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Commentary

G protein-coupled receptor signalling in astrocytes in health and disease: A focus on metabotropic glutamate receptors

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ABSTRACT

Work published over the past 10–15 years has caused the neuroscience community to engage in a process of constant re-evaluation of the roles of glial cells in the mammalian central nervous system. Recent emerging evidence suggests that, in addition to carrying out various homeostatic functions within the CNS, astrocytes can also engage in a two-way dialogue with neurons. Astrocytes possess many of the receptors, and some of the ion channels, present in neurons endowing them with an ability to sense and respond to an array of neuronal signals. In addition, an expanding number of small molecules and proteins have been shown to be released by astrocytes in both health and disease. In this commentary we will highlight advances in our understanding of G protein-coupled receptor signalling in astrocytes, with a particular emphasis on metabotropic glutamate (mGlu) receptors. Discussion will focus on the major mGlu receptors expressed in astrocytes, mGlu3 and mGlu5, how these receptors can influence different aspects of astrocyte physiology, and how signalling by these G protein-coupled receptors might change under pathophysiological circumstances.

1. Introduction and background

Two major classes of cells in the CNS are neurons and glia. While glial cells substantially outnumber neurons in the brains of rodents, these cell-types are present in approximately equal number in the human brain [1]. However, the ratio of glia to neurons differs between brain structures; varying from around 17:1 in thalamus to 1:10 in cerebellum, with the ratio being approx. 3:2 in cerebral cortex [1]. For many years, glial cells were considered to fulfil a structural/connective role and to provide homeostatic support for neurons, for example, by contributing to the regulation of the ionic (and nutrient) composition of the extracellular millieu. Today, while glial cells are unlikely ever to achieve status parity with neurons, at least in the eyes of the majority of neuroscientists, their importance in an ever increasing array of CNS functions is becoming much clearer. Cells too often dismissed in the past simply as "neuron-supporting" cells are at last beginning to engage and fascinate researchers, and to reveal a breadth of importance in many aspects of CNS function [2,3] that would have been inconceivable only a few years ago.

Glial cells can be sub-divided into microglia and macroglia. Microglia are generally considered to be the immune cells of the CNS, and are responsible for protecting the brain against infection and damage by participating in inflammatory responses (see [4] for recent review). Of the different types of macroglia, astrocytes are the predominant subtype in the CNS and can be divided into two main categories, fibrous and protoplasmic, which have different morphologies and localizations in the CNS, and may carry out both common and distinct functions [5]. Protoplasmic astrocytes are found primarily in the grey matter, and are closely associated with synapses and blood vessels. These cells have irregularly shaped cell bodies from which thousands of fine membranous processes emanate allowing them to occupy a large territory (e.g. 70,000 μm^3 in the case of a protoplasmic astrocyte in rat hippocampal CA1 region; [6]). In contrast, fibrous astrocytes are found primarily in the white matter, have fewer and better-defined processes and are usually associated with neuronal axons.

2. Established and emerging roles of astrocytes in the CNS

2.1. Homeostatic functions

The location of individual astrocytes within the CNS is highly organized, with each cell occupying a distinct functional domain. Astrocytes are often coupled to neighbouring astrocytes via gap junctions, but can uncouple from the syncytial network in response to changes in the extracellular environment (e.g. acidification). Astrocytes also possess a number of transporters and enzymes enabling them to regulate both intracellular and

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extracellular pH, including Na^+/HCO_3^- and $Na^+/Cl^-/HCO_3^-$ co-transporters, the vacuolar-type H^+-ATPase and members of the monocarboxylic acid transporter family [7]. Astrocytes express carbonic anhydrase, which facilitates conversion of CO_2, released from neurons, into HCO_3^- and H^+, and which can then efflux from astrocytes via transporters for these ions [7].

Other functions of astrocyte gap junctions include communication throughout the syncytium via Ca^{2+} waves [8], transport of nutrients from the bloodstream to neurons [9], and the buffering of ions accumulating in the extracellular space following neuronal activity [10]. For example, K^+ ion concentration can increase in the extracellular space during normal neuronal excitation, and this can lead to depolarization of neurons if not appropriately buffered. Astrocytes are proposed to possess a redistributive ability; taking up K^+ ions in areas where their concentration is rising and extruding them in regions where the concentration of K^+ ions is lower [11]. Astrocytes can take up K^+ ions via a number of uptake mechanisms, including Na^+,K^+-ATPases, transporters and K^+ channels, and express Na^+/K^+ and K^+/Cl^- co-transporters and high levels of inwardly rectifying K^+ channels at their endfeet.

Another well established function of astrocytes is the removal of neurotransmitters from the extracellular space, through the expression of an array of neurotransmitter transporters. Astrocytes are the principal cell-type expressing two high-affinity, Na^+-dependent glutamate transporters, EAAT1 (GLAST; SLC1A3) and EAAT2 (GLT-1; SLC1A2) [12]. While some synaptically released glutamate can be taken up by pre- or postsynaptic transporters, or can diffuse away from the synapse, the vast majority of glutamate uptake is carried out by these astrocyte transporters [12,13]. Glutamate taken up by astrocytes is rapidly converted by glutamine synthase to glutamine, which can be cycled back to neurons, where it is re-converted to glutamate by glutaminase. Astrocytes can adapt rapidly to changes in synaptic activity, through changes in the morphology of the cell (peripheral astrocyte processes) at the synapse [13] and metabolic adaptation (e.g. altered expression of glutamine synthase). This suggests that astrocytes have the capacity dynamically to adjust key components of the glutamate-clearance/recycling machinery according to demand. Astrocytes are also implicated in the clearance of GABA and glycine from the synaptic cleft through the expression of GABA (GAT-1 and GAT-3) and glycine transporters. However, the role of astrocytes in compartmentation of inhibitory neurotransmitters is likely to be contributory rather than pre-eminent [14].

2.2. Energy and metabolism

Astrocytes provide neurons with the substrates necessary for maintenance of neurotransmission (see [15] for review). As discussed above, astrocytes are responsible for transporting and metabolizing glutamate (and other transmitters) into precursors required for the re-synthesis of these neurotransmitters within neurons. The uptake of glucose is thought to occur via glucose transporters (primarily GLUT1). Astrocytes possess enzymes required for glycogen synthesis and breakdown, and in the adult CNS glycogen stores are found primarily in astrocytes. During periods of increased energy demand, or during hypoglycaemia, astrocytes glycolytically metabolize glycogen to lactate, which is released into the extracellular space, to be taken up by neurons for oxidative metabolism. Depletion of astrocytic glycogen stores, or inhibition of monocarboxylate transporters, results in accelerated compound action potential failure recorded from rat optic nerve following periods of high neuronal activity, even in the presence of normal levels of glucose. Using fluorescent glucose analogues, Rouach et al. [16] have shown that gap junctions can allow passage of glucose across astrocytic networks. In addition, glutamate released by spontaneous or evoked neuronal activity can stimulate AMPA receptor-dependent increases in the intercellular diffusion of glucose through connexon-dependent networks of hippocampal astrocytes [16]. The importance of metabolic cross-talk between astrocytes and neurons has been highlighted further by studies in EAAT1 knockout mice, showing that glucose utilization, lactate release and glutamate uptake by astrocytes in response to glutamate were reduced compared to wild-type mice. Recently, glial glycogenolysis and lactate export has been proposed to play a crucial role in long term memory formation [17] providing a complementary mechanism of astrocyte–neuronal interdependency alongside a D-serine release mechanism hypothesized to be crucial in establishing memory [18].

2.3. Formation and maintenance of functional synapses

Astrocytes play an important role in the formation of functional synapses, a process known as synaptogenesis (see [19] for review). During development, astrocytes have been shown to act as boundaries to guide neurites to their targets. When retinal ganglion cells are purified in the absence of glial cells, their synaptic activity is severely impaired, however, when co-cultured with astrocytes, but not with other cell-types, such as oligodendrocytes, their synaptic activity is significantly enhanced [20]. A study by Ullian et al. [21] demonstrated that astrocytes increase the number of mature and functional synapses between retinal ganglion cells: not only were astrocytes important for increasing the number of synapses, but they also played a role in augmenting their function in vitro. These and other studies highlight the importance of astrocytes and astrocyte-derived signals, in the development and function of synapses in the CNS.

Astrocytes are also implicated in the removal ('pruning') of synaptic connections, a process vital for controlling and refining the connectivity of mature neuronal circuits [22]. Elimination of unwanted synapses is mediated by the classical complement cascade, and complement component 1q (C1q) is a protein that initiates this pathway. In the mature CNS, little C1q protein is expressed, but postnatal neurons express C1q in response to immature astrocytes. Astrocytes release signals which effectively 'tag' synapses to be pruned with C1q, leading to the accumulation of complement component C3, which in turn leads to the recognition of synaptic terminals by microglia and subsequent phagocytosis [20]. In addition, astrocytes may also influence synaptic function by the release of growth factors. For example, on chronic blockade by tetrodotoxin, or by application of tumour necrosis factor-α (TNFα), the expression of AMPA receptor in hippocampal neurons is increased. TNFα is released from glial cells on chronic activity blockade to increase AMPA receptor expression and thereby increase synaptic strength [23].

Therefore, not only are astrocytes involved in the formation of synapses during development, the maintenance and pruning of synapses to form mature neural circuits, but glial cells are also implicated in the moment-to-moment regulation of synaptic function in response to increasing or decreasing neuronal activity.

2.4. Bi-directional communication with neurons

Considerable evidence now supports the concept that astrocytes can communicate with neurons in a bidirectional manner, giving rise to the term 'tripartite synapse', where a synapse possesses not only pre- and postsynaptic neuronal elements, but also an astrocytic component [24]. Release of neurotransmitters, such as glutamate and ATP, from presynaptic terminals, has been shown to lead to activation of GPCRs and ligand-gated ion channels in astrocytes. For example, glutamate stimulation of cultured hippocampal astrocytes has been shown to lead to Ca^{2+} oscillations, and these Ca^{2+} responses can propagate intercellularly [8]. In

addition, Ca^{2+} increases in astrocytes can lead to subsequent Ca^{2+} increases in neuronal cells [25,26].

Initial reports of glutamate release from astrocytes, following intracellular Ca^{2+} elevation, prompted further investigations into the release of a range of 'gliotransmitters' (e.g. glutamate, ATP, D-serine). The Ca^{2+}-dependent release of glutamate from astrocytes and consequent alterations in neuronal excitability has been reported by a number of groups. For example, stimulation of Schaffer collaterals induces not only a fast, excitatory postsynaptic current in CA1 hippocampal neurons, but also a delayed, slower inward current, thought to be mediated by glutamate release from astrocytes acting at extra-synaptic NMDA receptors [27]. Glutamate release from astrocytes not only can cause increases in neuronal excitability, but also can influence presynaptic neurotransmitter release: for example, release of glutamate from astrocytes following $P2Y_1$ purinoceptor activation, leads to an activation of presynaptic NMDA receptors promoting neurotransmitter release [28]. Glutamate release from astrocytes not only promotes neurotransmitter release in nearby neurons, but can also inhibit neurotransmitter release, leading to heterosynaptic depression [29].

Elevations in intracellular Ca^{2+} in astrocytes can also lead to release of D-serine, a co-agonist at the NMDA receptor and ATP. Release of D-serine from astrocytes is dependent on intracellular Ca^{2+} elevation and is attenuated by inhibiting components associated with the exocytotic machinery [30]. Similarly, an intimate link between astrocyte Ca^{2+} and ATP release has been long established [31]. Some studies provide support for the idea that astrocytic ATP is released via a vesicular mechanism: thus, Pangrsic et al. [32] found that glutamate stimulation of astrocytes leads to an increase in the fusion of ATP-containing vesicles with the membrane, while a recent study has implicated gliotransmitter release from both small vesicle and lysosomal organelles [33]. However, there is also evidence that ATP release may occur independently of a vesicle-dependent mechanism, but may instead involve the movement of ATP through pores formed by transmembrane protein complexes, including connexins and/or pannexins.

The presently available literature indicates that physiological and pathophysiological changes in neuronal activity can increase astrocytic Ca^{2+}, which can in turn promote 'gliotransmission' to evoke responses in adjacent neurons. While this demonstrates the existence of bidirectional communication between astrocytes and neurons, much of the mechanistic detail remains speculative and controversial, not least the processes regulating gliotransmitter release from astrocytes [34].

2.5. Regulation of cerebral blood flow

When neurons in the CNS are highly active, regional blood flow adjusts to match the increased energy demand; a process called functional hyperaemia. A number of studies have implicated astrocytes in this process (see [35] for review). Astrocytic processes form endfeet contiguous with blood vessels, with astrocytic endfeet ensheathing more than 99% of the cerebrovascular surface. Astrocytes are therefore able to form a conduit between neurons and blood vessels, enabling them to play a role in the tight coupling between neuronal activity and cerebral blood flow. As discussed earlier, astrocytes are capable of releasing gliotransmitters, and these include a number of vasoactive compounds, such as ATP, adenosine, nitric oxide, prostaglandins, and epoxyeicosatrienoates (EETs). Various groups have shown that $[Ca^{2+}]_i$ increases within astrocyte endfeet lead to increases in Ca^{2+}-dependent phospholipase A_2 activity, and the release of arachidonic acid. This diffusible metabolite can readily be transformed into an array of vasoconstrictors (e.g. 20-hydroxyeicosatetraenoic acid) within smooth muscle cells, or vasodilators (e.g. epoxyeicosatrienoic acids, prostaglandin E_2) within astrocytes [36]. Therefore, among many other functions, neuron-to-astrocyte communication plays a fundamental role in functional hyperaemia, and individual astrocyte endfeet can mediate contrasting actions on arterial smooth muscle tone.

3. Metabotropic glutamate (mGlu) receptors

As well as fulfilling a primary role in the clearance of glutamate from synapses, astrocytes also possess receptors that allow this excitatory neurotransmitter to influence astrocyte physiology and pathophysiology. In addition, astrocytes not only accumulate glutamate, but may also release it in a process referred to as gliotransmission (see above), although this remains a controversial and much debated area of research [34,37]. A range of glutamate receptors, both ionotropic and metabotropic, have been reported to be expressed by astrocytes. The ionotropic glutamate receptor complement, which includes isoforms of AMPA/kainate and NMDA receptors, has been reviewed recently by others (e.g. [38] for review) and will not be discussed in detail here.

Glutamate-stimulated increases in phosphoinositide turnover in astrocytes were first observed 25 years ago [39], with evidence for expression of $G_{i/o}$-linked metabotropic glutamate receptors being reported later [40]. Subsequent work has indicated a diversity of mGlu receptor subtypes (Table 1), some of which are expressed by astrocytes (Table 2), with the mGlu5 and mGlu3 receptor subtypes generally being reported to predominate. Group I (mGlu5) and II (mGlu3) mGlu receptor activation can independently or cooperatively regulate a number of aspects of astrocyte function, including glutamate transporter activity and gliotransmission to effect astrocyte–arteriolar and astrocyte–neuronal communication (see below).

3.1. mGlu5 receptor signalling in astrocytes

Group I mGlu receptors primarily couple to $G\alpha_{q/11}$ proteins to stimulate phospholipase Cβ activity, hydrolyse inositol phospholipids and generate diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3), which respectively activate DAG-dependent

Table 1
Classification and localization of mGlu receptor subtypes in the CNS.

	Receptor subtype	Splice-variation	Preferential signalling pathway	Location	Knock-out mouse
Group I	mGlu1	a, b, c, d	PLC	Postsynaptic (some presynaptic)	Yes [116]
	mGlu5	a, b	PLC	Postsynaptic (some presynaptic) astrocytes	Yes [117]
Group II	mGlu2	–	AC (inhibitory)	Presynaptic	Yes [118]
	mGlu3	–	AC (inhibitory)	Presynaptic astrocytes	Yes [119]
Group III	mGlu4	a, b	AC (inhibitory)	Presynaptic	Yes [120]
	mGlu6	–	($G_{i/o}$-coupling)	Retinal ON-bipolar cells	Yes [121]
	mGlu7	a, b	AC (inhibitory)	Presynaptic	Yes [122]
	mGlu8	a, b, c	AC (inhibitory)	Presynaptic	Yes [123]

Table 2
Regional heterogeneity of mGlu receptor-subtype expression in astrocytes.

mGlu receptor subtype	Expression in glial cells
mGlu1	Weak mRNA detection in the thalamus [50] Expression is detected in spinal cord astrocytes [124,125]
mGlu2	Not expressed in astrocytes
mGlu3	Strong detection of mRNA in cultured astrocytes from cortex, thalamus, tegmentum, hippocampus and striatum. Weak signal for mRNA expression in cerebellum [50,126]
mGlu4	Weak signal for mRNA expression in the tegmentum and striatum [50]
mGlu5	Strong detection of mRNA in cultured astrocytes from cortex, thalamus, tegmentum, hippocampus and striatum. Weak signal for mRNA expression in cerebellum [50,51]
mGlu6	Weak signal for mRNA expression in the tegmentum and striatum [50]
mGlu7	Weak mRNA detection in the cerebellum and tegmentum [50]
mGlu8	Weak mRNA detection in the cerebellum and tegmentum [50]

PKC isoenzymes and release Ca^{2+} from intracellular stores [41–43]. Despite this common signal transduction mechanism, mGlu1 and mGlu5 receptors can, nevertheless, elicit very different single-cell Ca^{2+} responses, with stimulation of the mGlu1 receptor generally causing peak-plateau-type changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), whereas stimulation of mGlu5 receptors often leads to robust oscillatory changes in $[Ca^{2+}]_i$ [44–46]. Importantly, mGlu5 receptor-stimulated Ca^{2+} oscillations have been shown to occur in parallel with, and as a consequence of, synchronous changes in IP_3 concentration [47]. A mechanism, termed "dynamic uncoupling" (Fig. 1), has been proposed that involves the rapid switching on and off of receptor-G protein coupling through PKC-dependent phosphorylation (uncoupling receptor from G protein) and protein phosphatase-dependent dephosphorylation (re-coupling receptor to G protein) [44,48]. Elegant work by [44] comparing mGlu1 and mGlu5 receptor coupling to Ca^{2+} responses demonstrated that a threonine residue at position 840 (Thr-840), within the membrane proximal *C*-terminal tail of the mGlu5 receptor was a potential site for rapid, reversible, PKC-dependent phosphorylation. However, more recently [46] have provided evidence for PKC-dependent phosphorylation of an adjacent serine residue (Ser-839) rather than Thr-840, with the latter residue now being considered to have a permissive role in PKC-dependent Ser-839 phosphorylation. Thus, mutation of Thr-840 to alanine in the mGlu5 receptor does not affect Ca^{2+} oscillations evoked by receptor stimulation; in contrast, when Ser-839 is mutated, Ca^{2+} oscillations are abolished, consistent with the conclusion that Ser-839 is the crucial residue for phosphorylation and receptor-G protein uncoupling [46].

In astrocytes, mGlu5 is the predominant or exclusive group I mGlu receptor subtype expressed [49,50] and, in culture, astrocyte mGlu5 receptor expression can be increased by growth factor supplementation [51,52]. Activation of astrocyte mGlu receptors causes Ca^{2+} oscillations [45,53–55] most likely via the dynamic uncoupling mechanism (see Fig. 1) previously established in mGlu5 receptor-expressing recombinant cell systems [44,48,55,56]. The relationship between receptor activation and Ca^{2+} oscillatory behaviour has also been explored in both astrocytes and recombinant cell systems [48,55–57]. A striking feature, first observed in an inducible mGlu5 receptor expression

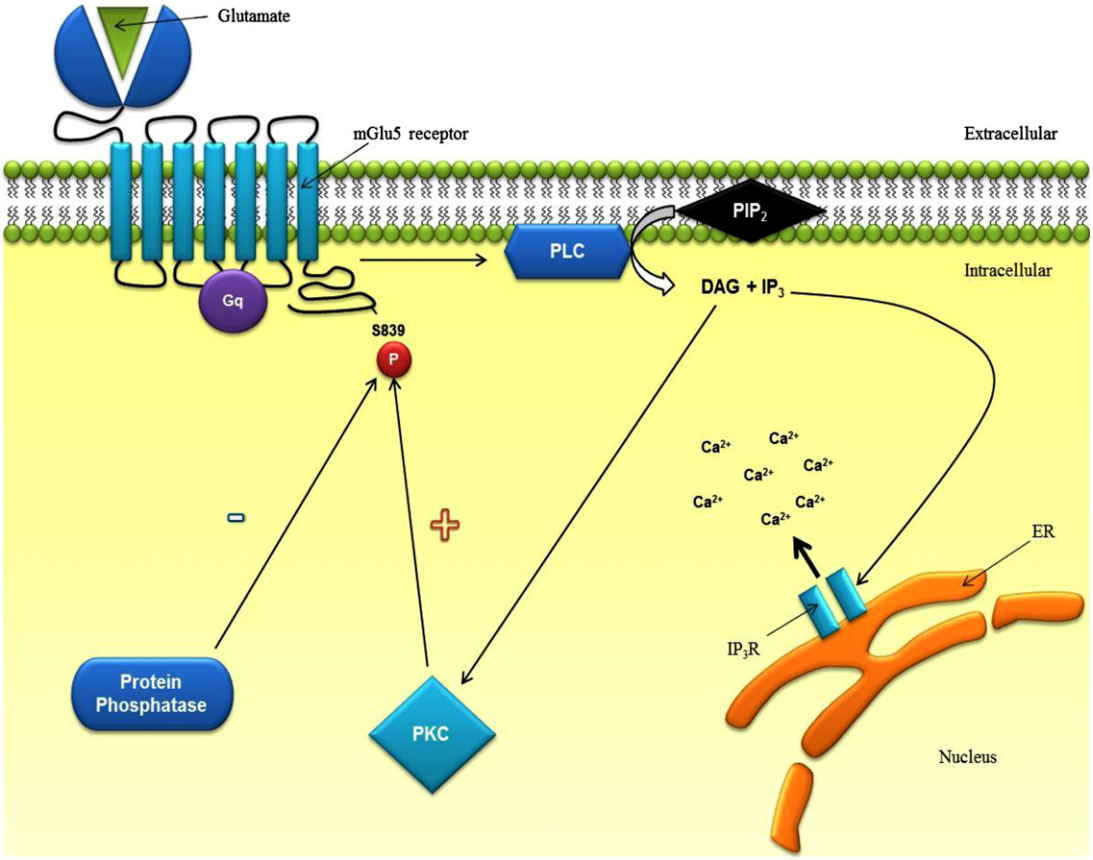

Fig. 1. 'Dynamic uncoupling' at the mGlu5 receptor. Activation of PKC(s) leads to phosphorylation of the mGlu5 receptor at Ser-839, leading to uncoupling of the receptor from associated G protein. Protein phosphatase(s) are proposed to restore G protein coupling, by dephosphorylating the receptor at Ser-839. Repeated cycles of phosphorylation and dephosphorylation give rise to Ca^{2+} oscillations.

system (CHO-*lac*-mGlu5a), was that once a sufficient glutamate concentration is present to evoke a Ca^{2+} response, neither the amplitude nor frequency of the Ca^{2+} oscillation changes significantly even if glutamate concentration is increased over a wide range (Fig. 2; and see [48]). In contrast, altering the level of mGlu5 receptor expression has a profound effect on the Ca^{2+} oscillatory frequency, with increasing mGlu5 receptor expression leading to higher Ca^{2+} oscillatory frequencies in both recombinant and native systems (Fig. 3; and see [48]). Although Ca^{2+} oscillation frequency in astrocytes is affected to some extent by changes in glutamate concentration, with high concentrations of glutamate often causing a transition from a Ca^{2+} oscillatory to a peak-plateau response, invariant Ca^{2+}-oscillatory amplitude and frequency can again be observed over a substantial glutamate concentration range [55] suggesting that an individual astrocyte has a defined Ca^{2+} oscillatory frequency that is, at least in part, determined by the level of mGlu5 receptor expression. Recently, a minimal simulation model has been reported that can recapitulate the key experimental observations and allows predictions to be made and experimentally tested regarding this dynamic uncoupling mechanism (see [58]).

3.2. Orthosteric and allosteric modulation of mGlu5 receptors

The activity of mGlu5 receptors can be manipulated pharmacologically by agents that act to mimic or block the actions of glutamate (orthosteric agonists and antagonists), or by agents that bind to other (allosteric) sites on the receptor to modulate orthosteric ligand–receptor interactions (Table 3). While it has proved difficult to generate orthosteric ligands that are selective between mGlu1 and mGlu5 receptors, some more recently discovered allosteric ligands show degrees of selectivity that make them essentially receptor subtype-specific [59].

When observed at the single-cell level, mGlu5 receptor-stimulated Ca^{2+} oscillation frequency in astrocytes can be concentration-dependently increased (by positive allosteric modulators (PAMs); Fig. 2) or decreased (by negative allosteric modulators (NAM)). This recapitulates pharmacologically the effect of increasing or decreasing receptor expression and offers the pharmacological potential to use PAMS and NAMs to not only modulate, but also to 'retune' mGlu5 receptor-evoked Ca^{2+} oscillation frequency in astrocytes [55,60]. This latter property may be of particular importance as pharmacologically resetting the frequency of astrocyte Ca^{2+} oscillations may "re-programme" the interpretation of initial glutamate-mGlu5 receptor binding event, for example through the activation/repression of a subtly different set of transcription factors [61–63].

3.3. Astrocytic targets of mGlu5 receptor activation

Astrocytes have the capacity to clear glutamate rapidly from the synaptic cleft through expression of high-affinity EAAT1/2 glutamate transporters. In addition, expression of mGlu receptors in astrocytes means that glutamate has the capacity to modulate synaptic activity by altering transporter activity (in the shorter term), or expression (in the longer term). Following acute stimulation of mGlu5 receptors in growth-factor treated astrocytes the activity of EAAT2 is enhanced showing that through this receptor glutamate can modulate its own clearance during periods of synaptic activity [64]. Thus, the mGlu5 receptor can act as a glial sensor acutely altering glutamate clearance rates. In contrast, chronic stimulation of mGlu5 receptors has been reported to result

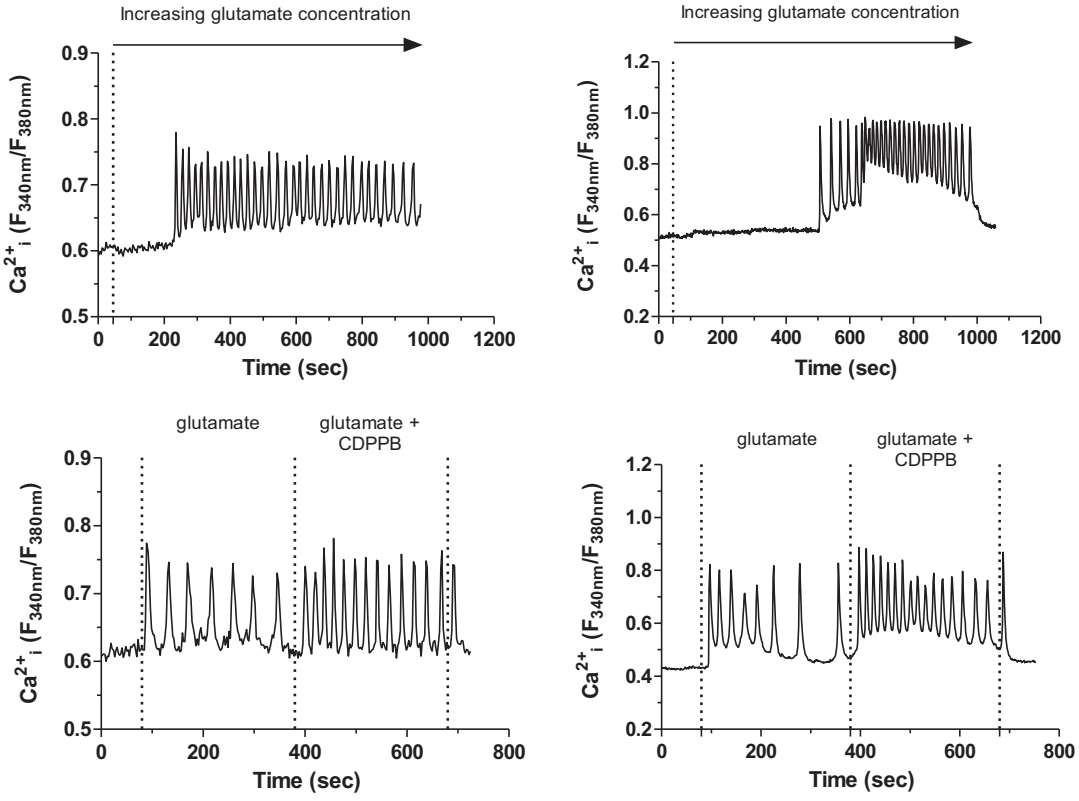

Fig. 2. Type 5 mGlu receptor-mediated single-cell calcium signalling. Effects of increasing glutamate concentration on single-cell Ca^{2+} oscillation frequency in CHO-*lac*-mGlu5a cells (A) or astrocytes (B). Glutamate concentration (0.3–300 μM) was incrementally increased every 150 s. Co-application of glutamate with a positive allosteric modulator (PAM: CDPPB, 10 μM) causes a significant increase in Ca^{2+} oscillation frequency, compared to that elicited by glutamate alone, in CHO-*lac*-mGlu5a cells (C) and astrocytes (D).

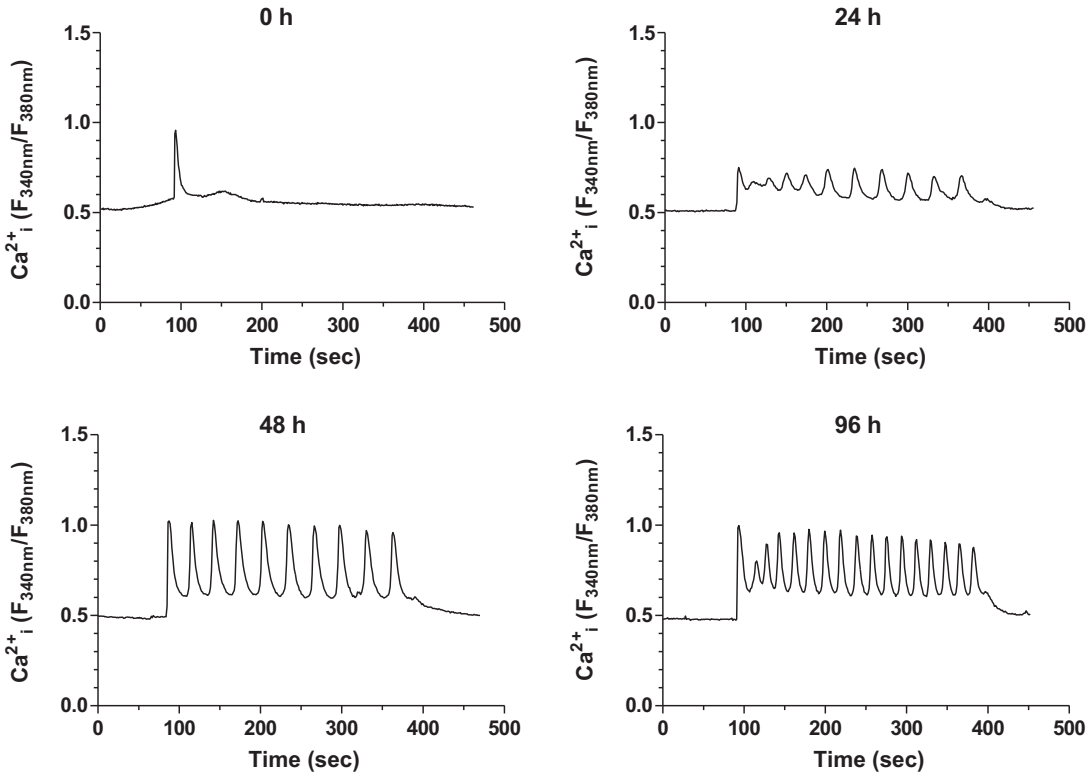

Fig. 3. Effects of growth factor treatment on mGlu5 receptor-mediated single-cell Ca^{2+} responses in astrocytes. Astrocytes were grown for approximately 8 days in vitro prior to the addition of G5 supplement for the indicated time-period. Changes in intracellular Ca^{2+} release following stimulation with a maximally effective concentration of glutamate (300 μM) are shown. Expression of mGlu5 receptor increases with time following G5 supplement treatment, leading the observed increases in glutamate-stimulated Ca^{2+} oscillation frequency.

in a down-regulation of both EAAT1 and EAAT2 levels in astrocytes [65].

Increasing evidence suggests that astrocyte mGlu5 receptors may also modulate synaptic activity by participating in bidirectional communication with neurons. Pasti et al. [53] showed that Ca^{2+} oscillations in astrocytes, triggered by neuronal stimulation, or by exogenous application of an mGlu receptor agonist, result in repetitive Ca^{2+} responses in neighbouring neurons, potentially due to Ca^{2+}-dependent release of glutamate (or other gliotransmitters) from astrocytes. The frequency of mGlu receptor-mediated Ca^{2+} oscillations in astrocytes appears to be dictated by the level of synaptic activity, suggesting that information may be transmitted between neurons and astrocytes in the frequency of oscillations [53]. In addition, activation of astrocyte mGlu5 receptors within the nucleus accumbens lead to Ca^{2+} oscillations and the appearance of slow, inward NMDA receptor-dependent currents in medium spiny neurons, potentially mediated by glutamate released from astrocytes [66]. Stimulation of astrocyte mGlu5

Table 3
Examples of orthosteric and allosteric ligands acting at mGlu5 and mGlu3 receptors.

	Orthosteric agonist	Competitive antagonist	PAM	NAM
mGlu5	(*S*)-DHPG (group I) [127] CHPG [128]	ACDPP [129]	DFB [130] CPPHA (group I) [131] CDPPB [132] ADX47273 [133] VU0092273 [134]	MPEP [135] Fenobam [136] M-5MPEP [137] CTEP [138] AFQ056 [139]
mGlu3	DCG-IV (group II) [140] NAAG [141] LY379268 (group II) [142] LY404039 (group II) [143]	LY341495 (group II) [144] MGS0039 [145]	LY487379 (group II) [146]	MNI-137 [147] RO4988546; RO5488608 [148]

(*S*)-DHPG, (*S*)-3,5-dihydroxyphenylglycine; CHPG, (*RS*)-2-chloro-5-hydroxyphenylglycine; ACDPP, 3-amino-6-chloro-5-dimethylamino-*N*-2-pyridinylpyrazinecarboxamide hydrochloride; DFB, [(3-fluorophenyl)methylene]hydrazone-3-fluorobenzaldehyde; CPPHA, *N*-[4-chloro-2-(phthalimidomethyl)phenyl]salicylamide *N*-[4-chloro-2-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]phenyl}-2-hydroxybenzamide; CDPPB, 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide; ADX47273, [*S*-(4-fluoro-phenyl)-{3-[3-(4-fluoro-phenyl)-[1,2,4]-oxadiazol-5-yl]-piperidin-1-yl}-methanone]; VU0092273, (4-hydroxypiperidin-1-yl)(4-phenylethynyl)phenyl)methanone; MPEP, 2-methyl-6-(phenylethynyl)pyridine hydrochloride; Fenobam; 1-(3-chlorophenyl)-3-(3-methyl-5-oxo-4H-imidazol-2-yl)urea; M-5MPEP, 2-(2-(3-methoxyphenyl)ethynyl)-5-methylpyridine; CTEP, 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1*H*-imidazol-4-yl)ethynyl)pyridine; AFQ056 (Mavoglurant), methyl (3a*R*,4*S*,7a*R*)-4-hydroxy-4-[(3-methylphenyl)ethynyl]octahydro-1*H*-indole-1-carboxylate; DCG-IV, (*1R,2R*)-3-[(*1S*)-1-amino-2-hydroxy-2-oxoethyl]cyclopropane-1,2-dicarboxylic acid; NAAG, *N*-acetyl-L-aspartylglutamate; LY379268, (*1S,2R,5R,6R*)-2-amino-4-oxabicyclo[3.1.0]hexane-2,6-dicarboxylic acid; LY404039, (−)-(*1R,4S,5S,6S*)-4-amino-2-sulphonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid; LY341495, 2-[(*1S,2S*)-2-carboxycyclopropyl]-3-(9*H*-xanthen-9-yl)-D-alanine, MGS0039, (*1R,2R,3R,5R,6R*)-2-amino-3-(3,4-dichlorobenzyloxy)-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid; LY487379, *N*-(4-(2-methoxyphenoxy)phenyl)-*N*-(2,2,2-trifluoroethylsulphonyl)pyrid-3-ylmethylamine; MNI-137, *N*-(4-(2-methoxyphenoxy)phenyl)-*N*-(2,2,2-trifluoroethylsulphonyl)pyrid-3-ylmethylamine; RO4988546, 5-[7-trifluoromethyl-5-(4-trifluoromethyl-phenyl)-pyrazolo[1,5-a]pyrimidin-3-ylethynyl]-pyridine-3-sulphonic acid; RO5488608, 3′-(8-methyl-4-oxo-7-trifluoromethyl-4,5-dihydro-3*H*-benzo[*b*][1,4]diazepin-2-yl)-biphenyl-3-sulphonic acid.

receptors may also lead to release of other gliotransmitters; for example, mGlu receptor-mediated Ca^{2+} increases in astrocytes during periods of high neuronal activity have been shown to release ATP, which acts on synaptic purinoceptors to increase the strength (“scaling”) of all synapses of the neuron [67].

Astrocytic mGlu5 receptors may also be involved in the regulation of cerebral blood flow. Zonta et al. [68] have shown that stimulation of neuronal afferents in freshly prepared cortical slices causes dilation of cerebral arterioles, and this process is dependent on mGlu receptor-mediated Ca^{2+} oscillations within perivascular endfeet. Inhibition of glutamate-stimulated increases in astrocyte $[Ca^{2+}]_i$ by application of an mGlu receptor antagonist impairs arterial vasodilation [68]. Increases in astrocyte $[Ca^{2+}]_i$ mediated by mGlu receptor activity can also lead to the opening of Ca^{2+}-activated maxi-K^+ channels in astrocyte endfeet, leading to the release of K^+ onto arterial smooth muscle cells, contributing to vasodilation [69].

3.4. mGlu3 receptor signalling in astrocytes

The group II metabotropic glutamate receptor, mGlu3, is widely expressed in the CNS, in both neurons and glia [70]. In common with astrocyte mGlu5 receptor expression, mGlu3 receptor expression in rodent and human astrocytes in vitro can be up-regulated by growth factors [65] and by proinflammatory cytokines, which, in contrast, down-regulate mGlu5 receptor expression [71]. Thus, the relative expression of the different mGlu receptor subtypes in glial cells appears to be plastic and dependent on the trophic support (and other factors, such as inflammation) within the microenvironment.

Compared to studies of the mGlu5 receptor, the signalling pathways modulated by astrocytic mGlu3 receptors has so far received less attention. Early studies in astrocytes indicated that mGlu receptors can couple negatively to adenylate cyclase via pertussis toxin-sensitive G proteins [40,70]. Subsequent studies showed that addition of agonists selective for group II mGlu receptors (such as 2*R*,4*R*-APDC and LY379268) inhibit forskolin-stimulated cyclic AMP accumulation in cultured astrocytes under Ca^{2+}-free conditions, but the same agonists increase this response in the presence of physiological Ca^{2+} concentrations [72]. A signalling mechanism consistent with these findings was proposed involving phospholipase C-dependent adenosine release and autocrine activation of G_s-coupled A_{2A} adenosine receptors [72]. Previously, a similar positive modulation of β-adrenoceptor-stimulated adenylate cyclase activity by co-activation of group II mGlu receptors had been shown in astrocytes [73]. Therefore, the effect of mGlu3 receptor activation on adenylate cyclase activity in astrocytes may depend on other coincident receptor-mediated inputs. In addition, the potential to stimulate the release of adenosine may not have only an autocrine action [74], but may also act on neuronal adenosine receptors to fine-tune synaptic transmission (see for example, [75]). Astrocyte mGlu3 receptors can also link to a variety of other signal transduction mechanisms, including phosphoinositide 3-kinase and ERK1/2 [74], and Akt/PKB and NF-κB [76]. mGlu3 receptor activation can also alter cyclic GMP turnover, perhaps via distinct signalling pathways in astrocytes compared to neurons [77].

Subtype-selective mGlu receptor activation can occur in vivo through the action of *N*-acetyl-L-aspartylglutamate (NAAG), a dipeptide abundant in the CNS [78]. Although NAAG has been reported to have a number of different actions, perhaps its clearest activity is as an mGlu3 receptor-selective agonist. While some uncertainties remain regarding the synthesis, degradation and neuronal/glia distribution of NAAG, and its role in intercellular communication [79], NAAG is likely to cause selective activation of mGlu3 receptor signalling in astrocytes. On tantalizing possibility is that NAAG is synthesized de novo in astrocytes from a precursor (*N*-acetyl-L-aspartate) released by neurons, with the NAAG produced being released to affect both the astrocyte itself, and perhaps neuronally located mGlu3 receptors in the immediate vicinity.

3.5. Astrocytic targets of mGlu3 receptor activation

Glial mGlu3 receptors have been implicated in a number of studies to have a neuroprotective functions in the CNS. A potential astrocyte mechanism to bring about this effect could be through regulation of glutamate transporter activity and/or expression. Exposure of astrocytes to group II mGlu receptor-specific agonists has been shown to increase glutamate transporter expression [65] and presumably to increase the capacity to clear glutamate from the extracellular space. Group II mGlu receptor activation in astrocytes can also prevent the down-regulation of astroglial glutamate transporters induced by neurotoxic agents, such as 1-methyl-4-phenylpyridium [80]. Recent studies have provided direct evidence for the neuroprotective potential of group II mGlu receptor stimulation in astrocytes against apoptosis of cultured neurons and astrocytes, by up-regulating glutamate uptake mechanisms in astrocytes [81].

Group II mGlu receptors can couple to phosphoinositide 3-kinase and positively or negatively to individual mitogen-activated protein kinase signalling pathways in astrocytes [74], leading to the release of trophic factors such as transforming growth factor-β (TGF-β), which in turn can protect striatal neurons against NMDA receptor-mediated neurotoxicity [65]. Others have also shown that increases in synthesis and release of TGF-β from astrocytes can protect surrounding neurons from excitotoxic cell death [82]. Stimulation of mGlu3 receptors in cultured astrocytes induces an increased synthesis and release of nerve growth factor and S-100β protein, important factors in neuronal survival [83]. The use of mGlu2 and mGlu3 receptor knockout mice has provided strong evidence that the protective actions of mGlu2/3 agonists against excitotoxic cell death are mediated solely by astrocytic mGlu3 receptors [84], and that this is the result of synthesis/release of neuroprotective agents, and/or through regulation of glutamate transporters in astrocytes.

4. Astrocyte mGlu receptor signalling in disease

In parallel with the emergence of new evidence for roles of astrocytes in neuropsychiatric disease has been evidence implicating mGlu receptors in many of the pathophysiological changes observed.

Astrocyte dysfunction likely contributes to the pathogenesis of epilepsy and reactive astrogliosis is often detected in the epileptic brain [85]. As already discussed (see Section 2.1) an important function of astrocytes is to buffer extracellular K^+, to prevent hyperactivity. A number of publications have highlighted a dysfunction in K^+ homeostasis within the epileptic brain. For example, Hinterkeuser et al. [86] showed that inwardly rectifying K^+ currents in astrocytes were down-regulated in the CA1 region of patients with epilepsy, suggesting that impairment in K^+ clearance by astrocytes might contribute to epileptic seizures. In addition, altered astrocyte Ca^{2+} signalling in epilepsy may contribute to paroxysomal depolarization shifts (abnormal and prolonged depolarizations with repetitive spiking, characteristic of seizures) and neuronal excitotoxicity after *status epilepticus* [87,88]. The former study also showed that anti-epileptic drugs inhibit Ca^{2+} increases in astrocytes, and therefore, astrocytes may be considered key targets in the treatment of epilepsy. Alterations in the expression and/or functions of glutamate transporters and mGlu receptors in astrocytes have also been implicated in the

pathogenesis of epilepsy. The expression of members of the mGlu receptor family (mGlu3, mGlu5, and possibly mGlu8) has been reported to be increased in hippocampal astrocytes in mouse models of epilepsy [89], and in vivo administration of a negative allosteric modulator (NAM) of the mGlu5 receptor has been shown to provide significant neuronal protection following the induction of *status epilepticus* in a mouse model [88].

Astrocytes can contribute to the death of motor neurons in familial [90,91] and sporadic [92] amyotrophic lateral sclerosis (ALS). Furthermore, expression of a disease-causing human superoxide dismutase mutation ($SOD1^{G93A}$) in astrocytes alone is sufficient to cause motor neuron degeneration and recapitulate many characteristics of ALS pathology [93]. In a $SOD1^{G93A}$ transgenic ALS rat model mGlu5 receptor expression in astrocytes has been shown to be up-regulated in late stage disease, but there was also a loss of the protein kinase C-dependent increase in EAAT2 transporter activity normally seen on mGlu5 receptor activation [94]. Such a loss of feed-forward regulation of astrocytic glutamate clearance might contribute to excitotoxic motor neuron death.

Evidence of a role for astrocytes in a number of neurodegenerative diseases has also accumulated, and it is known that reactive astrogliosis is a well-known feature of neurodegeneration in amyotrophic lateral sclerosis, Parkinson's disease and Alzheimer's disease (AD) [95,96]. Neuroanatomically AD is characterized by the hallmark deposition of extracellular amyloid-β (Aβ), which accumulates in focal plaques at a relatively early stage of the disease. Aβ-containing plaques often co-localize (and perhaps attract) microglia and astrocytes, suggesting that glia may play some role in the progression of AD [97]. Astrocytes have been shown to contain both full-length and *N*-terminally truncated forms of Aβ peptide, suggesting a clearance role for astrocytes. In addition, a study by Wyss-Coray et al. [98] demonstrated that cultured astrocytes have the capacity to remove and degrade Aβ deposits, but that this capability may be lost in disease, suggesting that a dysfunction of astrocytes may precede and/or coincide with the build-up of Aβ accumulation in the AD brain. Furthermore, disturbances of physiological Ca^{2+} signalling in astrocytes have been reported in a transgenic model of AD, with global increases in resting Ca^{2+} levels and Ca^{2+} waves being observed that originate near to plaques and propagate over considerable distances [99]. Activation of astrocyte mGlu receptors can promote the non-amyloidogenic processing of amyloid precursor protein [100] suggesting a potential to reduce β-amyloid formation. In addition, exposure of mouse astrocytes to β-amyloid has been reported to up-regulate the mGlu5 receptor (at both a mRNA and protein level) and to give rise to greater changes in $[Ca^{2+}]_i$ on mGlu receptor agonist addition [101]. Interestingly, in *post-mortem* human hippocampal tissue co-staining for mGlu5 receptor and β-amyloid has revealed increased receptor immunoreactivity co-localized with plaques [101]. A direct interaction between β-amyloid oligomers and the mGlu5 receptor at the cell-surface has also been demonstrated in vitro in cultured rat hippocampal neurons [102]. In the presence of β-amyloid oligomers, mGlu5 receptors cluster, show reduced lateral mobility and trigger increases in $[Ca^{2+}]_i$. This change in mGlu5 receptor stability and/or signalling eventually leads to synapse deterioration and potentially synaptic failure. Whether β-amyloid causes similar effects on mGlu5 receptors in astrocytes has yet to be investigated. Finally, Caraci et al. [103] have demonstrated complex, contrasting effects of mGlu 2 and mGlu3 receptor activation on the toxicity of β-amyloid oligomers in rat cortical neurons cultured in the absence or presence of astrocytes. This work has clearly demonstrated that astrocyte mGlu3 receptor activation is neuroprotective, at least in part through the increased release of transforming growth factor-β1.

Thus, pharmacologically targeting the mGlu5 and/or mGlu3 receptor in astrocytes, using either subtype-selective orthosteric or allosteric ligands may provide novel therapeutic strategies for the prevention of neuronal injury in Alzheimer's disease and in other pathological conditions.

5. Conclusions

Are we entering a golden age in glial cell research? Certainly the volume of research being published on all types of glia is growing year-on-year. Despite this, it is difficult not to conclude that our understanding of glial cell physiology and pathophysiology remains embryonic [20]. For example, while it is generally believed that astrocytes fulfil important synthetic and secretory roles there clearly remains disagreement on which transmitters/trophic factors they can release and, if they do, by what mechanism(s). In this context the publication of transcriptome data for astrocytes [104] has been illuminating, and has provided evidence implicating secretory lysosomes as a potential route by which ATP and D-serine might be released [105,106]. The case for glutamate release by astrocytes remains a matter of heated debate; however, this should not detract from the proven ability of these cells to respond to this transmitter and to clear it from the extracellular environment in a regulated manner [34].

In this context, mGlu3 and mGlu5 receptors appear to provide important mechanisms for astrocytes to sense and respond to changes in glutamate. In the case of the mGlu5 receptor it is presently unclear what, if any, significance there is to this receptor's unusual regulation by rapid, reversible phosphorylation and consequent propensity to generate a "hard-wired" Ca^{2+} oscillatory signal through dynamic uncoupling (see Section 3.1). Nevertheless, it is tempting to speculate that the ability to generate a cell-specific Ca^{2+} oscillatory frequency across an ascending and descending range of glutamate concentrations might be important in acute and/or longer term signalling. For example, a particular tuning of Ca^{2+} oscillatory frequency (defined by mGlu5 receptor expression level or by other factors) may allow the astrocyte to activate/repress particular subsets of genes through (Ca^{2+}-dependent) transcriptional regulation. Certainly, good evidence has accrued that altering either the Ca^{2+} signature (oscillatory versus peak-plateau) and/or oscillatory frequency can encode specific cellular outcomes [61–63]. However, little information on whether this occurs, or which facets of (patho)physiology might be regulated, has so far been obtained in astrocytes. It is worth noting that astrocytes express Ca^{2+}-dependent transcription factors, including NFAT [107,108] and it will be interesting to establish if this transcription factor is similarly regulated by dynamic uncoupling (via mGlu5 receptor-stimulated Ca^{2+} oscillations) as it is by other GPCRs mediating their actions through mechanistically distinct, but more common, regenerative Ca^{2+} oscillatory mechanisms (see [109]). An additional complication for astrocytes is the potential for Ca^{2+} changes (and perhaps Ca^{2+} oscillations) to spread through a syncytium of cells via gap junctions. Although mGlu5 receptor-initiated Ca^{2+} oscillations spreading from one cell to another has, to our knowledge, not been reported, the fact that oscillations in IP_3 concentration entrain Ca^{2+} oscillations makes this possibility more likely, as the former second messenger may be more free to cross between coupled cells to propagate the signal across a linked network, in contrast to the buffered, and consequently more spatially restricted Ca^{2+} ion. Indeed, an intriguing possibility arises that by going from local to global oscillatory signals a universal astrocyte transcriptional programme might be coordinated across wide territories of the CNS.

With respect to astrocyte mGlu5 receptor signalling, and Ca^{2+} homeostasis in this cell-type more generally, there remains so

much that has yet to be discovered. For example, we know almost nothing about the importance of Homer proteins [110] in mGlu5 receptor location and signalling, beyond the fact that astrocytes express some isoforms of Homer [111,112], and how this might influence mGlu5 receptor-Ca^{2+} signalling in health or disease. Exciting new discoveries are constantly being made that often cause us to reassess and reformulate our understanding of astrocyte Ca^{2+} homeostasis. Very recent discoveries in this respect include (i) the previously unexplored role of TRPA1 receptors as contributors to local basal $[Ca^{2+}]_i$ in astrocytes and the influence that modulating this plasma membrane ion channel can have on the kinetics of GABA clearance from the extracellular environment through altered astrocyte trafficking of GAT-3 [113]; (ii) the potential of astrocytes to sense the changes in extracellular $[Ca^{2+}]$ that occur at synapses and to release ATP (likely via connexin hemichannels) to modulate neural circuitry [114]; and (iii) the additional influence of microglia on astrocyte–neuron intercommunication to add an additional complexity to non-neuronal modulation of transmission [115]. It is hoped that through a growing appreciation of astrocyte Ca^{2+} homeostasis we can begin to understand how druggable targets, such as the mGlu5 and mGlu3 receptors, can be exploited therapeutically in an array of CNS disorders.

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