Preface for the Vladimir Parpura Honorary Issue of Neurochemical Research

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Vladimir Parpura

Special issue: In honor of Prof. Vladimir Parpura.

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Vladimir Parpura has joined academia at the particular time and at the particular place: he started his scientific career under the supervision of Phil Haydon who led one of the very few laboratories studying neuroglia in the late 1980s–early 1990s. Vlad (as he is known to all his close friends) went to US to start his PHD studies at the Department of Zoology of the Iowa State University in 1989 after receiving the medical degree from the University of Zagreb. He begun working with astrocytes, the cells which did not receive much attention at the time; nobody however knew that the Renaissance of glial research has already begun, and Vlad was to be a prominent actor. The glial spring was in the air; the year 1988 witnessed the creation of the first dedicated journal, the *Glia* under the ever-lasting leadership of Helmut Kettenmann and Bruce Ransom.

The neuroglia has been born in 1850s, after Rudolf Virchow introduced the concept of the connective tissue of the brain, into which “the nervous elements are embedded”. This *Nervenkitt* or neuroglia has been, according to Virchow, also “the most frequent seats of morbid change” ([1–3], for historic account on neuroglia see also [4–6]). The neuroglia rapidly gained interest, with every prominent neuroanatomist and neurologists of the late 19th and early twentieth centuries adding to neuroglial research. In 1850s Heinrich Müller, Max Johann Sigismund Schultze and Karl Bergmann characterised radial glia of the retina and the cerebellum; the cells which we know now as Müller and Bergmann glia [7–9]. In 1865 Otto Deiters isolated and visualised parenchymal glial cells, which we now know as astrocytes [10]. In 1870s Camillo Golgi introduced ‘*reazione nera*’ that allowed precise visualisation of neural cells. Golgi was the first to make detailed morphological description of multiple glial morphotypes and described glial endfeet in various parts of the brain. Golgi was also the first to contemplate the role of neuroglia in nurturing the brain; he regarded endfeet as an interface between the circulation and nervous tissue serving for distributing the nutritive material from the blood to the nervous parenchyma [11–14].

The term astrocyte (αστρον κητος; *astron*, *star*, *kytos*, *a hollow vessel*, later *cell* i.e. star-like cell) was introduced in 1895 by Michael von Lenhossek to define the stellate (as it appeared under Golgi staining) parenchymal glia [15]. This term gained universal acceptance within the next two decades being particularly popularised by Santiago Ramon y Cajal. Cajal developed the gold and mercury chloride-sublimation staining method specific for both protoplasmic and fibrous astrocytes [16], this technique labels intermediate filaments made from glial fibrillary acidic protein (GFAP), a protein used today as an astrocytic marker. Using this technique, Ramon y Cajal demonstrated origin of astrocytes from radial glia, and showed that astrocytes can divide in the adult brain, thus laying the basis for much later discoveries of the stem properties of astroglia [16–18]. Cajal considered astrocytes as versatile and multipurpose cells with many physiological functions. In particular, he regarded astrocytes as core regulators of functional hyperaemia, suggesting that contraction/relaxation of astroglial perivascular processes can increase or decrease the diameter of brain capillaries, thus regulating the blood flow [19]. Furthermore, Cajal contemplated (echoing the ideas of another glial visionary Karl Ludwig Schleich, see [20]) the perineuronal glia as a regulatory element of the inter-neuronal transmission and envisaged glial regulation as one of the principle mechanisms of sleep. In particular, Cajal suggested that astrocytes switch between active and passive states of the neuronal networks: retraction of astroglial processes allows information flow to promote wakefulness, whereas extension of astroglial processes applies a break on interneuronal connectivity, thus

inducing sleep [19, 21]. Secretory function of neuroglia was proposed by Hans Held and Jean Nageotte [22, 23]. In 1907 Ernesto Lugaro published a concise and prophetic paper “*Sulle funzioni della nevrogliia*” [24], in which he suggested that glia are involved in removal and catabolism of toxic substances, provide a special environment facilitating and guiding outgrowth of neuronal processes through secreting chemoattractants and proposed the role of glia in regulation of synaptic transmission through removal of chemical transmitters.

Discovery of neuronal ionic electrical excitability in 1940s and 1950s [25–29] has opened a new era in neuroscience: neurones can now be interrogated at a single cell level, and their electrical signals can be monitored over time in vitro, in situ and in vivo. Glial cells are non-electrically excitable; when probing with intracellular electrodes they show slow and small fluctuations in the membrane potential, which nonetheless may follow neuronal excitation [30–35]. These voltage responses generated following neuronal activity were generally considered of a passive nature reflecting redistribution of, mainly, K^+ ions. As the result, main focus of neuroscience shifted towards neuronal excitability and action-potential driven encoding in neuronal networks; for several decades glial cells went out of fashion. The tide begun to turn in 1980s when neurotransmitter receptors had been discovered in astrocytes and oligodendroglial cells [36, 37], while fluctuations in cytosolic Ca^{2+} as well as propagating Ca^{2+} waves had been imaged in cultured glia [38, 39], thus paving the way to the concept of glial ionic excitability [40–42].

This was the state of the glial art in 1989 when Vlad started his first experiments on mixed hippocampal cultures trying to reveal mechanisms of modulation of synaptic transmission. Then the serendipity, which often interferes with research, did emerge, when Srdija Jeftinija contacted Phil Haydon and Vlad with a proposal to look at Ca^{2+} -dependent glutamate release from glial cells. On May 5, 1992, the pilot experiments on DRG glia explants and hippocampal dissociated cultures were carried out. Perfusion with bradykinin (a potent metabotropic agonist) raised intracellular Ca^{2+} levels in dorsal root ganglia (DRG) glia and in astrocytes. It should be noted that these DRG glia explants were not only devoid of neurones, but were also depleted of fibroblasts; they contained mainly Schwann cells and some satellite glial cells. Having exciting initial results, Parpura, Jeftinija and Haydon designed the full set of experiments to test: (i) whether bradykinin-induced release of glutamate and aspartate from DRG glia operates via Ca^{2+} -dependent mechanism involving the endoplasmic reticulum store; and (ii) the existence of glutamate-mediated astrocyte-neurone signalling in hippocampal cultures. These two proposed studies took different paths to fruition.

The DRG glia study went smoothly and the paper was submitted to *Nature* on August 11, 1993. From there on things turned out rocky as the work was flatly rejected at the editorial level. It was then submitted to *Science*, where it was reviewed and deemed “interesting and important”, “show convincingly and for the first time”, but it was rejected. After an appeal to the editorial decision, the rejection was final. The paper was then submitted to *Neuron*, from where it was quickly returned without peer-review and with suggestion by two members of the editorial board that the work “would be most appropriate for another journal”. It was then submitted to the *Journal of Neuroscience* and after three revisions accepted and published in August of 1995 [43].

Meanwhile, Ca^{2+} imaging experiments on hippocampal astrocytes continued to work perfectly. Additionally, cortical astrocytes were also tested and showed robust BK-induced Ca^{2+} responses, while solitary neurones failed to respond to BK. Experiments using hippocampal neurones grown on a feeder layer of astrocytes worked the first time they were attempted on January 4, 1993, but in subsequent runs neuronal responses to BK (as per subsequent experiments due to glutamate release from astrocytes) became meek, which was certainly consistent with HPLC data on BK-induced glutamate release from hippocampal astrocytes. As fresh HPLC data showed robust BK-induced glutamate release from cortical astrocytes, results aligned with Ca^{2+} experiments, the decision was made to scrap out hippocampal culture data and repeat all experiments done thus far and focus solely on cultured cells obtained from cortex, more specifically from visual cortex. Such experiments started on May 28, 1993 and since then the study was fully executed with only minor problems. The paper was submitted to *Nature* on November 5, 1993 and after three revisions was published in July of 1994 [44].

Thus the concept of gliotransmission was born, to occupy gliologists minds since, with numerous debates and controversies [45–49]. The discovery of astrocytic Ca^{2+} -dependent secretion also was instrumental for the emergence of the concept of tripartite synapse that elevated glia to a legitimate neuronal partner in neurotransmission [50]. This concept forced the rethinking of glial role in the physiology of the brain and has been further advanced into the concept of brain active milieu [51]. Ensuing experiments on Ca^{2+} -dependent secretion in astrocytes revealed that this process differs to that in neurones; the kinetics of glial exocytosis is much slower [48, 52], this being likely a consequence of different sets of SNARE proteins mediating membrane fusion. Vlad and his colleagues discovered that instead of SNAP-25, astrocytes contain SNAP-23 [53], which defines the dynamics of exocytotic response [54]. Furthermore, Vlad contributed significantly to the understanding of the function of SNARE proteins in exocytosis by introducing atomic force spectroscopy [54] to study the interaction between proteins

forming the SNARE complex. In combination with single vesicle fusion studies and atomic force spectroscopy the results revealed that molecules interacting with the SNARE complexes, including the Munc18-1 protein, tune the interactions between SNARE proteins providing a safeguarding mechanism for vesicular tethering/docking to occur via the ternary SNARE complex, and through this permitting the transition of vesicle stages between states with a narrow fusion pore to states with wider exocytotic fusion pores leading to full fusion [55].

After successful years at Iowa, Vlad joined the University of California Riverside (UCR) as an Assistant Professor in July of 2000 and climbed to the rank of Associate Professor with tenure in July 2005. Then Vlad made a lateral move to The University of Alabama at Birmingham (UAB) in July 2007 where he is working since. At UAB, Vlad was promoted to the rank of full Professor in October of 2015. The main research topic remained, however, the same: the role of astrocytes in brain function.

It is impossible to even briefly overview all discoveries made by Vlad over last 30 years: he studied secretory molecules in astrocytes and glial signalling in *Caenorhabditis elegans*, he researched TRP channels and mitochondrial Na^+ – Ca^{2+} exchanger, he analysed pathophysiology of glia in animal models of neuropsychiatric and neurodegenerative diseases, and he studied interactions between carbon nanotubes and neurones and astrocytes. All these were published in more than 180 journal write-ups, 8 books and numerous book chapters, and cited in excess of 14,000 times.

The papers included in this honorary issue reflect in many ways the significance of the work of Vlad and many of the authors have benefitted from his contributions.

Alexei Verkhratsky,
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Guest Editors

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