Progress in glial cell studies in some laboratories in China

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Glia in the central nervous system (CNS) consist of a heterogeneous population of cell types, each characterized by distinct morphological features, physiological properties, and specific markers. In contrast to the previous view that glial cells were passive elements in the brain, accumulating evidence suggests that glial cells are active participants in various brain functions and brain disorders. This review summarizes recent progress of glial cell studies from several groups in China, ranging from studies about the mechanisms of neuron-glia crosstalk to investigations on the roles of glial cells in various CNS disorders.

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Glial cells constitute a large proportion of brain cells—from 25% in Drosophila to 90% in human, suggesting that glial cells may play important roles in high level brain functions. However, due to lacking synaptic transmission and spiking activity in response to various stimuli, glial cells had been long considered as “passive cells” that play only supportive rather than signaling roles in the brain. Starting from the 1980s, the patch clamp technique was utilized in glial cell studies and it became clear that many types of channels and receptors, originally found in neurons, were similarly expressed in glial cells. Neuroscientists thus realize that glial cells may not be real “inert cells”, rather that they sense and respond neuronal signaling. It has been found that astrocytes are able to create propagating Ca^{2+} waves among astrocytes as well as between neurons and astrocytes. Because intracellular Ca^{2+} signaling is critical for various cell functions, it is thus plausible to consider the propagating Ca^{2+} wave as a unique form of excitability and signal transmission in glial cells. Research concerning neuron-glia interactions has been one of the most active fields in neuroscience in the past decade.

There is also increasing interest in glial cell studies in China. More than 1000 papers are listed when searching with the key words ‘glia’ and ‘China’ in Medline, an online database. This review does not attempt to summarize all of these publications, which would be impractical in a single review article. Instead, I will review the last 10 years’ studies from a few groups in China, whose major efforts have been focused on the properties and functions of glial cells in the brain.

1 Neuron-glial crosstalk

Because astrocytes intimately associate with neuronal synapses, neuroscientists have been particularly interested in elucidating whether or not and how astrocytes modulate neuronal activity at the synaptic level [1–4]. Duan ShuMin and his group from the Institute of Neuroscience, Chinese Academy of Sciences (CAS), China, made several significant findings in the field. In addition, they disclosed some
unique properties and mechanisms of signal processing in NG2 glial cells.

1.1 Heterosynaptic modulation mediated by astrocyte released ATP

Early studies suggest that astrocytes may release glutamate to induce a slow inward current (SIC) in neurons [1]. The finding that most CNS synapses are closely associated with astrocyte processes and the idea that astrocytes may constitute an integrative part of synapses has prompted research about neuron-glia crosstalk at the synaptic level. Using dual whole-cell recordings from pairs of interconnected hippocampal neurons in mixed neuron-astrocyte co-cultures, Duan and his colleagues found that glutamatergic, but not GABAergic synaptic activity induced a significant presynaptic suppression of the reciprocally connected neurons. Further analysis indicated that this heterosynaptic inhibition was caused by the activation of presynaptic P2Y receptors. This ATP-mediated heterosynaptic suppression was not detected in pure neuronal cultures, suggesting involvement of astrocytes. Similar heterosynaptic suppression induced by glutamatergic synaptic activities were also detected in CA1 hippocampal neurons using slice preparations, although this suppression was mediated by presynaptic A1 receptors, rather than P2Y receptors. However, a P2Y receptor-mediated tonic and activity-induced heterosynaptic inhibition was detected in the presence of inhibitors of the ecto-nucleotidase activity in brain tissue, but not in neural cultures, caused the rapid conversion of extracellular ATP into adenosine, which in turn induced A1-receptor-mediated presynaptic suppression. Using multiple approaches including Ca$^{2+}$ imaging and pharmacological tools, Duan et al. [5,6] were able to identify a pathway of astrocyte-mediated heterosynaptic suppression. Thus, besides the spillover of neurotransmitters from synapses, signaling molecules released from astrocytes, e.g., ATP and adenosine, also mediate neuronal activity-dependent heterosynaptic modulation. These findings were further confirmed by another group using a transgene mouse model with a deficiency in astrocyte exocytosis [7].

1.2 Contribution of glial cells to synaptic plasticity

Long-term potentiation (LTP) of synaptic transmission has been studied extensively in various neuronal synapses for more than 30 years. Although both the presynaptic and postsynaptic mechanisms of LTP induction and expression have been intensively explored, the potential contribution of parasympatic astrocytes to neuronal LTP had not been examined. Using two types of hippocampal cultures-neurons cultured on a layer of astrocytes (mixed cultures) or in a glia conditioned medium (GCM) without direct contact with astrocytes (GCM cultures), Duan et al. [8] found that LTP was evoked in mixed cultures but not in GCM cultures. Further results from both hippocampal slices and cultured neurons indicated that, by providing extracellular D-serine which facilitated activation of NMDA receptors, astrocytes directly supported the induction of LTP. As LTP is believed to be a cellular basis for learning and memory, the finding that astrocyte-derived D-serine is critical for LTP induction indicates that glial cells, through their contribution to neural signaling processing and information storage, are involved in various higher brain functions. A correlation of astrocyte-derived D-serine and neuronal synaptic plasticity was revealed by a French group in the hypothalamic supraoptic nucleus, in which the extent of astrocytic ensheathing of neurons was found to be changing during lactation. They found that the degree of astrocytic coverage of neuronal synapse control the activation level of the glycine binding site on the NMDA receptor by D-serine, and thus the activity dependence of long-term synaptic plasticity [9]. Another group further showed that astrocyte-derived D-serine supporting the induction of hippocampal LTP was released in a Ca$^{2+}$ dependent manner [10].

1.3 Plasticity of neuron-glia signaling

Glia cells express various receptors and channels and thus sense and response to the signal molecules released from neurons. One of the most significant properties of neuronal synaptic transmission is plasticity, which has been related to learning and memory. It is of great interest to examine whether or not the signal transmission from neuron to glia also exhibits plasticity. Duan et al. [11] addressed this topic by whole-cell recordings from astrocytes and NG2 glial cells in the CA1 region of hippocampal slices. Glial cell membrane currents induced by stimulation of the Schaffer collaterals (Sc) were used to monitor neuron-glia signaling. Persynaptic astrocytes respond to neuronal activity by slow membrane depolarizations, due to extracellular accumulation of K\(^+\) ([K\(_o\)],) or glutamate secreted by neurons. The NG2 cells, also classified as oligodendrocyte precursor cells (OPCs), are widely distributed in both developing and adult brains and may represent a distinct population of macroglia-like cells. These NG2 cells in the CA1 area receive direct glutamatergic and GABAergic synaptic inputs from neurons, although the structure of neuron-glia synapses found in NG2 cells differs from that of neuronal synapses by having a less well-defined postsynaptic density and smaller presynaptic boutons which contain fewer vesicles [12,13]. The Sc-evoked membrane depolarizations in both NG2 cells and persynaptic astrocytes exhibited LTP-like enhancement following high-frequency Sc stimulation that induced LTP at Sc-CA1 pyramidal cell synapses, indicating that neuron-glia signaling underwent activity-dependent plasticity similar to that found at neuronal synapses. However, the underlying mechanisms of LTP induction and expression in the two types of glial cells are different. The LTP-like enhancement of the slow depolarization in astro-
cytes was found to be a passive response to the increased extracellular K⁺ accumulation accompanying the LTP of neuronal synapses [11], whereas the induction and expression of LTP in neuron-NG2 cell synapses involved Ca²⁺-permeable AMPARs in NG2 cells [14]. These findings demonstrate the activity-dependent long-term enhancement of neuron-glia signaling and suggest additional sites for information storage in the brain.

1.4 Vesicular release in astrocytes

Astrocytes release various signaling molecules to mediate intercellular communication. However, the mechanisms of signaling molecule release from astrocytes are not established. Using FM dye labeling and electrochemical amperometric detection of preloaded dopamine in cultured astrocytes, Zhou et al. [15] from Peiking University found that a population of large vesicles exhibited ‘kiss and run’ exocytosis. Although glutamate release from these vesicles was suggested, the exact nature of the vesicles was not identified with specific markers of various vesicles. Duan et al. [16] found that lysosomes in astrocytes were specifically labeled with FM dyes and exhibited two modes of Ca²⁺-dependent exocytosis: partial release in response to mild extracellular stimuli (ATP, glutamate) and full release triggered by ischemic insult (KCN). Further studies showed that lysosomes contained abundant ATP and its partial exocytosis resulted in a low-level ATP release, whereas full exocytosis led to the release of lysosome enzymes together with a larger amount of ATP. Disruption of lysosomes abolished stimulation-induced ATP release and Ca²⁺ wave propagation in astrocytes [16]. Similar Ca²⁺-dependent lysosomal exocytosis in astrocytes was subsequently reported from two other groups [17,18]. These findings revealed a novel mechanism of ATP release in astrocytes and indicated that lysosome release from astrocytes may contribute to intercellular signaling under physiological conditions. The ischemic insult-induced full lysosomal exocytosis may have pathological implications due to the release of a large amount of ATP as well as lysosomal enzymes. Indeed, the inhibitors of lysosomal enzymes have been reported to exert protective roles in preventing ischemic damage [19], while a high concentration of extracellular ATP is known to induce large pores on the cell membrane through activation of P2X7 receptors and thus to cause the leak of intracellular glutamate and ATP and the damage to the cell [20,21]. Thus, lysosome exocytosis may be a potential therapeutic target for neural protection against ischemic injury.

Although it is not clear how ATP is accumulated in lysosomes, a mechanism similar to the ATP accumulation in synaptic vesicles may be responsible for lysosomal ATP storage. Many types of synaptic and secretory vesicles are reported to contain different levels of ATP [22]. Proton pump activity which maintains an inside-positive potential of the vesicle membrane is critical for the vesicular uptake of the negatively charged ATP through the nucleotide transporter expressed on synaptic and secretory vesicles [22]. Although we do not know whether similar nucleotide transporters are expressed in the lysosomal membrane, putative ATP-binding cassette (ABC) transporters and multidrug-resistant proteins, which mediate ATP efflux from the cell [23,24], have been reported to be expressed on the lysosomal membrane [25,26]. Thus the high-level activity of the proton pump in the lysosome membrane may maintain a higher inside-positive membrane potential in the lysosome [27] than that in the synaptic vesicle, allowing lysosomes to accumulate a high concentration of ATP through ABC transporters or multidrug-resistant proteins expressed in the lysosomal membrane.

1.5 Ca²⁺ signaling in NG2 glial cells

NG2 glial cells have many unique properties, including receiving direct glutamatergic and GABAergic inputs from neurons [13,28] and expressing voltage gated Na⁺ channels without producing regenerative action potentials [14,28]. The physiological roles of Na⁺ channels in these cells are thus unclear. Activation of GABA₁ receptors induces hyperpolarization in adult neurons but evokes depolarization in immature neurons, due to the differential concentration of intracellular Cl⁻ in the two types of neurons maintained by the differential expression level of Cl⁻ transporter KCC2 and NKCC1. Similar to that found in immature neurons, GABA also induces depolarizing responses in NG2 cells through the activation of GABAₐ receptors. The depolarization induced by the activation of GABAₐ receptors in immature neurons is considered to be excitatory, because it activates voltage dependent Na⁺ channels to trigger action potentials, opens the voltage-dependent Ca²⁺ channels (VGCC) to elevate [Ca²⁺], and removes a Mg²⁺ blockade of NMDA receptors to enhance Ca²⁺ influx through NMDA receptors [30]. However, all of these 3 forms of excitatory effects found in immature neurons seem to be not applicable to NG2 glial cells. Except for a subpopulation of NG2 cells in the cerebellar white matter [31,32], most NG2 cells in the brain do not fire action potentials [14,33,34], nor do they express NMDA receptors [14]. No detectable VGCC was recorded in NG2 cells [35]. The functional consequence of the GABA-induced depolarization in NG2 cells is thus unclear. Duan et al. [29] found that similar to that found in immature neurons, perfusion with GABA also induced Ca²⁺ elevation in NG2 cells through activation of GABAₐ receptors. However, the mechanisms underlying this Ca²⁺ elevation in NG2 cells is different from that disclosed in immature neurons. Instead of activating the VGCC found in immature neurons, GABA-evoked depolarization induces Na⁺ influx in NG2 cells through the voltage-gated persistent Na⁺ channel, which in turn causes intracellular Ca²⁺ elevation.
through the Na+/Ca2+ exchanger. Further evidence indicated that this unconventional pathway is involved in GABA-induced NG2 cell migration [29]. Thus, the GABAergic signaling mediated by the collaboration of GABA_A receptors, non-inactivating Na+ channels, and Na+/Ca2+ exchangers may play a key role in the development and function of NG2 glial cells in the brain.

2 Glial cells and pathological pain

Pathological pain, also known as chronic pain, is usually associated with neural injury or tissue inflammation and is characterized by hyperalgesia, allodynia, and spontaneous pain. Activation of astrocytes and microglia has been reported in various animal models of pathological pain since the 1990s. Zhao, Zhang et al. [36,37] in Fudan University have made a number of contributions in the field of studies on the correlation of glial cell activation and pathological pain. LTP of C fiber-evoked field potential in a rat spinal dorsal horn induced by tetanic stimulation of the sciatic nerve is accompanied by long-lasting mechanical allodynia and thus is regarded as an animal model of neuropathic pain. Zhao et al. [36] found that intrathecal application of fluorocitrate, a glial metabolic inhibitor, blocked tetanic sciatic stimulation-induced mechanical allodynia and spinal LTP of C fiber-evoked field potentials, an effect that was prevented by treatment with antagonists of ionotropic glutamatergic receptors. Similarly, intrathecal administration of the inhibitor of GLT-1, a subtype of glial glutamate transporter, impaired the LTP of C fiber-evoked field potentials and decreased Fos expression in the spinal cord induced by tetanic stimulation of the sciatic nerve [38], suggesting that astrocytes may modulate the sensitization of spinal nociceptive neurons through glutamate uptake. An earlier report from this group showed that chronic morphine treatment led to a significant increase in GFAP immunostaining density in the spinal cord, posterior cingulate cortex, and hippocampus, whereas treatment with fluorocitrate partially attenuated spinal tolerance to morphine analgesia [39].

A rat model of rheumatoid arthritis that accompanies hyperalgesia, allodynia and spontaneous pain are induced by intrarticular injection of complete Freund’s adjuvant (CFA). Using this chronic pain model, Zhang and her colleagues found a robust microglial activation preceded astrocytic activation in the bilateral spinal cord. Evidence using intrathecal injection of astrocyte inhibitor fluorocitrate or microglial inhibitor minocycline suggests that activation of microglia and astrocytes may respectively be responsible for the initiation of facilitated pain and the maintenance of the persistent pain following CFA-induced arthritis [40–44]. Further study suggests that a chemotactic cytokine fractalkine, which is primarily expressed in neurons, may be responsible for CFA-induced glial activation and pain hypersensitivity via activating its receptor CX3CR1, which is chiefly expressed on microglia [40,44].

3 Astrocyte injury and apoptosis

Astrocytes are thought to be more resistant to ischemic damage than neurons. However, astrocytes are the first cells to suffer ischemic insult among all types of neural cells, because their end feet directly contact capillaries. Astrocytic reaction to and dysfunction from the insult may influence the responses of other neural cells to the ischemia. Indeed, it has been reported that dying glia killed neighboring neural cells through gap junctions [45], further indicating the significance of astrocytes in brain injury. Dr. Yu of the Peking University School of Medicine has studied the mechanisms of ischemia-induced astrocytic cell death using an in vitro ischemic model. Although it is widely believed that ischemic astrocytes die from necrosis, Yu et al. [46] found that cultured astrocytes exhibited distinct morphologic and biochemical features of apoptosis under ischemia, indicating that astrocytes are capable of undergoing apoptosis without the presence of other neural cells. The 14-3-3 gamma protein, a subtype of the 14-3-3 family of proteins that bind to and regulate the functions of serine/threonine-phosphorylated proteins to regulate cell division, cell cycle, and apoptosis, was originally reported to be neuron-specific [47]. Yu et al. [48,49] found that this protein was also expressed in cultured astrocytes. The endogenous 14-3-3 gamma in astrocytes was found to bind the phosphorylated Raf, a critical serine/threonine kinase controlling cell growth, differentiation, and death [49]. It was found that ischemia induced both the upregulation and phosphorylation of Bcl-2-associated death protein (Bad) in astrocytes through the MAPK/ERK pathway, whereas 14-3-3 gamma binds the phosphorylated Bad to prevent it from entering mitochondria to initiate apoptosis [50,51]. The expression level of 14-3-3 gamma in cultured astrocytes was upregulated by ischemic insult but not by scratch wounds or heat shock injuries [49], suggesting a specific role for this protein in ischemia-induced astrocytic injuries. Yu et al. [52] further showed that 14-3-3 gamma associates with GFAP in a phosphorylation- and cell-cycle-dependent manner, which may contribute to the regulation of the dynamics of GFAP filaments and the stability of the cytoskeleton. It will be of interest to examine whether 14-3-3 gamma is involved in the ischemia-induced astrocytic apoptosis and brain injury in vivo.

4 Cell cycle regulation, glial cell activation and neural injury

Neuropathological disorders and neural injuries usually induce reactive responses in astrocytes and microglia characterized by cell cycle activation leading to excessive glial
cell proliferation and eventually glial scar formation, which may be devastating to the recovery of neuronal function. Wang and his colleagues in the Tongji Medical College of the Huazhong University of Science and Technology, demonstrated that the application of cell cycle inhibitors suppressed reactive astrogliosis, microglial proliferation, and cytokine/chemokine production and thus had therapeutic benefits in both the cerebral ischemic model and neural injury model studies [53–59]. Aberrant re-entry into the cell cycle is also frequently detected in neurons in CNS injury including stroke and trauma. However, the injury-induced cell cycle activation in neurons usually causes apoptosis rather than proliferation, probably due to the incomplete activation of the cell cycle ending at the G1–S phase. Thus, application of cell cycle inhibitors may also have direct effects on neuronal survival and neurogenesis following neural injuries and neurodegenerative disorders. It will be of interest to clarify the issue in future studies by interfering with the cell cycle specifically in neurons or glial cells using transgenic animal models.

5 Olfactory ensheathing cells and CNS repair

Repair of CNS injury, especially spinal cord injury, remains a major challenge for neuroscientists. The olfactory receptor neurons are unique in retaining their ability to regenerate. Olfactory axons spontaneously grow within the adult olfactory bulb throughout life, both in response to injury and as part of normal turnover. The major difference in regenerating capability between the olfactory system and the rest of the CNS seems to be the presence of olfactory ensheathing glial cells (OECs) in the olfactory system. Thus, OECs transplantation has emerged as a promising therapy for CNS axonal injuries and demyelinating diseases [60,61]. The migrating ability of OECs is thought to be essential for pioneering the olfactory nerve pathway during development and for promoting axonal regeneration by accompanying the regenerating nerve fibers through the unfavorable CNS environment. He et al. [62] in the Second Military Medical University, Shanghai, has studied the properties and mechanisms of OECs migration and explored approaches for promoting OECs migration and CNS repair.

OECs are unique in that they exist in both the peripheral and the central nervous system, sharing the phenotypes of both astrocytes and Schwann cells. Cultured OECs have been classified as the Schwann cell-like OECs and the astrocyte-like OECs, respectively based on their bipolar shape and flat sheet-like shape. He and his colleagues further classified the astrocyte-like OECs into astrocyte-like types 1 and 2 OECs, respectively based on their morphology of either fan-like or round shapes. The different morphological phenotypes of OECs may be determined by the distinct patterns of cytoskeletal organization mediated by the differential activity level of the Rho family of small G-proteins. He et al. [62] found that the three subpopulations transform into each other within 20–40 min, indicating that the three subtypes of OECs may originate from a single cell type representing different functional states. They found that Schwann cell-like OECs were more motile than astrocyte-like type 1 OECs, while type 2 OECs were the least motile. Accordingly, Schwann cell-like OECs exhibited a more apparently attractive response toward a gradient of lysophosphatidic acid (LPA), while astrocyte-like type 2 OECs failed to respond to the LPA gradient [62]. These results are consistent with earlier reports that Schwann cell-like OECs are more effective in promoting axonal regeneration than astrocyte-like OECs [63,64].

Neurotrophic factors have been extensively studied for their function in promoting neuronal survival, nerve fiber outgrowth, and neural injury repair. He et al. [65] found that after being transplanted into the injured spinal cord of adult rats, genetically modified OECs overexpressing GDNF (glial cell line derived neurotrophic factor) were capable of producing GDNF and significantly improving the recovery of spinal cord injury. Such an approach that combines the outgrowth promoting property of OECs with the neuroprotective effects of the additionally overexpressed neurotrophic factors may facilitate the treatment of spinal cord injury. Further study showed that GDNF also promoted OECs migration both in vitro and in vivo [66].

Schwann cells are well-known for their ability in promoting axonal regeneration and remyelination. However, the migration of the transplanted Schwann cells into CNS seems to be restricted by surrounding astrocytes and the regenerated neurites failed to traverse the Schwann cell-astrocyte interface. Combining Schwann cell bridges with OECs grafts have been reported to have additive effects for promoting axonal regeneration and locomotor recovery after spinal cord injury [67,68], although the underlying mechanisms are not clear. He et al. [69] found that although wild type OECs produce very limited GDNF, these cells produce a significant amount of nerve growth factor (NGF), enough to promote the migration of Schwann cells in an astrocyte environment. He et al. [70] also found that OEC-secreted factors could improve the migration of neural progenitors in RMS to the olfactory bulb, indicating that OEC-secreted factors may attract neural stem/progenitor cells toward the lesion sites after OECs transplantation.

Damage to the adult CNS usually results in the formation of a complex hostile environment, including inhibitory molecules in the CNS myelin as well as proteoglycans associated with astroglial scarring, which may present a major hurdle for axon regeneration. After transplantation into the intricate glial environment, the biological and regenerative properties of OECs may be influenced. It is well documented that Nogo is one of the prominent components of CNS myelin inhibitory activity for adult axon-regeneration. He et al. [71] showed that Nogo enhanced the adhesion of OECs and inhibited their migration both in vitro and in vivo.
In addition, the reactive astrocytes in a glial scar formed after spinal cord injury are also reported to attract OECs migration towards the lesion site of the spinal cord by secreting TNF-α [72].

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Biographical Sketch

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