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Glial heterogeneity: the increasing complexity of the brain

"How does the brain work?" is one of the most frequently asked questions. By and large, the human brain consists of approximately 160 billion cells that mainly represent two types, classified as neurons and glia, each contributing about 50 % to the total cell number. Modern imaging techniques such as functional magnetic resonance imaging (fMRI) or diffusion-tensor imaging (DTI) show us many functional details of our brain's plastic activities and have provided exciting new insights of how and where the brain processes specific information (◘ **Fig. 1a**). Interestingly, neither fMRI nor DTI are mechanistically based on the electrical activity of neurons, classically thought to be exclusively responsible for information processing in the brain. Instead, these techniques enable the visualization of brain activity based on changes of cerebral blood flow, i.e. the oxygen content supplied by blood capillaries, or by anisotropic water diffusion. The cellular building blocks of the smallest fMRI and DTI signals have already been identified as the neurovascular unit and the myelin-axon unit, respectively (◘ **Fig. 1b** and **c**). A single fMRI voxel integrates the oxygen consumption of several cell types with vascular, glial and neuronal origin: endothelial cells, neutrophils, pericytes, astrocytes, microglia, NG2 glia, oligodendrocytes and neurons. The myelin-axon unit that gives rise to the DTI signal appears much less complicated: neuronal fiber tracts, the axons, are surrounded by the insulating and support-providing myelin sheaths of the oligodendrocytes. Both imaging techniques take thus advantage of the strategic positions of glial cells which enable them to power neurons and to maintain long-range connections, highlighting the prominent role of neuroglia in neuronal performance.

The two-dimensional space of histological or vital brain sections already demonstrated that cells were organized in functional and morphological networks. Among these are the columnar modules of the cortex that contribute to sensory integration and cognition, the layers of the hippocampus involved in learning and memory, the rhythmic centers of the brain stem which regulate breathing, or the cerebellar circuits responsible for fine-tuning motor coordination. While excitatory and inhibitory neurons are the main relay stations for the input, processing and output of electrical signals, the macroglial cells execute quite different tasks. Astrocytes are polarized cells that represent a bridge between blood vessels and neurons. They take up nutrients from the blood, metabolize them and provide them to neurons. Astrocytes also regulate extracellular ion and transmitter homeostasis from the so-called "tri-partite" synapse where they are in direct contact with neuronal synapses. Furthermore, by secreting transmitters and peptide hormones, they can directly modulate synaptic transmission. Oligodendrocytes insulate neuronal axons with a lipid-rich structure, the myelin sheath, to accelerate action potential propagation and to electrically insulate axons. Recent data has additionally demonstrated that oligodendrocytes metabolically support axons, the long-range links of neural circuits. Glial cells expressing the proteoglycan NG2 (NG2 glia) are a relatively novel class of macroglia and were originally identified as oligodendroglial progenitor cells, but appear to represent a more versatile cell reservoir in the adult brain.

Present-day research provides compelling evidence that a neuron-centered picture of the brain is way too simplistic, indicating that each class of glial cells is much more diverse than commonly thought. Glial cells appear to have distinct physiological properties in different brain regions, at different developmental stages and at different activity levels of the organism. These observations suggest that functional specializations of glia might have developed to meet the specific requirements of distinct networks which might as such be critical determinants of brain activity. This new concept will change the way we think about brain function and put glial cells into an even more prominent focus of attention.

Astrocytes are probably the most versatile class of neuroglia. Functionally positioned between the pia mater, blood vessels and neuronal synapses, they display a plethora of properties. Astrocytes contribute to the blood-brain barrier [3], take up nutrients from the blood, metabolize them and provide energy substrates to neurons [26]. They link neuronal activity to blood circulation [2, 15], promote synapse formation [5, 8], and determine the properties of the extracellular matrix [9, 16, 27]. Furthermore, they regulate extracellular ion and transmitter levels thereby regulating synaptic transmission. Last but not least, astrocytes secrete compounds which modulate neurotransmission [1, 22]. This impressive list demonstrates via how many routes astrocytes interact with neurons and influence brain activity. Astrocytes must be particular-
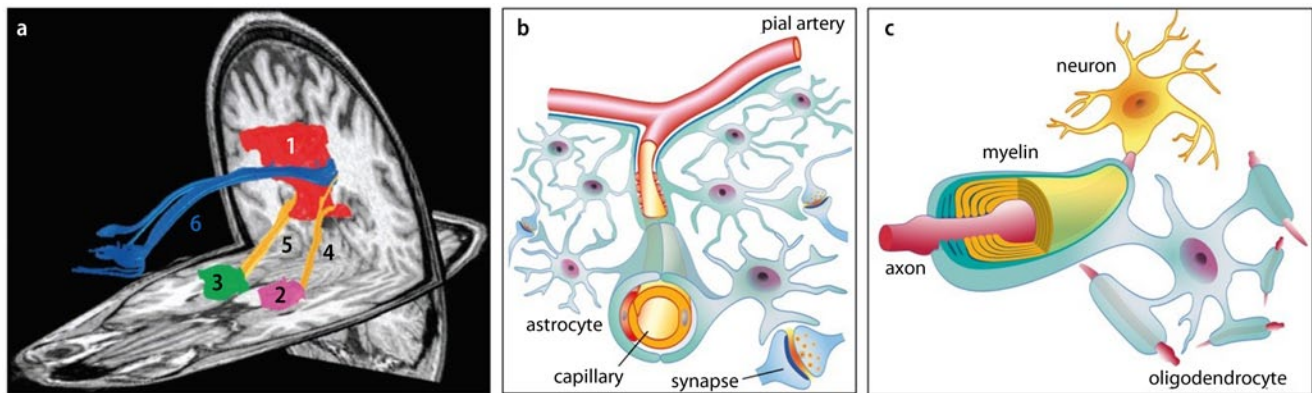
Fig. 1 ▲ Modern brain imaging techniques are mechanistically based on activity of macroglial cells. **a** Functional magnetic resonance imaging (*fMRI*) is able to visualize different functional units (1–3) of the brain in its idle mode (default mode network). A variant of fMRI, diffusion tensor imaging (*DTI*) can selectively visualize connecting fibre tracts between these centres (4–6). **b** The cellular basis of the fMRI signal is the change in oxygen levels in the neurovascular unit composed of capillaries, astrocytes and neurones. **c** The axon-myelin unit of fibre tracts causes anisotropic water diffusion that gives rise to DTI signals. Figure A modified from [13]

ly tailored to fulfil these functions and be able to adapt to the developmental stage, the brain region and the activity phases. Indeed, numerous examples of glial heterogeneity exist. This is especially conspicuous in the morphological specialization of astrocytes (◘ **Fig. 2**). In some regions such as the cerebellar cortex and the retina, they exhibit a radial orientation. In contrast, astrocytes in the cortex or hippocampus extend processes in all directions displaying a star-like appearance, while those of the white matter are less frequently branched and largely lack thin membrane protrusions. Not surprisingly, first profiling studies of astrocytes isolated from different brain regions display substantial differences in gene expression [4, 7, 23]. These include cell surface glycoproteins and components of the extracellular matrix, ion channels, neurotransmitter receptors and transporters, connexins, Eph receptors, and many more [10, 20, 28]. More recent studies show that astroglia sense and compute neuronal activity to feed back to neurones, thereby even modulating the most visible brain output, the behaviour. Astroglial cannabinoid receptors in the hippocampus, for example, are involved in the acquisition of spatial working memory [14], and in the cerebellum, Bergmann glial AMPA receptors are important determinants of fine motor coordination [25].

The second class of macroglia, the **oligodendrocytes**, are the myelin-forming cells of the CNS. A single oligodendrocyte enwraps up to 50 axons, and myelinating segments can vary in length from 50 to 400 μm. Their morphological heterogeneity has already been described by Rio-Hortega, who distinguished four types. In white matter fiber tracts such as the optic nerve or the corpus callosum, axons are mainly oriented in parallel, and so are the processes of the oligodendrocytes. In contrast, in grey matter regions where axons traverse the parenchyma irregularly, oligodendroglial processes point into all directions. The functional characterization of oligodendroglial heterogeneity is still in its infancy. They are equipped with a variety of receptors to sense the extracellular level of transmitters released by neurones [17]. As a consequence, myelination is regulated by neuronal activity, but is also determined by axon diameter. In addition to their role in myelination, recent studies highlight the important role of oligodendrocytes in supporting axons also metabolically [11, 19].

NG2 glial cells constitute less than ten percent of glial cells in the developing and adult CNS [6]. They have originally been identified by the expression of the chondroitinsulfate proteoglycan NG2, and functionally been characterized as oligodendrocyte progenitor cells. Recent studies demonstrate that outside of neurogenic niches, NG2 cells are the most proliferative cells of the adult CNS. NG2 glial cells are the only glial cells directly innervated by neurons. While in the hippocampus and cerebellum NG2 cells receive both glutamatergic and GABAergic input, NG2 cells in the medial nucleus of the trapezoid body only receive excitatory glutamatergic synaptic input in parallel to the Calyx of Held [21]. The neuron-glia synapses even persist during cell division. Although all NG2 cells can develop into oligodendrocytes, there exist strong differences between white and grey matter. While in the corpus callosum, almost two third of NG2 glia become oligodendrocytes, in the cortex 90 % remain NG2 glia. Cell proliferation was suggested to be regulated by voltage-gated sodium and potassium channels heterogeneously expressed on NG2 glia during differentiation [12, 18].

The Deutsche Forschungsgemeinschaft acknowledges the demand for further research on the heterogeneity and funds the special priority program SPP 1757 "Functional specializations of neuroglia ascritical determinants of brain activity". The Priority Program will pave the way for a better understanding of the molecular and cellular role of glia in brain pathologies that is urgently needed to develop novel, more customized and targeted strategies for the treatment of brain injury and disease.

In this special issue of Neuroforum members of the SPP 1757 will present recent highlights of glia research addressing the heterogeneity of astrocytes (Christian Henneberger), NG2 glia (Dirk Dietrich and Christian Steinhäuser) and oligodendrocytes (Leda Dimou and Michael Wegner). These reviews will be complement-
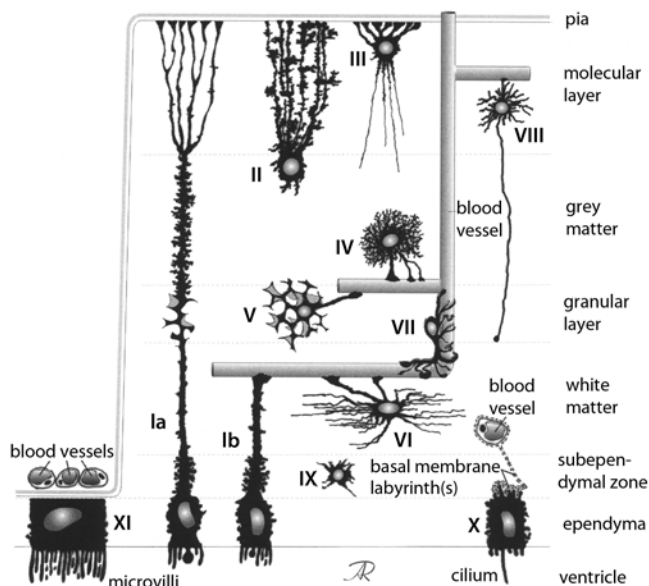
Fig. 2 ▲ Heterogeneity of human astroglia. Semi-schematic survey of the main types of astroglia and related glial cells, and their localization in different layers/specialized regions of the human brain. *I*: tanicyte (a: pial; b: vascular); *II*: radial astrocyte (Bergmann glial cell); *III*: marginal astrocyte; *IV*: protoplasmic astrocyte; *V*: velate astrocyte; *VI*: fibrous astrocyte; *VII*: perivascular astrocyte; *VIII*: inter-laminar astrocyte; *IX*: immature astrocyte/glioblast; *X*: ependymocyte; *XI*: choroid plexus cell. From: Reichenbach and Wolburg [24]

ed by an article of Daniela Dieterich and Moritz Rossner who describe novel high-throughput approaches with significant impact for the identification of cellular specializations in the brain and among glial cells. The SPP 1757 moreover will serve as a platform to enhance inter-lab communication not only at the national, but also at the international level. Especially strong ties have been established with glial research colleagues in Japan. Kazuhiro Ikenaka will describe the Japanese program to foster glial research.

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