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REVIEW

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Astrocyte dysregulation as an epileptogenic factor: a systematic review

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Abstract

Background Epilepsy initiation involves multifactorial etiologies, including genetic susceptibility, structural anomalies, and glial cell dysregulations, particularly in astrocytes. Despite advancements in understanding various factors, the mechanisms of astrocyte dysregulation in epilepsy, critical for neural homeostasis, remain elusive, requiring comprehensive evaluation of molecular pathways and cellular interactions for future targeted interventions.

Methods A systematic search of PubMed, ScienceDirect, and the Cochrane databases up to January 1st 2024 identified relevant studies predominantly from experimental models, forming the basis for an in-depth analysis of astrocytic contributions to epileptic pathophysiology. The aims, subjects, epilepsy induction techniques, assessment methods, and findings of each studies were presented.

Results A total of 24 clinical trials met the inclusion criteria and were included in the systematic review. Altered potassium buffering compromises extracellular potassium regulation, fostering hyperexcitability. Aquaporin dysfunction disrupts water homeostasis, aggravating seizure susceptibility. Disturbances in glutamatergic transmission, marked by changes in glutamate transporter function, contribute to excitotoxicity, fueling epileptogenesis. Intricacies in calcium signaling and disruptions in calcium-binding proteins tip intracellular calcium balance towards hyperexcitability. Dysfunctional GABA transporters compromise inhibitory neurotransmission, upsetting excitatory–inhibitory balance. Gap junction protein dysregulation disrupts astroglial networks, impacting neuronal synchronization in epileptogenic circuitry. Compromised BBB allows entry of epileptogenic factors, exacerbating the epileptogenic milieu.

Conclusions Collectively, these astrocytic dysregulations unveil intricate contributors to epilepsy onset and progression.

Keywords Astrocyte, Dysregulation, Epilepsy, Glial cell

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Introduction

A seizure is a transient and paroxysmal event characterized by abnormal and synchronous neuronal activity in the brain, leading to various manifestations encompassing motor, autonomic, and sensory domains. Motor manifestations may include tonic, clonic, tonic–clonic, or atonic movements, presenting as convulsions or jerking motions. Autonomic manifestations may manifest as changes in heart rate, blood pressure, pupillary size, or gastrointestinal symptoms, reflecting dysregulation of the autonomic nervous system. Sensory manifestations encompass alterations in perception, such as visual

disturbances, auditory hallucinations, olfactory sensations, or sensations of tingling, numbness, or abnormal sensations in the skin, reflecting aberrant sensory processing within the brain [1]. A seizure can also cause brief behavioral alterations. In seizures, a few abnormal neurons prompt changes in nearby neurons, leading to progressive synchronization and altered behavior [2]. Epileptogenesis constitutes a multifaceted process encompassing the development, progression, and maintenance of epilepsy, a chronic neurological disorder characterized by recurrent seizures. Epilepsy is increasingly recognized as a brain network disease, implicating aberrant interactions and dysregulation within neural circuits rather than isolated neuronal dysfunction [3]. Epilepsy manifests as paroxysmal, transient, and stereotyped clinical events, comprising idiopathic, symptomatic (secondary or acquired), and cryptogenic forms. Symptomatic epilepsy, the most common type, results from central nervous system lesions or abnormalities, including structural issues or disturbances in brain function [4]. The pooled lifetime prevalence of epilepsy was 7.60 per 1000 persons (95% CI 6.17–9.38), with a higher incidence observed in low/middle-income countries (LMIC) compared to high-income countries (HIC), 139.0 (95% CI 69.4–278.2) and 48.9 (95% CI 39.0–61.1), respectively [5, 6]. The prevalence of active epilepsy exhibited age-related increases, reaching peaks at 5–9 years and beyond 80 years, while global mortality rates for idiopathic epilepsy were 1.74 per 100,000 population for women and 2.09 per 100,000 population for men [7]. The complex nature of epilepsy, marked by transient electrical surges and various diagnostic criteria, underscores the significance of understanding its diverse manifestations.

An unprovoked seizure occurs without identifiable triggers and includes events without recognized causes, as well as those associated with stable or progressive CNS abnormalities [8]. Current evidences show that the initiation of epilepsy is multifactorial, involving genetic susceptibility, structural anomalies, traumatic brain injuries, chemical exposures, hypoxia, infections, metabolic imbalances, immunologic factors, stroke, and glial cells dysregulations among its diverse etiological factors [9]. Glial cells, especially astrocytes, enhance individual neuronal activity through gliotransmission and the tripartite synapse. While they normally play a crucial role in maintaining blood–brain barrier integrity and addressing inflammation and oxidative stress, these functions become impaired in epilepsy [10]. Despite advancements in understanding various etiological factors contributing to epilepsy, the precise mechanisms underlying astrocyte dysregulations remain elusive. One reason is that in humans, chronic temporal lobe epilepsy usually follows a seizure-free latent period that could last years, during which crucial pathophysiological changes occur. Animal models are essential to study this latent phase, vital for understanding astrocytic alterations preceding epileptic neuronal activity [11]. Studies have shown that astrocytes play pivotal roles in ion and water uptake, glucose metabolism, and communication with neurons, making these functions integral to neural homeostasis. Disruptions in these crucial astrocytic activities have been intricately linked to the pathophysiology of epilepsy [12]. Astrocytes play a crucial role in preserving the integrity of the blood–brain barrier and mitigating inflammation and oxidative stress. However, in epilepsy, these functions are compromised [13]. Epileptic conditions lead to disruptions in astrocytic communication through gap junctions, impacting ion and water balance. Activated astrocytes contribute to altered neuronal excitability by decreasing glutamate uptake and increasing adenosine metabolism. Moreover, their heightened adenosine metabolism may play a role in DNA hypermethylation and other epigenetic changes associated with epileptogenesis [10, 13]. In the context of drug-resistant epilepsy (DRE) and refractory status epilepticus (RSE), astrocytic dysfunction assumes heightened significance. Astrocytes play a crucial role in the mechanisms underlying resistance to antiseizure medications (ASMs), contributing to reduced drug efficacy and treatment failure [9]. Their involvement in pharmacoresistance encompasses various mechanisms, such as impaired drug transport across the blood–brain barrier, enhanced drug metabolism, and altered expression of drug targets or efflux transporters [14]. Moreover, astrocytic gliosis and neuroinflammation in DRE and RSE further exacerbate neuronal hyperexcitability and perpetuate seizure activity [14, 15].

As our understanding of the brain's non-neuronal elements expands, glial cells emerge as central figures in epilepsy pathogenesis [16]. Recognizing their role as key organizers of homeostasis and contributors to inflammation and brain excitability opens avenues for innovative therapeutic approaches [17]. By evaluating the molecular pathways and cellular interactions underlying astrocyte dysregulation, this systematic review aims to provide a comprehensive overview of the current state of knowledge. Ultimately, this synthesis of evidence is crucial for guiding future research directions and developing targeted interventions that may effectively modulate astrocytic function, thereby mitigating the epileptogenic process.

Methods

Study design and inclusion criteria

In strict adherence to the PRISMA guidelines, we conducted a systematic review characterized by high methodological rigor. The study protocol underwent

registration and approval in the PROSPERO database (ID: CRD496570) before the commencement of the systematic search. Inclusion criteria were defined to encompass studies addressing dysregulations in astrocytes associated with epilepsy, with no language restrictions imposed. The selected criteria included studies examining both animal and human subjects, evaluating the involvement of astrocyte components in epileptogenesis and their roles in pathological conditions predisposing to epilepsy development. Dysregulations in astrocytes were categorized, with a focus on but not limited to dysfunctions involving potassium buffering, water homeostasis, glutamatergic transmission, calcium signaling and calcium-binding proteins, gamma-aminobutyric acid (GABA) transporter, gap junction proteins, and blood–brain barrier (BBB). Studies concentrating on astrocyte roles in brain diseases unrelated to epileptogenesis or not exploring the molecular mechanisms of seizure onset were excluded.

Literature search and selection

A comprehensive literature search, following the PRISMA flowchart, was conducted on PubMed, ScienceDirect, and the Cochrane databases to identify studies investigating the impact of astrocyte dysfunctions on epileptogenesis and epilepsy-related brain diseases. The search encompassed studies published up to January 1, 2024, without backward limits, employing MeSH terms. To mitigate the potential omission of pertinent studies, the reference lists of included papers and previous reviews on similar topics were manually screened. Duplicate articles were eliminated using Microsoft Excel 16.37 (Redmond, WA, USA). The research strategy relied on the analysis of titles and abstracts. The full text of an article was retrieved if the title and abstract met the inclusion criteria. No automated tools were employed during this phase.

A total of 583 papers were identified, of which 47 underwent full-text screening due to indications in their titles or abstracts suggesting a discussion of astrocyte dysregulations in the pathogenesis of epilepsy. Furthermore, only primary research articles were included, while several reviews were consulted for general information. Among the 23 papers, the majority did not specifically address astrocyte dysregulations but rather focused on therapeutic approaches related to astrocyte regulation or epilepsy in general. Ultimately, only papers explicitly discussing the dysfunctions of astrocyte components in epilepsy or a model thereof were included, amounting to a total of 24 papers (Fig. 1).

Qualitative data extraction

A comprehensive and systematic data extraction process was diligently executed to acquire comprehensive demographic, baseline clinical, and outcome-related data from the selected studies. This methodological rigor facilitated a nuanced evaluation of the effects of dysregulations in each astrocyte component on the pathogenesis of epilepsy. The categorization of mechanisms of action in astrocyte dysregulation afforded a comprehensive understanding of epilepsy pathophysiology, thereby enhancing the precision and depth of the research findings.

In accordance with the aforementioned criteria, all articles underwent screening and identification by two reviewers. Disagreements were resolved through discussion and consensus, and when discussion failed to lead to consensus, a third researcher mediated. Extracted qualitative data included authorship, publication year, study objectives, and principal findings.

Results

Study selection

From the literature search conducted with the abovementioned queries, 583 articles were identified, which were subsequently reduced to 171 after the removal of duplicates. Following exclusion based on title and abstract, 47 papers were obtained for eligibility assessment. Subsequently, these were screened for relevance, culminating in the inclusion of 24 in accordance with our inclusion criteria and the overarching objective of the review. The full text was accessible for all 24 included studies [18–41], all of which were incorporated into our qualitative analysis (Fig. 1).

Findings of included studies

The synthesized evidence from diverse studies presents a comprehensive landscape of astrocytic dysregulations implicated in epileptogenesis (Table 1). These findings collectively showcase the intricate interplay between astrocyte dysfunction and key molecular components, shedding light on potential mechanisms underpinning the development and progression of epilepsy. Three studies [18–20] demonstrated that increased potassium levels lead to frequency-dependent synaptic facilitation, contributing to neuronal hyperexcitability. In addition, the downregulation of astrocytic inward rectifier potassium channels 4.1 (Kir4.1) channel in the Leucine-Rich Glioma-Inactivated 1 (Lgi1)-related seizure model suggests a functional impairment, potentially heightening seizure susceptibility by compromising astrocytes' ability to maintain extracellular homeostasis. In addition, four studies [21–24] found that downregulation of Aquaporin 4 (AQP4) disrupts astrocytic homeostasis, potentially

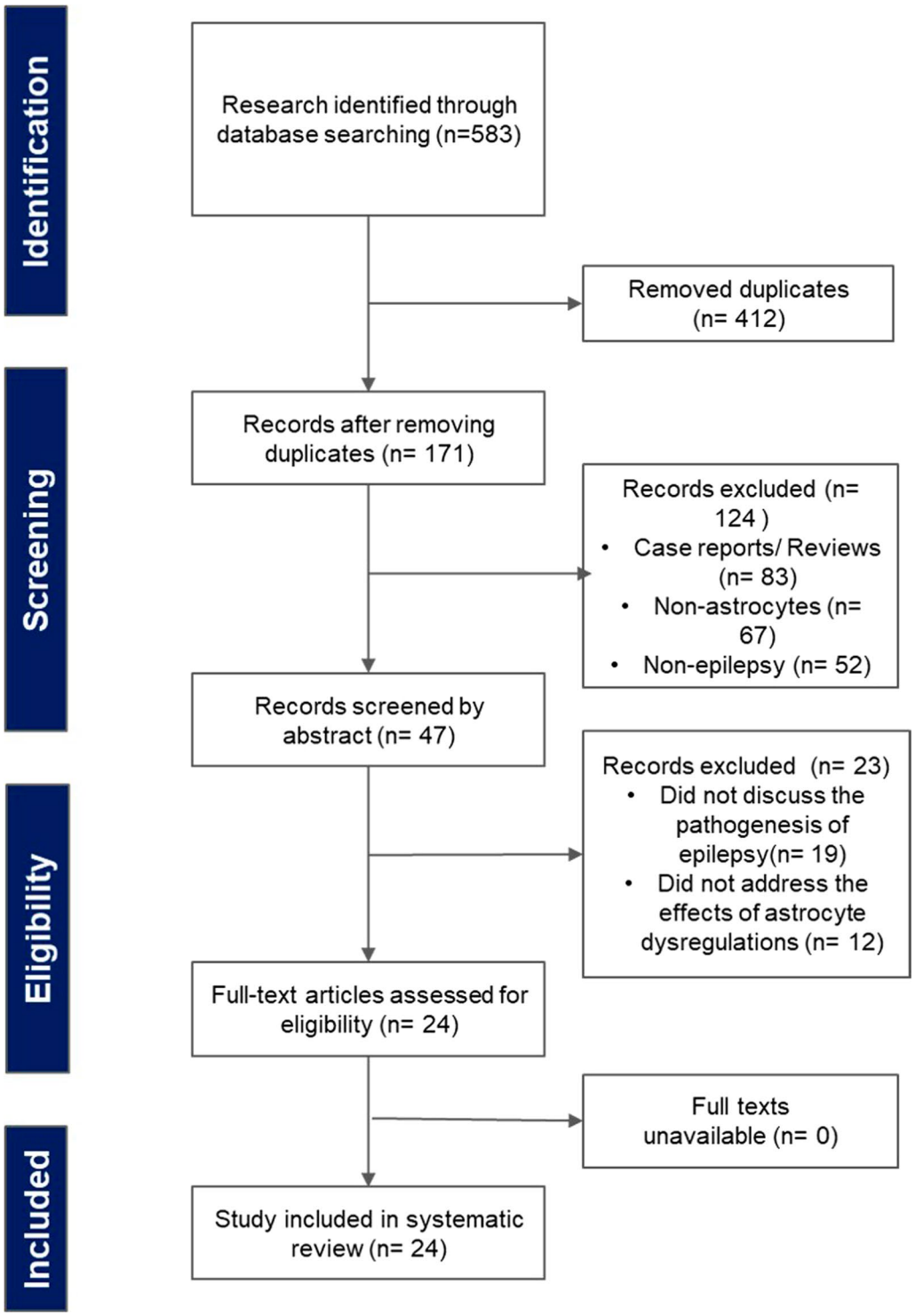

Fig. 1 PRISMA flowchart

leading to powerful epileptogenic and cognitive effects. AQP4 plays a protective role in post-traumatic seizures by promoting astrogliosis and preventing microgliosis, with dysregulation involving specific upregulation and mislocalization in post-traumatic epilepsy. Three studies [25–27] showed that Glutamate Transporter 1 (GLT-1)

Table 1 Astrocyte dysregulations in epileptogenesis

Study, Year	Aim	Subjects	Epilepsy Induction	Assessment Methods	Findings
Potassium buffering					
David and colleagues, 2009 [18]	To investigate how altered potassium hemostasis of astrocytes may alter neuronal excitability and contribute to epileptogenesis	Adult male Wistar rats (120–250 g)	A BBB-disrupting agent, deoxycholic acid sodium salt (DOC, 2 mM, Sigma-Aldrich)	To study altered extracellular potassium ([K+]o), each compartment accumulated potassium, with diffusion into the bathing solution or astrocytes exhibiting KIR kinetics. Influx into "astrocytes" was determined by local potassium gradients, governed by KIR channel conductance proportional to [K+]o	Potassium accumulation induces frequency-dependent (10–50 Hz) and NMDA-dependent synaptic facilitation. This decrease in extracellular potassium clearance contributes to frequency-dependent neuronal hyperexcitability and network synchronization
Konboshi and colleagues, 2019 [19]	To clarify the role of astrocytic Kir4.1 channel in Lgi1-related epileptogenesis	WT rats and Lgi1 mutant rats	Acoustic priming stimulation (130 dB, 10 kHz, 5 min) for 8 weeks	Verified Kir4.1 expression in WT F344 rats through immunofluorescent double-staining. Topographical mapping assessed changes in Kir4.1 and GFAP expression by counting IR-positive cells stained with ABC method	This study reveals a reduction in astrocytic Kir4.1 expression in the Lgi1-related seizure model. The findings suggest that the down-regulation of Kir4.1 channel in astrocytes is implicated in the audiogenic epileptogenesis resulting from Lgi1 mutation
Méndez-González and colleagues, 2020 [20]	To ascertain the occurrence of downregulated functional astrocytic Kir4.1 channel in the brains of type 2 diabetic mice and evaluate their potential impact on hippocampal neuronal hyperexcitability	Diabetic homozygous (db/db) and non-diabetic heterozygous (db/+) male mice	100 µM 4-aminopyridine (4-AP; Sigma) added to the ACSF	Patched astrocytes were superfused with ACSF containing 100 µM barium, a selective Kir channel blocker, for 10 min, and barium-sensitive Kir currents were obtained by subtracting currents with and without barium. Whole-cell voltage-clamp recordings revealed Kir-dependent inward currents, obtained by subtracting currents with barium from those without, in response to external solution changes from 2.5 mM to 10 mM K+	Astrocytic Kir4.1 channel displayed functional downregulation, as indicated by depolarized membrane potential, diminished barium-sensitive Kir currents, and compromised potassium uptake in hippocampal astrocytes. The resulting astrocytic dysfunction, stemming from Kir4.1 downregulation, may heighten seizure susceptibility by impeding astrocytes' capacity to uphold extracellular homeostasis effectively

Table 1 (continued)

Study, Year	Aim	Subjects	Epilepsy Induction	Assessment Methods	Findings
Water hemostasis					
Hubbard and colleagues, 2016 [21]	To examine GFAP, GLT1, and AQP4 regulation during epileptogenesis in a temporal lobe epilepsy mouse model	7–8-week-old CD1 male mice	IHKA injections	Dorsal hippocampus was microdissected from the mouse brain at 1, 4, 7, and 30 days	GLT1 and AQP4 are downregulated during the early epileptogenic period, associated with increased GFAP expression; early disruption of astrocytic water, potassium, and glutamate homeostasis could have powerful epileptogenic and cognitive effects
Heuser and colleagues, 2010 [22]	To identify variants of the AQP4 and KCNJ10 genes associated with TLE and subgroups of this condition	218 Norwegian patients with TLE and 181 ethnically matched healthy controls		Comprehensive search for DNA variation within the AQP4 gene was carried out by PCR	Dysregulations and polymorphism in AQP4 and KCNJ10/KCNJ9 genes are associated with temporal lobe epilepsy
Lu and colleagues, 2021 [23]	To examine the differences of post-traumatic seizure susceptibility between AQP4-deficient mice (AQP4−/−) 1 month after CCI and wild-type sham injury control mice	40 wild-type mice and 40 AQP4-deficient mice	PTZ injections	Epitopes exposed with citrate buffer; sections blocked (3% BSA), incubated with AQP4, CD11b, or GFAP antibodies (4 h, room temperature; Millipore). Immunolabeling visualized in brown with diaminobenzidine	Protective role of AQP4 in post-traumatic seizure susceptibility by promoting astrogliosis, formation of a glial scar, and preventing microgliosis
Szu and colleagues, 2020 [24]	To characterize changes in AQP4 expression in CCI injury model of PTE	95 Adult male CD1 WT between the ages of 8–10 weeks old	Using a high-speed drill and ¼ mm bur, TBI was induced with a CCI device using a 2 mm flat impactor tip at 5 m/s velocity, 1 mm depth, and 200 ms contact time	Sections underwent PBS washing, followed by blocking. Incubation with T-lectin, AQP4 (1:200, Millipore AB2218), and GFAP occurred in 0.3% Triton X-100 for 2 days at 4 °C. After PBS washing, sections were treated with Alexa 594 and Alexa 647-conjugated secondary antibodies then mounted using the ProLong Antifade kit without DAPI	AQP4 levels significantly increased in the frontal cortex and hippocampus of PTE mice compared to those without PTE. Dysregulation of AQP4 in the PTE model was characterized by specific upregulation and mislocalization of perivascular AQP4, exclusively observed in mice that developed PTE
Glutamatergic transmission					
Sun and colleagues, 2021 [25]	To assess the loss of neogenin (NEO1), a coreceptor for various ligands such as netrins and bone morphogenic proteins, in the onset of epilepsy	Male Neogeninf/f (Neof/f), GFAP-Cre (#004600), PV-Cre (#017320), and Ai9 (#007907) mice	PTZ injections	Conducted liquid chromatography–tandem mass spectrometry to identify altered plasma membrane proteins in primary cultured Neo1 KO astrocytes, elucidating the impact of astrocytic NEO1 deficiency on GABAergic transmission and epileptic response	Astrocyte-specific KO of Neo1 diminished inhibitory synaptic vesicles and GABAergic synaptic transmission in the hippocampus, impairing the GLAST-mediated glutamate–glutamine cycle. This study highlights NEO1's role in hippocampal astrocytes, functioning to safeguard the brain from epilepsy

Table 1 (continued)

Study, Year	Aim	Subjects	Epilepsy Induction	Assessment Methods	Findings
Peterson and colleagues, 2021 [26]	To investigate the therapeutic capacity of astrocyte-specific AAV-mediated GLT-1 expression in the IHKA model of TLE	58 Charles River CD1 male mice, 8–10 weeks	Intrahippocampal kainic acid (IHKA) injections	Western blot analysis, immunohistochemistry, and long-term-video EEG monitoring to demonstrate that cell-type-specific upregulation of GLT-1 in astrocytes	In epilepsy, GLT-1 is diminished; however, upregulating GLT-1 in astrocytes proves neuroprotective during early epileptogenesis, decreasing seizure frequency and duration, and abolishing large behavioral seizures in the IHKA epilepsy model
Ramandi and colleagues, 2021 [27]	The purpose of this study is to investigate the neuroprotective role of astrocytic glutamate uptake via the GLT-1 in the processes related to TLE	Male Wistar rats (200–280 g)	30 min after pilocarpine injection	GLT-1 and GAPDH gene expression levels were measured following RNA extraction, where the concentration and integrity of the extracted RNA were assessed through UV spectrophotometry and gel electrophoresis. Subsequently, cDNA was synthesized using the RevertAid First Strand cDNA Synthesis Kit	Four weeks post-Status Epilepticus, glutamate levels and GLT-1 expression decreased compared to controls, highlighting the impact of astrocytic glutamate uptake on hippocampal epileptogenesis and associated cognitive deficits
Ca2+ signaling and Calcium binding protein					
Zhang and colleagues, 2019 [28]	To investigate the role of astrocytic Ca2+ signaling in epilepsy, particularly in the context of neurovascular coupling (NVC)	14 male Charles-River mice, postnatal 8 weeks old, 20–25 g weight	Potassium channel blocker 4-aminopyridine (4-AP; Sigma; 15 mM, 0.5 µL) injection	OGB-1 was used to monitor dynamic changes in intracellular Ca2+	Despite the mechanism by which astrocytic endfoot Ca2+ was elevated during epileptic events, this study observed that increases in Ca2+ were linked to vasodilation in the focus during each individual ictal event. In the remote area, Ca2+ increases correlated with vasoconstriction at the onset of the seizure and vasodilation during the later part of the seizure
Umpierre and colleagues, 2019 [29]	To investigate the functional signaling properties and downstream consequences of astrocyte metabotropic mGluR5-mediated Ca2+ signaling during the development of epilepsy	Adult male and female mice between 8 and 14 weeks of age	Kainic acid intraperitoneally at a dose of 7.5 mg/kg every 30 min	Calcium activity was assessed using the genetically encoded calcium indicator GCaMP5G. Application of aCSF consistently produced negative calcium responses, while ATP application yielded positive responses, as measured through the dye-spread ROI calcium analysis technique	During epileptogenesis, mGluR5-mediated Ca2+ signaling re-emerges. The selective and conditional knock-out of mGluR5 signaling from astrocytes in epilepsy development slows the rate of glutamate clearance

Table 1 (continued)

Study, Year	Aim	Subjects	Epilepsy Induction	Assessment Methods	Findings
Szokol and colleagues, 2015 [30]	To investigate whether Ca2+ signaling in astrocytic endfeet could contribute to epileptogenesis by triggering a sequence of events leading to the disassembly of the DAPC in the endfoot plasma membrane	Male Charles River C57BL/6N mice of 2–4 months of age	Deep cortical (juxtahippocampal) kainate injection	Recorded astrocytic GCaMP5 fluorescence signals during simultaneous neuronal stimulation using a two-photon microscope. Compared responses on the kainate-injected side with the non-injected (control) side using a 25× water-immersion objective at 900–910 nm laser pulses	The disassembly of endfoot protein complexes, initiated by dystrophin cleavage mediated by Ca2+-dependent proteases, is identified as one of the mechanisms through which astroglia may contribute to hyperexcitability and epileptogenesis
Khamis and colleagues, 2023 [31]	To measure the correlation of the serum levels of S-100B protein in pediatric cases with epilepsy	90 pediatric patients		S-100B samples were collected within 1 h of seizure onset, centrifuged at 5000 rpm for 10 min, and stored at −20 °C until analysis. Serum S-100B protein levels were assessed using the DRG® S-100B (Human) ELISA (EIA-4555) kit from DRG International, Inc., USA	Elevated serum S-100B protein levels may suggest neuronal damage in the brains of children with epilepsy
GABA transporter					
Mermer and colleagues, 2022 [32]	To investigate the patho-mechanisms associated with mutations in the GAT-1-encoding SLC6A1 gene, particularly in the context of MAE	4 patients		Utilizing various machine learning tools, the study predicted the impact of the variant on the GAT-1 protein. Tertiary structures of both the wildtype and mutated GAT-1 proteins were modeled using I-TASSER and analyzed through the MAESTRO web platform	SLC6A1 variants occurring at different locations within the protein peptides can induce F24 MAE with consistent seizure phenotypes and EEG features. The diminished GABA uptake is a result of decreased functional GAT-1, particularly in thalamic astrocytes. This impairment leads to heightened extracellular GABA accumulation and intensified tonic inhibition, ultimately contributing to the occurrence of seizures and abnormal EEG patterns

Table 1 (continued)

Study, Year	Aim	Subjects	Epilepsy Induction	Assessment Methods	Findings
Mazaud and colleagues, 2019 [34]	To investigate the glutamate/GABA/glutamine cycle in major glial developmental determinant, Repo, in adult Drosophila glia and its continuous requirement for the transcriptional regulation of neurotransmitter recycling	*Drosophila* fruitflies	Heat-induced seizure assay	anti-GFP (1/100, mouse, Roche, RRID:AB_390913), anti-GFP (1/500, chicken), anti-Repo (1/100, mouse DSHB 8D12, RRID:AB_528448), anti-Elav (1/500, rat, DSHB 758A10, RRID:AB_528218 or 1/2000 mouse, DSHB, 9F8A9, RRID: AB_528217), and anti-GABA (1/1000, Sigma-Aldrich A2052, RRID:AB_477652)	The transient loss of Repo in Drosophila significantly shortens the lifespan, induces motor deficits, and increases susceptibility to seizures. This effect is attributed, at least in part, to the impairment of the glutamate/GABA/glutamine cycle. The findings underscore the critical role of transcriptional regulation of genes related to the glutamate/GABA/glutamine cycle in glial cells, influencing neurotransmitter levels in neurons and consequently shaping behavioral outcomes
Pirttimaki and colleagues, 2013 [35]	To investigate the GABA transporter current in thalamic astrocytes of the GAERS, a well-established model of absence seizures	8–25-day-old male and female GAERS and NEC rats	Genetically modified epilepsy rats from Strasbourg	GABA transporter currents were elicited through the swift, localized application of a 100 µl solution containing 10 mm GABA using a pipette in close proximity to the targeted astrocyte. The quantification of charge transfer associated with the GABA transporter current was conducted using the Event detection protocols in pCLAMP 9	The study reveals a dysfunction in the astrocytic thalamic GAT-1 transporter in the context of absence epilepsy, indicating an abnormal astrocytic influence on thalamic ambient GABA levels. In addition, it observes that while glutamatergic astrocyte–neuron signaling remains unchanged in the GAERS thalamus, alterations in certain aspects of GABAergic astrocyte–neuron signaling in this epileptic strain may play a role in the onset of absence seizures
Gap junction protein					
Kékesi and colleagues, 2015 [36]	To investigate the role of astrocytic signaling in epileptiform activity, focusing on the global control exerted by the astrocytic syncytium over neuronal networks	12–14 weeks old male Wistar rats	MgSO4 was eliminated and 2 mM KCl was added (low-[Mg2+] ACSF)	Cx43 antibody (Abcam, #ab11370) was applied at 7.5 µg/ml (1:100 dilution) after the first seizure-like event, with perfusion briefly paused and then resumed after a 10-min exposure, while control measurements followed the same protocol without antibody addition	Inhibiting astrocytic gap junction proteins with CBX or Cx43 antibody increased the interictal interval and completely prevented recurrent seizure-like activity in 41% of slices. In addition, CBX induced unsynchronized Ca2+ transients, correlating with a decreased incidence of epileptiform discharges afterward

Table 1 (continued)

Study, Year	Aim	Subjects	Epilepsy Induction	Assessment Methods	Findings
Yoon and colleagues, 2010 [37]	To investigate the role of Cx43 gap junctions in lesion spread and cell loss following epileptiform activity, exploring the dose-dependent protective effect of a Cx43 mimetic peptide in an ex vivo model of epileptiform lesion	6–8-day-old Wistar rats	100 µM bicuculline methochloride	Peptides were synthesized by solid-phase Fmoc chemistry using a Protein Technologies Symphony Instrument and purified by HPLC. The mimetic peptide, with the amino acid sequence VDCFLSRPTEKT, targeted the extracellular loop two of Cx43	The study suggests that during periods of excessive neuronal firing and epileptic stress, Cx43 gap junction communication is essential for tissue survival, but the opening of hemichannels may be damaging. Following epileptiform insult, cx43 gap junction communication becomes crucial for the spread of neuronal damage
Deshpande and colleagues, 2020 [38]	To investigate the impact of astrocytic gap junction disconnection, specifically involving Cx channels, on the development and progression of MTLE with HS	Male C57Bl6/J mice (Charles River) and transgenic mice lacking Cx30 and Cx43 in GFAP-positive cells aged 90–120 days	70 ml of a 20 mM solution of kainate injection	Microglial activation was assessed by quantifying the numbers of BrdU-positive microglia and measuring the areas occupied by Iba1 staining. The degree of astrogliosis was estimated by calculating the area covered by GFAP immunoreactivity. Continuous telemetric EEG recordings and video monitoring performed over a period of 4 weeks	The study observed significantly increased seizure and interictal spike activity during the chronic phase in mice deficient for astrocytic Cx compared to wild-type mice. Interestingly, seizure-induced neurogenesis in the adult dentate gyrus was found to be independent of astrocytic Cxs, suggesting that the constitutive loss of gap junction coupling between astrocytes enhances neuronal hyperexcitability while mitigating seizure-induced histopathological outcomes
Volvona and colleagues, 2022 [39]	To investigate the impact of the GJ blocker CBX on epileptic activity both in vitro and in vivo	Rat	4-aminopyridine	The study conducted a comparative analysis of the instantaneous repetition rate of spike waves within the composition of SWA episodes in experimental groups of rats	The findings suggest that the astrocytic syncytium formed by GJ-associated astrocytes influences the development of epileptic activity in astrocytes in vitro and contributes to the onset of epileptic seizures. The study concludes that this effect is likely an important, though not the sole, mechanism by which CBX suppresses epileptic activity

Table 1 (continued)

Study, Year	Aim	Subjects	Epilepsy Induction	Assessment Methods	Findings
BBB and TGF-βR					
Ivens and colleagues, 2007 [33]	To investigate the mechanisms underlying epileptogenesis in the injured brain, particularly focusing on the role of albumin uptake into astrocytes mediated by TGF-βRs	Wistar rats	ACSF the BBB-disrupting agent DOC, 2 mM or BSA, 0.1 mM, corresponding to 25% of serum albumin concentration	To check whether TGF-βRs mediate albumin uptake into brain astrocytes, cortical slices were exposed to inhibitors targeting TGF-βR1 kinase activity (SB431542) and/or antibodies against TGF-βR2	Direct exposure of the brain to serum albumin leads to albumin uptake into astrocytes, facilitated by TGF-βRs. This process results in activity-dependent increased extracellular potassium accumulation, leading to neuronal hyperexcitability and eventually epileptiform activity. In vivo blocking of TGF-βR reduces the likelihood of epileptogenesis in albumin-exposed brains to 29.3%
Bar-Klein and colleagues, 2014 [40]	To investigate the role of TGF-β signaling in epileptogenesis following vascular injury and to test the efficacy of losartan, an angiotensin II type 1 receptor antagonist known to block TGF-β signaling, in preventing epilepsy	79 adult male Wistar rats (160–240gr)	BSA (0.2 mM in ACSF) or DOC (2 mM)	The study employs a combination of in vitro and in vivo methods, including biochemical assays, gene expression analysis, magnetic resonance imaging, and direct optical imaging to assess blood–brain barrier permeability and vascular reactivity. Long-term electrocorticographic recordings are conducted in freely behaving animals to investigate the impact of losartan on the development of recurrent spontaneous seizures following vascular injury	The study reveals that serum-derived albumin activates the activin receptor-like kinase 5 pathway of TGF-β receptor I in astrocytes. In addition, the angiotensin II type 1 receptor antagonist, losartan, known for blocking peripheral TGF-β signaling, effectively inhibits albumin-induced TGF-β activation in the brain. Most notably, losartan prevents the development of delayed recurrent spontaneous seizures, indicating that TGF-β signaling activated in astrocytes by serum-derived albumin is implicated in epileptogenesis
Prager and colleagues, 2019 [41]	To investigate the impact of recurrent seizures on neurovascular coupling, BBB integrity, and pericytic membrane currents	Wistar rats and heterozygous transgenic mice expressing GFP at the PDGFRβ promoter	4-aminopyridine	Labeling of astrocytic endfeet by calcein-AM allowed for reconstruction of the vessels, whereas pericytes were visualized by the accumulation of MitoSox or by GFP fluorescence in slice cultures obtained from PDGFRβ-bac-GFP mice	The study reveals that recurrent seizures lead to diminished vasodilation responses, increased BBB permeability, and capillary constriction. This suggests that pericyte injury plays a crucial role in disrupting capillary integrity during epilepsy, providing direct observations of neurovascular decoupling during seizures and highlighting pericytic injury as a key factor in vascular dysfunction in epilepsy

4-AP 4-aminopyridine, *ABC* Avidin–biotin complex, *ACSF* Artificial cerebrospinal fluid, *AQP4* Aquaporin 4, *ATP* Adenosine triphosphate, *BBB* Blood–brain barrier, *BSA* Bovine serum albumin, *CA* Cornu Ammonis, *CBX* Carbenoxolone, *CCI* Controlled cortical impact, *CNS* Central nervous system, *Cx43* Connexin 43, *DAPC* Dystrophin-Associated Protein Complex, *DOC* Deoxycholic acid sodium salt, *EEG* Electroencephalography, *GABA* Gamma-aminobutyric acid, *GAT-1* GABA transporter 1, *GCaMP5G* Genetically encoded calcium indicator, *GFAP* Glial fibrillary acidic protein, *GLAST* Glutamate aspartate transporter, *GLT-1* Glutamate transporter 1, *GPCR* G protein-coupled receptor, *IHKA* Intrahippocampal kainic acid, *IR* Immunoreactive, *KO* Knockout, *MAE* Myoclonic atonic epilepsy, *MRI* Magnetic resonance imaging, *NMDA N*-methyl-D-aspartate, *NVC* Neurovascular coupling, *PDGFRβ* Platelet-derived growth factor receptor β, *PTE* Post-traumatic epilepsy, *PTZ* Pentylenetetrazole, *ROI* Region of interest, *S-100B* S100 calcium-binding protein B, *SWA* Slow-wave activity, *TGF-β* Transforming growth factor β, *TGF-βR* TGF-β receptor, *TLE* Temporal lobe epilepsy, *WT* Wild type

dysfunction, characterized by impaired clearance of synaptic glutamate in astrocytes, fosters epileptogenesis by elevating extracellular glutamate levels. This disruption leads to heightened neuronal excitability, synchronization, and increased susceptibility to seizures. Furthermore, four studies [28–31] suggest that dysregulation in Ca2+ signaling contributes to epilepsy by disrupting the delicate balance of intracellular calcium concentrations, leading to abnormal neuronal excitability and synaptic transmission. The involvement of calcium-binding proteins such as S-100B further exacerbates epileptogenesis by influencing processes, such as neuroinflammation, oxidative stress, and neuronal plasticity. Three studies [32, 34, 35] showed that dysregulation of the GABA transporter 1 (GAT-1) in epilepsy leads to impaired GABAergic synaptic transmission, resulting in reduced inhibitory control and heightened neuronal excitability. Four studies [36–39] found that dysregulation of gap junction protein Connexin 43 (Cx43) in astrocytes induces a breakdown in intercellular communication, leading to impaired astrocytic network coordination. This disruption in astrocyte coupling hinders the efficient spread of potassium ions and other signaling molecules, contributing to an imbalance in extracellular homeostasis and increased neuronal hyperexcitability, ultimately promoting epileptogenesis. Finally, three studies [33, 40, 41] showed that impaired BBB integrity allows the infiltration of pro-inflammatory molecules and immune cells into the brain parenchyma, fostering a neuroinflammatory environment. Concurrently, dysregulated transforming growth factor β receptor (TGF-βR) signaling disrupts the astrocytic response to inflammation, compromising their neuroprotective functions and exacerbating neuronal hyperexcitability, thereby contributing to the onset and progression of epilepsy. Each facet of astrocytic dysfunction contributes synergistically to the heightened neuronal excitability characteristic of epileptogenesis. These studies underscore the integral role of astrocytes in the pathophysiology of epilepsy.

Discussion

The following discussion section systematically organizes clinical studies focused on various dysregulations within astrocytes, covering a spectrum of dysfunctions. This categorization prioritizes critical aspects, including potassium buffering, water homeostasis, glutamatergic transmission, calcium signaling, calcium-binding proteins, GABA transporter, gap junction proteins, and BBB integrity. This structured approach aims to provide a thorough analysis of the intricate involvement of astrocytes in epileptogenesis. Each category serves as a discrete analytical framework, elucidating the impact of astrocytic dysregulation on epilepsy, elucidating potential mechanisms, pathways, and therapeutic targets associated with each facet (Fig. 2).

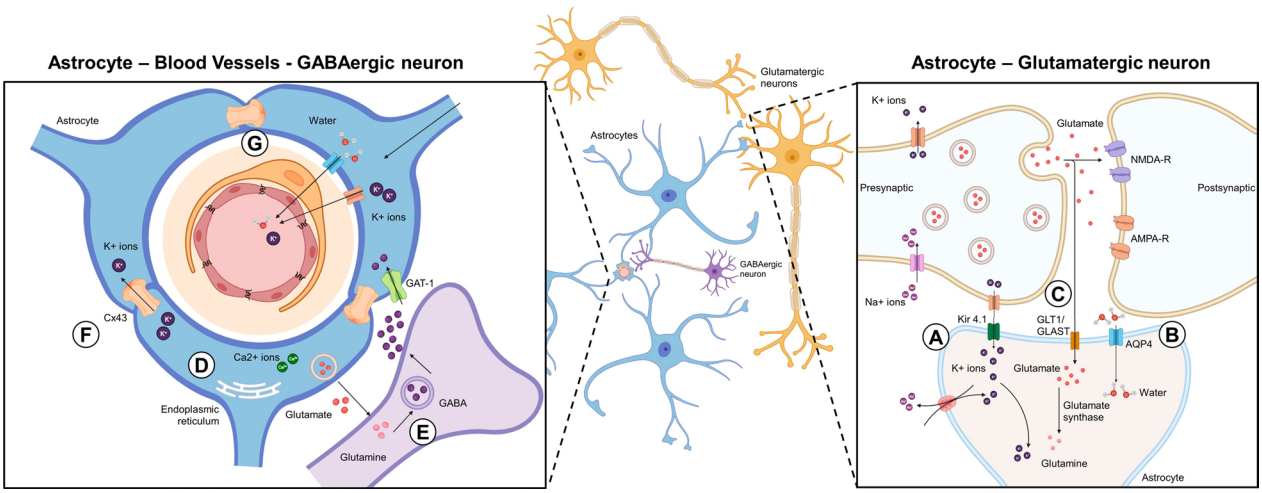

Fig. 2 Astrocytes fulfill diverse functions crucial for neuronal homeostasis. **A** In the context of potassium buffering, astrocytes employ Kir4.1 channels to facilitate the entry of K+ released during neuronal activity, promoting its distribution into capillaries. **B** Addressing water homeostasis, the astrocytic water channel Aquaporin-4 (AQP4) orchestrates water flow between the extracellular space and the blood, maintaining osmotic balance. **C** In glutamatergic transmission, astrocytes utilize glutamate transporters (GLT-1 or GLAST) to uptake glutamate, subsequently converting it to glutamine, a process critical for neurotransmission. **D** In calcium signaling, voltage-gated channels and Ca2+ waves stimulate gliotransmitter release from astrocytes, influencing neuronal excitability. **E** Gamma-aminobutyric acid (GABA) transporter GAT-1 facilitates the removal of GABA from the synaptic cleft, impacting synaptic transmission. **F** Astrocytic gap junction proteins, such as Cx43, enable intercellular communication, contributing to spatial buffering of ions. **G** Astrocytes play a role in the blood–brain barrier (BBB) integrity, where disruptions, as seen in neuroinflammatory conditions, activate microglia and lead to neuroinflammation

Potassium buffering

Potassium buffering, a crucial homeostatic function performed by astrocytes, is integral to the regulation of extracellular potassium concentrations within the central nervous system. Under normal physiological conditions, neurons generate action potentials, leading to potassium efflux into the extracellular space. Astrocytes actively participate in maintaining the ionic balance by swiftly taking up excess potassium ions through various mechanisms [42]. This intricate process involves the activity of Kir channels, particularly Kir4.1, abundantly expressed on astrocytic endfeet. Kir4.1 channels enable astrocytes to effectively clear extracellular potassium, preventing its accumulation and ensuring a stable neuronal environment [43].

These channels play a crucial role in regulating the brain's extracellular potassium ($[K^+]_o$) and water fluxes. During physiological activation and repetitive activation, nonhomogeneous increases in $[K^+]_o$ occur, particularly in specific cortical layers, such as the pyramidal layer of Ammon's horn in the hippocampus or deep layers in the neocortex. The localized increase in $[K^+]_o$ depolarizes astrocytes, leading to spatial buffering of $[K^+]_o$ [44, 45]. Dysregulation in astrocytic Kir4.1 channels has been implicated in epileptogenesis. Studies suggest that downregulation or functional impairment of Kir4.1 channels results in decreased potassium buffering capacity, leading to elevated extracellular potassium levels [46]. This heightened potassium concentration in the extracellular milieu has profound effects on neuronal excitability, potentially contributing to the generation and propagation of epileptic events. Elevated extracellular K^+ concentration ($[K^+]_o$), resulting from defective K^+ regulation, is strongly associated with seizure initiation during hypersynchronous neuronal activities when $[K^+]_o$ reaches peaks of 10–12 mM [47].

Astrocytic Kir4.1 channel dysfunction, induced by KCNJ10 gene mutations or downregulation, elevates extracellular potassium and glutamate levels, triggering neuronal hyperexcitation in epileptogenesis [48]. Research involving glial-specific conditional Kir4.1 KO mice confirmed disrupted K+ homeostasis, offering additional mechanistic evidence supporting the role of abnormal Kir4.1 expression and function in the epileptic phenotype [49, 50]. Linkage analysis conducted in patients presenting with seizures, ataxia, sensorineural deafness, mental retardation, and electrolytic imbalance (SeSAME or EAST syndrome) implicated KCNJ10. Sequencing of the affected KCNJ10 gene in individuals with SeSAME or EAST syndrome revealed loss-of-function mutations within the channel, and subsequent heterologous expression experiments confirmed the functional impact of these mutations on Kir4.1, leading to depolarization [51, 52]. The compromised potassium buffering capacity of astrocytes may induce frequency-dependent synaptic facilitation. The aberrant accumulation of potassium, particularly in the context of epileptic events characterized by increased neuronal activity, may enhance synaptic transmission [53]. This heightened synaptic facilitation, dependent on *N*-methyl-D-aspartate (NMDA) receptor activity, further contributes to neuronal hyperexcitability and network synchronization. Consequently, the dysregulation of potassium buffering in astrocytes emerges as a pivotal factor in the pathophysiological cascade leading to epileptogenesis [53, 54].

Water hemostasis

Dysregulation of water homeostasis, specifically involving AQP4, constitutes a pivotal aspect of astrocyte dysfunction in the epileptogenic process. AQP4, a water channel protein predominantly expressed in astrocytes, plays a fundamental role in maintaining water balance within the central nervous system [55]. In normal physiological conditions, AQP4 facilitates the movement of water across astrocytic membranes, ensuring the precise regulation of extracellular fluid volume. This process is particularly critical in the context of ion and water homeostasis within the brain, where astrocytes actively participate in the intricate maintenance of the microenvironment [56]. During epileptogenesis, dysregulation of AQP4 emerges as a significant contributor to pathological alterations in water homeostasis. Experimental studies have demonstrated that changes in AQP4 expression and function are associated with epileptic activity [21–24]. The sensitivity of neuronal excitability to osmolarity and changes in the extracellular space is manifested through reductions in the extracellular space, resulting in increased concentrations of extracellular ions and neurotransmitters, thereby intensifying ephaptic interactions among closely interacting neurons and fostering heightened synchronous firing and bursting activity, contributing significantly to the complexities of epileptogenesis [57, 58].

Roles in the etiology of water homeostasis dysfunction in astrocytes encompass AQP4 misexpression, AQP4 mislocalization, dysregulation of AQP4 isoforms, loss of AQP4 polarity, and inadequacy in phosphorylation of AQP4 [59]. Following systemic kainic acid-induced status epilepticus in rats, hippocampal AQP4 expression was found to be mislocalized, with reduced density in the adluminal endfeet of astrocytes during the latent phase before chronic epileptic seizures [60]. In a mouse model of post-traumatic epilepsy, AQP4 subcellular redistribution was observed [24]. Furthermore, the pivotal protein in anchoring AQP4 to perivascular end feet astrocytes is dystrophin, a component of the dystrophin-associated

protein complex (DAPC) [61]. This complex interacts with AQP4 via α-syntrophin. The anchoring system is vital for AQP4's physiological role in fluid circulation and ion homeostasis between blood and brain tissue [62]. Through the deletion of α-syntrophin, a considerable and consistently ranging proportion (79–94%) of the perivascular AQP4 pool is eliminated and could precedes the occurrence of chronic seizures [59, 63]. In addition, dysregulation of AQP4 phosphorylation, triggered by CaM activation and PKA-mediated phosphorylation at S276, plays a crucial role in epileptogenesis. The resulting AQP4 translocation to the plasma membrane contributes to seizure initiation, presenting a promising avenue for therapeutic intervention in epilepsy [64].

Glutamatergic transmission

Astrocytes play a pivotal role in maintaining glutamatergic homeostasis within the central nervous system, primarily through the glutamate transporters GLAST (Glutamate Aspartate Transporter) and GLT-1. GLAST and GLT-1, localized on astrocytic processes ensheathing synapses, function to efficiently clear the neurotransmitter glutamate from the extracellular space, preventing excitotoxicity [65]. Dysregulation of these transporters can lead to aberrant glutamate levels, thereby influencing neuronal excitability and contributing to the pathogenesis of epilepsy. In the intrahippocampal kainic acid model of temporal lobe epilepsy, an early upregulation of astrocyte glutamate transporters GLT-1 and GLAST occurs, implying their potential involvement in epilepsy development [66]. Downregulation or impaired function of these transporters results in compromised glutamate clearance, leading to an accumulation of glutamate. Elevated synaptic glutamate concentrations induce overstimulation of postsynaptic glutamate receptors, precipitating hyperexcitability and even excitotoxic neuronal death [67]. Thus, alterations in GLAST and GLT-1 expression, function, or localization can disrupt the delicate balance of glutamatergic neurotransmission.

GLT-1, an Na+-dependent transmembrane symporter, serves as the predominant astrocytic glutamate transporter in the adult human brain, responsible for more than 90% of synaptic glutamate clearance and exhibiting expression levels surpassing GLAST by four to six times in astrocytes [68, 69]. It plays a pivotal role in maintaining the synaptic glutamate gradient, and its dysregulation has been associated with excitotoxicity, neuronal death, and neurological disorders, as evidenced by studies on GLT-1 knockout mice experiencing lethal spontaneous seizures and significant neuronal loss, while functional GLT-1 prevented post-traumatic seizures in a rat traumatic brain injury (TBI) model [70–72]. Various factors, such as genetic predisposition, trauma, or inflammation, may instigate changes in glutamate transporter activity. Jen and colleagues demonstrated that a heterozygous mutation in GLT-1 resulted in reduced glutamate uptake, contributing to neuronal hyperexcitability and leading to manifestations, such as seizures, hemiplegia, and episodic ataxia [73].

Moreover, dysfunction in astrocytic glutamate transporters can impact synaptic plasticity and long-term potentiation, processes crucial for normal neuronal function [74]. Dysregulated glutamate transport may contribute to hyperexcitability and the generation of abnormal neuronal circuits, fostering a conducive environment for the initiation and propagation of seizures [75]. Glutamate transporters tightly control synaptic transmission, influencing long-term plasticity by regulating the spatiotemporal profile of glutamate transients and potentially determining the sensitivity of synapses to various plasticity paradigms [76]. In addition, the altered expression or function of GLAST and GLT-1 in epilepsy may not only affect synaptic transmission but also influence the surrounding microenvironment. The intricate interplay between astrocytic glutamate transporters and neuronal activity underscores their significance in epileptogenesis.

Ceftriaxone, functioning as a transcriptional activator, has demonstrated the capacity to enhance GLT-1 expression during the initial stages of epileptogenesis. This effect may potentially ameliorate cognitive impairments associated with epilepsy by addressing the deficit in glutamate uptake [27, 77]. Although, administration of ceftriaxone has demonstrated negative effects on hippocampal synaptic plasticity and memory recognition [78]. A study conducted by Sha and colleagues found that inhibition of Hsp90 increases GLT-1 levels by disrupting the association between Hsp90β and GLT-1, preventing GLT-1 degradation and suggesting a potential therapeutic target for epilepsy and excitotoxicity through up-regulation of GLT-1 in reactive astrocytes [79].

Ca2+ signaling and calcium binding protein

The dysregulation of Ca2+ signaling and calcium-binding proteins represents a pivotal aspect of astrocyte dysfunction in the pathogenesis of epileptogenesis. Ca2+ serve as crucial intracellular messengers, participating in diverse cellular processes, including neurotransmitter release, gene expression, and modulation of astrocytic function [80, 81]. In the context of astrocyte dysfunction in epileptogenesis, aberrations in Ca2+ signaling are integral to the intricate interplay of cellular events leading to the development and perpetuation of epilepsy [82]. Astrocytes actively engage in bidirectional communication with neurons, and alterations in Ca2+ dynamics profoundly influence this intercellular signaling [83]. Calcium waves within astrocytic networks contribute to the

regulation of neuronal activity, with disturbances in these waves being implicated in epileptic pathophysiology [84]. Intracellular Ca2+ oscillations in astrocytes are tightly regulated by various mechanisms, including purinergic signaling and release from intracellular stores, such as the endoplasmic reticulum [85]. In various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and epilepsy, calcium ion (Ca2+) serves as a crucial second messenger, influencing neuronal excitability. The interplay of voltage-dependent calcium channels (VDCCs), intracellular calcium-binding proteins, and calcium channels within intracellular stores contributes to epileptogenesis [86].

Furthermore, calcium-binding proteins play a pivotal role in shaping astrocytic responses to changes in Ca2+ levels. These proteins, such as calmodulin and S100B, exert modulatory effects on intracellular signaling cascades [87]. S100B exerts its effects on astrocyte function through interactions with various cellular targets. S100B, featuring a helix–loop–helix structure and a calcium-binding domain, plays a crucial role in neurological disorders by activating the MAPK pathway, inducing increased NF-kB expression, and influencing cellular processes, such as survival, proliferation, and gene upregulation [88]. Notably, S100B modulates intracellular calcium levels, acting as both a sensor and effector in calcium-mediated signaling pathways within astrocytes [89]. Dysregulation of calcium-binding proteins in astrocytes has been associated with altered synaptic transmission, compromised neuronal homeostasis, and heightened susceptibility to seizures [31]. Moreover, S100B is intricately linked to neuroinflammatory processes, further exacerbating astrocyte dysfunction in epilepsy [90]. The release of S100B from astrocytes into the extracellular space can activate microglia and perpetuate a proinflammatory milieu. This inflammatory cascade, coupled with disruptions in astrocytic calcium homeostasis mediated by S100B, creates a conducive environment for epileptogenic changes within the neural circuitry [91, 92]. In the epileptogenic milieu, the dysregulation of S100B extends beyond its role in calcium signaling and inflammation. S100B has been implicated in gliosis, contributing to the reactive astrocytic phenotype observed in epilepsy [93, 94]. The sustained activation of astrocytes, characterized by altered morphology and function, is a hallmark of epileptogenic processes, and S100B appears to play a modulatory role in this regard.

GABA transporter

GABA, the primary inhibitory neurotransmitter, modulates excitatory neurotransmission upon release from interneurons. Astrocytic glutamine is transported to GABAergic neurons, where it undergoes conversion to glutamate and then promptly to GABA via glutamate decarboxylase (GAD) [95]. GABA, crucially reliant on the tricarboxylic acid (TCA) cycle intermediaries, faces deficits in transmitter production during cellular energy metabolism inefficiencies, often observed after compromised tissue perfusion and heightened neuronal metabolic demand [96]. Presynaptically stored GABA is released onto various postsynaptic terminals, and the majority is reclaimed by its transporter, GAT-1, into the presynaptic neuron for recycling into vesicles. Under normal conditions, GAT-1 is responsible for the majority of GABA reuptake in astrocytes, preventing excessive accumulation in the synaptic cleft and maintaining inhibitory tone. However, in epileptogenesis, alterations in the expression and function of GAT-1 lead to impaired GABAergic neurotransmission [97]. This dysfunction may manifest as reduced GABA uptake, resulting in prolonged GABAergic signaling and increased susceptibility to seizures [98, 99]. GABA transporter dysregulation, encompassing various isoforms, such as GAT-1, plays a pivotal role in the complex process of astrocyte dysfunction during epileptogenesis [99]. GABA, a major inhibitory neurotransmitter in the central nervous system, is actively reuptaken by astrocytes through GABA transporters, ensuring precise regulation of its extracellular concentrations [100]. The dysregulation of GABA transporters, notably GAT-1, disrupts this delicate balance, contributing to aberrant inhibitory signaling within neuronal networks [101]. Moreover, dysregulated GABA transporters influence the availability of GABA for extrasynaptic signaling, impacting tonic inhibition. The heightened tonic inhibition stems from compromised GABA uptake by the GABA transporter GAT-1 in the tested genetic models, playing a crucial role in seizure genesis [102]. The altered GABAergic tone can contribute to hyperexcitability in neuronal circuits, fostering a pro-epileptic environment. The intricate interplay between GABA transporters and the homeostatic control of inhibitory neurotransmission underscores their significance in the epileptogenic process.

Gap junction protein

Gap junction protein dysregulation, particularly involving Cx43, stands as a pivotal factor in the intricate process of astrocyte dysfunction contributing to epileptogenesis [103]. Astrocytes, integral to maintaining neuronal homeostasis, express Cx43, forming gap junctions that facilitate direct intercellular communication [103]. In epileptic conditions, alterations in Cx43 expression and function disrupt normal signaling cascades between astrocytes, compromising their regulatory roles [104]. Astrocytic Cx channels have been implicated in epilepsy, particularly in the sclerotic hippocampus of temporal

lobe epilepsy (TLE) patients, where astrocytic gap junction (GJ) coupling is lost despite sustained expression of Cx isoforms [105]. Dysregulated Cx43 can result from genetic mutations, aberrant post-translational modifications, or environmental triggers, precipitating an array of pathological consequences [106, 107]. The dysregulation of Cx43 in astrocytes significantly impacts potassium buffering, a crucial function in the prevention of extracellular potassium accumulation [44]. Disrupted gap junctional communication due to Cx43 abnormalities compromises the spatial buffering capacity of astrocytes, leading to elevated extracellular potassium concentrations [44, 108]. This heightened potassium environment fosters neuronal hyperexcitability and synchronous firing, which are hallmark features of epileptic activity. Moreover, dysregulated Cx43 contributes to altered calcium wave propagation among astrocytes, influencing neurotransmitter release and perpetuating the epileptic milieu [109].

Astrocytes, with their extensive network, play a pivotal role in immune response modulation. Cx43 dysregulation further intertwines with neuroinflammation, a prominent aspect of epileptogenesis [110]. Dysfunctional Cx43 hampers astrocytic coordination in responding to inflammatory cues, potentially exacerbating neuroinflammatory processes [111]. In addition, impaired Cx43-mediated gap junctions hinder the formation of astrocytic scar tissue, crucial for containing aberrant neuronal activity [112, 113]. This deficiency may contribute to a persistent pro-epileptic microenvironment. Beyond localized effects, Cx43 dysregulation extends its impact to long-range network dynamics. Altered astrocytic connectivity disrupts the synchronization of neural networks, fostering hypersynchrony associated with epileptic seizures [36]. Compromised gap junctions may impede the propagation of antiepileptic signals, further tipping the balance towards hyperexcitability [114]. The intricate interplay of Cx43 dysregulation in both local and network-level astrocytic functions underscores its multifaceted role in the pathogenesis of epilepsy.

BBB and TGF-βR

BBB dysfunction and dysregulation of TGF-βR signaling contribute significantly to astrocyte dysfunction in epileptogenesis. The BBB, a dynamic interface between the blood and the CNS, maintains homeostasis by selectively restricting the passage of substances [115]. In epilepsy, compromised BBB integrity leads to the infiltration of blood-derived factors into the brain parenchyma [116]. This breach triggers a cascade of events, including activation of astrocytes, which respond to the altered microenvironment. Upon exposure to blood-derived factors, astrocytes undergo phenotypic changes characterized by increased reactivity and altered expression of various transporters and receptors [117, 118]. Notably, dysregulated TGF-βR signaling is implicated in these astrocytic alterations [33, 40]. TGF-βR modulates astrocyte function in response to BBB disruption. Dysfunctional TGF-βR signaling exacerbates astrocyte reactivity, contributing to a pro-inflammatory environment conducive to epileptogenesis [119]. This altered astrocytic state, in turn, can further compromise BBB integrity, creating a positive feedback loop [40, 120]. This altered state contributes to network hyperexcitability, excitatory–inhibitory (E/I) imbalance, and cognitive deficits, with implications for synaptic remodeling and increased expression of c1q, a complement protein associated with synapse elimination [121–124]. In addition, TGF-βR dysregulation may influence the expression of tight junction proteins at the BBB, exacerbating permeability changes and allowing increased entry of inflammatory mediators [125].

Several limitations must be acknowledged in the scope of this comprehensive review. First, the majority of evidence is derived from experimental models, primarily rodents, and translation to human epileptogenesis necessitates caution. Human astrocytic heterogeneity, particularly in pathological conditions, adds complexity, and the extent to which findings from animal models accurately reflect human astrocyte behavior remains a subject of ongoing investigation. Furthermore, the diversity of epilepsy etiologies and patient populations introduces variability that challenges the generalizability of specific astrocytic dysregulations across different forms of epilepsy. In addition, the interdependence of various astrocytic functions and the intricate crosstalk with other cell types in the brain necessitate further elucidation. The evolving landscape of astrocyte research might bring forth new insights beyond the current scope, prompting continual reassessment and refinement of our understanding.

Conclusions

Astrocytic dysfunctions in epilepsy encompass disruptions in potassium buffering, water homeostasis, glutamatergic transmission, calcium signaling, calcium-binding proteins, GABA transporters, gap junction proteins, and BBB integrity. These disruptions collectively contribute to epileptogenesis by fostering a hyperexcitable environment. Dysregulated potassium buffering and impaired water homeostasis exacerbate seizure susceptibility, while altered glutamatergic transmission and calcium signaling promote excitotoxicity and hyperexcitability. Dysfunctional GABA transporters disrupt inhibitory neurotransmission, and gap junction protein dysregulation impacts neuronal synchronization,

contributing to epileptogenic circuitry. Compromised BBB integrity allows entry of epileptogenic factors into the brain parenchyma. A comprehensive understanding of these astrocytic dysfunctions is essential for unraveling epilepsy's complex pathogenesis, emphasizing the need for holistic approaches, particularly in human studies, to show their interplay effectively.

Abbreviations

AQP4	Aquaporin 4
BBB	Blood–brain barrier
Cx43	Connexin 43
GABA	Gamma-aminobutyric acid
GLAST	Glutamate aspartate transporter
GLT-1	Glutamate transporter 1
Kir4.1	Astrocytic Inward Rectifier Potassium Channels 4.1
TGF-βR	Transforming growth factor β receptor

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The initial concept for this literature review was hatched by KTS. The text was written by BGL, and KTS with guidance from INMA, IPEW, and NML. KTS, BDL, INMA, IPEW and NML completed, copyedited and revised the manuscript. All authors assisted in reviewing, composing the manuscript, creating the figures and reviewing the final manuscript.

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