Astroglial cradle in the life of the synapse

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Astroglial persynaptic sheath covers the majority of synapses in the central nervous system. This glial coverage evolved as a part of the synaptic structure in which elements directly responsible for neurotransmission (exocytotic machinery and appropriate receptors) concentrate in neuronal membranes, whereas multiple molecules imperative for homeostatic maintenance of the synapse (transporters for neurotransmitters, ions, amino acids, etc.) are shifted to glial membranes that have substantially larger surface area. The astrocytic persynaptic processes act as an ‘astroglial cradle’ essential for synaptogenesis, maturation, isolation and maintenance of synapses, representing the fundamental mechanism contributing to synaptic connectivity, synaptic plasticity and information processing in the nervous system.

\section{1. Multiple components of the central synapse}

The power of the brain lies in the intercellular connections; some tens of trillions of these connections create the neural web that is the substrate for information processing. The connectivity of neural networks is immensely plastic and it is constantly remodelled under the influence of the environment and experience; this remarkable plasticity underlies learning. Intercellular connections in the nervous system are represented by chemical and electrical synapses, with the former predominantly established between neurons and the latter between neuroglia. Chemical transmission in the brain is not confined to synapses, it also operates in a ‘volume transmission’ mode when neurotransmitters diffuse through interstitial space, finding their multiple targets between neurons and neuroglial cells.

Synaptic structures evolved over the last approximately 600 million years, after the first diffuse nervous system appeared in primitive animals such as hydras and comb jellies; this first nervous system was composed from cells of a single cell type, the neurons, which establish a neuronal net through synaptic contacts. Subsequent evolution of the nervous system progressed by the way of great diversification and specialization of cells. The first glial-like cells emerged in nematodes, they became specialized in annelids and attained a high degree of morphological and functional heterogeneity in arthropods. In basal chorbrates, the new type of glial cells, the radial glia, replaced parenchymal glial cells, which signalled an advent of layered organization of the central nervous system (CNS). Increase in the size of the CNS instigated re-emergence and diversification of astrocytes and appearance of myelin [1]. The brain and the spinal cord of mammals contain hundreds of distinct types of neurons and many types of neuroglia. Similarly, there are many types of synapses established between neuronal terminals and effector organs in the periphery or between neuronal terminals and other neurons and between neurons and some NG2 glial cells [2] in the CNS as well as in sympathetic ganglia or enteric plexuses.

Synapses in the CNS are composed of several distinct components (figure 1), which include (i) the presynaptic terminal, (ii) the postsynaptic element that can be represented, for example, by the dendritic spine, (iii) the persynaptic process of the astrocyte, (iv) the process of a neighbouring microglial cell that periodically contacts the synaptic structure and (v) the extracellular matrix (ECM), which is present in the synaptic cleft and also extends extra-synaptically. The concept of this multi-partite synaptic assembly evolved over a recent decade, when it became clear that complex multidirectional relations exist between all the

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components outlined above [3–8]. In physiology, the concept of the multi-partite synapse (mainly in its ‘tripartite’ version) is linked to the idea of fast ‘gliotransmission’, which postulates that Ca\(^{2+}\)-regulated exocytotic release of neurotransmitters from astrocytes directly contributes to synaptic transmission. The gliotransmission has recently been overviewed in detail [9], although its importance for synaptic physiology remains a controversy [7,10–13]. Debates on this matter, which have generated much emotion in the neuroglial field, are outside the scope of the current narrative, although we are favouring the notion that fast gliotransmission plays little role in physiological activity of multi-partite synapse.

Each and everyone of the synaptic structural components plays a distinct role in the life of the synapse, from its appearance and maturation to its maintenance and, if needed, to its extinction. The microglia cells appear in the CNS from the very early embryonic period [14], and, being essentially the first glial cells existing in the developing CNS, possibly contribute to early synaptogenesis; at the later developmental stages, microglial cells are fundamental for shaping synaptic connectivity through, for example, synaptic pruning/stripping [15,16]. Similarly, the ECM regulates synaptogenesis, interconnected pre- and postsynaptic protein complexes, controls receptor trafficking through integrins-mediated signalling and modulates postsynaptic excitability [5,17–20]. In this brief essay, we shall overview the contribution of astroglia to the life of a synapse in the CNS.

2. Astroglial synaptic coverage

(a) Morphology

The majority of synapses in the CNS are covered with perisynaptic astroglial sheaths that emanate from peripheral astroglial processes (known as PAPs); these latter are identified by immunoreactivity to glutamate synthetase and glutamate transporters and are devoid of glial fibrillary acidic protein (GFAP) [21,22]. In total, about 60% of synapses in the CA1 area of the hippocampus are enwrapped with membranes of protoplasmic astrocytes; furthermore, this coverage seems to vary between different synapses: astroglial processes surround approximately 90% of all large mushroom spines and perforated synapses [23], whereas only 50% of small macular synapses are enclosed with astroglia. In the cerebellum, almost all of the synapses formed by parallel fibres on the dendrites of Purkinje neuron are tightly covered by terminal processes of Bergmann glial cells; these glial structures are quite complex, sprouting from specialized appendages that protrude from the main shaft of Bergmann glial processes [24]. The perisynaptic astroglial sheaths covering synapses are exceedingly thin, the profiles of these perisynaptic structures being on average less than 200 nm (and often less than 100 nm) in diameter [22]. Importantly, astroglial PAPs demonstrate remarkable morphological plasticity, when through retracting or extending astroglial membranes the degree of synaptic coverage can be dynamically regulated [25]. It has to be noted, however, that the data on synaptic coverage by astroglial processes remain generally insufficient with only a few brain regions being investigated; the detailed cartography of astroglial synaptic profiles throughout the CNS is very much longed for.

(b) Physiology

The perisynaptic astroglial membrane represents the major part of the cell surface area, accounting for approximately 80% of the total astroglial plasmalemma, and yet it contributes only a minor fraction (approx. 4–10%) to the cellular volume, this being reflected by an extremely high surface-to-volume ratio (around 25\(^{-1}\) \(\mu\)m [26]). This in essence means that the
perisynaptic astroglial membrane sheath is limited to plasmalemma with very little cytosol. Such a morphological arrangement provides an excessive space for receptors, channels, transporters and pumps that contribute to dynamic neuronal–glial signalling and accomplish glial homeostatic function; at the same time, shifting of all homeostatic molecules to glial membranes allows maximal density of exocytotic machinery in the presynapse and neurotransmitter receptors in the postsynaptic membrane. Astrocytes possess a highly heterogeneous complement of neurotransmitter receptors (both iono- and metabotropic), expression of which varies between brain regions, being most probably regulated by the local chemical environment [27]. Astroglial ionotropic receptors are activated by neurotransmitters released in the course of synaptic transmission and create local transient microdomains of high concentration of cytosolic Ca\(^{2+}\) and Na\(^{+}\) that represent a substrate for glial excitability. The perisynaptic processes also contain numerous transporters critical for homeostatic transport of ions, neurotransmitters, glutamine, lactate, etc. (figure 2). These transporters create substantial ion fluxes; the PAPs and perisynaptic astroglial compartments are generally poor in organelles [22], and in particular they are almost completely devoid of endoplasmic reticulum [28] making transmembrane ion fluxes fundamental for local ion signalling mediated by both Ca\(^{2+}\) and Na\(^{+}\) [29,30].

3. Astrocytes cradle: fostering and maintaining synaptic connectivity

The intimate relationship between perisynaptic astroglial compartments and central synapses is synthesized in a concept of the astroglial cradle [7], which appears as a fundamental element in the life of a synapse, being involved in synaptic genesis and maturation and providing lifelong support for the synaptic function (figure 1).

(a) Synaptogenesis and synaptic maturation

The life of the synapse progresses through several stages that include (i) formation of an initial contact between the terminal and postsynaptic neuron, (ii) maturation of the synapse, (iii) its stabilization and maintenance and (iv) elimination.

Figure 2. Homeostatic molecular cascades localized in astroglial perisynaptic processes. (Online version in colour.)
Once formed, synapses survive for several hours in the early postnatal period and for days and month in the adult CNS [31]. The major wave of genesis of glutamatergic synapses in the mammalian brain occurs in the early postnatal period (postnatal weeks 1–3 in rodents); this wave of synaptogenesis immediately follows massive astrogliogenesis [32]. Astroglia contribute to synaptogenesis through secreting various factors, in particular cholesterol [33] and thrombospondins; the latter are an astroglia-derived component of ECM. Thrombospondins exert their effects through an accessory subunit of voltage-activated calcium channels identified as α2/δ1 protein (or CaCn22d1) [18]. Astrocytes may also promote synaptogenesis through oestradiol, γ-protochadernins, integrins and protein kinase C [34]. Not all central synapses require glia, and for instance inhibitory GABA-ergic contacts develop before the profuse appearance of astrocytes [35].

Similarly, factors produced and secreted by astrocytes enhance and facilitate maturation of synapses affecting both pre- and postsynaptic neuronal compartments. These astroglia-derived molecules include activity-dependent neurotrophic factor and tumour necrosis factor α which regulate the trafficking of glutamate receptors into postsynaptic membranes, whereas cholesterol may enhance neurotransmitter release from presynaptic terminals [36].

Astrocytes may also regulate the number of synapses on the given neuron by denying presynaptic terminals the contact with postsynaptic membranes and can be involved in synapse elimination. In particular, astrocytes in vitro have been found to label synaptic terminals with complement factor C1q, this tag being recognized by microglia with subsequent selective phagocytotic removal of the synaptic contact [36,37]. Other mechanisms may also exist, although the overall role and contribution of astrocytes to synaptic extinction remains controversial [38].

(b) Synaptic isolation

A fundamental function of the astroglial cradle is synaptic isolation, when perisynaptic astroglial sheath shields the synapse from the outer space, preventing both ‘spill-over’ of the transmitters from the synaptic cleft and ‘spill-in’ of the transmitters into the cleft from the extra-synaptic structures [7]. This idea of synaptic isolation or insulation has a long history, being invented by Santiago Ramon y Cajal (who credited his brother Pedro with the suggestion [39]). The astroglial cradle isolates synapses both mechanically (by enwrapping synaptic structures with perisynaptic membranes) and functionally by active neurotransmitter uptake, although of course not all the synapses are fenced by astroglial membranes and the functional consequences for these ‘open’ synapses remain to be investigated.

(c) Ion homeostasis

Ion homeostasis of the extracellular space is of paramount importance for CNS function; fluctuations in extracellular ion concentrations rapidly affect excitability of neurons that may cause numerous adverse effects. This is particularly important for K⁺ ions that travel across neuronal membranes firing action potentials and postsynaptic membranes activated by neurotransmitters.

(i) Potassium buffering

Astrocytes are central for extracellular K⁺ homeostasis in the CNS, their critical role being recognized by Leif Hertz in 1965 [40]. The astroglial K⁺ removal system is represented by several complementary molecular mechanisms that include Na⁺/K⁺ ATPase, inward rectifying K⁺/Cl⁻ co-transporter KCC1 (SLC12A4) and Na⁺/K⁺/Cl⁻ co-transporter NKCC1 (SLC12A2) [41,42]. More recent data suggest that inhibition of Na⁺/K⁺ ATPase reduced post-stimulus clearance of K⁺ transients, whereas inhibition of K⁺/Cl⁻ channels and NKCC1 had minor effects [43]. The transporters are regulated by [Na⁺], and (indirectly) by [Ca²⁺]: for example, increase in astroglial Ca²⁺ activates the forward mode of the Na⁺/Ca²⁺ exchanger that mediates substantial Na⁺ influx, which in turn stimulates Na⁺/K⁺ ATPase and alters the Na⁺ electro-driving force that maintains functional activity of relevant SLCs [44]. Potassium buffering not only removes excess K⁺ but also affects short-term plasticity for example in hippocampal synapses [44,45]. Potassium ions, accumulated by astrocytes, can subsequently dissipate through astroglial syncytium through the mechanism known as spatial K⁺ buffering [46].

(ii) Proton homeostasis and pH regulation

Astrocytes are indispensable for regulation of pH in the CNS interstitium by providing transmembrane transport for H⁺ (by sodium–proton exchanger, SLC9A1/NHE-1) and bicarbonate (through sodium–bicarbonate co-transporter SLC4A4/NBC). In addition, astrocytes contribute to extracellular H⁺ levels through the lactate transporter SLC16A1/MCT-1, which co-transport H⁺ with lactate, and through glutamate transporters, which remove H⁺ from the extracellular space together with each glutamate molecule (see [47] for details).

(iii) Other ions

Astrocytes contribute to homeostasis of other biologically relevant ions. In particular, activation of astroglial anion channels trigger Cl⁻ efflux (because of high cytosolic Cl⁻ concentration) that is activated for example in hypo-osmotic stress. Astrocytes can also accumulate Cl⁻ by NKCC1. Astrocytes participate in Zn²⁺ homeostasis [48], being endowed with ZnTs/SLC30 transporter; incidentally the latter may mediate astroglial Zn²⁺ release in hypo-osmotic conditions [49]. Finally astrocytes, may, at least in principle, contribute to restoration of extracellular Ca²⁺ in the cleft after periods of intense neuronal activity that may substantially decrease [Ca²⁺]i [50]. Lowering extracellular Ca²⁺ can trigger astroglial intracellular Ca²⁺ release [51]; and Ca²⁺ may subsequently leave the astrocyte through the Na⁺/Ca²⁺ exchanger.

(d) Neurotransmitter homeostasis

(i) Glutamate

Astroglial cells are fundamental elements of glutamatergic transmission in the CNS that control the extracellular distribution of glutamate and maintain the neuronal glutamate pool by supplying glutamine. Astrocytes regulate extracellular glutamate concentration by balancing glutamate uptake through Na⁺-dependent astroglia-specific glutamate transporters (SLC1A3/EAAT1 and SLC1A2/EAAT2) and glutamate release mainly through the cysteine–glutamate exchanger or system Xc⁻ (SLC7A11) [52,53]. These two types of transporters are differentially expressed in the astroglial membranes so that glutamate uptake dominates around the synaptic cleft, whereas cysteine–glutamate exchangers are concentrated in extra-synaptic regions. In the synaptic cleft at rest, glutamate
is kept at low nanomolar levels (that prevents desensitization of glutamate receptors) and a high density of glutamate transporters ascertains rapid clearance of glutamate upon synaptic transmission. Extra-synaptically, however, glutamate is maintained at low micromolar concentrations that provide for tonic modulation of synaptic transmission and plasticity through extra-synaptic metabotropic (and possibly NMDA) glutamate receptors [53].

Besides providing for glutamate clearance and controlling interstitial glutamate distribution, astrocytes maintain glutamatergic transmission through supplying neurons with glutamate precursor, glutamine. The latter is synthesized solely by astroglia-specific glutamine synthetase and is fed to neurons through a coordinated amino acid transporting system. Astrocytes express Na\(^+\)/H\(^+\)-dependent sodium-coupled neutral amino acid transporters SN1/SNAT3/SLC38A3 and SN2/SNAT5/SLC38A5, which mediate glutamine efflux, whereas neurons specifically express the sodium-coupled neutral amino acid transporters ATA1/SNAT1/SLC38A1 and ATA2/SNAT2/SLC38A2, which act as influx transporters mediating glutamine accumulation into the neuronal compartment [54]. Glutamate uptake, glutamate conversion by glutamine synthetase and glutamine transport together represent the glutamate–glutamine shuttle that maintains a releasable pool of neuronal glutamate and sustains glutamatergic transmission [55,56]. Release of glutamine by astrocytes is linked to synaptic activity; increase in synthetically released glutamate increases astroglial release of glutamine, this process possibly being mediated through activation of glutamate transporters and consequent increase in cytosolic Na\(^+\) concentration in perisynaptic astroglial processes [57,58].

Inhibition of this pathway affects glutamatergic transmission differentially in different brain regions: for example, in the retina neuronal responses disappear in 2 min after inhibition of glutamine synthetase, whereas in hippocampus glutamatergic activity lasts for some hours after cessation of glutamine supply [59].

(ii) GABA and glycine

Similar to glutamate, inhibitory transmission mediated by GABA and glycine is terminated through transmitter uptake. Astrocytes express Na\(^+\)-dependent GABA transporters 1 and 3 (SLC6A1/GAT1 and SLC6A11/GAT3); GABA taken up by astroglia is converted to glutamine or is metabolized by GABA-transaminase to succinic semialdehyde [52]. Astroglial GABA transporters can reverse by moderate depolarization or relatively minor (by 5–7 mM) increase in [Na\(^+\)], thus turning an astrocyte into a GABA source [60]; GABA can possibly leave astrocytes also through plasmalemmal channels [61]. Astroglial glutamine is critical for maintenance of GABAergic transmission through the glutamate–glutamine/GABA shuttle; inhibition of the latter rapidly occludes GABA-mediated inhibitory transmission [62]. Astroglia predominantly express glycine transporter GlyT1 (SLC6A9), which, in contrast to neuronal GluT2 (SLC6A5), can be reversed in response to physiological fluctuations of membrane potential and/or [Na\(^+\)] [52].

(iii) Adenosine

Astrocytes are primary regulators of adenosine levels in the CNS through the astroglial adenosine cycle; adenosine is transported to astroglia via equilibrative ENT1 (SLC29A1) transporters and is catabolized intracellularly by adenosine kinase that in the CNS is expressed exclusively in astrocytes [63]. Whether astrocytes or neurons represent the main source for extracellular adenosine (either released directly or derived from degradation of ATP) remains a matter of debate [64,65]. Nonetheless, astrocytes are most likely the main sink for adenosine because adenosine kinase activity keeps cytosolic adenosine levels low, thus allowing equilibrium transporter to generate adenosine influx. This is a vital function, and transgenic deletion of adenosine kinase is lethal [63].

(e) Water transport and synaptic cleft volume regulation

Astrocytes provide the main conduit for water movements in the CNS by the virtue of dense expression of aquaporin AQP4 (that is the main water channel in the nervous system); these channels are specifically concentrated in the perivascular and subpial endfeet and, to a lesser extent, in the perisynaptic astroglial membranes. The AQP4 channels are co-localized with K\(_v\)4.1 channels, thus coupling water and K\(^+\) movements [66,67]. While AQP4 displays a highly polarized expression in astrocytic vascular endfeet, K\(_v\)4.1 is more uniformly distributed across the cell body and perisynaptic processes. The difference in spatial distribution of the two proteins questions the coupling between water and K\(^+\) movement through AQP4 and K\(_v\)4.1. It has to be noted, however, that recent observations on AQP4\(^{-/-}\) mice found facilitated spatial K\(^+\) buffering further supporting the role for AQP4 in regulation of K\(^+\) homeostasis. The water fluxes are relevant for synaptic transmission, because synaptic activity is linked to a transient decrease of the volume of extracellular space and the synaptic cleft, this decrease being chiefly a function of astroglia. The transient shrinkage of the cleft contributes to an increase of effective concentration of neurotransmitter and further limits neurotransmitter spillover. It is postulated that the transient volume change is triggered by astroglial accumulation of glutamate, K\(^+\) and CO\(_2\) (the latter being taken up by Na\(^+\)/HCO\(_3\)\^-\(\) co-transporter SLC4A4/NBC). Accumulation of these molecules increases local osmotic pressure and stimulates AQP4-mediated water intake; removal of extracellular water leads to shrinkage of the synaptic cleft. Water accumulated onto the astroglial perisynaptic compartment is subsequently redistributed through the glial syncytium and is extruded distantly [68,69].

(f) Energy support

Neurons do not have direct access to the vasculature and depend on astrocytes for supply of energy metabolites. The many glucose transporters, primarily GLUT1, present in the astrocytic plasma membrane [70] facilitate diffusion of glucose to energy demanding synapses, which are often located at a considerable distance from the vasculature. Diffusion in the interstitial space is a relatively slow process in complex brain tissue [71]. It is therefore an open question whether simple diffusion is sufficient to ensure that synapses located at a distance of 20–50 \(\mu\)m from the vasculature [72] receive sufficient supply of glucose during periods of high neural activity. For example, in vivo imaging of NADH has shown that oxygen diffusion is inadequate to supply oxygen to distant tissue, resulting in the appearance of watershed regions that experience hypoxia during physiological activity (e.g. in response to whisker stimulation) [73]. Glucose is more than 10-fold larger than
4. Translational outlook: disruption of astroglial cradle triggers synaptopathy and contributes to the loss of synaptic connectivity

The astroglial cradle, with all its highly developed homeostatic pathways, is critical for normal synaptic transmission. Any disturbances to these molecular cascades that coordinate fluxes of ions, neurotransmitters, their precursors, amino acids, water, etc., as well as remodelling of astroglial synaptic coverage may profoundly affect synaptic strength, alter synaptic connectivity and thus contribute to neuropathology. For example, impaired astroglial K⁺ buffering is a central event in numerous brain pathologies from spreading depression and migraine to hepatic encephalopathy [82,83]. Altered glutamate uptake represents a key pathogenetic step in toxic encephalopathies (such as Wernicke encephalopathy in which severe downregulation of astroglial glutamate transporters expression results in massive neuronal death [84]) or in amyotrophic lateral sclerosis, with remarkable downregulation of EAAT1/2 identified in various animal models of the disease [85,86]. Alternatively, deregulation of glutamate homeostasis through downregulation of cystine/glutamate Xc⁻ exchange is induced by chronic nicotine or cocaine abuse, which might be responsible for the development of addiction [87]. Reduced synaptic coverage, possibly responsible for disrupted synaptic connectivity and synaptic loss, is documented in neurodegenerative diseases [88,89]. Astroglia seem to be involved in the pathogenesis of major psychiatric disorders such as schizophrenia and major depression, which are characterized by a loss of astrocytes that can impair neurotransmitter balance which in turn is regarded as a main pathogenetic event in these disorders [90,91].

Accordingly, molecules involved in astroglial homeostasis represent potential therapeutic targets, and indeed several drugs capable of enhancing functional activity of astrocytic glutamate transporters are already identified. These, for example, include beta lactam antibiotics such as ceftriaxone, which showed neuroprotective potential and positive outcomes in several animal models of neurodegenerative diseases, stroke, and addictive and psychiatric disorders [92–95].

5. Conclusion

The concept of the astroglial cradle unifies multiple astroglial functions that assist in genesis and support of the normal function of synaptic transmission. This is achieved by numerous molecules expressed in perisynaptic processes of astrocytes that provide for homeostatic control over the synaptic cleft, catalyse metabolism of neurotransmitters and ascertain synaptic isolation. These ‘homeostatic’ molecular cascades present in the astroglial membrane regulate synaptic connectivity and synaptic plasticity in wide temporal domains and may constitute a fundamental mechanism of astroglial contribution to information processing and higher brain functions. These molecular cascades are subject to pathological remodelling and represent a new class of pharmacological targets that may underlie novel therapeutic strategies for various classes of neurological diseases.

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**Glossary**
(Appropriate gene names are given in parentheses)

**Glu** glutamate

**GABA** γ-aminobutyric acid

**GLY** glycine

**ADO** adenosine

**Potassium homeostasis:**

NKA Na⁺/K⁺ ATPase or ATP-dependent Na⁺/K⁺ pump, the α2 subtype (ATP1A2) is predominantly expressed in astrocytes

K⁺,4.1 inward rectifier K⁺,4.1 channels

KCC K⁺/Cl⁻ co-transporter (SLC12A4)

**Neurotransmitter homeostasis:**

EAAT1,2 excitatory amino acid transporters 1 (SLC1A3) and 2 (SLC1A2)

GAT1,3 GABA transporters 1 (SLC6A9) and 3 (SLC6A11)

GlyT1 glycine transporter 1 (SLC6A9)

SN1,2 Na⁺/H⁺-dependent sodium-coupled neutral amino acid transporters 1 (SLC38A3) and 2 (SLC38A5)

Xc⁻ cysteine–glutamate exchanger (SLC7A11)

ENT1 equilibrative adenosine transporter 1 (SLC29A1)

**pH homeostasis:**

NHE sodium-proton exchanger 1 (SLC9A1)

NBC sodium-bicarbonate co-transporter (SLC4A4)

**Metabolic support:**

MCT-1 monocarboxylase transporter 1 (SLC16A1)

MCT-4 monocarboxylase transporter 4 (SLC16A3)

MCT-2 monocarboxylase transporter 2 (SLC16A7)

**Reactive oxygen species homeostasis:**

NAAT Na⁺-dependent ascorbic acid transporter (SLC23)