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Authors: Petrelli F, Bezzi P

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mGlu5-mediated signalling in developing astrocyte and the pathogenesis of autism spectrum disorders

Francesco Petrelli, Paola Bezzi

Department of Fundamental Neurosciences, University of Lausanne, CH-1005, Lausanne, Switzerland
Corresponding author: Bezzi, Paola (paola.bezzi@unil.ch)

Highlights

- Bidirectional glutamatergic communication between neurons and astrocytes during post-natal development are poorly understood
- mGlu5-mediated signalling may be important for developmental maturation of astrocytes
- Astrocyte maturation may be important for a proper neuronal development

Abstract

Astrocytes, the largest glial population in human and murine brains, are crucial to the regulation of synaptic connectivity. During the first three weeks of post-natal development, immature astrocytes express mGlu5 and expands several fold while undergoing a transition towards their mature phase. Although mGlu5-mediated signalling in astrocyte functions has been extensively studied in the last decades, whether this signalling is implicated in the mechanisms governing their development, as well as the effects of dysregulated astrocytic development on neurodevelopmental disorders, are still unclear. The aim of this review is to examine what is known about the mGlu5-mediated signalling in the developing astrocytes and its possible contribution to the pathophysiology of autism spectrum disorders.

Overview

Mature astrocytes are integral components of the "tripartite synapse" [1] in which perisynaptic

astrocyte processes regulate neurotransmitter homeostasis and recycling, provide basic substrates for neuronal metabolism, sequester ions, promote synaptogenesis and synaptic remodelling, and modulate synaptic activity and plasticity [2,3]. Immature astrocytes express high levels of metabotropic glutamate receptor 5 (mGlu5) [4] and with their numerous small peri-synaptic processes are in strategic position to monitor and, eventually, influence developing synaptic activity. Number of studies in the last decades established a bidirectional glutamatergic communication between neurons and astrocytes [2,5]. The interaction of synaptically released glutamate with G-protein-coupled receptors (GPCRs) in astrocytes, in particular with group I mGlu, leads to transient elevations of intracellular calcium (Ca^{2+}) levels [6,7,8] which have been linked to the release of neuroactive substances called gliotransmitters [2] that in turn can modulate diverse physiological phenomena [9,10], including synaptic transmission and plasticity [5]. Despite the putative importance of mGlu5-mediated signalling on post-natal maturation of astrocytes and the associated synapses, surprisingly little is known about this astrocytic signalling on the cellular and molecular mechanisms regulating post-natal maturation phase of astrocytes and the associated neuronal circuits.

Excellent reviews have recently explored the role of astrocyte-secreted or –expressed factors on the formation and maturation of synaptic circuits [*11,12,13,14], therefore in this review we intend to examine what is known about the glutamatergic signalling on developing astrocytes and to discuss the involvement of this signalling in the pathophysiology of autism.

mGlu5-mediated signalling in the post-natal developmental of astrocytes

Even though peak gliogenesis occurs about E17-18 [15], astrocytes are mainly generated during the post-natal period, when they expand 6-8 fold [16, 17]. A recent study has shown that the local proliferation of already differentiated astrocytes is the major source of astroglia in post-natal cortex [16]. The fact that early astrocytes continue to divide while differentiating and maturing during the second and the third post-natal week implies that they may have progenitor status and, during the first three post-natal weeks of their maturation, they undergo dramatic molecular and structural changes (Fig 1). For example, genes regulating proliferation such as *MKI67* are progressively down-regulated whereas a number of important astroglial genes, including those coding for glutamate transporter GLT1, GABA transporters (GATs), connexin 30 and 43 (Cx30 and Cx43) and the inwardly rectifying potassium channel Kir4.1, are progressively up-regulated [18,**19].

These astroglial genes represent some of most characteristic and important functions of astrocytes in the central nervous system (CNS): GATs and GLT1 are crucial for maintaining the proper termination of GABA or glutamate signalling at synapses, and Kir1.4 is critical for maintaining the K^+ gradient for glutamate uptake and buffering activity-dependent K^+ release. The induction of these genes during post-natal development suggests that astrocytes undergo developmental maturation from the first week to the second and third weeks after birth.

The genes that are developmentally regulated in immature astrocytes also include those controlling glutamatergic signalling. The expression of *GRM5* and *Homer1* genes, which respectively encode metabotropic glutamate receptor 5 (mGlu5) and the Homer1 scaffold proteins, is high in developing astrocytes during the first post-natal week but have dramatically decreased by the third [18,**19,4]. It has recently been found that Homer1 proteins modulate mGlu5 calcium (Ca^{2+}) signalling in developing astrocytes [*20]. Given the close association of astrocytes with synapses from the early post-natal phase, the expression pattern of mGlu5 Ca^{2+} signalling indicates that developing astrocytes sense neuronal activity during their post-natal maturation. This is particularly intriguing because it indicates that astrocytes can detect neuronal activity even before the major wave of synaptogenesis occurs (i.e. during second and third post-natal week), and suggests that the activity-dependent induction of astroglial genes may take place from the early stages of post-natal development. The importance of synaptically released glutamate in the post-natal maturation of astrocytes is not clear, but several papers have shown that neuronal activity has an important role in the regulation of astrocyte morphology [21], specification [22] and function [23]. For example neurons can induce the classic stellate morphology in astrocytes, resembling their appearance *in vivo* [21] and neuron-derived Notch signalling is necessary and sufficient to promote the expression of several plasma membrane transporters, including GLT1 [23]. Many of these results have been obtained in cultured cells and the cellular mechanisms by which early formed synapses signal to immature astrocytes to regulate their development *in vivo* remain, however, poorly understood.

The role of mGlu5-mediated signaling on the post-natal maturation phase of astrocytes, at the moment, has never been investigated. Interestingly, Homer1 proteins have been recently found to modulate the mGlu5 Ca^{2+} signalling in cortical astrocytes since the second post-natal week [*20]. The long form Homer 1b/c is constitutively expressed in immature astrocytes; its

immunoreactivity is visible in the soma and main processes as well as in the perivascular end feet of astrocytes, emphasizing the widespread distribution of this scaffold protein across cellular compartments. At the subcellular levels Homer1b/c has a punctate distribution and clusters with mGlu5 and endoplasmic reticulum (ER) tubules to form sub-plasmalemmal microdomains. Ca^{2+} events in astrocytes may occur both in the form of global cytoplasmic increase and of local events along astrocytic processes [24,25] and the expression of Homer1b/c in astrocytic processes facilitates both [*20]. Indeed, when Homer1b/c is replaced by the short form Homer1a, the physical link between mGlu5 and ER is lost and the drop in localized events leads to a strong reduction of global cytosolic Ca^{2+} signalling [*20]. Regulation of the global levels of Ca^{2+} by Homer1 proteins may have a crucial importance in developing cells where cytosolic Ca^{2+} signaling plays a key role in the regulation of both proliferation and differentiation [23].

The existence of localized Ca^{2+} events in astrocytic process suggests the presence of a subcellular specialization controlling the spread of cytosolic Ca^{2+} within individual astrocytic compartments. Electron microscopy studies have indeed indicated that astrocyte architecture is characterized by the prevalence of processes containing ER tubules and mitochondria [25,2]. This architecture could favour the organization of subcellular structural domains where the close interaction among ER and mitochondria may have a crucial role in the modulation of cytosolic Ca^{2+} signals or in the regulation of mitochondrial activities, as already reported in a number of cells including neurons [26]. Given that mitochondrial Ca^{2+} handling through the mitochondrial Ca^{2+} uniporter (MCU) can impact diverse aspects of cellular physiology including mitochondrial metabolism [26, 27, 28] and the expression of MCU gene is particularly high in immature astrocytes [**19], investigate the role of MCU in the modulation of astrocytic Ca^{2+} signals would be an important next step to understand the role of mGlu5-mediated Ca^{2+} signaling in the post-natal maturation of astrocytes.

The gene of fragile mental retardation 1 (*FMR1*), which encodes the fragile X mental retardation protein (FMRP) [29], is also part of group I mGlu signalling. Like mGlu5 and Homer1, *FMR1* is highly expressed in the first post-natal week but its expression decreases over the next two weeks. It has been reported that *FMR1* has many physiological functions, including the control of local protein translation [30]. Accumulating evidence suggests that it plays a central role in regulating GLT1 expression in developing astrocytes [31], thus indicating that it also plays a role in post-natal astrocyte maturation. The functional role of GLT1 transporter occurs at perisynaptic processes of astrocytes. Developing astrocytes sprout cellular processes during the first week of

postnatal development, and most processes appear filopodial (i.e. actively growing) in nature.
At this developmental time, astrocyte borders are quite ragged and long processes extend well beyond them but, during subsequent weeks, the developing processes become ramified and there is an increasing formation of fine distal processes [32], also known as peripheral astrocyte processes (PAPs) [33], which express a number of proteins including plasma membrane transporters. Although a significant number of astrocytes are generated during the first post-natal week, PAPs are not induced in until several weeks later, thus suggesting that a sort of morphological maturation also occurs during the first 3-4 postnatal weeks. As perisynaptic processes are responsive to neural activity, it is likely that synaptic activity drives the morphological development of astroglial PAPs towards newly-formed synaptic contacts, although the role of neuronal activity in the formation and modulation of PAPs in developing astrocytes has never been investigated directly. Indeed, the synapse association of astrocytic peri-synaptic processes is known to be a dynamic process that can be altered by neuronal activity [34,35,36] and, in agreement with this possibility, astrocyte coverage of synaptic contacts is altered during development, in response to injury and in various physiological conditions, such as partition, starvation, and satiety [*11,37,38]. Most of these structural changes occur over a slow timescale, and it is likely that the cue for altered astrocyte-synapse interaction is a direct sensing of alterations in neuronal activity rather than an additional signal released by neurons. Despite, these indications, the role of glutamatergic signalling in the formation and modulation of PAPs in developing astrocytes, however, has never been investigated directly. A recent study of the role of Cx30 in modulating behavioural and cognitive processes has proposed a new role for this channel in the modulation of glutamate signalling and astrocyte morphogenesis. Cx30 is one of the two main astroglial gap-junction subunits, and seems to control excitatory synaptic transmission through modulation of astroglial glutamate transport, which directly laters synaptic activity [*39]. However, unexpectedly, the role of Cx30 in modulating glutamate transport is mediated by its ability of keeping astrocytic PAPs restricted to perisynaptic regions and is independent of its channel function. Cx30 seems indeed to be very similar to that of a cell adhesion protein. Thus, by controlling the migration of processes towards the clefts of developing excitatory synapses, Cx30 regulates the efficacy of glutamate transport and, consequently, the strength of excitatory synapses.

Possible involvement of post-natal astrocytes in the pathogenesis of autism spectrum

disorders

Over the last ten years, growing evidence has emerged to suggest that astrocytes may play an important role in the pathophysiology of autism spectrum disorders (ASDs) [40]. The genes associated with ASDs are highly expressed during development [41] and many of those whose variation confers susceptibility to ASDs play a fundamental role in brain development [42]. Unfortunately, there is still a lack of detailed molecular studies of developmental events within the brain areas involved in the etiology of ASDs, and very little is known about the characteristics of astrocytes in ASDs, although recent transcriptome analyses have indicated that they contain many of the genes associated with ASDs [18,**19].

As mentioned above, developing astrocytes highly express mGlu5 signalling, a pathway that plays an important roles in normal brain development and in disorders such as Phelan-McDermid syndrome [43], fragile X syndrome [44] and some other isolated ASDs [45]. In neurons and developing astrocytes, the mGlu5 Ca^{2+} signalling generated by inositol 1,4,5-trisphosphate receptor (IP3R) is regulated by the expression of Homer1 scaffolding proteins [*20,46], whose rare variants have been associated with autism [47]. These proteins act by modulating the physical link between the plasma membrane of mGlu5 and IP3R located in the endoplasmic reticulum, thus governing the local and global cytosolic IP3R-derived Ca^{2+}. It is generally accepted that IP3R-mediated Ca^{2+} signals in astrocytes is coupled to the release of neuroactive compounds called gliotransmitters [2,3,5]. The functional role of Homer1 proteins by regulating intracellular Ca^{2+} go beyond a simply permissive effect on the detection of neuronal activity. The organization of structural microdomains could effectively transduce specific and localized signals to tailored outputs, such as the release of gliotransmitters. In fact, Homer1 proteins while extering a tight control on Ca^{2+} signaling, influence astrocytic glutamate release [*20]. The existence of a mGlu5- and Ca^{2+}-dependent glutamate secretion process in the developing astrocytes suggests the competence of astrocytes to interact with synaptic activity during assembly of synaptic circuits, as already reported [6,8]. It is therefore possible that glutamatergic gliotransmission plays an important role in the stregthening of synaptic connections and the establishment of neuronal pathways during post-natal development. Consistent with this idea, by manipulating secretion of a specific gliotransmitter, notably D-serine [48], it is possible to direct modify crucial steps of the development of new neurons beyond the stem or progenitor cell stage [49]. In particular, inhibition of vesicular release of D-serine from astrocytes in the hippocampal dentate gyrus has been reported

to reduce synapse formation and network integration of adult-born neurons, which in turn affect neuronal survival and net adult neurogenesis [49]. In addition to astrocyte-secreted gliotransmitters, immature astrocytes can control the formation and maturation of synaptic circuits by a number of secreted and contact-mediated factors [*11,12,13,14]. Despite the fact the initial data have been obtained mainly in culture models *in vitro* [50,51], the role of glial cells in the regulation of synapse formation and maturation of synapses has been replicated across species, such as in *C. elegans* [52], *Drosophila* [53], *Xenopus* [54], and human [55], thus indicating that cultured cells can be a valuable model to study the contribution of glial cells on mechanisms regulating synaptogenesis.

Homer1 proteins in neurons act synergistically with SH3 and multiple ankyrin repeat domain proteins (Shanks) in functionally linking mGlu5 and IP3R [56,57], and the *SHANK1*, *SHANK2* and *SHANK3* genes that encode Shank1, 2 and 3 post-synaptic scaffolding proteins are also expressed by astrocytes according to the recent transcriptome analysis [18,**19]. Interestingly, mutations in *SHANK* family genes have been associated with syndromic and idiopathic ASDs and other neurodevelopmental disorders [56, 57,**58], and mutations in these genes in mice often give rise to marked behavioural phenotypes resembling those found in some human neuropsychiatric disorders [59]. Pharmaceutical treatments that increase mGlu5 activity ameliorate several behavioral deficits in mouse models of ASDs [56,57,60]. Although treatments have been performed in adult animals it is likely that they will show similar beneficial effects when administered during post-natal development. According to the recent transcriptome analysis *SHANK 2* and *3* expression is particularly high in immature astrocytes [**19], thus suggesting that, like Homer1, the Shank2 and 3 coding scaffold proteins may be important in regulating post-natal astrocyte maturation. Given the role Homer1 and Shank proteins play in regulating cytosolic Ca^{2+} signalling, it is not surprising that alterations in the activity of this IP3R/Ca^{2+} signalling system contribute to the onset of ASDs [61,62]. The IP3R-mediated Ca^{2+} signalling modulated by scaffold proteins may be important also in the modulation of mitochondrial Ca^{2+} homeostasis. Mitochondrial Ca^{2+} entry of IP3R-released is mediated by a macromolecular complex composed by the pore forming subunit, the MCU, and several regulatory subunits including MICU1, MICU2 and EMRE [26,27] and is a fundamental step to support oxidative phosphorylation and ATP production necessary for cell metabolic needs during proliferation and maturation of differentiating cells [63,64,65]. A fascinanting aspect surrounding mitrochondrial Ca^{2+} entry

supporting a possible involvement of this mechanism in the regulation of post-natal maturation of astrocytes and of associated neural circuits is the observation that human patients carrying loss-of-function mutations in MICU1 or null mutation in MICU2 exhibit learning disability and a sever neurodevelopmental disorder [66,67].

Significant progress in our understanding of the role of astrocytes in ASDs has been made using mouse models of fragile X syndrome (FXS), which is caused by the transcriptional silencing of FMRP expression [68,69]. Astrocytes from *FMR1* knock out (KO) animals induce development delays in the dendrite maturation of hippocampal neurons [70,71], and many studies of the same animals have identified an abnormal increase in mGlu5 signalling [72] that mirrors the typical abnormalities observed in patients with FXS [73]. Interestingly, genetic or pharmacological inhibition of mGlu5 activation significantly reduces the phenotypes of *FMR1* KO mice [74,75], thus suggesting that abnormal mGlu5 signalling may contribute to the etiology of ASDs, but it is not clear whether mGlu5 signalling is dysregulated in developing astrocytes. However, it is likely that abnormal astrocyte maturation occurs in the absence of *FMR1*.

Recent findings have shown the down-regulation of GLT1 expression and reduced glutamate uptake in the astrocytes of *FMR1* KO mice during post-natal development [31]. As it is known that the proper expression of GLT1 is essential for normal brain development [76], it is not surprising that GLT1 KO mice experience severe seizures from the second post-natal week and show behavioural phenotypes often observed in ASDs [77].

Conclusions and perspectives

Astrocytes have recently emerged as critical regulators of neuronal development and synapse formation [*11,12,13,14] and an increasing number of studies show that astrocytes are likely contributing to the neuronal and synaptic deficits reported in ASDs. However, despite the importance of the post-natal maturation of astrocytes, very little is known about the cellular and molecular mechanisms regulating the transition towards the mature phenotype, or the reciprocal mechanisms by means of which developing synapses might signal to developing astrocytes in order to ensure proper neural circuit formation, which therefore remain crucial challenges in the field. A more in depth and unbiased analysis and description of alterations/dysfunctions in astrocytic features during post-natal maturation of neuronal circuits will be of fundamental importance not only for deepening our understanding of the mechanisms governing astrocyte-

controlled synaptogenesis, but also for developing new and unexplored therapeutic strategies for ASDs.

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**of outstanding interest

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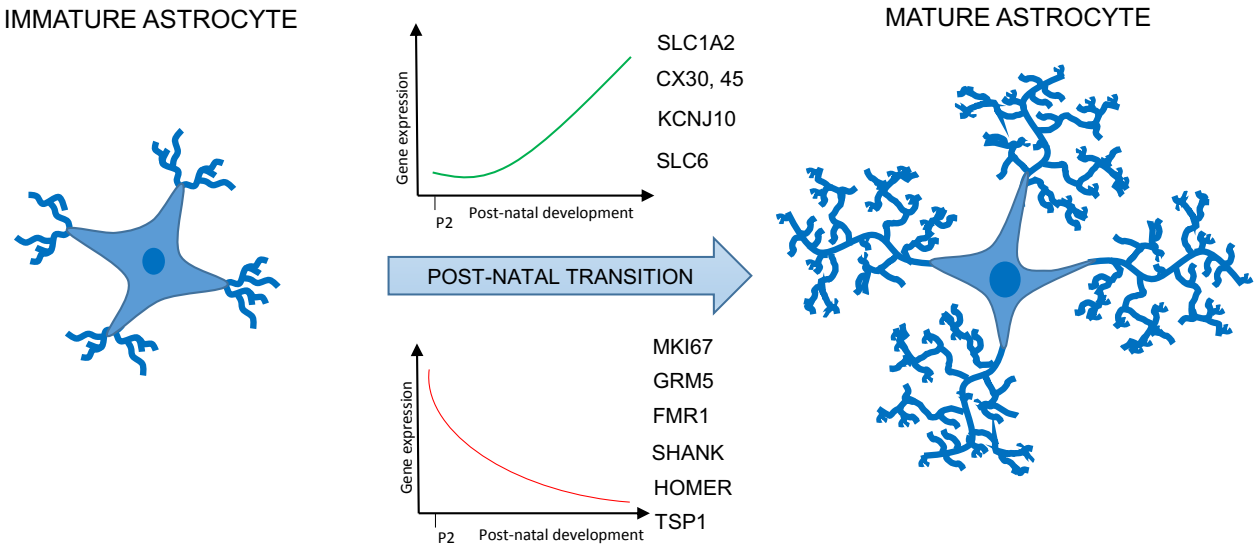

Fig 1. Astrocytes in the post-natal phase of development undergo dramatic molecular and structural changes. Genes regulating proliferation (i.e. *MKI67*) are progressively down-regulated whereas the so called "astroglial genes" (i.e. *SLC1A2*, *Cx30* and *Cx43*, *KCNJ10* and *SLC6* coding for GLT1, Cx30 and 43, Kir4.1 and GABA transporter, respectively) are progressively up-regulated. Other genes such as *GRM5*, *FRM1*, *SHANK1*,*2,3*, *HOMER1* and *TSP1* respectively coding for SHANK1,2,3, Homer1 and thrombospondin1, are also progressively down-regulated. During the first post-natal week astrocytes start of developing peripheral astrocyte processes that become progressively ramified three weeks later. Many cellular and molecular mechanisms regulating post-natal maturation phase of astrocytes are still to be established.