NEURONAL-GLIAL NETWORKING

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***Prof. Oleksii Verkhratsky**, MD, PhD, Dr.Sci., Member of Academia Europaea, born in 1961 in the city of Stanislav, Western Ukraine. He graduated from Kyiv Medical Institute in 1983, and received PhD in Physiology from Bohomolets Institute of Physiology (Kyiv, Ukraine) in 1986. In 1993 he was awarded the degree of Doctor of Science from the same Institute. From 1990 to 1995 O. Verkhratsky was head of Laboratory of cellular signaling at Bohomolets Institute of Physiology. In 1992-1995 he also was a Deputy Director of the International Centre of Molecular Physiology, Ukraine. Between 1995 and 1999 O. Verkhratsky was a research scientist at Max Delbrück Centre of Molecular Medicine in Berlin. He joined the Division of Neuroscience in Manchester in 1999, became a Professor of neurophysiology in 2002 and served as head of the said Division from 2002 to 2004. From 2007 he assumed a second appointment of head of Department of cellular and molecular neurophysiology at the Institute of Experimental Medicine, Prague.*

Prof. O. Verkhratsky was elected to the membership of Academia Europaea in 2003, and from 2006 he is Chairman of the Physiology and Medicine section. He is Editor-in-Chief of Cell Calcium and Membrane Transport & Signalling-Wiley Interdisciplinary Reviews and member of editorial boards of Pflugers Archiv European Journal of Physiology, Journal of Molecular & Cellular Medicine, Acta Physiologica (Oxford), Acta Pharmacologica Sinica (2005), Glia (2008), Frontiers in Neuropharmacology, Frontiers in Aging Neuroscience (2009) and Cell Death and Disease (2010). He has delivered more than 150 international invited lectures and seminars.

Prof. Oleksii Verkhratsky is an internationally recognised scholar in the field of cellular neurophysiology. His research is concentrated on the mechanisms of inter- and intracellular signaling in the CNS, being especially focused on two main types of neural cells, on neurones and neuroglia. He made important contributions to understanding the importance of chemical and electrical routes in reciprocal neuronal-glial communications and on the role of intracellular Ca^{2+} signals in the integrative processes in the nervous system. Many of O. Verkhratsky's studies are dedicated to investigations of cellular mechanisms of neurodegeneration. O. Verkhratsky was the first to perform intracellular Ca^{2+} recordings in old neurones in isolation and in situ, which provided direct experimental support for "Ca^{2+} hypothesis of neuronal ageing. Currently, he is extending these investigations to characterisation of neuronal and glial pathology in Alzheimer disease. In collaboration with Dr. P. Fernyhough, A. Verkhratsky has demonstrated that experimental diabetes is associated with disruption of Ca^{2+} homeostasis and mitochondrial function; both these systems appear to be regulated by insulin-receptor-dependent signaling cascades.

Scientometry: Prof. Verkhratsky has authored and edited 7 books, edited 16 special issues, and published 210 papers and chapters. His papers have been cited more than 5000 times, H-index 40 (ISI, 09/08/2009).

The concept of neuroglia

The concept of neuroglia as a connective tissue into which all elements of the central nervous system (CNS) are embedded was introduced by Rudolf Virchow (Virchow, 1856, Virchow, 1858, Kettenmann and Verkhratsky, 2008). Virchow never considered the cellular nature of glia; for him neuroglia was not more than a sort of extracellular binding element, and he often referred to it as a "Nervwenkitt" (i.e. nerve cement). Very soon, however, the cellular nature of glial cells was identified and many types of neuroglial cells were described. In the course of late 19th — early 20th century the cellular nature and morpho-functional heterogeneity of neuroglial cells were firmly established (Kölliker, 1889, Andriezen, 1893, Lenhossek, 1893, Retzius, 1894-1916, Golgi, 1903). In the recent decades the functional relevance and versatility of neuroglia which is involved in all activities of the brain, from structural and metabolic support to information processing has started to be fully appreciated (Verkhratsky and Kettenmann, 1996, Kettenmann and Ransom, 2005, Volterra and Meldolesi, 2005, Verkhratsky, 2006b, Verkhratsky, 2006a, Verkhratsky and Toescu, 2006, Verkhratsky and Butt, 2007, Kettenmann and Verkhratsky, 2008, Verkhratsky, 2009). Furthermore, the evolutionary uniqueness of human glial cells (Oberheim et al., 2009, Verkhratsky, 2009) indicates their specific role in the formation of human intelligence.

Glia in the CNS

Glial cells in the CNS are represented by several main types: astroglia, NG2 glia, oligodendroglia, and microglia (Kettenmann and Ransom, 2005, Verkhratsky and Butt, 2007). Phylogenetically, glial cells appeared early in evolution, most likely simultaneously with the first conglomerates of neurones. The emergence and progression of glia paralleled an increase in neuronal mass, which called for efficient system of subsistence and collecting cellular waste. At this stage the primordial brain-blood barrier (BBB) was also created, which, in its ancestral form, was made solely by endfeet of proto-astrocytes (Abbott and Pichon, 1987, Abbott, 2005, Bundgaard and Abbott, 2008). Later in evolution the BBB function shifted to the endothelial cells, which, nevertheless, remained under tight astroglial control. From the ancient times glial cells were divided into "neuronal carers", such as e.g. giant glia in leech ganglia, which is a singular example of the proto-astrocyte; and myelinating cells, which appeared first in a periphery in the form of Schwann cells when an increase in the length of axons demanded dramatic rise in action potential conduction velocity. Evolution of vertebrates was associated with a remarkable phylogenetic advance of glial cells, which increased in number, dimension, shape and, most importantly, in functional importance.

Indeed, an increase in complexity of glia, and especially astroglia, along the phylogenetic ladder is quite extraordinary. Overall the relative number of glial cells in the CNS increased from ~2-10% in invertebrates to ~ 50% in rodents, and to >90% in humans (Oberheim et al., 2006, Verkhratsky and Butt, 2007). In the cortex of rodents the ratio of astrocytes to neurones is about 0.3 to 1, while in humans this ratio is much higher being ~1.65 : 1. Similar increase in astroglial population is found in evolving primates (Sherwood et al., 2006), the highest astroglia to neurone ratio being observed in human brain. Furthermore, evolution dramatically increased the complexity of glial cells in the CNS. Human protoplasmic astrocytes are, for example, about ~3 times larger and their volume is about ~27 times greater than for these cells in the mouse brain. The number of processes and their complexity is significantly higher in human protoplasmic astrocytes when compared to rodents. As a result, every human protoplasmic astrocyte contacts and enwraps ~2 million of synapses compared to only ~100,000 synapses covered by the processes of mouse astrocytes (Oberheim et al., 2006, Verkhratsky and Butt, 2007). In addition the specific types of astroglia, the interlaminar astrocytes and polarised astrocytes, which are to all probability involved in cortical integration, are exclusively present in the brain of primates (Oberheim et al., 2006, Verkhratsky and Butt, 2007).

Signaling in neuronal-glial networks

Probably the most ancient type of neuronal-glial signaling was mediated by potassium channels, invariably expressed in all types of glia (Verkhratsky and Steinhauser, 2000). Neuronal activity produces an increase in extracellular K^+, which in turn depolarises glial membranes; this process reflecting K^+ accumulation by glia, which controls extracellular K^+ homeostasis thus preserving neuronal excitability. Evolution elaborated upon neuronal-glial signaling, which, in the course of CNS development, acquired new qualities associated with an increased complexity of astrocytes and the appearance of astroglial syncytium. Protoplasmic astrocytes in mammalian brain became true integrative cellular elements. These astrocytes divided the gray matter into clearly demarcated domains, as every astroglial cell occupies its own territory (Oberheim et al., 2006, Verkhratsky and Butt, 2007). Astroglial processes cover neuronal membranes and contact nearby capillary by a perivascular process forming an endfoot (Bushong et al., 2002, Nedergaard et al., 2003). Thus the neuronal-glial-vascular unit is formed, where astrocyte integrate neurones with brain capillaries (Verkhratsky and Butt, 2007). This link is functionally active, as astrocytes control activity-dependent local blood flow (Zonta et al., 2003, Mulligan and MacVicar, 2004). These neuronal-glial-vascular units are further integrated into more general assemblies through astroglial syncytium, created by gap junctions, which connect distal astroglial processes. The gap junctions are formed by closely (2-2.5 nm) apposing mem-

branes of adjacent cells, penetrated with intercellular channel connexons constructed by extended family of connexins (Verkhratsky and Butt, 2007). These connexon-based trans-cellular channels mould large pores, permeable to molecules with m.w. up to 1 KD, allowing for intracellular diffusion of many ions, metabolites, and second messengers.

Neurotransmitter receptors in glia

Astrocytes are potentially capable to express virtually every known neurotransmitter receptor (Verkhratsky and Kettenmann, 1996, Verkhratsky et al., 1998, Verkhratsky and Steinhauser, 2000, Verkhratsky and Kirchhoff, 2007b, Verkhratsky and Kirchhoff, 2007a, Verkhratsky et al., 2009). This expression, however, is restricted *in vivo*, and astrocytes from different brain regions have strictly limited and distinct complements of receptors. Physiologically astrocytes are extremely heterogeneous, being specifically tuned to the signaling requirements of different brain regions. For example, expression of NMDA receptors has been hitherto described in cortex and spinal cord, but not in the other brain regions (Lalo et al., 2006, Verkhratsky and Kirchhoff, 2007b). Similarly, ionotropic P2X receptors are operative in cortical astroglia but are absent in hippocampal astrocytes (Jabs et al., 2007, Lalo et al., 2008). Likewise, glycine receptors are present only in the spinal cord (Kirchhoff et al., 1996), where glycine acts as the main inhibitory mediator. The same exclusive localization was found for dopamine receptors, which are present only in basal ganglia (Miyazaki et al., 2004). In cerebellar Bergmann glial cells several functional receptors are expressed, which include α_1 adrenoreceptors, H_1 histamine receptors, AMPA glutamate receptors, GluR5 metabotropic glutamate receptors, P2Y purinoceptors and GABA receptors, all of them exactly matching the modality of receptors expressed in adjacent Purkinje neurone, and being congruent to the neurotransmitters released in their vicinity (Kirischuk et al., 1995a, Kirischuk et al., 1996a, Kirischuk et al., 1996b, Kirischuk et al., 1999). Fundamentally, it means that immediate neurotransmitter environment controls selective expression of glial neurotransmitter receptors.

Many glial receptors are specifically concentrated in perisynaptic processes, where they are involved in the signaling to the glial part of tripartite synapse, which comprises the presynaptic neuronal terminal, postsynaptic membrane, and astroglial membrane, enwrapping the former elements (Araque et al., 1999). The astroglial compartment provides for structural and functional isolation of every synaptic input and for integration of synaptic activity within a neuronal-glial-vascular unit and further within an astroglial syncytium (Verkhratsky and Butt, 2007). In addition, astrocytes are responsible for the delivery of energy substrates through astroglial neuronal-lactate shuttle (Magistretti, 2006).

Signaling in glial syncytium

Contrary to neurons, glial cells are not electrically excitable. In many glial cells excitable molecules, e.g. voltage-gated ion channels, are expressed (Kirischuk et al., 1995b, Verkhratsky and Steinhauser, 2000); however, their low density combined with high resting K^+ permeability prevents glia from firing action potentials. Glial excitability results from intracellular routes associated with endomembrane and volume transmission through gap junctions. The endomembrane which forms an endoplasmic reticulum (ER) acts as an intracellular excitable medium. Its excitability is build around several sets of intracellular Ca^{2+} channels and Ca^{2+} pumps residing in the ER membrane (Solovyova and Verkhratsky, 2002, Solovyova et al., 2002, Verkhratsky, 2002, Verkhratsky and Petersen, 2002, Verkhratsky, 2005). Intracellular Ca^{2+} channels are generally regulated by cytosolic Ca^{2+} concentration and by Ca^{2+} gradient across the ER membrane (Burdakov et al., 2005, Burdakov and Verkhratsky, 2006). This feature, together with the internal continuity of the ER lumen (Solovyova and Verkhratsky, 2003, Petersen and Verkhratsky, 2007) supports propagating Ca^{2+} waves. The leading role in glial Ca^{2+} signaling belongs to the $InsP_3$ receptors, the functional relevance to ryanodine receptors, although clearly present in glial cells. Thus, intracellular Ca^{2+} release forms the basis for glial excitability, termed "Ca^{2+} excitability" (Verkhratsky and Kettenmann, 1996, Verkhratsky et al., 1998).

Ca^{2+} excitability also provides for intercellular signaling between glial cells, conveyed by Ca^{2+} waves crossing cell-to-cell boundaries (Verkhratsky and Butt, 2007). The mechanisms of intercellular Ca^{2+} waves propagation are complex; they involve diffusion of $InsP_3$ through gap junctions, release of ATP or combination of the above. These mechanisms vary among brain regions: for example, deletion of connexin Cx43 completely inhibits Ca^{2+} wave propagation in neocortex but not in hippocampus or corpus callosum of mice (Haas et al., 2006), suggesting that in the former case the wave propagation relies exclusively on the gap junction connectivity, whereas in the latter case on the release of gliotransmitters.

Release of gliotransmitters

The ability to perform regulates exocytosis, which forms the basis for extracellular chemical transmission was over many years believed to be the sole prerogative of neurones. This neurocentric view, however, was recently overturned as numerous experiments have demonstrated that astrocytes contain vesicles, which are in turn endowed with transporters ensuring transmitter accumulation. Further, all the protein components of exocytotic release were found in astroglia, and vesicular release of several transmitters (including glutamate, D-serine and ATP) was directly detected (Volterra and Meldolesi, 2005, Verkhratsky and Butt, 2007).

Conclusion: neuronal-glial networks are the substrate for the brain function

The neuroglia represents an elaborated cellular circuit, endowed with sophisticated communication resources. Importantly, signaling within glial networks relies on the intercellular volume transmission mediated by many molecules represented by second messengers, metabolites, and modulators. Evolution of the brain, which eventually created human intellect, was accompanied by a remarkable increase in complexity of glial circuitry, both morphologically and functionally. Both cellular networks, neuronal and glial, act in concert and cannot be considered in separation as indeed every aspect of neuronal function is controlled by glial cells and every neuronal signal is instantly perceived by glia. Glial signaling, although being slower and less precise than neuronal, adds further complexity to the brain function and can be intimately involved in production of thoughts.

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