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NMDA receptors in astrocytes

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Abstract

Astrocytes support glutamatergic neurotransmission in the central nervous system through multiple mechanisms which include: (i) glutamate clearance and control over glutamate spillover due to operation of glutamate transporters; (ii) supply of obligatory glutamate precursor glutamine via operation of glutamate-glutamine shuttle; (iii) supply of L-serine, the indispensable precursor of positive NMDA receptors neuromodulator D-serine and (iv) through overall homoeostatic control of the synaptic cleft. Astroglial cells express an extended complement of ionotropic and metabotropic glutamate receptors, which mediate glutamatergic input to astrocytes. In particular a sub-population of astrocytes in the cortex and in the spinal cord express specific type of NMDA receptors assembled from two GluN1, one GluN2C or D and one GluN3 subunits. This composition underlies low Mg^{2+} sensitivity thus making astroglial NMDA receptors operational at resting membrane potential. These NMDA receptors generate ionic signals in astrocytes and are linked to several astroglial homoeostatic molecular cascades.

Key words: astrocyte, neurotransmitters, glutamate, NMDA receptors

Astrocytes as homoeostatic element of neural networks

Astroglia represent heterogeneous (Fig. 1) class of neural cells primarily responsible for the homeostatic control [1; 2] as well as for defence [3-6] of the central nervous system (CNS). Astrocytes are classified into several subtypes, which include protoplasmic astroglia of the grey matter, fibrous astrocytes of white matter, radial astrocytes (Bergmann glia of cerebellum, Müller glia of the retina, radial glia-like stem cells of the neurogenic niches and tanycytes of hypothalamus), specialised astrocytes such as velate astrocytes, marginal astrocytes, Gomori astrocytes, etc. as well as ependymocytes, choroid plexus cells or retinal pigment epithelial cells (Fig. 1). Despite prominent morphological and functional heterogeneity all astrocytes possess several common features: they are electrically non-excitabile, they have highly hyperpolarised (about -80 mV) resting membrane potential and they express numerous membrane transporters fundamental for homeostatic support of neural tissue. Although being unable to generate regenerative action potentials, astroglial cellular signalling relies on a complex, spatially and temporally coordinated fluctuations in the intracellular concentrations of ions (most notably Ca^{2+} , Na^{+} and Cl^{-}) ; these ionic fluctuations provide the substrate for astrocyte excitability; often referred to as “ionic” excitability of glia [7-15]. Spatially and temporally coordinated ionic fluxes triggered by neurochemical stimulation or occurring spontaneously regulate functional responses of astrocytes and tune numerous homeostatic cascades expressed in these cells. Astrocytes regulate CNS homeostasis on all levels of CNS organisation from molecular (for example astrocytes control ionostasis and neurotransmitter turnover and catabolism) and cellular (astrocytes support synaptogenesis, synaptic maturation and maintenance, while astroglia like stem cells provide for adult neurogenesis) to organ (astroglia support brain glymphatic system and participates in operation of blood-brain barrier) and systemic (some astrocytes, especially those localised in hypothalamic structures act as chemosensors for oxygen, CO_2 or Na^{+} in circulating blood). Astrocytes signal to other cells in the CNS through releasing various types of signalling or neuroactive molecules from neurotransmitters and their precursors to active forms of hormones and pro- or anti-inflammatory factors [16-18]. Astroglial contribution to neurological diseases is multi-faceted ranging from evolutionary conserved protective astrogliosis to astroglial pathological remodelling and astrodegeneration resulting in atrophy and loss of function; to a large extend astroglial responses define the progress and outcome of neuropathologies [3; 19-24].

Neurotransmitter receptors in astrocytes

The first electrophysiological studies of astroglia in the preparations of the brain and the spinal cord or in organotypic brain cultures described these cells as “unresponsive” or even “idle” because they were not able to generate action potentials [25-29]; similarly these cells were not sensitive to major neurotransmitters such as noradrenaline or serotonin. In some of these early observations, however, depolarisation of astrocytes in response to iontophoretic injections or high concentrations of γ -aminobutyric acid (GABA) or acetylcholine has been noted [28]. Somewhat later depolarisations were recorded from astrocytes in organotypic cultures of medulla oblongata, pons and spinal cord challenged with glutamate and aspartate [30] or with GABA, glycine, β -alanine and taurine [31]. These electric responses of astrocytes were interpreted either as reflecting activity of ion pumps [28] or as being

secondary, reflecting release of K^+ ions from neighbouring neurones activated by neurotransmitters [31]. This latter explanation was very much in line with K^+ buffering by astroglia that has been already discovered [32; 33], formalised and acknowledged [34; 35].

Establishment of reliable techniques for preparation and maintenance of purified cell cultures of astrocytes and oligodendrocytes [36] allowed electrophysiological studies of glial cells in isolation in the absence of possible interference from other cell types. Direct patch clamp and microelectrode recordings from cultured astrocytes devoid of neuronal contamination, demonstrated functional expression of glutamate, GABA and glycine receptors [37; 38], all of which mediated depolarisation of individual astroglial cells. These initial observations have been promptly confirmed and both whole-cell and single channel ion currents associated with activation of astroglial ionotropic receptors have been characterised biophysically and pharmacologically [39-46]. Slightly later, application of fluorescent Ca^{2+} recording techniques to cultured astrocytes revealed functional expression of multiple metabotropic G-protein coupled receptors, linked to phospholipase C, production of inositol-4,5-trisphosphate ($InsP_3$) and subsequent $InsP_3$ -induced Ca^{2+} release from the endoplasmic reticulum. Operation of this metabotropic pathway has been described and characterised in astrocytes *in vitro*, *in situ* and *in vivo*; with a particular role played by $InsP_3$ receptor type II [47-56]. All in all these experiments have unequivocally demonstrated that astrocytes potentially (i.e. *in vitro*) can express all types of receptors to neurotransmitters, neuromodulators and neurohormones present in the CNS; this expression however is tightly regulated and restricted *in vivo* [10; 14; 57]. It appeared that the modality of astroglial neurotransmitter receptors is controlled by their immediate neurochemical environment and generally matches the modality of receptors present in their neuronal neighbours. In the cerebellum, for example, receptors expressed in the Bergmann glial cells have the same modality as receptors expressed in the adjacent Purkinje neurone; and these receptors sets are congruent to the neurotransmitters released onto this cellular pair [14]. In the spinal cord, where glycine acts as the major inhibitory neurotransmitter, astrocytes express glycine receptors [58]. Dopamine receptors are expressed in astroglial cells in basal ganglia, whereas astroglial serotonin receptors are usually concentrated near serotonergic terminals.

Astrocytes and glutamatergic transmission

Synaptic transmission in the CNS is heavily regulated and supported by astrocytes; through the astroglial cradle formed by perisynaptic processes (Fig. 2A) astroglia control synaptogenesis, provide for synaptic isolation, maintenance and contribute to synaptic extinction [59; 60]. One of the fundamental functions of astroglia is control over the synaptic cleft, which is accomplished by numerous types of solute carrier (SLC) transporters that translocate neurotransmitters and ions, regulate extracellular pH, supply neurones with scavengers of reactive oxygen species, and provide for many more homeostatic supports [2].

Glutamate is the main excitatory neurotransmitter in the CNS of mammals; glutamatergic transmission is fundamental for executive function as well as for higher brain functions, such as learning, memory and cognition. Glutamatergic transmission occurs in specific synapses that contain releasable pool of synaptic vesicles endowed

with vesicular glutamate transporters VGLUT 1 - 3. These concentrative glutamate transporters driven by H^+ gradient (established by vacuolar H^+ ATPase) underlie accumulation of high (10 - 100 mM) glutamate concentration in the vesicles. Invasion of the action potential and opening of voltage-gated Ca^{2+} channels in the membrane of presynaptic terminal triggers formation of local Ca^{2+} microdomains that in turn instigate exocytosis of glutamate-containing synaptic vesicles, which elevates intracleft glutamate to millimolar levels thus activating postsynaptic glutamate receptors [61; 62].

The role of astrocytes in maintaining glutamatergic transmission is distinct and fundamental: firstly, astroglial cells remove glutamate from the synaptic cleft thus terminating the neurotransmission and preventing glutamate excitotoxicity; secondly, astroglial perisynaptic processes reach in glutamate transporters prevent glutamate spillover thus providing synaptic isolation and thirdly, astrocytes supply neurones with glutamine, the obligatory precursor for glutamate and GABA (Fig 2B and [2; 63-66]. Glutamate clearance is mediated by glutamate transporters, which are preferentially expressed in astrocytes: the latter are responsible for about 80% of glutamate removal following physiological neurotransmission. Astrocytes express two types of glutamate transporters classified as EAAT1/SLC1A6 and EAAT2/SLC1A2; both transporters utilise Na^+ gradient for translocation of glutamate [63]. Anatomically the EAAT1 are predominantly expressed in cerebellum, in the retina and in circumventricular organs [67-69]; the EAAT2 transporters demonstrate higher expression in astrocytes in the rest of the brain. At the ultrastructural level EAAT1/2 are concentrated in perisynaptic astroglial processes [70].

Translocation of a single glutamate molecule by EAAT1/2 is thermodynamically coupled with co-transport of 3 Na^+ and 1 H^+ and with counter-transport of 1 K^+ [71; 72]. Glutamate transporters are also associated with thermodynamically uncoupled Cl^- flux through an aqueous pore inbuilt into the transporter molecule [73]. The reversal potential of the transporter (E_{EAAT}) is determined by transmembrane ion gradients and by concentration of glutamate in the cytosol and in the synaptic cleft [71; 74]. The extracellular concentration of glutamate ranges between 25 nM at rest and > 1 mM at the height of transmission event [61; 75] whereas cytosolic glutamate concentration in astrocytes probably does not exceed 0.3 - 0.5 mM because of a continuous activity of glutamine synthetase [76]. Changes in the concentrations of ions and glutamate affect the E_{EAAT} in the following manner: 10 fold change in $[Na^+]_o$ shifts the equilibrium potential by 100 mV; whereas 10-fold changes in $[Glu^-]_o$, $[H^+]_o$ and $[K^+]_o$ shift E_{EAAT} by 31, 25 and -31 mV respectively [71]. In physiological context however the E_{EAAT} is primarily determined by $[Glu^-]_o$, which increases by 5 - 6 orders above resting concentration during synaptic activity; thus at rest the E_{EAAT} is ~ 9 mV, whereas at 1 mM $[Glu^-]_o$ the E_{EAAT} is ~ 145 mV. This $[Glu^-]_o$ dependence ensures that transporter never reverses unless upon terminal failure of ionic homeostasis. The transports velocity of EAAT1/2 is slow with a single cycle requiring ~ 30 ms [77].

Astroglial role in supplying neurones with glutamine is again of fundamental importance for neurotransmission, because neurones are incapable of synthesising glutamate; synthesis of glutamate from glucose occurs solely in astrocytes, which specifically express the key enzyme pyruvate carboxylase that produces an obligatory intermediate α -ketoglutarate [65; 66; 78]. The second astroglia-specific enzyme fundamental for glutamate catabolism is glutamine synthetase [79; 80], which

converts glutamate (either synthesised *de novo* or imported by transporters) into glutamine, a direct precursor of glutamate. In addition glutamine synthetase is critical for detoxifying ammonium (NH_4^+), which is converted into glutamine [81]; of note NH_4^+ is secreted in the course of physiological neurotransmission with subsequent accumulation and conversion in astroglia [82]. Glutamine is exported from astrocytes and redirected to neurones by coordinated A and N systems of sodium-coupled neutral amino acid transporters or SNATs; the N system (SNAT3/SLC38A3 and SNAT5/SLC38A5) is present in astrocytes and is tailored for glutamine efflux, whereas the A system (SNAT1/SLC38A1, SNAT2/SLC38A2 and SNAT4/SLC38A4) is expressed in neurones and is tuned for glutamine import [83; 84]. The glutamine entering neurones is deaminated by phosphate activated glutaminase to glutamate thus accomplishing the glutamine-glutamate shuttling. In addition to glutamine astrocytes supply neurones with L-serine, the obligatory precursor for endogenous NMDA receptor positive neuromodulator D-serine [85; 86].

Astroglial NMDA receptors

Functional expression of NMDA receptors in astroglia have been, for a long time, controversial; the NMDA receptors that require depolarization-dependent relief from Mg^{2+} block and are implicated in synaptic plasticity as coincidence detectors were believed to be expressed exclusively in neurones [87; 88]. These controversies have been addressed and solved in the course of recent two decades, with several lines of evidence indicating presence of fully functional NMDA receptors in astroglia.

Evidence from gene expression

The first comprehensive transcriptome database for neurones, astrocytes and oligodendrocytes was produced by the group of late Ben Barres [89] in 2008 [90]. A subpopulation of astrocytes bearing enhanced green fluorescence protein expressed under s100B promoter was isolated using fluorescence-activated cell sorting (FACS). Analysis of gene expression demonstrated that astroglial cells, from neonatal as well as young mice express NMDA receptor subunits GluN1, GluN2C, and GluN3A. In astrocytes acutely isolated from cortices of 10, 20, 30, and 50 days old transgenic mice expressing EGFP under the control of GFAP promoter (GFAP/EGFP mice) single-cell transcriptome revealed expression of GluN1, GluN2A, GluN2B, GluN2C, GluN2D, and GluN3A receptor subunits [91]. Similarly in primary cultured human astrocytes expression of all seven NMDA receptor subunits (GluN1, GluN2A, GluN2B, GluN2C, GluN2D, GluN3A and GluN3B) has been detected at mRNA and protein levels [92]. Expression of *Grin*1-3 genes have been identified in astrocytes isolated from young (2.5 months) and old (15–18 months) mouse astrocytes labelled (for FACS isolation) with fluorescent antibodies for glutamate transporter-1 [93]. In cortical astrocytes isolated based on their expression of EGFP under aldehyde dehydrogenase (*Aldh* 1 l1) promoter expression of all 7 NMDA receptor subunits was detected (GluN2C (*Grin2c*) > GluN3A (*Grin3a*) > GluN1 (*Grin1*) > GluN2B (*Grin2b*) > GluN2D (*Grin2d*) > GluN3B (*Grin3b*) > GluN2A (*Grin2a*), see [94]).

Structural evidence

Several immunohistochemical studies have detected presence of NMDA receptors in astroglia. The GluN1 and several GluN2 subunits have been identified at mRNA and

protein level in cultured or isolated astrocytes [95; 96]. In the basolateral amygdala about 20% of profiles (revealed by electron microscopy) stained with antibody against GluN1 receptor subunit has been identified as astroglial distal processes [97]. Similarly distal processes and sometimes even somata of adult rat cortical astrocytes were immunopositive for GluN1 and GluN2A/B subunits [98; 99]. Glial distal and perisynaptic processes labelled with GluN1 and GluN2A/B were found in the dorsal cochlear nucleus [100]; GluN1 immunopositive astroglial processes have been detected in the *nucleus coeruleus* [101] and in the basal nuclei of the amygdala [102]. Using recently developed fluorescent marker model (in which EGFP was linked to GluN2C) astroglial expression of GluN2C subunit has been detected in the cortex, striatum, hippocampus or amygdala but not in the thalamus and basal ganglia [103].

Functional evidence

The very first electrophysiological recordings of NMDA-induced currents have been performed on cultured and freshly isolated human Müller glial cells. These NMDA currents were sensitive to specific antagonists APV and MK-801, and were somewhat inhibited by Mg^{2+} , although the voltage-dependence of Mg^{2+} block was shifted towards hyperpolarisation, which allowed receptor activation at resting membrane potential [95]; the NMDA currents were subsequently recorded in parenchymal astrocytes [104; 105]. The NMDA-induced Ca^{2+} signals (sensitive to NMDA receptor antagonists) have been also monitored from astrocytes in primary culture [106; 107] and in acute hippocampal slices from young rats [108]. At the same time in many cells in slice preparation NMDA responses were suppressed by tetrodotoxin suggesting indirect action mediated through neuronal activity [108].

The first *in situ* electrophysiological recordings of NMDA-receptor mediated ion currents were performed on astrocytes from acutely isolated slices of spinal cord of rats [109]. In these experiments applications of NMDA (2 mM) induced ion currents in 96% of all cells studied (Fig. 3); amplitudes of these NMDA currents were, on average, about 50 pA. Currents mediated by NMDA receptors in spinal cord astrocytes were insensitive to AMPA/Kainate receptors antagonists and were not modulated by extracellular Mg^{2+} (Fig. 3). Several years later functional NMDA receptors were identified in astrocytes from cortical slices of wild type and GFAP/EGFP transgenic mice; NMDA at 100 μ M concentration triggered ion currents in 72% of all astrocytes examined [110].

The NMDA-activated currents were subsequently recorded from single astrocytes acutely isolated (by employing non-enzymatic vibro-dissection technique) from cortices of GFAP/EGFP transgenic mice [111; 112]. Challenge of isolated cortical astrocytes with 10 μ M glutamate triggered the biphasic ion current with distinct pharmacological profile: the fast current component was suppressed by AMPA receptor blocker 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), whereas the slow component was inhibited by D-2-amino-phosphonopentanoic acid (D-AP5) or dizocilpine (MK-801) (Fig. 4A,B). Application of NMDA alone triggered, in concentration-dependent manner, monophasic currents (Fig. 4C); these NMDA-induced currents were positively modulated by glycine (Fig. 4D) and were inhibited by specific antagonists D-2-amino-phosphonopentanoic acid (D-AP5) and MK-801 ([111]). The voltage-dependence of Mg^{2+} block was shifted towards hyperpolarisation; with almost 100% availability of receptors for activation at resting

membrane potential of -80 mV [111; 113]. Astroglial NMDA receptors have intermediate Ca^{2+} permeability ($P_{\text{Ca}}/P_{\text{monovalent}} \sim 3$) thus mediating, upon activation, influx of both Ca^{2+} and Na^{+} [114]. Astroglial NMDA receptors contribute to glial postsynaptic currents triggered by stimulation of neuronal inputs into cortical layer III [111]; the density of astroglial NMDA receptors in mice is age-dependent with maximal expression between 3 and 6 month of age [115].

The precise regional mapping of astrocytes expressing NMDA receptors is yet to be accomplished. In particular, the data on expression of NMDA receptors in astrocytes remain rather controversial. Electrophysiological studies of hippocampal astrocytes in vitro and in situ failed to detect NMDA-induced currents or NMDA-evoked Ca^{2+} responses [43; 116; 117]. At the same time application of NMDA-glycine mixture to hippocampal astrocytes co-cultured with neurones evoked substantial depolarisation of the former; this depolarisation was suppressed by NMDA receptor antagonist MK-801 [118]. This finding was further corroborated in experiments on genetically modified mice, in which NR1 subunit was specifically deleted from astroglial cells. Depolarisation of astrocytes in acute slices isolated from these animals in response to NMDA was significantly reduced compared to the wild type controls [118]. At the same time activation of neuronal NMDA receptors in hippocampal synapses causes significant K^{+} efflux which instigates astroglial K^{+} buffering associated with K^{+} inward currents sensitive to NMDA antagonists [119].

Subunit composition

Astroglial NMDA receptors differ from neuronal ones in their sensitivity to Mg^{2+} block and in relatively low Ca^{2+} permeability (for neuronal NMDA receptors $P_{\text{Ca}}/P_{\text{monovalent}}$ is around 10, being thus about 3 times higher [120]). These features reflect specific subunit composition. Astroglial NMDA receptors are sensitive to memantine and the GluNR2C/D subunit-selective antagonist UBP141 [113]; whereas low Mg^{2+} sensitivity at resting potential arguably indicates the presence of GluNR3 subunit [121; 122]. In conclusion, the astroglial functional NMDA receptor may therefore be a heterotetramer assembled from two GluN1, one GluN2C or D and one GluN3 subunits [123].

NMDA receptors and astroglial function

Physiology

Probably the most obvious function of NMDA receptors is to generate influx of Na^{+} and Ca^{2+} for generating ionic signals associated with various glial functions. Indeed activation of NMDA receptors either by application of NMDA or by synaptic stimulation results in Ca^{2+} signals in both processes and somata of isolated astrocytes and astrocytes in cortical slices (Fig. 5, [114]). The actual mechanisms of this Ca^{2+} signals could be complex and may reflect direct entry of Ca^{2+} through NMDA-gated channels or indirect elevation of Ca^{2+} through, for example, reversed $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger following an increase in cytosolic Na^{+} associated with Na^{+} influx through the NMDA receptor. Of note astroglial NMDA receptors can be activated not only by glutamate but also by mechanical stimulation delivered as fluid shear stress in the absence of agonist [124]. Stimulation of astroglial NMDA receptors may even contribute to heterosynaptic plasticity [118]

Astroglial NMDA receptors, however, may trigger astroglial Ca^{2+} signalling in an alternative and rather peculiar way, by direct link to Ca^{2+} release from the endoplasmic reticulum (ER) Ca^{2+} store. It appeared that NMDA triggered Ca^{2+} signals in rat cortical astrocytes co-cultured with endothelial cells. These NMDA Ca^{2+} responses were blocked by APV and ifenprodil (the GluN2B antagonist) and were almost completely suppressed by the depletion of the ER [125]. Thus, the possible metabotropic mode of operation of NMDA receptors in astroglia was suggested. This metabotropic pathway was subsequently described in cultured cortical rat astrocytes in which NMDA Ca^{2+} signals were blocked by inhibitors of the ER Ca^{2+} release Xestospognin C and ryanodine; authors also presented evidence for NMDA-dependent mitochondria depolarisation which may be associated with Ca^{2+} release [126]. The NMDA-evoked Ca^{2+} responses were found to be sensitive to the inhibition of phospholipase C with U73122 again indicating possible link to a classical InsP_3 - mediated Ca^{2+} release pathway [126].

Activation of astroglial NMDA receptors triggered homeostatic astroglial responses; in particular NMDA receptors were reported to couple to antioxidant transcriptional activation nuclear factor-erythroid 2-related factor-2 (Nrf2), which stimulates release of glutathione precursors, thus supporting neuronal glutathione synthesis and boosting antioxidant neuroprotection [127]. Astrocytic NMDA receptors were also shown to stimulate secretion of pro-inflammatory cytokines; this secretion was blocked by memantine [128].

Pathology

Pathobiological changes of astroglia (manifested in multiple forms from astroglial reactivity to degeneration with loss of function) are indispensable components of neurological diseases [3; 129; 130]. Expression of astrocytic NMDA receptors is generally up-regulated in reactive astrocytes. In hippocampus subjected to transient ischemia astrocytes increased expression of GluN2A and GluN2B subunits; this increase was maximal at 28 days after the lesion [131]. In rat healthy hippocampi the GluN2B subunits were not present in astrocytes; however transient ischemia boosted their expression, which started 3 days after ischaemic attack and reached its maximum in 28 days [132]. Similarly, *in vitro*, astroglial expression of GluN2B was induced by 5 minutes of anoxia [132]. In mouse primary cultured astrocytes exposed to a medium equilibrated with 85% N_2 and 0% O_2 and lacking glucose and serum expression of GluN1 decreased, while expression of GluN2A and GluN2B demonstrated some increase [133]; this *in vitro* model however is somewhat difficult to interpret. The up-regulation of NMDA receptors in reactive astrocytes could be a part of neuroprotective response associated for example with (already alluded to) NMDA-dependent release of glutathione precursors [127].

Activity of NMDA receptors was also found to connect with expression of another important homeostatic astroglial molecule, the inward rectifying $\text{K}_{\text{ir}}4.1$ K^+ channel critical for physiological K^+ buffering. Prolonged exposure (3 days) of rat cortical astrocytes to 1 mM glutamate or 100 μM NMDA down-regulated astroglial expression of $\text{K}_{\text{ir}}4.1$ at both mRNA and protein levels; this down-regulation was specifically prevented by NMDA receptor antagonists MK-801 or AP-5 [134]. Very similar down-regulation of astroglial $\text{K}_{\text{ir}}4.1$ channels was observed in the *in vivo* model of hepatic encephalopathy; expression of $\text{K}_{\text{ir}}4.1$ was restored by intraperitoneal

1 injections of a competitive NMDA receptor antagonist memantine [134]. In addition,
2 long-lasting activation of astroglial NMDA receptors was found to down-regulate
3 expression of glutamine synthetase and aquaporin-4 [135]. Of note, NMDA receptors
4 may also regulate expression of kynurenic acid (powerful endogenous modulator of
5 acetylcholine and glutamate receptors) in astroglia; pharmacological inhibition of
6 astroglial NMDA receptors by memantine facilitated synthesis of kynurenic acid in
7 the context of hepatic encephalopathy [136].
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9

10 **Conclusions**

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12
13 Astrocytes in different areas of the brain and of the spinal cord express specific type
14 of NMDA receptors characterised by low sensitivity to Mg^{2+} at resting potential and
15 by relatively low Ca^{2+} permeability. These biophysical idiosyncrasies arguably reflect
16 specific configuration of astroglial NMDA receptors likely assembled as
17 heterotetramers from 1 GluN1, 2 GluN2 (C/D?) and 1 GluN3 subunits. Activation of
18 NMDA receptors trigger astroglial Ca^{2+} and Na^{+} signalling by mediating influx of
19 these ions; there are also indication that NMDA receptors in astrocytes can be coupled
20 to Ca^{2+} release from the intracellular stores. Astroglial NMDA receptors may
21 contribute to neuropathology; their over-activation can affect astroglial homeostatic
22 mechanisms.
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Figure legends

Figure 1. Classification of astroglia

Figure 2. Astrocytes in glutamatergic neurotransmission.

A: The concept of astroglial cradle. Synapses in the brain and in the spinal cord are composed of several components that include the presynaptic terminal the postsynaptic part and the perisynaptic process of the astrocyte. Astroglial perisynaptic sheath enwraps synaptic structures and regulate, influence and assist synaptogenesis, synaptic maturation, synaptic maintenance and synaptic extinction. Modified from [60].

B: Astrocytes support and regulate glutamatergic neurotransmission. Astrocytes are central component of glutamate-glutamine shuttle; glutamate taken up through glutamate transporters or synthesised *de novo* from glucose is converted to glutamine; glutamine is translocated to the neurone via N and A system of SNAT transporters. After entering neuronal terminal glutamine is converted into glutamate and accumulated into synaptic vesicles. Astrocytes also provide neurones with L-serine, an obligatory precursor for NMDA receptor endogenous positive modulator D-serine.

Figure 3. NMDA receptors mediated currents in astrocytes from rat spinal cord slices.

A: Membrane currents recorded from an astrocyte in acute spinal cord slice. The membrane was clamped for 50 ms to increasing de- and hyperpolarizing potentials ranging from -160 mV to +20 mV with 10 mV increment, which revealed classical passive electrophysiological signature of an astroglial cell.

B: Representative traces demonstrating ion current evoked by application of 2 mM NMDA.

C: NMDA (2 mM) -evoked current was not blocked by CNQX (100 μ M) in contrast to kainate-evoked inward current recorded from the same cell.

D. E: Neither 5 min preincubation with Ca^{2+} -free media, nor 5 minutes preincubation with 3 mM of Mg^{2+} significantly affected NMDA-evoked inward currents.

Membrane currents (B - E) were recorded in dorsal horn of P6-7 animals at a holding potential of -70 mV.

Modified from [109]

Figure 4. NMDA receptor-mediated currents in cortical astrocytes.

A: NBQX inhibits the fast component of glutamate-induced current. Representative traces illustrate the current before, during and after application of 30 μ M NBQX (left panel), and the NBQX-sensitive current obtained by subtraction (right panel). The concentration-dependence of the block of the fast component for four cells ($\text{IC}_{50} = 2.2 \pm 0.4 \mu\text{M}$, Hill coefficient = 1.9) is shown in the inset.

B: D-AP5 inhibits the slow component of glutamate-induced current. Representative traces demonstrating the effect of 1 μ M D-AP5 (left panel) and the D-AP5-sensitive component obtained by subtraction (right panel). The concentration-dependence of the block for five cells ($IC_{50} = 0.64 \pm 0.1$ μ M, Hill coefficient = 1.6) in the inset.

C: NMDA-induced (2 s application) currents in a single astrocyte and concentration-response curve constructed from six such experiments (EC_{50} 0.34 ± 0.06 μ M, Hill coefficient = 1.5).

D: Glycine-dependent potentiation of astrocyte NMDA response. NMDA-induced currents in glycine-free normal extracellular solution are shown on the top; NMDA-induced currents in the presence of different glycine concentrations (30 nM, 1 μ M, 10 μ M and 30 μ M) are displayed below. The concentration-response curve (ΔI_{norm} represents the amplitudes of current increase normalised to the maximal increase at 30 μ M glycine) constructed from seven experiments is shown on the right (EC_{50} 1.1 ± 0.07 μ M, Hill coefficient = 1.2).

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Figure 5. NMDA-receptor mediated Ca^{2+} signalling in astrocytes *in vitro* and *in situ*.

A: NMDA receptor-mediated Ca^{2+} -signalling in cortical astrocytes isolated from GFAP/EGFP mice. Acutely isolated astrocytes were loaded with Fluo-4 via a patch pipette. Fluorescent images were recorded simultaneously with transmembrane currents evoked by application of 30 μ M NMDA. $[Ca^{2+}]_i$ transients are represented as the $\Delta F/F_0$ ratio averaged over the cell soma. Holding potential was -80 mV.

On the top the images of Fluo-4 loaded astrocyte at rest (left) and EGFP fluorescence image from the same cell taken prior to the establishment of whole-cell configuration. Bottom panel shows Fluo-4 image of the cell at the peak of the response; representative $[Ca^{2+}]_i$ transient and membrane current are shown on the right.

B: Cortical layer II astrocytes were loaded with Fura-2 *in situ* via patch pipette. Fluorescent images were recorded simultaneously with glial currents evoked by neuronal afferent stimulation (single stimuli or high-frequency (HFS) stimulation) in presence of CNQX (control) and after application of 30 μ M D-AP5. Representative images (pseudo-colour, pipette image subtracted) are shown on the left and glial synaptic currents on the right.

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Fig. 1

Figure 2

Fig. 3

Fig. 4

Fig. 5

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Supplementary material

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