and ATP as neurotransmitters; *CF* and *PF* climbing and parallel fibers, respectively, major neurotransmitter glutamate; *BA* and *ST* basket and stellate cells which delivers GABA to Purkinje neurone layer. Reproduced with permission from [86]

Fig. 6 Synaptic currents in cortical astrocytes. **a** Synaptic currents mediated by NMDA receptors in astrocytes. Astrocytes in layer II of the slice were identified by EGFP fluorescence and voltage-clamped; electrical stimulation of synaptic inputs was in layer IV. Synaptically evoked currents are inhibited by MK801 (10 μ M), and the residual current is partially blocked by DL-TBOA (100 μ M). Each point on the time graphs represents the mean $\pm$ SEM for five EPSCs; illustrative EPSCs are shown below. **b** and **c** Miniature spontaneous excitatory currents in cortical astrocytes mediated by AMPA and NMDA glutamate receptors. **b** Representative whole-cell recordings in control and after application of NBQX (30 μ M). **Right panels** represent generalized waveform of spontaneous currents (average of 50 events).

Lower graph shows probability density function of spontaneous currents in control (*solid line*) and in the presence of NBQX (*dots*). **c** Representative whole-cell recordings from astrocytes in cortical slice in control and after application of D-AP5 (30 μ M). **Right panels** show generalized waveform of spontaneous currents (average of 50 events). **Lower graph** shows probability density function of spontaneous currents in control (*solid line*) and in the presence of D-AP5 (*dots*). All recordings were made at holding potential -80 mV in the presence of TTX (1 μ M), picrotoxin (100 μ M) and DL-TBOA (100 μ M). Note the significant decrease in the amplitude of spontaneous current as well as the leftward shift of amplitude distributions under action of glutamate receptor antagonists. Reproduced with permission from [52]

review) initiating the hypothesis of active role of glia in the information exchange in the nervous system.

In parallel, the cytoplasmic calcium fluctuations were intensively studied in cultured glial cells loaded with fluorescent Ca^{2+} indicators. These studies have found that (1) stimulation of glia with neurotransmitters or hormones

almost invariably resulted in generation of intracellular Ca^{2+} signals driven predominantly through the metabotropic route, which involved phospholipase C/InsP₃-evoked Ca^{2+} release from the endoplasmic reticulum Ca^{2+} store [30, 46, 72, 85] and (2) that the Ca^{2+} signals triggered in one glial cell may travel through the syncytium formed within

monolayers of cultured glial cells connected with gap junctions [21–24, 34]. These findings led to formulation of hypothesis of special form of glial excitability, which is governed by Ca^{2+} release from the intracellular excitable media, represented by the membrane of the ER [86].

In 1989, another important technical step was made, when patch-clamp recordings were applied to the neurones in situ, in brain slices [28, 74]. This technique was almost immediately applied to glial cells, thus eliminating the artefacts of cell culture (especially important for astrocytes, which demonstrated remarkable plasticity when being maintained in cell culture conditions) and permitting direct investigations of neuronal–glial interactions within their natural environment. These experiments (e.g. [10–12, 82]) have confirmed that glial cells in situ express voltage-gated channels and neurotransmitter receptors, yet their distribution and regulation revealed important peculiarities. It turned out that in situ, the expression of many voltage-gated channels is developmentally regulated; e.g. calcium and sodium channels are predominantly expressed in glial precursors and immature glial cells [1, 11, 16]. Second, it was realised that the expression of neurotransmitter receptors in situ is strictly controlled by the local environment. It was established that glial cells express receptors sensitive to neurotransmitters released in their vicinity; moreover, the receptors expressed in glial cell and in its neuronal neighbour have matching modalities [85, 86]. Initially, this relation was shown for Bergmann glial cells in the cerebellum (Fig. 5). These cells express receptors for noradrenalin, glutamate, GABA, histamine and ATP, i.e. the receptors activated by neurotransmitters released from terminals present in the Purkinje layer of cerebellum [43, 45, 47, 62, 63]; the closest neighbour of the Bergmann glial cell, the Purkinje neurone, also expressed receptors for these neurotransmitters [44]. Subsequently, similar matching relations between neuronal and glial receptor expression was found in the spinal cord, where both astrocytes and neurones possess glycine receptors acting as a main neurotransmitter in this region [42, 69].

Neuronal–glial circuitry

The experiments in situ also directly demonstrated the existence of neurones to glia signalling by showing that stimulation of axons or synaptic inputs trigger active responses in glial cells either in electrical form or in the form of Ca^{2+} signals. Stimulation of peripheral nerves triggered intracellular Ca^{2+} responses in both periaxonal and perisynaptic Schwann cells [36, 53, 73]; in the latter, Ca^{2+} signals resulted from the activation of glial receptors by synaptically released neurotransmitter. Similarly, stimulation of central axons induced Ca^{2+} responses in oligoden-

drocytes (e.g. in optic nerve [49]). In the grey matter, neuronal activity also often triggered astroglial activation. In hippocampus and visual cortex, for example, electrical stimulation of synaptic afferents triggered both Ca^{2+} elevations [25, 68, 71] and receptor-mediated ion current responses [35, 52] in astroglia (Fig. 6a). Most notably, electrophysiological recordings from identified cortical astrocytes detected spontaneous glutamate-mediated currents (Fig. 6b), very similar to neuronal ‘miniature’ EPSCs [52], suggesting that parts of glial membranes are located very close to the site of presynaptic release and astroglia is involved into the background neurotransmission.

In cerebellum, stimulation of parallel fibres induced a complex membrane current response in Bergmann glial cells (which intimately enwrap synapses formed by parallel fibres on Purkinje neurones), which was mediated by both activation of ionotropic AMPA-type glutamate receptors and activation of Na^{+} -dependent glutamate transporters [22]. Most interestingly, the glial AMPA receptor-mediated responses show both short-term and long-term plasticity. The former is manifested by prominent paired-pulse facilitation [8], whereas the latter appears in a form of long-term depression of synaptically induced glial responses following low-frequency repetitive stimulation of parallel fibres [9]. Bergmann glial cells also demonstrated clear elementary ‘postsynaptic’ currents in response to quantal release of glutamate from presynaptic terminals [56], suggesting very close apposition of glial and presynaptic membranes. Electrical stimulation of parallel fibres also triggered cytoplasmic calcium responses in Bergmann glia; these calcium responses were very much localised, being limited to a small compartments within glial cell processes [32]. It is interesting to note that the size of these compartments, where local Ca^{2+} signals occurred, was similar to morphologically distinct appendage-like structures, protruding from the main process and enwrapping a cluster of synapses formed by parallel fibres. This high compartmentalisation of synaptically induced glial Ca^{2+} responses can be important for spatial discrimination of the incoming signals.

Further experiments revealed yet another important fact: glial cells can signal back to neurones. This type of glia to neurone signalling was detected in both glial–neuronal co-cultures and in acutely isolated brain slices. Using the in vitro model, several groups have demonstrated that stimulation of astrocytes evoked direct excitation of nearby neurones [34, 75] and affected spontaneous [4] and evoked [2] postsynaptic currents. Inhibition of astroglial calcium signals (either by intracellular administration of BAPTA or by suppression of ER Ca^{2+} release by thapsigargin) eliminated all neuronal responses. In acute slices, activation of glia was also shown to trigger direct neuronal responses. Spontaneous Ca^{2+} oscillations in astrocytes, for example,

excited neighbouring neurones in hippocampus, cortex and thalamus [66, 68]. When astrocytes in CA1 hippocampal slices were activated by prostaglandin E₂, adjacent neurones generated Ca²⁺ signals; this action was mediated through glutamate receptors [14]. Activation of hippocampal astrocytes was also reported to produce tonic suppression of synaptic transmission; this inhibition was mediated by adenosine accumulated after the release of ATP from the astroglia [67].

Quite recently, it also became clear that the main mechanism of astroglial to neuronal signalling involves secretion of classical neurotransmitters, such as glutamate or ATP from the glial cells; this secretion can occur through several independent routes, most importantly though regulated exocytosis. It is interesting to note that the first indication that glial cells (e.g. Schwann cells and astrocytes) are capable of secreting neurotransmitters such as acetylcholine and GABA were obtained already from mid-1970s [27, 59, 78], although these data were not somehow generally acknowledged. In recent years, however, the concept of neurotransmitters release from glia gained firm support. Glial cells were found to contain secretory vesicles endowed with glutamate transporters and reach in glutamate; it was also shown that astroglial cells express numerous proteins responsible for exocytotic vesicles fusion, including cellubrevin (VAMP3), SNAP23, complexin 2, Munc18a and synaptotagmin IV [15, 61, 92, 94]. Finally, the Ca²⁺-induced exocytosis of glutamate from astrocytes was directly identified by total internal reflection fluorescence imaging [15], and exocytotic fusion after [Ca²⁺]_i signals was measured by membrane capacitance recordings [48]. Besides glutamate and ATP astrocytes are able to release several other 'glial' transmitters, most notably taurine [20], aspartate [60] and D-serine [64, 76]. All these transmitters can be secreted either by exocytosis or through membrane channels, such as volume-sensitive Cl⁻ channels or pores associated with activation of P2X₇ purinoreceptors, or indeed through reversal of neurotransmitter transporters.

All these discoveries led to a complete rethinking of the role and place of neuroglia in brain circuits and initiated an appearance of a concept of a 'tripartite' synapse; this concept regards glial cell as legitimate part of the synapse, which is built from three functionally important compartments: presynaptic, postsynaptic and glial [3, 70, 91].

Brain integration as a function of neuronal–glial networking: can neuronal doctrine hold?

Our understanding of glial function changed dramatically over the last 15 years. This change concerns not only the physiology of glia but also the whole concept of the way

how the brain is organised and how the development, life and death of neural circuits are controlled. There is a compelling evidence demonstrating that the radial glial cells serve as pluripotent neural progenitors and the cells with astroglial phenotype are the brain stem elements underlying adult neurogenesis. The mature astroglia defines the microarchitecture of the grey matter by dividing it into separate units, where the astrocyte rules over all neuronal membranes and synaptic contacts within this clearly defined territory. Within this territory, astrocytes control almost everything from regulating birth, maintenance and elimination of synapses to providing active neurones with energy substrates and linking neurones with local microcirculation. Although clearly delineated, the astrocytic domains are tightly integrated into the syncytium by gap junctions connecting the terminal processes of glial cells. This syncytial structure permits long-range signalling and intercellular exchange of metabolites and second messengers. At the same time, astrocytes are fully capable of extracellular signalling by virtue of 'glial' transmitters secreted in highly regulated fashion. These multiple signalling abilities, which include both intra- and extracellular routes, can potentially be very important for integrative processes within astroglial networks. Finally, the failure of astrocytes to perform their duty invariably results in neuronal death, and many types of brain pathology, from stroke to Alzheimer disease may be primarily glial diseases.

All in all, glial cells weave the canvass on top of which neurones perform their rapid signalling. The extremely elaborated panglial syncytium resembles the 'reticular theory' of brain organization so much championed by Golgi and Kolliker, which now can be safely reconciled with 'neuronal' theory, as both internally connected glial syncytium and discreet neuronal networks peacefully coexist within the brain. Shall we anticipate even more exciting discoveries which eventually can challenge the role of neurones as sole origins of cognition? Can neuronal doctrine withstand the challenge from glial cells? These questions are still open. Nonetheless, after 20 years of patching the glia, we already elevated these cells from a mere 'nerve putty' to an active element of the brain circuitry.

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