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REVIEW

Glutamate-mediated neuronal–glial transmission

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Abstract

The brain is the most complex organ of the human body. It is composed of several highly specialized and heterogeneous populations of cells, represented by neurones (e.g. motoneurons, projection neurons or interneurons), and glia represented by astrocytes, oligodendrocytes and microglia. In recent years there have been numerous studies demonstrating close bidirectional communication of neurons and glia at structural and functional levels. In particular, the excitatory transmitter glutamate has been shown to evoke a variety of responses in astrocytes and oligodendrocytes in the healthy as well as the diseased brain. Here we overview the multitude of glutamate sensing molecules expressed in glia and describe some general experiments which have been performed to identify the glutamate-responsive molecules, i.e. the ionotropic and metabotropic glutamate receptors as well as the glutamate transporters. We also discuss a transgenic mouse model that permits detailed and specific investigations of the role of glial glutamate receptors.

Key words AMPA; astrocytes; glia; glutamate transporters; glutamate; kainate; neuronal–glial interactions; NMDA; oligodendrocytes.

Introduction: neuronal–glial circuits

The neuronal doctrine, which from the 1890s shaped the development of neuroscience (von Waldeyer, 1891; Exner, 1894), is giving way to more inclusive theories, which regard the brain as a system of interconnected neuronal and glial networks (Haydon, 2001; Perea & Araque, 2005; Volterra & Meldolesi, 2005; Verkhratsky, 2006a). Numerous studies have demonstrated that all glial cell types, as diverse as the neuroectoderm-derived astrocytes, oligodendrocytes and NG2 glia or the mesoderm-derived microglia, express the same variety of neurotransmitter receptors as neurons in both culture systems and in *in situ* preparations (Verkhratsky & Kettenmann, 1996; Verkhratsky et al. 1998; Verkhratsky & Steinhauser, 2000). Many of these receptors, when activated by neural activity, initiate glial Ca^{2+} responses, which produce long-range interglial Ca^{2+} waves (Cornell-Bell et al. 1990; Cornell-Bell & Finkbeiner, 1991; Dani et al. 1992; Verkhratsky, 2006b). Glial Ca^{2+} signals, in turn, activate release of 'glio'transmitters (which include glutamate, ATP, taurine, D-serine and probably many others) that can signal back to neurons, thus functionally integrating neuronal and glial circuitries (Bezzi et al. 1998, 2004; Zhang et al. 2004a,b; Volterra & Meldolesi, 2005). Glial cells perform a multitude of functions, which to a large extent control neuronal development, appearance, maintenance and plasticity of synaptic contacts, energy support, and integration into neuron–glia vascular units (Alvarez-Buylla et al. 2001; Malatesta et al. 2003; Nedergaard et al. 2003; Newman, 2003; Zonta et al. 2003; Hirrlinger et al. 2004; Mulligan & MacVicar, 2004; Gotz & Huttner, 2005; Magistretti, 2006; Verkhratsky, 2006b). Moreover, probably the most numerous glial cells, the astrocytes, can form an extended physically connected syncytium endowed with sophisticated mechanisms of intercellular 'volume' transmission (Dermietzel, 1998), which may allow them to play an important, albeit yet unknown, role in supporting high cognitive functions.

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Glutamate as an excitatory neurotransmitter

Probably the first contemplation of glutamate as an excitatory transmitter in the central nervous system was made by Hayashi (1954), based on his findings that injection of glutamate in the brain provokes convulsions. By that time it was well known that glutamate is present in the nervous system at very high concentrations, yet it was firmly believed that glutamate is primarily involved with brain metabolism, rather then with synaptic transmission. The first direct experiment, which gave birth to the theory of glutamatergic transmission, was reported by Curtis et al. (1959), who discovered that iontophoretically delivered glutamate excited neurons in the spinal cord; they, however, found that other amino acids had the same ability and concluded that glutamate was most probably a non-specific agent not connected with interneuronal signalling. This view of non-specificity of glutamate prevailed for almost 20 years, before the appearance of specific agonists and antagonists helped to identify neuronal glutamate receptors (Evans et al. 1979; Watkins & Evans, 1981). In the ensuing 25 years the concept of glutamate as a main excitatory neurotransmitter in the brain was fully developed.

Incidentally, glutamate excites not only neurons but also glial cells, which became apparent in 1984 when microelectrode recordings revealed glutamate-mediated depolarization of astrocytes and oligodendrocytes (Bowman & Kimelberg, 1984; Kettenmann et al. 1984a,b). Further experiments have clearly demonstrated that glutamate acts as a mediator of both interneuronal and neuronal–glial signalling.

Overall, the glial cells interact with glutamate through three distinct molecular systems (Fig. 1), represented by ionotropic and metabotropic glutamate receptors as well as glutamate transporters. This review provides a concise overview of the diverse glutamate signalling pathways within the various glial cell types.

Ionotropic glutamate receptors

Three families of ionotropic glutamate receptors, defined by specific pharmacology and with distinct molecular structures, are present in the nervous system. These three families are AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), kainate and NMDA (*N*-methyl-D-aspartate) receptors, which all belong to a broad family of cationic ligand-operated channels (for reviews see Wisden & Seeburg, 1993; Mayer & Armstrong, 2004; Mayer, 2005). All three families are expressed and functionally operational in the brain glia, although their distribution shows remarkable region- and cell-type specificity.

AMPA receptors are the most common in glia, being detected in various astroglial cells throughout the brain (Condorelli et al. 1999; Verkhratsky & Steinhauser, 2000; Seifert & Steinhauser, 2001), in white matter oligodendrocytes (Gottlieb & Matute, 1997; Bergles et al. 2000; Alberdi et al. 2005) and in NG2 glia (Lin & Bergles, 2002). Recently, they were also detected in microglia (Noda et al. 2000). The functional diversity of AMPA receptor-mediated membrane currents is determined by a variable assembly of four receptor subunits, GluR1 to GluR4, encoded by distinct genes (Wisden & Seeburg, 1993; Hollmann & Heinemann, 1994) and further post-transcriptional modification by alternative splicing and mRNA editing (Seeburg et al. 1998). AMPA receptors constructed from various subunits assemblies were identified in astroglial cells from virtually all brain areas, including hippocampus, cerebellum, neocortex and retina (Gallo & Ghiani, 2000; Verkhratsky & Steinhauser, 2000; Seifert & Steinhauser, 2001; Matthias et al. 2003) (Fig. 2). The comparative physiological analysis of glial cells from different brain regions was greatly facilitated by the use of transgenic mice with glia-specific expression of fluorescent proteins (Zhuo et al. 1997; Nolte et al. 2001; Mallon et al. 2002; Yuan et al. 2002; Hirrlinger et al. 2005). Importantly, in many types of glial cells, AMPA receptors are Ca^{2+} permeable, due to the relatively low abundance of the GluR2 subunit, which induces the Ca^{2+} impermeability of the receptor. The Ca^{2+} signals originating from Ca^{2+} influx through AMPA receptors were identified in several types of astrocytes, both in culture and in brain slices (Enkvist et al. 1989; Glaum et al. 1990; Burnashev et al. 1992; Muller et al. 1992; Jabs et al. 1994; Porter & McCarthy, 1995).

The second type of glutamate receptors, the kainate receptor, is assembled from five distinct subunits, the KA1 and KA2 and GluR5–7 (Huettner, 2003; Lerma, 2003). All these subunits were identified in certain types of astroglial cells (e.g. in bovine corpus callosum or in rodent perivascular astrocytes) at either the mRNA or the protein level (Garcia-Barcina & Matute, 1996; Brand-Schieber et al. 2004). Unfortunately, we still lack the evidence for functional kainate receptors determined as ion fluxes through the receptor pore. Incidentally,

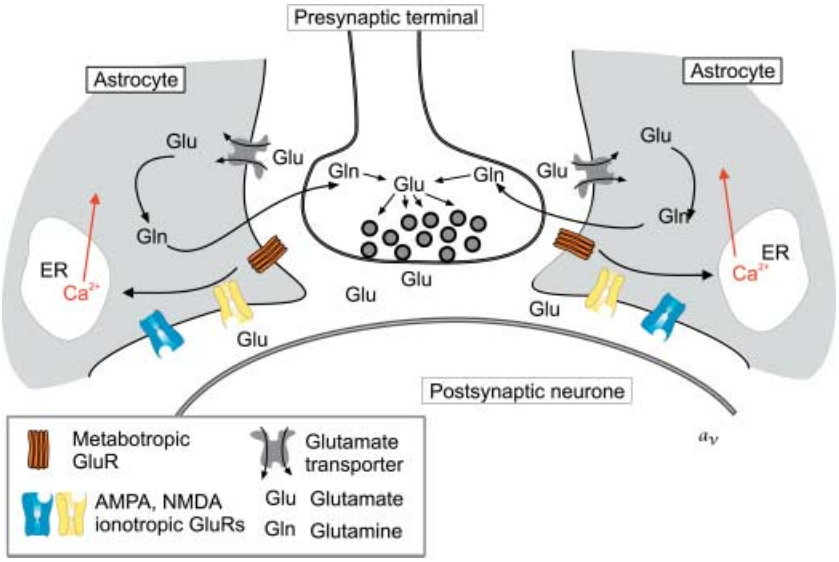

Fig. 1 Glutamate-mediated neuronal–glial signalling. Synaptically released glutamate activates glial ionotropic (AMPA and NMDA) and metabotropic receptors. Activation of group I metabotropic receptors initiates phospholipase C-dependent synthesis of $InsP_3$, which in turn triggers Ca^{2+} release from the endoplasmic reticulum (ER) Ca^{2+} store. The majority (~80%) of glutamate released during synaptic transmission is taken up by astroglial Na^+/glutamate transporters; subsequently, glutamate is converted into glutamine, which is transported back to neurons, where it acts as a main source of newly synthesized glutamate ('glutamate–glutamine shuttle').

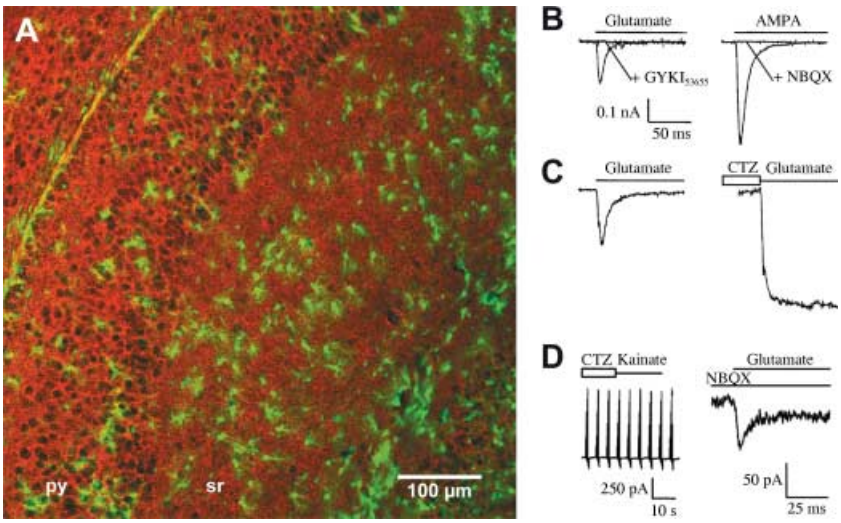

Fig. 2 Glutamate responses in astroglial cells of the hippocampus. Within the hippocampus of TgN(GFAP-EGFP) transgenic mice two different astroglial cell populations can be distinguished by fluorescence intensities due to different levels of green fluorescent protein (EGFP) expression. These two cell populations respond to glutamate either by activation of glutamate transporters or AMPA-type glutamate receptors. (A) Confocal laser scanning microscopy of a hippocampal section obtained from TgN(GFAP-EGFP) mice and immunostained for the glutamate transporter GLT-1 (red). Astroglial cells (green) with different morphology can be readily distinguished, isolated and electrophysiologically characterized. (B) In two of the EGFP-positive astrocytes, rapid application of glutamate (left, postnatal day 9) or AMPA (right, postnatal day 15) evoked fast transient and completely desensitizing [τ (inactivation time constant) = 6 and 8.2 ms, respectively] inward currents that were completely inhibited by AMPA receptor antagonists GYKI53655 or NBQX. (C) In another EGFP-positive cell (postnatal day 13), the control response to glutamate (left) was enhanced two-fold in the presence of cyclothiazide (CTZ; right). (D) Cells with numerous processes and brightest fluorescence levels do not express functional AMPA receptors but possess functional glutamate transporters, since kainate cannot elicit currents while an inward current can still be observed in the presence of the selective AMPA receptor blocker NBQX. A: F. Kirchhoff, personal observations; B–D: modified from Matthias et al. (2003).

the GluR5 receptors are important for astroglial–neuronal signalling in hippocampus, where glutamate, released from the astroglia, signals to inhibitory neurons precisely through activation of neuronal GluR5 kainate receptors (Frerking, 2004; Liu et al. 2004). Kainate receptors were also identified in oligodendrocytes, where they may be important for mediating glutamate excitotoxicity (Alberdi et al. 2005, 2006).

The third type of glutamate receptor are the NMDA receptors encoded by seven genes, NR1, NR2A–D, and NR3A and B (Kew & Kemp, 2005). Similar to the AMPA or kainate receptors a heteromeric assembly of four subunits forms the ion-permeating pore of the NMDA receptor. The NR1 subunit is essential. NR2 or NR3 subunits alone are not sufficient to form a functional channel. NMDA receptors were for a long-time considered to be specifically confined to neurons. NMDA receptors display several important features, which conceptually made them relevant for neuronal integration and regulation of synaptic plasticity. These features include a very high Ca^{2+} permeability ($P_{Ca}/P_{Na} > 10$; Mori & Mishina, 1995) and a specific Mg^{2+}-dependent voltage-dependent block, which renders NMDA receptors barely active at hyperpolarized (–80 to –70 mV) membrane potentials (Mayer et al. 1984; Nowak et al. 1984). As a consequence, NMDA-mediated Ca^{2+} fluxes occur only when Mg^{2+} block is removed by sufficient depolarization of neurons, which provides for coincidence detection, relevant for various forms of synaptic plasticity (Collingridge & Bliss, 1995). As glial cells typically maintain a high degree of membrane polarization (resting potential ~ –80 mV) and are almost devoid of depolarizing ion permeabilities, it was firmly believed that NMDA receptors do not have any room for operation. Yet some time ago NMDA receptors subunits were detected in several types of astrocytes by immunohistochemistry, RT-PCR or *in situ* hybridization. In addition, NMDA-induced Ca^{2+} and membrane current responses were recorded from astroglia (e.g. Muller et al. 1993; Conti et al. 1996, 1997, 1999; Ziak et al. 1998; Schipke et al. 2001). Very recently, a series of papers substantiated the presence of functional NMDA receptors in cortical astrocytes (Fig. 3; Lalo et al. 2006) and demonstrated NMDA receptor expression and their fatal involvement in ischaemic insults in oligodendrocytes (Karadottir et al. 2005; Salter & Fern, 2005; Micu et al. 2006). Interestingly, in hippocampal astrocytes an up-regulation of the NMDA receptor subunit NR2B could be detected a few days after ischaemia and anoxia (Krebs et al. 2003). Incidentally, glial NMDA receptors are much less sensitive to Mg^{2+} block, which may result from a specific molecular composition (Lipton, 2006) being, as result, perfectly operational at the glial resting potentials.

All in all, glial cells have a wide complement of ionotropic glutamate receptors, which have different sensitivity to glutamate, different kinetics and different ion permeability properties, thus allowing glial cells a high degree of sophistication in deciphering signals carried by external glutamate.

Metabotropic glutamate receptors

Metabotropic glutamate receptors are abundantly present in glial cells. These receptors, represented by eight different genes (mGluR1–8), belong to the family of seven-transmembrane-domain, G-protein-coupled receptors (Nakanishi, 1994; Kew & Kemp, 2005; Ferraguti & Shigemoto, 2006). Metabotropic glutamate receptors 1 and 5 (also known as group I mGluRs) are functionally linked to phospholipase C and synthesis of 1,4,5-inositol-trisphosphate ($InsP_3$) and diacylglycerol (DAG); by contrast, mGluRs 2 and 3 (group II) and mGluRs 4 and 6–8 (Group III) are coupled to adenylate cyclase. Although it is assumed that glial cells can express all types of mGluRs, mGluR 3 and 5 remain the most abundant and best characterized glial mGluRs. These two types of mGluRs were also identified *in situ*, in astroglial processes at the ultrastructural level (Petralia et al. 1996; Aronica et al. 2000; Tamaru et al. 2001). Immunohistochemical identification of mGluR subtypes in oligodendroglia has not yet been shown. Both mGluR 3 and 5 could be demonstrated on cultured oligodendrocyte progenitor cells by Western blot analysis (Deng et al. 2004). Functionally, the group I mGluRs are important for triggering cytosolic Ca^{2+} signalling through stimulation of $InsP_3$-induced Ca^{2+} release from the endoplasmic reticulum (ER) calcium store (Verkhratsky, 2005). Conceptually, the ER calcium store acts as a main source for glial calcium signalling; furthermore, it forms an intracellular excitable medium, which is instrumental for generation of long-range propagating glial Ca^{2+} waves (Verkhratsky & Kettenmann, 1996; Verkhratsky et al. 1998; Verkhratsky, 2006b). Stimulation of mGluR5 triggers elevation of cytosolic Ca^{2+} concentration in glia; these Ca^{2+} signals do not require extracellular Ca^{2+} and are sensitive to inhibitors of ER Ca^{2+} accumulation (thapsigargin or cyclopiazonic acid) and to heparin, which blocks $InsP_3$ receptors residing in the ER membrane (Fig. 4; Kirischuk et al. 1999). Furthermore, at least in Bergmann glial cells, the mGluRs represent the main route for Ca^{2+} signal generation, as Ca^{2+} entry through AMPA receptors is limited by rapid desensitization of the receptors when activated by glutamate (cf. Fig. 2; Kirischuk et al. 1999).

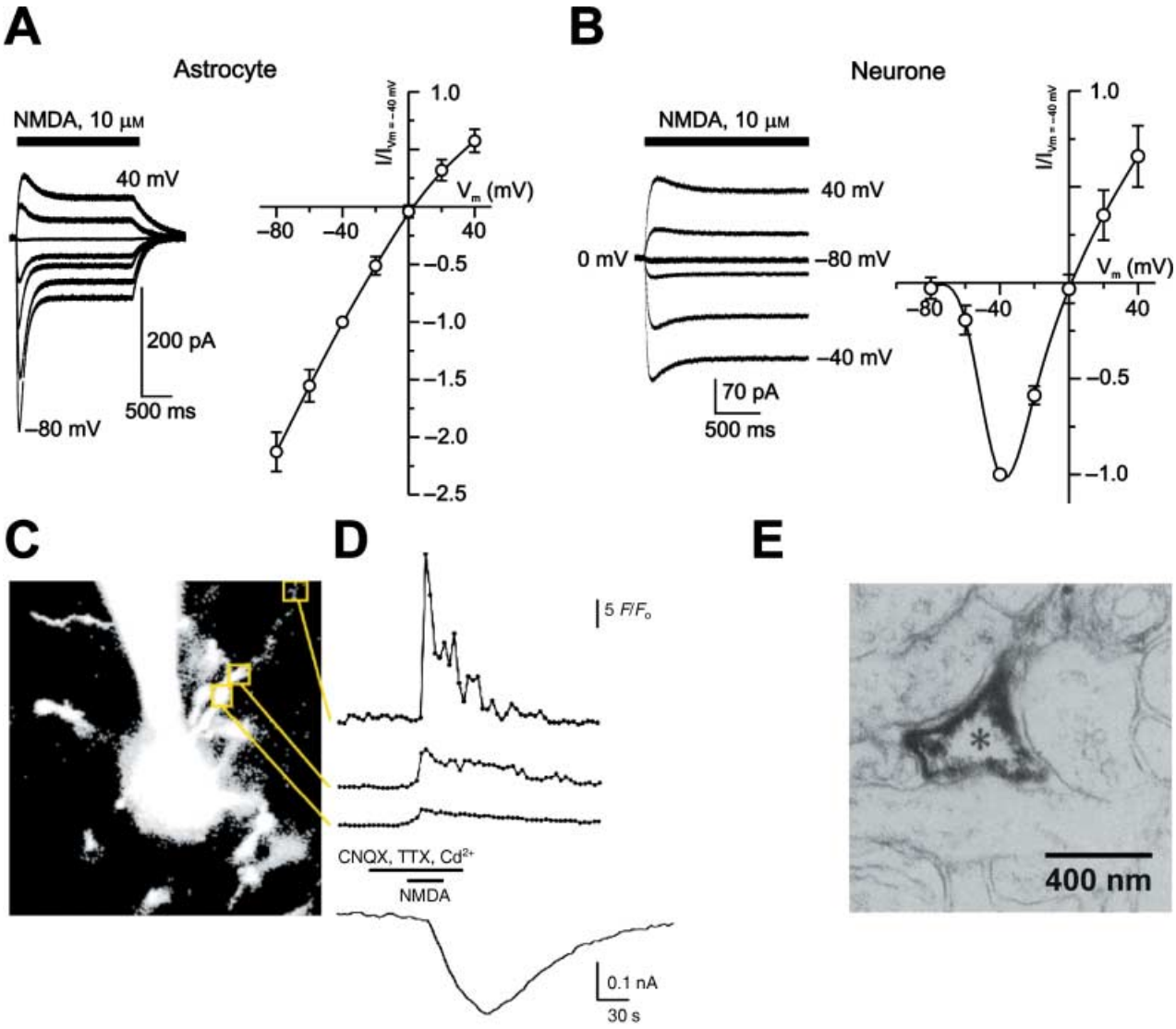

Fig. 3 NMDA responses in astrocytes and in neurons. (A,B) Comparison of NMDA activated currents and corresponding *I–V* curves measured from an astrocyte (A) and a neuron (B) acutely dissociated from the same mouse cortical slice. The recordings were performed in normal physiological solution, containing 1 mM Mg^{2+}. Note clear voltage-dependent relief from Mg^{2+} block in neurons vs. linear *I–V* curve in astrocytes. (C) A cell with the morphological features of an astrocyte was dialysed with Fluo-4 via the patch pipette in a cortical slice acutely isolated from a non-transgenic FVB/N mouse. Note the recording pipette approaching the cell from top. Changes in fluorescence (F/F_o) were analysed in the areas delineated by squares and are displayed in the top three traces in D; the lower trace shows simultaneously recorded membrane current response. NMDA (100 μM), CNQX, TTX and Cd^{2+} were applied as indicated. The largest fluorescent intensity changes, i.e. the highest Ca^{2+} influx, demonstrates high level of NMDA receptor expression in distal astroglial processes rather than in somatic regions. (E) Immunoelectron micrograph visualizing the expression of the NR1 subunit in astroglial processes of the rat cortex. A, B: reproduced from Lalo et al. (2006); C, D: modified from Schipke et al. (2001); E: modified from Aoki et al. (1994).

Glutamate transporters and glial Na^+ homeostasis

Astroglial cells represent the main sink of glutamate in the brain. From the bulk of glutamate released during synaptic transmission, about 20% is accumulated into postsynaptic neurons and the remaining 80% is taken up by perisynaptic astrocytes (Swanson, 2005). This transport is achieved by specific glutamate transporters, represented, in the human brain, by five types, classified as EAAT1 to EAAT5 (where EAAT stands for excitatory amino acid transporter). EAAT1 and EAAT2 (analogues

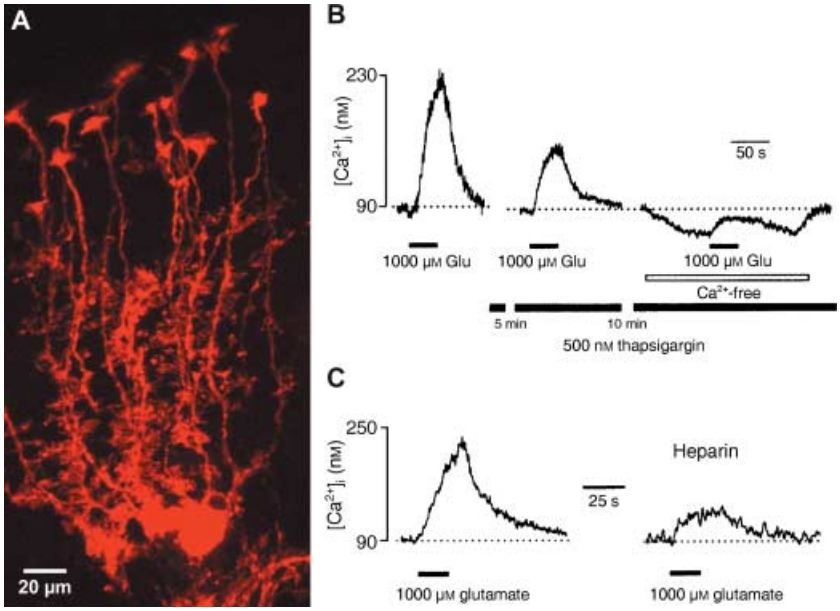

Fig. 4 Metabotropic glutamate receptors in Bergmann glial cells. (A) Confocal laser scanning micrograph of single Bergmann glial cells in parasagittal brain sections of TgN(GFAP-mRFP1) mice, where Bergmann glial cells are selectively labelled by the expression of the red fluorescent protein mRFP1. (B) Incubation of slices with thapsigargin inhibits glutamate-induced $[Ca^{2+}]_i$ transients. The $[Ca^{2+}]_i$ transients were recorded from the Fura-2/AM 'bulk' loaded Bergmann glial cells; after recording the control response (first trace on the left) slices were incubated with 500 nM thapsigargin for 15 min before recordings re-commenced. (C) Heparin inhibits glutamate-triggered $[Ca^{2+}]_i$ transients. The $[Ca^{2+}]_i$ transient shown on the left panel was recorded from Fura-2/AM 'bulk' loaded Bergmann glial cell. Subsequently the cell was approached with patch pipette and the whole-cell patch clamp configuration was established. After 5 min of cell dialysis with the intrapipette solution containing 10 μM heparin, glutamate was applied for the second time. Note that heparin significantly reduced the amplitude of glutamate-triggered $[Ca^{2+}]_i$ transient. A: from Scheller and F. Kirchhoff, own observations; B,C: modified from Kirischuk et al. (1999).

of which in rodent brain are known as glutamate/aspartate transporter, GLAST, and glutamate transporter-1, GLT-1) are expressed almost exclusively in astrocytes (Danbolt, 2001). Translocation of glutamate is powered by transmembrane ion gradients, and transport of a single glutamate molecule requires influx of three Na^+ ions and one H^+ ion together with efflux of one K^+ ion. As a result Na^+/glutamate transporter has an electrogenic effect, manifested in a net inward sodium current, and may substantially affect intracellular Na^+ concentration.

Future experimental strategies: astroglia-specific and inducible gene deletion

Many studies have been performed to investigate the repertoire of glutamate signalling in glial cells. It has been shown that glial cells are sophisticated sensors of the excitatory transmitter glutamate, which is achieved through various response pathways. Remarkably, glial cells do not necessarily express significantly different glutamate receptors than their neighbouring neurons. Therefore, it was almost impossible to analyse the unique contribution of a given receptor subunit expressed in glia to the function of the whole brain or the behaviour of the freely moving animal. Any pharmacological intervention would have immediately affected both cell types, neurons and glia.

Very recently a series of transgenic mice have been developed which allow the selective ablation of defined genes either in astrocytes or in oligodendrocytes. Transgenic mice were generated by oocyte injection (non-homologous recombination) of transgenes composed of the human glial fibrillary acidic protein (GFAP) promoter (to achieve astroglia-specific expression), the coding sequence for a fusion protein of the DNA recombinase Cre and the mutated ligand binding domain of the human oestrogen receptor (Fig. 5; Hirrlinger et al. 2006). A different mouse line has been generated by direct targeting (homologous recombination) of the CreERT2 coding sequence into the astroglia-specific locus of the glutamate transporter GLAST (Mori et al. 2006). Inducible recombination in myelinating

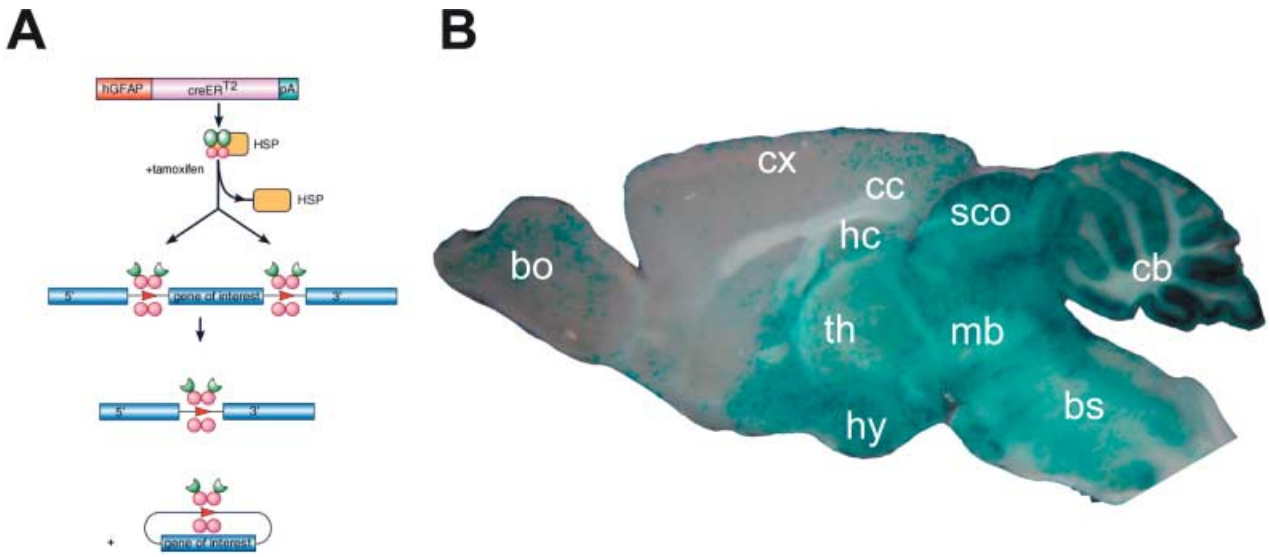

Fig. 5 Temporal control of astroglia-specific gene deletion in TgN(GFAP-CreERT2) mice by tamoxifen. (A) Principles of inducible gene recombination. Astrocytes of TgN(GFAP-CreERT2) mice express a fusion protein (CreERT2) of the DNA recombinase Cre and a mutated ligand binding domain of the oestrogen receptor. Under normal conditions CreERT2 is trapped in the cytoplasm by binding to heat shock proteins. The oestrogen receptor antagonist tamoxifen can liberate CreERT2 from this complex. After subsequent translocation into the nucleus, CreERT2 selectively excises DNA regions, which were flanked by loxP sites. (B) In cross-breeds of GFAP-CreERT2 mice with appropriate lacZ reporter mice widespread gene recombination in astrocytes can be achieved. The highest recombination rates are observed in the cerebellum (cb) and midbrain (mb). bulbus olfactorius (bo), brainstem (bs), corpus callosum (cc), cortex (cx), hippocampus (hc), hypothalamus (hy), and thalamus (th). Modified from Hirrlinger et al. (2006).

glia (oligodendrocytes and Schwann cells) is also possible by using the TgN(PLP-CreERT2) mice in which CreERT2 is targeted by the mouse proteolipid protein promoter into oligodendrocytes (Doerflinger et al. 2003; Leone et al. 2003).

The transgenic CreERT2-mice can now be cross-bred to genetically modified mice in which relevant target genes, i.e. glutamate receptor genes, are flanked by loxP sites. The functional role of a given gene in a defined glial cell population can now be tested in the whole animal (e.g. by *in vivo* physiology or behavioural experiments) by temporal control of gene ablation via intraperitoneal injection of tamoxifen. Several mouse lines with appropriate modification of glutamate receptor genes are already available, for example to study different AMPA receptor genes GluR1–4 or the NR1 subunit of NMDA receptors (Tsien et al. 1996; Zamanillo et al. 1999). Unfortunately, similar models are not yet available for the metabotropic glutamate receptors.

Conclusions

Glutamate mediates neuronal–neuronal and neuronal–glial signalling. Synaptic release of glutamate triggers a complex response in glial cells, which involves activation of several distinct types of ionotropic and metabotropic receptors as well as glutamate transporters. These molecules, in turn, initiate a variety of intracellular signalling processes, most notably those associated with propagating cytoplasmic Ca^{2+} signals. Extended complement of glutamate sensors expressed in glial cell membranes allows the glia to decode neuronal activity and to synchronize and integrate neuronal and glial circuitries. Deployment of transgenic animal models with conditional deletion of single signalling components promises new discoveries of the pivotal functions of glial cells.

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