Distinct Types of Astroglial Cells in the Hippocampus Differ in Gap Junction Coupling

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ABSTRACT Previous studies have shown that two subpopulations of cells with astrocytic properties coexist in the mouse hippocampus, which display distinct morphological and functional characteristics, specifically a nonoverlapping expression of either AMPA-type glutamate receptors (GluR cells) or glutamate transporters (GluT cells). Use of transgenic mice with hGFAP promoter-controlled EGFP expression and patch-clamp recordings allow reliable identification of the two cell types in hippocampal slices. Extending functional characterization, we report here the complete lack of gap junctional tracer coupling in GluR cells, while GluT cells are shown to be extensively coupled. This distinction is valid in immature as well as adult animals. Analysis of transgenic mice expressing β-Gal under regulatory elements of the Cx43 promoter revealed the absence of Cx43 in GluR cells. Experiments using gap junction blockers demonstrated that passive currents, displayed primarily by GluT cells, do not reflect intercellular coupling but are attributable to intrinsic membrane properties of individual cells. This study supports the notion that the two subpopulations of hGFAP-EGFP-positive cells represent distinct cell types with contrasting physiological properties. Since GluR cells do not participate in the astrocytic gap junctional network, their functional role must be different from spatial buffering of ions or signaling molecules, i.e., properties generally assigned to astrocytes. © 2004 Wiley-Liss, Inc.

INTRODUCTION

Increasing evidence suggests a coexistence of different astroglial cell types in the central nervous system (CNS). In the hippocampus, Matthias et al. (2003) recently demonstrated nonoverlapping expression of either AMPA-type glutamate receptors or glutamate transporters in cells with astroglial properties, using a transgenic mouse with hGFAP promoter-controlled expression of EGFP (Nolte et al., 2001). While functional expression of glutamate receptors correlated with a complex pattern of voltage-dependent K+ currents, the presence of glutamate uptake currents was associated with cells revealing a passive current phenotype. In addition, the two populations of cells displayed strikingly different morphologies. The authors’ suggestion that these cells represent two distinct astroglial cell types is opposite to the classical notion that astrocytes comprise a uniform cell population (Walz, 2000).

Extensive gap junctional coupling is considered a prominent feature of astrocytes. Three isoforms of connexins, the protein subunits forming gap junctions, Cx43, Cx30, and Cx26, have been reported to be expressed in astrocytes of rodent brain (Nagy and Rash, 2000; Nagy et al., 2003). Gap junctions connect the cytoplasm of adjacent cells and are permeable to ions and a variety of small molecules. Although the exact

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role of gap junctional communication is still obscure, astrocytes take part in processes that seem to rely on the existence of a multicellular syncytium, e.g., intercellular distribution of energetic substrates throughout the brain (Giaume et al., 1997), clearance of ions and neurotransmitters from the extracellular space (Zahs, 1998; Hansson et al., 2000), or spreading of intercellular Ca\(^{2+}\) waves (Giaume and Venance, 1998).

In this study, we report that the glutamate receptor-bearing cells with astroglial properties in the hippocampus (GluR cells) completely lack gap junctional coupling, as assessed by dye transfer experiments. This contrasts with the extensive coupling of the astrocytes expressing glutamate transporters (GluT cells) and supports the notion that the two cell types represent distinct cellular populations with different physiological properties and functions.

**MATERIALS AND METHODS**

**Slice Preparation**

Hippocampal slices (300 \(\mu\)m) were prepared as described previously (Steinhäuser et al., 1992). Transgenic hGFAP-EGFP mice (Nolte et al., 2001) aged 9–14 (p9), 30–65 (p30), or 230–460 (p230) days were kindly provided by Dr. H. Kettenmann. The mice were anesthetized, decapitated, the brains were removed and cut perpendicularly to the main hippocampal axis using a cryotome (Microm HM560, Walldorf, Germany), had resistances of 3.5–6 \(M\)\(\Omega\) when filled with a solution consisting of (in mM) 126 NaCl, 3 KCl, 2 MgSO\(_4\), 1 Na-pyruvate, 10 glucose, 10 HEPES, 90 sucrose, pH 7.4. The slices were stored in artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 3 KCl, 2 MgSO\(_4\), 2 CaCl\(_2\), 10 glucose, 1.25 NaH\(_2\)PO\(_4\), 26 NaHCO\(_3\), equilibrated with 95% O\(_2\) and 5% CO\(_2\) to a pH of 7.4 at room temperature.

**Patch-Clamp Recordings**

Slices were transferred to a recording chamber and were constantly perfused with ACSF at room temperature. Whole-cell recordings were obtained using an EPC9 amplifier (HEKA Elektronik, Lambrecht, Germany). Pipettes, fabricated from borosilicate capillaries (Hilgenberg, Malsfeld, Germany), had resistances of 3.5–6 \(M\)\(\Omega\) when filled with a solution consisting of (in mM) 130 K-glucuronate, 1 MgCl\(_2\), 3 Na\(_2\)-ATP, 20 HEPES, 10 EGTA, pH 7.2. Voltages were corrected for liquid junction potential. Series resistance compensation was routinely performed (up to 60%). Recordings were sampled at 30 kHz, filtered at 10 kHz, and monitored with TIDA software (HEKA). Cells with hGFAP promoter activity in the CA1 stratum radiatum of the hippocampus were selected using their EGFP fluorescence. Visual control was achieved with a microscope (Axioskop, Zeiss, Oberkochen, Germany) at \(\times600\) magnification. Only one cell was recorded per individual slice. For intracellular labeling, biocytin (Ne-biotinyl-L-lysine; Sigma, Taufkirchen, Germany) was added to the pipette solution (0.5%). During recording (exactly 20 min), the membrane was stepped to potentials between \(-160\) and 70 mV.

The experiments with gap junction blockers were performed by adding 100 \(\mu\)M carbenoxolone (Sigma) or 1 mM octanol (solved in ethanol at a final concentration of 0.48%; Sigma) to ACSF. In some cases, to block nonjunctional currents, pipettes were filled with (in mM) 110 CsCl, 20 TEA, 3 MgCl\(_2\), 0.5 CaCl\(_2\), 10 HEPES, 5 EGTA, 3 Na\(_2\)-ATP. In those cases, ACSF was replaced by an oxygenated, HEPES-buffered solution consisting of (in mM) 3 CsCl, 20 TEA, 0.2 BaCl\(_2\), 5 4-aminopyridine, 1.5 CoCl\(_2\), 1 MgCl\(_2\), 1 CaCl\(_2\), 10 HEPES, 110 NaCl, 10 glucose, to which the gap junction blockers were added.

**Tissue Processing and Staining for Biocytin**

Immediately after recording, slices were immersion-fixed in a solution of 4% paraformaldehyde in 0.1 M sodium phosphate saline, pH 7.4, at 4°C. Consecutive cryosectioning (60 \(\mu\)m), biocytin detection with the Elite ABC kit (Vector, Burlingham, CA), and the DAB reaction were performed as described (Theis et al., 2003). To localize biocytin while keeping the intrinsic EGFP fluorescence, in some of the experiments, cryosections were rinsed in 0.1 M phosphate buffer (pH 7.4), then in 0.1 M Tris-buffered saline (TBS), pH 7.4, and subsequently incubated in Texas Red Avidin D (Vector) diluted 1:100 in TBS (1 h).

**Visualization of Cx43 Expression in hGFAP-EGFP\(^{+}\) Cells**

Mice expressing \(\beta\)-galactosidase (\(\beta\)-Gal) as a reporter for Cx43 in hGFAP-Cre\(^{+}\) cells as well as hGFAP-EGFP were generated by interbreeding of Cx43flu1 (the floxed coding region of Cx43 (one allele) in 0.1 M sodium phosphate saline, pH 7.4, at 4°C. Consecutive cryosectioning (60 \(\mu\)m), biocytin detection with the Elite ABC kit (Vector, Burlingham, CA), and the DAB reaction were performed as described (Theis et al., 2003). In the hGFAP-Cre\(^{+}\) offspring, Cre-mediated deletion of the floxed coding region of Cx43 (one allele) in hGFAP-Cre\(^{+}\) cells leads to nuclear \(\beta\)-Gal expression under control of regulatory elements of the Cx43 gene (Theis et al., 2003). In addition, these mice express EGFP under the control of the hGFAP promoter.

Immunohistochemistry was performed using antibodies directed to \(\beta\)-Gal (polyclonal rat anti-\(\beta\)-Gal, Molecular Probes, Leiden, The Netherlands). Briefly, hGFAP-Cre\(^{+}\) offspring (p90; \(n = 3\)) were transcardially perfused with 4% PFA in 0.1 M PBS, pH 7.4. After cryoprotection (30% sucrose in PBS), 25 \(\mu\)m sections were cut on a cryostat (Microm HM560, Walldorf, Germany), followed by washing in PBS and a blocking step with 0.5% Triton X100 and 5% BSA in PBS. Incubation with primary antibodies (1:500 in PBS with 0.1% Triton, 1% BSA) was carried out overnight at room temperature (RT). Consecutively, the slices were washed,
blocked with 5% NGS in PBS, and incubated with secondary antibodies (Alexa Fluor 594 goat anti–rabbit; 1:1,000 in PBS with 1% NGS; Molecular Probes) for 2 h at RT. The tissue was inspected in a Zeiss Axiophot equipped with fluorescence optics. Images were taken with a digital SPOT camera (Diagnostic Instruments, Sterling Heights, MI) and MetaView software (Universal Imaging, West Chester, PA). Optical sections at 1 μm intervals through the depth of the slice were digitally combined to yield the final images. Alternatively, fluorescent images were obtained using a confocal laser scanning microscope (Leica TCS, Pulheim, Germany; Fig. 3).

Reagents were purchased from Sigma unless otherwise stated. Input resistance (Ri) was calculated from the currents elicited at the end of a 50-ms voltage step from −70 to −60 mV, corrected for series resistance (Schroeder et al., 1999). Data are given as mean ± SD. Differences were tested for significance using the Student’s t-test (P < 0.05).

RESULTS

Two types of cells with astroglial characteristics were identified in the hippocampus of transgenic hGFAP/EGFP mice (GluR- and GluT-cells) (Matthias et al., 2003). Here, we tested these cells for differences in gap junction coupling. GluR and GluT cells were reliably distinguished in acute slices from mice at p30 according to their intrinsic EGFP fluorescence and different morphological properties, as reported in the later study. Patch-clamp recordings confirmed that cells with oval or elongated somata bearing tapering primary processes branching into many fine processes always displayed large time- and voltage-independent (passive) membrane currents, properties characteristic of astrocytes of the GluT type (Fig. 1A). Biocytin filling of these cells during recording and subsequent tissue processing always revealed abundant dye coupling (Fig. 1B). Quantitative analysis demonstrated tracer spread from single injected astrocytes (p30; n = 8) to a total of 120 ± 42 encircling cells (recording period: 20 min). The resting potential (Vr) of these cells was −69 ± 3 mV (t = 0) and remained stable during recording (alteration at t = 20 min: 2 ± 4 mV). In accordance with previous findings (Konietzko and Muller, 1994), the tracer spread from the stratum radiatum to strata oriens and lacunosum moleculare (Fig. 1B).

GluR cells possessed spheric somata with few thin processes, displaying voltage- and time-dependent whole-cell currents (Fig. 1C) (Matthias et al., 2003). Intriguingly, biocytin filling of these cells revealed a complete lack of dye coupling (Fig. 1D). In all cases (p30; n = 13), the tracer was confined solely to the recorded cell. Intermediate or weak coupling was never found (VR at t = 0: −70 ± 3 mV; alteration at t = 20 min: −2 ± 3 mV).

To determine whether GluR cells lack gap junction coupling throughout life, we performed experiments with older mice (p230). As for p30 animals, EGFP+ cells with fine processes displayed complex current patterns and lacked junctional coupling (t = 0: VR = −72 ± 1 mV; shift at t = 20 min: 0.2 ± 3 mV; n = 9).
As expected, injection of GluT cells again led to abundant tracer spread into adjacent astrocytes (69 ± 39 coupled cells; \( V_R = -70 \mp 1 \text{ mV} \), shift at \( t = 20 \text{ min} \): 0.3 ± 3 mV; \( n = 3 \)). Comparable experiments were also performed at more immature developmental stages (p9). Biocytin injection of GluR cells (\( n = 6 \)) revealed a complete lack of dye coupling similar to cells in mature mice. In contrast, immature GluT cells always displayed biocytin coupling, mostly to hundreds of cells (234 ± 182; \( n = 8 \)). However, we noted one exceptional case with a very low degree of coupling (Fig. 3B). Another astrocyte in the juvenile hippocampus, morphologically being classified as a GluT cell, was coupled and endowed with a large resting conductance, but in addition showed voltage-gated current components. We have previously reported that in the juvenile hippocampus, GluT cells sometimes display voltage-dependent currents (Matthias et al., 2003). This was confirmed by excising outside-out patches from cells with such properties in situ (\( n = 3 \)). Indeed, using KSCN-based pipette solution, rapid application of glutamate in all cases evoked transporter currents but no receptor responses (not shown). Together, these data further substantiate the notion that GluR and GluT cells in the hippocampus represent independent types of cells with astroglial properties.

Although biocytin injection of GluR cells did not reveal dye coupling, the tracer might have entered them when injecting a GluT cell due to unidirectional dye transfer. Such asymmetric coupling has been described for glial cells in the retina (Robinson et al., 1993; Zahs and Newman, 1997). Therefore, experiments were performed in which EGFP fluorescence was preserved during biocytin visualization. After filling single GluT cells (p30; \( n = 4 \)), double fluorescence analysis revealed that all morphologically identified EGFP-positive GluT cells in the vicinity of the injected cells (\( n = 106 \)) were biocytin-positive and hence part of the coupled syncytium. In contrast, none of the morphologically identified GluR cells, localized within the circular area of coupled cells (\( n = 51 \)), appeared to be positive for biocytin (Fig. 2). We noted that even GluR cells that virtually touched biocytin-positive GluT cells lacked the tracer (Fig. 2C, inset). On the other hand, some of the biocytin-positive cells, morphologically resembling GluT cells, were EGFP-negative, indicating that some coupled astrocytes express very low or no hGFAP promoter activity, which is consistent with earlier observations (Nolte et al., 2001). Biocytin-positive processes of EGFP-positive GluT cells as well as EGFP-negative cells were often seen in close contact with the vasculature (Fig. 2), evidently representing perivascular endfeet, which are characteristic for cerebral astrocytes (Kacem et al., 1998). In contrast, processes of GluR cells did not form endfeet-like structures.

Complete lack of dye coupling in GluR cells prompted us to compare expression of Cx43, the major astrocytic gap junctional protein, in both cell types. Assigning Cx43 immunopositive puncta to individual cells proved to be difficult. Therefore, we indirectly analyzed Cx43 expression in hGFAP+ cells. By interbreeding of hGFAP-EGFP mice (Nolte et al., 2001) and hGFAP-Cre Cx43fl/fl mice (Theis et al., 2003), we generated mice expressing β-Gal under the regulatory elements of the Cx43 gene in hGFAP-Cre+ cells (Fig. 3A). The pattern of β-Gal-positive cells was as described in the latter study. In the hippocampus, the density of β-Gal/Cx43-expressing cells matched the density of coupling cells, with a high density in the stratum lacunosum-moleculare and a lower density in the stratum radiatum (Fig. 3A) (Konietzko and Müller, 1994; and this study). The EGFP fluorescence enabled us to discriminate GluR cells versus GluT cells. Detection of β-Gal by immunohistochemistry implied Cx43 expression in almost all EGFP+ GluT cells (\( n = 47 \) of 49; Fig. 3B). GluR cells that also expressed hGFAP-EGFP were always found to be negative for β-Gal (\( n = 111 \)) and hence did not express Cx43 (Fig. 3B). It should be mentioned that β-Gal immunoreactivity was also present in many EGFP-negative cells. Activation of hGFAP-Cre at one time point is sufficient to delete floxed DNA, thereby creating the β-Gal open reading frame under control of the Cx43 regulatory elements. Thus, at the time of analysis, these cells express Cx43/β-Gal but not hGFAP-EGFP. These findings are consistent with the previously mentioned biocytin-positive, coupled, but EGFP-negative GluT cells. We noticed that the number of EGFP+ cells in these heterozygous mice was generally low (data not shown).

Based on the observation that the highly coupled GluT cells show a significantly lower \( R_i \) compared to the noncoupled GluR cells (11 ± 6 MΩ, \( n = 8 \), vs. 88 ± 62 MΩ, \( n = 13 \), at p30), we wanted to assess whether junctional currents contribute significantly to the membrane conductance of GluT cells. Actually, the existence of intrinsic passive currents in astrocytes has been doubted (Walz, 2000). As none of the available gap junction blockers are completely specific, we used two structurally unrelated compounds and tested them for their efficacy in uncoupling GluT cells in our preparation. Exposure to carbenoxolone (\( n = 3 \)) or octanol (\( n = 3 \)) prior to biocytin filling significantly reduced tracer transfer to neighboring cells. However, no additional current components were unmasked, and \( R_i \) was not significantly affected (Fig. 4A). In other experiments, gap junction blockers were applied during whole-cell recording for direct comparison. No significant changes in \( R_i \) were observed after 3 min of incubation in carbenoxolone (9.9 ± 4 MΩ vs. 8.2 ± 5 MΩ in control, \( n = 4 \)) or octanol (7.5 ± 2 MΩ vs. 6.3 ± 3 MΩ in control, \( n = 4 \); Fig. 4C, bar graph). Additional evidence for passive currents being largely independent of the degree of coupling was gathered from the finding that GluT cells with unequal numbers of coupled cells displayed a similar resting conductance (Fig 4B). To avoid nonspecific modulation of voltage-dependent channels by gap junction blockers, we reduced nonjunctional currents by applying K+ and Ca2+ channel blockers (Tamalu et al., 2001). This incompletely blocked the resting current in GluT cells (\( R_i = 77 ± 29 \text{ MΩ}, n = 18 \)). In contrast,
currents of GluR cells were completely abolished (n = 7; Fig 4C). Application of the K\(^+\) and Ca\(^{2+}\) channel blocking cocktail led to a significant positive shift of resting potential in GluR cells (\(V_r = -5.6 \pm 3\)) but not in GluT cells (\(V_r = -72 \pm 10\) mV). In the presence of the blocking cocktail, carbenoxolone did not significantly increase \(R_i\) of GluT cells (124 ± 44 M\(\Omega\) vs. 95 ± 30 M\(\Omega\), n = 8; Fig. 4C). Together, these data confirm the notion that GluR and GluT cells exhibit distinct intrinsic membrane properties, and that the large resting conductance of GluT cells is not primarily due to currents through gap junction channels.

**DISCUSSION**

The identification of two distinct cell types with astroglial properties in the hippocampus displaying different morphological, functional, and molecular profiles (Matthias et al., 2003) led us to look for differences in their gap junctional coupling. A high degree of gap junctional communication is considered an important feature of astrocytes and a prerequisite for proper brain function. Using biocytin as a tracer, we show that the GluR cells completely lack coupling. Moreover, asymmetric dye coupling from GluT to GluR cells, which occurs from astrocytes to Müller cells in rat retina (Zahs and Newman, 1997), was never observed.

Konietzko and Müller (1994) performed biocytin injections with sharp microelectrodes, demonstrating abundant coupling among astrocytes in the hippocampus, with the pattern of coupled cells closely corresponding to the distribution of GFAP. This let the authors suggest that the entire astrocyte population is functionally interconnected by gap junctions. This conclusion was in apparent contradiction with other work combining functional characterization with dye coupling analysis in the same brain region (Steinhäuser et al., 1994; Kressin et al., 1995). Specifically, the latter authors noted that presumed GFAP-negative astrocytes with complex current patterns were mostly uncoupled, and later on, the same group reported that these cells express functional glutamate receptors of the AMPA subtype (Seifert and Steinhaüser, 1995). In view of the current results, it seems plausible to assume that Konietzko and Müller (1994) injected astrocytes of the GluT type, while the two former patch-clamp studies investigated GluR cells. Abundant coupling among the GluT cells, which obviously represent classical protoplasmic astrocytes, is in line with their presumed role as a sink for neuronally released glutamate and K\(^+\) (Orkand et al., 1966; Anderson and Swanson, 2000).

![Fig. 2. Lack of asymmetric tracer coupling from GluT cells to GluR cells. A: hGFAP promoter-driven EGFP expression in hippocampal astrocytes in the vicinity of the injected cell. GluT cells (blue arrows) could be identified based on the many branched processes which mostly appeared like a halo surrounding the soma. GluR cells (white arrowheads) usually displayed very faint fluorescence; processes were barely visible. B: In the same area, biocytin spread was visualized with Texas Red Avidin. All GluT cells were positive for biocytin; all GluR cells lacked the tracer. In the middle, a blood vessel was highlighted by biocytin-positive processes of contacting GluT cells. C: Overlay. Note that a number of presumed biocytin-positive GluT cells were EGFP-negative. The inset reveals lack of biocytin in a GluR cell despite its close association with a biocytin-positive GluT cell. Scale bar = 25 \(\mu\)m.](image)
Fig. 3. Cx43 reporter mice reveal the absence of Cx43 in GluR cells. A: β-Gal+ nuclei denoting Cx43 expression in hGFAP-Cre+ cells are present throughout the brain (see inset, DIC optics, for better orientation). The density of β-Gal/Cx43 expressing cells is particularly high in strata lacunosum-moleculare (lm) and moleculare (m) and lower in stratum radiatum (r). B: Confocal images of a GluT cell, identified by its highly branched processes (left), and of two GluR cells (right) in the stratum radiatum (r). The overlay of EGFP fluorescence (green) with β-Gal immunofluorescence (red) reveals β-Gal/Cx43 expression in the GluT cell (yellow, left) and absence of β-Gal/Cx43 in the GluR cells (right). Processes of the GluT cell can be seen to encircle a blood vessel (arrow head). In both panels, additional β-Gal/Cx43+ cells are found that lacked detectable hGFAP-EGFP activity at the time point of analysis. Scale bars = 100 μm (A) and 25 μm (B). alv, alveus; o, stratum oriens; p, stratum pyramidale.
Although regional differences in astrocyte coupling were reported for the hippocampus (D’Ambrosio et al., 1998), our data provide the first evidence for the existence of a subpopulation of cells with astroglial properties that completely lack tracer coupling in situ. Despite the fact that biocytin is a very sensitive coupling tracer, weak electrical coupling might occur in the absence of dye coupling (Gibson et al., 1999). However, in the present study, the cells were filled over extended periods and not a single GluR cell displayed dye transfer to a neighboring cell, which makes electrical coupling unlikely. Moreover, analysis of transgenic mice with hGFAP-Cre/hGFAP-EGFP expression evidenced the absence of Cx43, the major astroglial connexin subunit, in the GluR cells. The noncoupled GluR cells and the highly coupled GluT cells were found to coexist in immature, adult, and aged mice. No intermediate, weakly coupled cells occurred in mature animals. Even in the aged hippocampus, high numbers of S100β+ GluR cells persist. In view of the low mitotic activity in the postnatal CA1 region (Rietze et al., 2000) and the lack of hGFAP-EGFP-positive cells with intermediate functional properties (Matthias et al., 2003), it seems unlikely that GluR cells merely represent an immature form of GluT cells. Rather, the present findings add evidence to the concept of discrete functional classes of cells with astroglial characteristics in the hippocampus.

There is a controversy in the literature concerning the existence of passive currents in astrocytes (Walz, 2000). We noted previously that acutely isolated GluT cells often display reduced passive currents (Matthias et al., 2003). This might indicate that the corresponding channels are primarily located on processes that are lost during preparation. It is unlikely that the reduced resting conductance in isolated cells reflected loss of junctional currents because we show here that gap junction blockers do not significantly enhance $R_i$ of passive GluT cells in situ. Thus, most likely, the gap junctional conductance is very small compared to the intrinsic nonjunctional conductance of the cells. Presumably, gap junctions are mainly localized at the tips of astrocyte processes and therefore only minimally impact somatic input resistance measured with whole-cell recordings. Our suggestion that a passive current profile is attributable to intrinsic properties of a single cell was also corroborated by the observation that GluT cells show passive currents insensitive to blockers of voltage-dependent $K^+$ channels, which are insensitive to carbonoxolone. These current were sufficient to set the resting membrane potential to negative values and did not exist in GluR cells, which were strongly depolarized by the blocking cocktail. Members of the two-pore-domain $K^+$ channel family reveal time- and voltage-independent currents that are relatively insensitive to blockers of other $K^+$ channels (Lesage and Lazdunski, 2000), which might indicate their contribution to the GluT cell current phenotype.

GluR cells were shown to express the astroglial markers S100β and GFAP, but subsets of these cells...
coexpressed the proteoglycan AN2/NG2 and transcripts for the neuronal glutamate transporter EAAC1 and hence did not match the classical definition of an astrocyte (Matthias et al., 2003). Certainly, the identity of these cells needs further investigation, and their physiological functions have to be defined. The apparent lack of gap junctional coupling, together with the lack of functional glutamate transporters, seems to exclude them from spatial buffering of $K^+$ or glutamate. Intercellular communication enclosing GluR lack of gap junctional coupling, together with the lack of functional glutamate transporters, seems to exclude them from spatial buffering of $K^+$ or glutamate. Intercellular communication enclosing GluR

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