

REVIEW ARTICLE

Written in the Stars: Astrocyte Biology From Evolution to Disease

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ABSTRACT

In the 21st century, neuroglial research has entered a period of Renaissance, extending the views of prominent neuroanatomists and neurologists of the 19th and early 20th centuries, who assigned to glial cells numerous physiological functions and highlighted their fundamental role in the pathophysiology of nervous system diseases. Astrocytes are highly diversified in structure and function; they control brain homeostasis, support synaptic connectivity, and enable information processing in neural networks. Evolutionary diversification of astrocytes, initially emerging as supportive cells of primitive sensory organs, drove a continuous expansion of astroglial complexity and functional versatility, ultimately making them indispensable neuroprotectors and homeostatic regulators. The large, morphologically elaborate astrocytes of the human brain arguably reflect an evolutionary response to increased neuronal homeostatic demands. Astrocytes are indispensable for synaptic function, serving as the principal regulators of neurotransmitter turnover and neuronal excitability. Astrocytes also govern brain energy metabolism, mitochondrial dynamics, and calcium signaling, thereby actively shaping cortical plasticity and circuits. Astrocytes are fundamental elements of the pathophysiology of neurological, neuropsychiatric, and neurodegenerative diseases, including Alzheimer's and Parkinson's disease, amyotrophic lateral sclerosis, Rett syndrome, genetic astrocytopathies, and neurotrauma, where they demonstrate complex reactive changes directed at tissue preservation and regeneration, but which can also contribute to disease progression. Advances in single-cell transcriptomics, calcium imaging, chemogenetics, and iPSC-based models have transformed our understanding of astrocyte diversity and disease-specific dysfunction, opening new avenues of investigation. Given that no CNS disorder is known to occur without astrocyte involvement, multiple astrocyte-specific molecules represent compelling targets for cell-directed therapeutic strategies.

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Practioner Points

- Astrocytes are active regulators of brain homeostasis and synaptic function throughout a variety of species, and their dysfunction drives pathology across the full spectrum of neurological, neuropsychiatric, and neurodegenerative diseases.
- Reactive astrogliosis is double-edged, initially neuroprotective, but capable of contributing to disease progression in chronic conditions including Alzheimer's disease, ALS, and Parkinson's disease.
- Multiple astrocyte-specific molecules represent compelling targets for cell-directed therapeutic strategies across CNS disease.

1 | Introduction

Astrocyte research has greatly benefited from the convergence of technical advances including single-cell RNA sequencing, spatial transcriptomics, advanced imaging, chemogenetics, and human iPSC-based models, revealing layers of molecular complexity, functional diversity, and dynamic regulation within these glial cells that were previously inaccessible. In this review, we discuss key aspects of astrocyte biology spanning their evolutionary origins, developmental programs and diversity, physiological roles in brain metabolism and neural circuits, and their involvement in pathological conditions including neurodegeneration, neurodevelopmental disorders, and neurotrauma.

We trace the evolutionary trajectory of astroglia from primordial supportive cells in flatworms and nematodes to the morphologically elaborate protoplasmic astrocytes of the primate brain, with each human astrocyte contacting up to two million synapses per cell. Astrocytogenesis is driven by lineage-intrinsic programs and local neuronal cues, generating region-, layer-, and circuit-specific molecular identities that are established early and maintained throughout life, with individual astrocyte processes potentially functioning as compartmentalized units at distinct synapse types. Astrocyte physiology encompasses brain energy metabolism, mitochondrial dynamics, Ca^{2+} signaling, cortical plasticity, and homeostatic control of feeding circuits. In pathological contexts, astrocyte dysfunction emerges as an active contributor to disease onset and progression across hormone-related mood disorders, Rett syndrome, Alzheimer's and Parkinson's disease, amyotrophic lateral sclerosis (ALS), genetic astrocytopathies, and neurotrauma.

Together, these sections build toward a central thesis: that the evolutionary diversification and progressive specialization of astrocytes (from primitive homeostatic cells to the morphologically and molecularly elaborate glia of the primate brain) is exactly what made them indispensable neuroprotectors, and consequently central contributors to the pathophysiology of neurological, neuropsychiatric, and neurodegenerative diseases. Understanding astrocyte biology in its full evolutionary and functional complexity therefore represents one of the most consequential frontiers in modern neuroscience, with direct

implications for therapeutic development across the entire spectrum of brain disorders.

2 | Astrocyte Evolution

Across evolution, astroglia—a broad class of homeostatic cells of the CNS encompassing astrocytes, radial astrocytes, neural stem cells and ependymoglia [1]—diversified in parallel with expanding cellular architecture of the nervous tissue. The emergence of neuroglia as the class of supportive cells reflects evolutionary specification and segregation of functions associated with ever-increasing complexity of neural circuitry and neural tissue [2]. The supportive neural cells, which may be defined as primeval neuroglia, are found in flatworms Acoelomorpha and in nematodes [3]. This primeval neuroglia (probably of mesenchymal origin) are associated with terminals of sensory neurons and form sensory organs known as sensilla [4–6]. Such glial-neuronal association is evolutionarily conserved all the way to humans; as all organs of special senses (taste buds, organ of Corti, olfactory systems, etc.) contain supportive glial cells, which are indispensable for the function of these organs [7]. The nervous system of *Caenorhabditis elegans* also contains four cephalic glial cells that project to neuronal cell bodies, providing them with homeostatic support [6]. Platyhelminths developed a centralized nervous system composed of the anterior brain (a pair of cephalic ganglia), the longitudinal nerve cords, and several plexi distributed throughout the body. The cephalic ganglia of platyhelminths contain glial cells of mesenchymal origin that control the homeostasis of the neuropil [8, 9]. In Planaria glial cells of the cephalic ganglia have characteristic morphology with multiple processes and are endowed with glutamate, GABA, and glucose transporters and glutamine synthetase, which altogether indicate their contribution to neurotransmitter homeostasis [9].

In Annelida, glial cells further diversify and contribute to numerous homeostatic functions. In the earthworm, four distinct types of neuroglial cells have been distinguished: neurilemmal, subneurilemmal, supporting-nutritive, and periaxonal sheath-forming glia [10]. Neurilemmal glia are elongated with long processes; subneurilemmal glial cells are small, spindle-shaped, and sparsely branched; supporting-nutritive glia are glial fibrillary acidic protein (GFAP)-immunopositive (GFAP being a widely used marker of mammalian astrocytes), and form brush-like arrays along neuronal somata; sheath-forming glia surround the giant axons [10]. In medicinal leech, each segmental ganglia and both anterior and posterior brains (essentially fused segmental ganglia) contain two giant glial cells, along with two connective glial cells, which ensheath axons, and six packet cells that cover neuronal cell bodies [11, 12]. The giant glial cells, which dwell in the central neuropil, have large somata ($>100\mu\text{m}$ in diameters) and extensively branched processes that appose synaptic structures. These glial cells express a substantial variety of receptors to neurotransmitters (including ionotropic and metabotropic glutamate receptors, nicotinic acetylcholine receptors, ionotropic and metabotropic serotonin receptors, metabotropic purinoceptors, and metabotropic receptors to myomodulin), ion channels and transporters, therefore, exerting homeostatic

control of synaptic transmission, particularly by regulating extracellular glutamate, choline and pH [11]. Packet glia buffer extracellular K⁺, and giant glial cells extend processes that contact neuronal dendrites and provide homeostatic control over the neuropil [13–15]. All glial cells in the leech nervous system are connected with innexin-based gap junctions thus forming panglial syncytium [16].

In insects, neuroglial diversity is quite exceptional; they are characterized by a wide array of names that reflect their location and roles across the CNS, peripheral nervous system (PNS) and sensory organs. A unifying modern framework groups them into: surface glia, encompassing perineurial and subperineurial layers that form the hemolymph-brain barrier; cortex (cell-body/satellite) glia that ensheath neuronal somata; neuropil glia, including epithelial, marginal, ensheathing, and astrocyte-like types; peripheral wrapping glia that resemble nonmyelinating Schwann cells. Classic lamina terminology also includes “pseudocartridge” and “fenestrated” surface glia, while other historical descriptors capture region-specific morphologies such as “interface” glia. In parallel, sensory structures are supported by accessory cells—trichogen, tormogen, and thecogen in olfactory sensilla—and specialized chordotonal-organ cells such as cap and scolopale cells; in the eye, pigment and Semper (cone) glia-like cells provide additional support [17]. Together, this classification captures a genuine diversity rather than mere nomenclature, further reinforced by developmental and single-cell studies in *Drosophila* [18–25]. In locusts the ultrastructural analysis of the metathoracic ganglion revealed seven major forms (together with subdivisions) of neuroglia, illustrating how morphological specializations map to function. These encompass transport glia, subperineurial (barrier) glia, cell-body/satellite glia—including a subset closely associated with large motor neurons—axon-hillock glia, “glial-glia” that primarily contact other glia, neuropilar glia within synaptic regions, and tracheal-associated glia that ensheath tracheoles coursing through nervous tissue [26].

In *Drosophila melanogaster*, a population of astrocytes-like glia (ALG) cells that are somewhat similar to vertebrate astrocytes has been characterized. ALG are part of the neuropil glia, as they extend their processes deep into the neuropil where they closely contact synapses to modulate their formation and function [27]. Their cell bodies, however, are mostly located in the cortex [reviewed in [28]]. *Drosophila* ALG cells exhibit major similarities to vertebrate astrocytes across morphological, functional and molecular level, strongly suggesting a close similarity between these cells [reviewed in [29]]. Specifically, like vertebrate astrocytes, *Drosophila* astrocytes-like glia cells bear a complex spongioform morphology characterized by an intricate network of fine processes that tightly contact synapses, tile the neuropil and occupy distinct territories with minimal overlap of their projections [27, 30, 31]. Functionally, *Drosophila* ALG cells play similar roles to those of astrocytes in vertebrates, including the maintenance of ionic homeostasis [32], regulation of the balance between inhibitory and excitatory neurotransmitters, and clearance of glutamate and GABA [33, 34], both neurotransmitters being inhibitory in *Drosophila* [35]. Additionally, ALG cells are involved in synapse formation and refinement during development [36]. In both larvae and adults, ALG cells express

molecular markers also found in vertebrate astrocytes including GABA transporter (GAT) [33], excitatory aminoacidic transporter 1 (EAAT1) [31] and the glutamine synthetase 2 (Gst2) that converts glutamate to glutamine [22]. Some crucial mechanisms underlying gliogenesis, such as the role of Notch signaling in the specification of both vertebrate and *Drosophila* astrocytes, are evolutionary conserved [37]. Moreover, the generation and expansion of astrocyte populations depend on intermediate neural progenitors (INPs), derived from Type II neuroblasts in *Drosophila*. These progenitors undergo amplification during development to then differentiate into ALG cells [38].

Despite these similarities, notable differences between vertebrate astrocytes and ALG cells must be considered when using *Drosophila* as a model for investigating astrocyte development and function. The most significant distinctions arise during early phases of astrocyte development, including progenitor expansion, stereotyped production, cellular migration and maturation. These differences stem primarily from two factors. First, *Drosophila* brain has a markedly different morphology from that of vertebrates, and the position and migration pattern of neuropil-glia precursors in the embryo are well characterized and highly stereotyped [38]. Second, *Drosophila* neurodevelopment occurs in three different stages encompassing the embryonic, larval and pupal stages. Larval astrocytes-like glia cells originate from embryonic progenitors but undergo apoptosis during early stages of metamorphosis [36, 38, 39]. While the exact origin of adult ALG cells remains largely unclear, Type II neuroblasts in the larval brain generate INPs that can differentiate into adult ALG cells [37]. Additionally, some larval neuropil glia, namely ensheathing glia (EG), dedifferentiate during the pupal stage to give rise to adult ALG cells [40].

While *Drosophila* studies have revealed conserved mechanisms of glial development, circuit assembly, and behavior—and even on models that are relevant to disease—these translational aspects are only briefly addressed here. Similarities between insect astrocyte-like cells and vertebrate astrocytes are best viewed as analogies: developmental control differs fundamentally (e.g., insect gliogenesis hinges on *glial cells missing/gcm*, encoding a key transcription factor without a comparable CNS role in mammals), supporting the view that glia most likely underwent divergent evolution across major metazoan lineages [41, 42].

In vertebrates, the emergence of radial glia represents an evolutionary novel ancestral astroglial form. Radial glia are phylogenetically ancient, emerging in echinodermata (sea urchin, starfish, and sea cucumber, among the most ancient chordata) [43, 44] and persisting as the principal glia in many lineages, where they serve as progenitors and execute key astrocyte-like homeostatic functions [45]. From their evolutionary origins in early chordates, where radial glia perform many of the functions attributed to astrocytes, glial cells underwent significant diversification in parallel with an increase in cortical thickness and neural circuit expansion. In amniotes such as lampreys and teleost fish, radial glia persist throughout life, functioning as both progenitors and homeostatic cells, often carrying astrocyte-like functions including neurotransmitter clearance, metabolic support, and water homeostasis

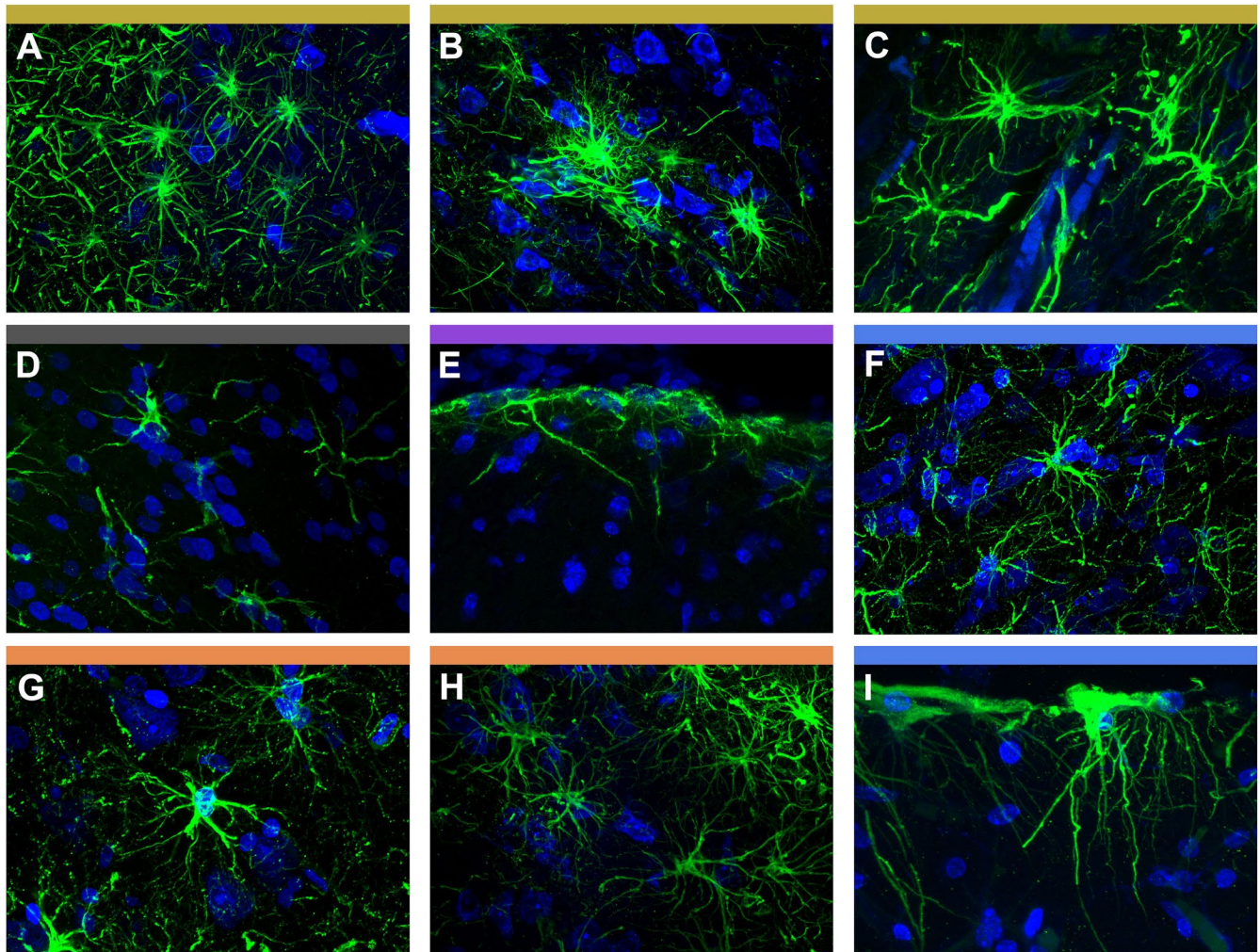


FIGURE 1 | Astrocytes from different mammals. GFAP immunostaining tested on a wide variety of mammalian species (i.e., *Mus musculus* (mouse), *Macaca mulatta* (Rhesus macaque), *Macaca fascicularis* (crabeating macaque), *Homo sapiens* (human), *Pan troglodytes* (chimpanzee), *Carollia perspicillata* (Seba's short-tailed bat), *Bos taurus* (cattle), *Tursiops truncatus* (common bottlenose dolphin), *Panthera leo* (lion), *Panthera tigris* (tiger), *Panthera pardus* (leopard)). On the right, indication of the correspondent orders. Color coded is as follows: Rodentia in gray; Primates in dark yellow; Chiroptera in purple; Artiodactyla in blue; Carnivora in orange. (A–I) In green, immunostaining for Glial fibrillary acidic protein (GFAP) labeling astrocyte intermediate filament-III; in blue, NeuroTrace labeling nuclei. Species of the brain sections are indicated per each micrograph. Each micrograph is color labeled depending on the order the species belongs to. Scale bars = 20 μ m.

[43, 46, 47]. In amphibians (like the African clawed frog) and reptiles (like turtles), transitional forms emerge, where protoplasmic astrocyte-like cells coexist with radial glia in the postnatal brain, suggesting a gradual shift in glial identity and anatomy [48].

In mammals, astrocytes diversify not only into classical protoplasmic and fibrous types (Figure 1), but also into specialized radial derivatives (Bergmann and Müller glia) and regionally distinct parenchymal forms, supporting synaptic integration, metabolic coupling, and neurovascular regulation in a domain-organized manner [49–53]. Astrocyte size, complexity, and molecular programs then scale with brain expansion. Electron microscopy in monotremes (platypus, echidna) first highlighted how early-diverging mammals differ from therians: immature monotreme brains contain a single macroglial population with dense cytoplasm/nucleoplasm and microtubule organization unlike therian oligodendrocytes (not circumferential) or astrocytes

(not filament-associated), suggesting a hybrid phenotype with functions of both major glia; however, contemporary follow-up data remain scarce [54]. In marsupials, the opossum (*Didelphis virginiana*) exhibits astrocytes and “transitional” glia but lacks typical adult ependymal cells; opossum astrocytes vary in soma shape and extend processes to vessels, neurons, and neighboring glia with region-specific distributions across hippocampus, neo-cortex, and other areas [55]. Studies in cattle and horse similarly emphasize expansive networks of protoplasmic and perivascular astrocytes contacting pyramidal neurons and the vasculature in the cortex and hippocampus, alongside “transitional” forms with intermediate features between protoplasmic and fibrous astrocytes, or even between protoplasmic astrocytes and oligodendrocytes as described in classic histology [56, 57]. In carnivores, ferret visual cortex astrocytes are roughly twice the size of rodent cortical/hippocampal astrocytes yet smaller than human. Moreover, ferret astrocytes also deviate from the canonical “tiling” model by showing substantial overlap among neighboring territories [58].

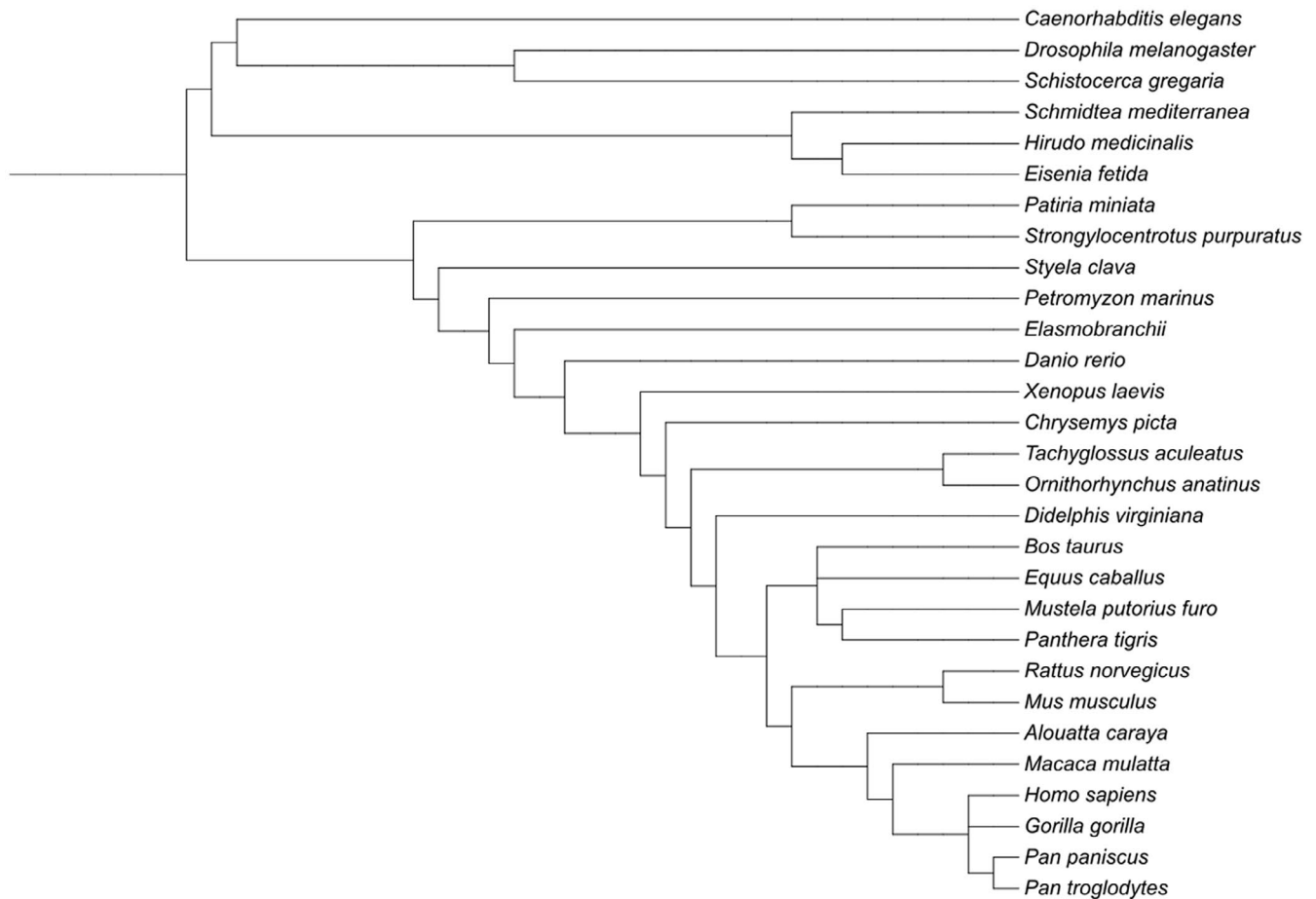


FIGURE 2 | Phylogenetic overview of the species mentioned in the review. Phylogenetic tree of the species referenced in the “Astrocyte evolution” section. Evolutionary tree made with <https://phylot.biobyte.de/>.

In primates, and particularly humans, astrocytes are structurally and functionally more complex than in other mammals. Human protoplasmic astrocytes are significantly larger than those in rodents, with up to 2.5-fold more primary processes and a tenfold greater volume, allowing them to contact and potentially coordinate activity across hundreds of thousands of synapses [51, 59]. Certain astrocyte subtypes, such as interlaminar astrocytes that extend radially through multiple cortical layers show primate-specific features in terms of density, morphological complexity, molecular markers and developmental trajectories [49, 50]. These cell types arguably support long-range coordination across cortical layers and columns, reflecting an adaptation to the expanded and folded neocortex. Finally, varicose-projection astrocytes had been thought to exist only in humans and chimpanzees [51]; however they have been recently shown to be present not only in other apes, including bonobo, gorilla, and gibbon, but they have also been observed in other mammals such as rodents (i.e., mice), and carnivores (i.e., tigers) [60]. Recent advances in induced pluripotent stem cell (iPSC)-based systems demonstrated that evolutionary changes in astrocyte biology can be modeled in vitro [59]. Comparative analyses of human, chimpanzee, and macaque iPSC-derived astrocytes revealed that human astrocytes recapitulate key evolutionary features observed in vivo, including increased cell size and morphological complexity. Mechanistically, these phenotypes were linked to evolutionary activation of Hippo-TEAD signaling, as inhibition of TEAD activity selectively reduced the complexity of

human astrocytes [59]. Human astrocytes have also been shown to produce increased numbers of extracellular vesicles (EVs), which carry signals that promote astrocyte growth and complexity in recipient cells, suggesting that long-range intercellular communication has contributed to astrocyte evolution [59]. At the transcriptional level, genes associated with intellectual disability and chromatin regulation underwent pervasive changes in expression during recent primate astrocyte evolution, indicating extensive remodeling of neuron-supportive and regulatory programs [59]. Importantly, these genes encode factors essential to maintain proper chromatin three-dimensional arrangement, indicating an important role of proper DNA-regulatory dialogue in primate brain evolution [59]. The iPSC-based models allow the isolation and culture of astrocytes, which in turn enable detailed profiling of regulomes and the identification of key DNA elements that control gene expression. The recent comparative epigenomic analyses identified an evolutionary gain in enhancer activity linked to the acquisition of binding sites for broadly acting “stripe” transcription factors, suggesting that rewiring of the cis-regulatory architecture contributed to the emergence of human-specific astrocyte states [59]. The iPSC-derived astrocytes (iAstrocytes) system offers the unique possibility to study how human and nonhuman primate astrocytes impact neuronal development and activity. The next steps in this research line are to leverage iAstrocytes to investigate the potential implications of evolutionary changes in human astrocyte biology for the regulation of how neuronal development unfolds over time.

TABLE 1 | Astrocyte-like glial cells across species: Evolutionary overview.

Scientific name	Common name	Taxonomic group	Summary of astrocyte/astrocyte-like cells information
<i>Caenorhabditis elegans</i>	Roundworm/ Nematode	Nematoda	Four cephalic glial cells projecting to neuronal cell bodies, providing homeostatic support. Glial-neuronal sensilla associations in sensory organs.
<i>Schmidtea mediterranea</i>	Freshwater planarian	Platyhelminthes	Cephalic ganglia glia: characteristic morphology with multiple processes; glutamate, GABA, glucose transporters; glutamine synthetase; all indicate contribution to neurotransmitter homeostasis. Glial cells of mesenchymal origin controlling neuropil homeostasis.
<i>Lumbricus terrestris</i>	Earthworm	Annelida (Oligochaeta)	Four types of neuroglial cells: neurilemmal, subneurilemmal, supporting-nutritive (GFAP-immunopositive), and periaxonal sheath-forming glia. Supporting-nutritive glia form brush-like arrays along neuronal somata.
<i>Hirudo medicinalis</i>	Medicinal leech	Annelida (Hirudinea)	Each segmental ganglion: 2 giant glial cells (neuropil), 2 connective glia (ensheath axons), 6 packet cells (cover cell bodies). Giant glia: > 100 μ m soma, branched processes, diverse neurotransmitter receptors (ionotropic/metabotropic GluR, nAChR, 5-HT, purinoceptors, myomodulin), regulate extracellular glutamate, choline, and pH. Connected by innexin gap junctions forming panglial syncytium.
<i>Schistocerca gregaria</i>	Desert locust	Insecta (Orthoptera)	Metathoracic ganglion: seven major glial forms including transport glia, subperineurial barrier glia, cell-body/satellite glia, axon-hillock glia, glial-glia, neuropilar glia, and tracheal-associated glia.
<i>Drosophila melanogaster</i>	Fruit fly	Insecta (Diptera)	Astrocyte-like glia (ALG): spongiform morphology, tile neuropil, contact synapses, minimal territory overlap. Express EAAT1, GAT, Gs2. Regulate ionic homeostasis, glutamate/GABA balance, synapse formation. Gliogenesis depends on gcm transcription factor (not conserved in mammals). Notch signaling conserved with vertebrates.
<i>Strongylocentrotus purpuratus</i>	Purple sea urchin	Echinodermata	Radial glia-like cells present; among most ancient chordates to show radial glia, serving progenitor and homeostatic functions. Evidence for phylogenetically ancient origin of radial glia.
<i>Patiria miniata</i>	Bat star (starfish)	Echinodermata	Radial glia documented in nervous system; part of evidence base for ancient origin of vertebrate-type radial glia.
<i>Holothuria</i> sp.	Sea cucumber	Echinodermata	Radial glia-like cells present; contributes to evidence of radial glia emerging in echinoderms.
<i>Petromyzon marinus</i>	Sea lamprey	Agnatha (Petromyzontidae)	Radial glia persists throughout life; functions as progenitor and homeostatic cell with astrocyte-like roles including neurotransmitter clearance, metabolic support, water homeostasis.
<i>Danio rerio</i>	Zebrafish	Actinopterygii (Teleostei)	Teleost radial glia persists into adulthood; performs astrocyte-like functions (neurotransmitter clearance, metabolic support, water homeostasis). Model for radial glia-astrocyte continuum.
<i>Elasmobranchii</i> (subclass)	Sharks and rays	Chondrichthyes	GFAP, S-100, and glutamine synthetase immunohistochemistry used to characterize astroglia. Evolutionary changes in astroglia compared to amniotes documented [47].
<i>Xenopus laevis</i>	African clawed frog	Amphibia (Anura)	Transitional forms: protoplasmic astrocyte-like cells coexist with radial glia in postnatal brain; suggests gradual evolutionary shift in glial identity.
<i>Chrysemys picta</i>	Painted turtle	Reptilia (Testudines)	Transitional glial forms; protoplasmic astrocyte-like cells coexist with radial glia, indicating gradual shift from radial glia to astrocyte-type glia characteristic of amniotes.

(Continues)

TABLE 1 | (Continued)

Scientific name	Common name	Taxonomic group	Summary of astrocyte/astrocyte-like cells information
<i>Ornithorhynchus anatinus</i>	Platypus	Mammalia (Monotremata)	Immature monotreme brains contain a single macroglial population with dense cytoplasm/nucleoplasm and microtubule organization unlike therian oligodendrocytes or astrocytes; may represent a hybrid phenotype.
<i>Tachyglossus aculeatus</i>	Short-beaked echidna	Mammalia (Monotremata)	Same hybrid macroglial phenotype as platypus; contemporary follow-up data remain scarce.
<i>Didelphis virginiana</i>	Virginia opossum	Mammalia (Marsupialia)	Exhibits astrocytes and “transitional” glia but lacks typical adult ependymal cells; opossum astrocytes vary in soma shape and extend processes to vessels, neurons, and neighboring glia with region-specific distributions.
<i>Bos taurus</i>	Cattle	Mammalia (Artiodactyla)	Expansive networks of protoplasmic and perivascular astrocytes contacting pyramidal neurons and vasculature in cortex and hippocampus; “transitional” forms with intermediate features described.
<i>Equus caballus</i>	Horse	Mammalia (Perissodactyla)	Protoplasmic and perivascular astrocyte networks documented; transitional forms between protoplasmic astrocytes and oligodendrocytes noted in classic histology.
<i>Mustela putorius furo</i>	Ferret	Mammalia (Carnivora)	Visual cortex astrocytes roughly twice the size of rodent cortical/hippocampal astrocytes but smaller than human. Deviate from canonical “tiling” model: substantial overlap among neighboring territories.
<i>Panthera tigris</i>	Tiger	Mammalia (Carnivora)	Varicose-projection astrocytes recently reported [60], expanding their known distribution beyond great apes to carnivores.
<i>Mus musculus</i>	Mouse	Mammalia (Rodentia)	Primary model organism. Astrocytogenesis from E16; layer-specific transcriptional profiles; multiple molecularly defined subtypes. Varicose-projection astrocytes also observed. Well-characterized astrocyte lifecycle from birth to adult.
<i>Rattus norvegicus</i>	Rat	Mammalia (Rodentia)	Widely used in astrocyte physiology; rat cortical astrocytes used in metabolic, Ca2+ signaling, and pharmacological studies.
<i>Macaca mulatta</i>	Rhesus macaque	Primates (Cercopithecidae)	iPSC-derived astrocytes compared with human and chimpanzee: reveals evolutionary changes in astrocyte size, morphological complexity, and Hippo-TEAD signaling.
<i>Symphalangus syndactylus</i>	Siamang gibbon	Primates (Hylobatidae)	Varicose-projection astrocytes documented [60]; expanding known distribution across apes.
<i>Gorilla gorilla</i>	Western gorilla	Primates (Hominidae)	Varicose-projection astrocytes documented [60].
<i>Pan paniscus</i>	Bonobo	Primates (Hominidae)	Varicose-projection astrocytes documented [60].
<i>Pan troglodytes</i>	Chimpanzee	Primates (Hominidae)	Varicose-projection astrocytes originally thought unique to humans and chimps [51]. iPSC-derived astrocytes compared with human and macaque for evolutionary analysis of Hippo-TEAD signaling.
<i>Homo sapiens</i>	Human	Primates (Hominidae)	Protoplasmic astrocytes: 2.5× more primary processes, 10× greater volume than rodents; contact up to 2 million synapses per cell. Interlaminar and varicose-projection astrocyte subtypes are primate-enriched. Evolutionary activation of Hippo-TEAD signaling linked to increased complexity. Genes for intellectual disability and chromatin regulation underwent pervasive changes in primate astrocyte evolution.

The evolutionary relationships among the species discussed in this section are illustrated in Figure 2, and the key features of their astrocyte-like glial cells are summarized in Table 1. Together, these lines of evidence argue that the evolutionary trajectory from invertebrate astrocyte-like glia to vertebrate radial glia and, ultimately, to diversified mammalian astrocytes reflects both convergent solutions to core homeostatic challenges and lineage-specific innovations tuned to circuit scale and architecture. Building on this evolutionary foundation, the sections that follow examine how developmental programs sculpt astrocyte diversity across species (with a focus on mammals) and how these programs bear on function in health and disease.

3 | Decoding Astrocyte Diversity: Developmental Origins and Functional Implications

3.1 | Developmental Origins

Astrocytogenesis begins around embryonic day (E) 16 in mice and continues postnatally [61]. This process involves progenitor migration from the ventricular zone (E16 to postnatal day 3, P3) to the cortical plate, clonal expansion of immature astrocytes (P0 to P7), and their progressive maturation into functional subtypes (P7 to P21) [62, 63]. Single-cell RNA sequencing (scRNA-seq) has revealed extensive inter-regional heterogeneity, with astrocytes from different brain regions, such as the cortex, striatum, and hippocampus, exhibiting distinct molecular identities [64–66]. This inter-regional diversity may reflect transcriptional adaptation to local environmental cues, as demonstrated by the role of Sonic Hedgehog signaling in establishing astrocyte identity, but may equally arise from the distinct progenitor pools from which regionally specified astrocytes originate, as evidenced by fate-mapping studies and cortical layering mutants [64–67]. Of note, astrocytes partially align with the transcriptional profiles of nearby neurons, further supporting the idea that their molecular identity is closely linked to local neuronal context and may influence their capacity for selective neuronal reprogramming [68, 69].

Beyond regional differences, astrocytes also display pronounced intra-regional heterogeneity. In the mouse cortex, protoplasmic astrocytes exhibit layer-specific transcriptional profiles, distinct morphologies, and defined spatial organization [70, 71]. Integration of scRNA-seq with spatial transcriptomics has further refined this view, identifying multiple molecularly defined cortical astrocyte subtypes with consistent spatial distributions across cortical layers [72]. Among these, a proliferative white matter-associated population persists into adulthood [73]. Notably, the spatial organization and transcriptional identity of these subtypes are already evident at early postnatal stages and remain stable into adulthood, suggesting that astrocyte molecular identity is established early during development and maintained over time.

Despite progress in characterizing cortical astrocyte diversity, the mechanisms underlying its establishment during development remain unresolved. Two mutually nonexclusive scenarios have been proposed: cell-intrinsic mechanisms which pre-determine astrocyte identity through fate-restricted

progenitors, or cell-extrinsic factors that shape astrocyte identities by guiding immature astrocytes to adapt locally, as shown in *Drosophila* [74] and mice [70, 71]. While classical lineage tracing yielded conflicting evidence [62, 75], recent findings using TrackerSeq—a method combining genetic barcoding with scRNA-seq [76] provided new insights into astrocyte development. This approach revealed that cortical astrocytes arise from two distinct lineages: S100a11 and Olig2 [72]. The S100a11 lineage originates from multipotent Emx1⁺ progenitors, sequentially generating neurons and astrocytes through the neurogenic-to-gliogenic switch. In contrast, the Olig2 lineage gives rise to a specific subset of astrocytes. Notably, knockout of Olig2 in progenitors leads to a shift toward S100a11 lineage at both the molecular and morphological levels, highlighting the role of lineage-specific mechanisms in maintaining astrocyte diversity. Understanding how astrocyte heterogeneity emerges during development is fundamental to deciphering their diverse roles in the CNS. Recent findings indicate that astrocyte subtypes originating from S100a11 and Olig2 lineages may have different regulatory functions at synapses. Indeed, Olig2⁺ astrocytes preferentially express genes that promote synaptogenesis, whereas S100a11⁺ astrocytes express factors that suppress synapse formation [72]. These observations challenge the notion of astrocytes as functionally redundant, instead highlighting their subtype-specific contributions to neural circuit maturation.

Despite these advances, several fundamental questions remain. How do intrinsic lineage programs interact with extrinsic signals to establish astrocyte identity? How do different subtypes adapt to environmental changes, and how does their dysfunction contribute to neurological disorders? Addressing these gaps is critical, particularly as growing evidence links astrocyte dysfunction to neurodevelopmental and neuropsychiatric conditions. Deciphering astrocyte diversity is not merely an academic pursuit but a crucial step toward targeted therapeutic strategies. Integrating single-cell multi-omics with functional studies will be essential for defining astrocyte subtype specification, plasticity, and dysfunction. Ultimately, harnessing astrocyte diversity may open new avenues to modulate neural circuits in disease, positioning these glial cells at the center of efforts to restore brain function and homeostasis.

Beyond developmental lineage, astrocytes are shaped by their local environments and neuronal partners, acquiring distinct molecular identities and functional roles across the brain. The following section explores how astrocytes achieve such fine-scale specialization—even down to individual processes within a single cell.

3.2 | Diversification and Functional Specialization

Astrocytic diversity is perhaps best described in the mouse cortex, where we arguably have the most comprehensive picture of the astrocyte life-cycle from birth to adult. Our understanding of astrocyte diversity has been revolutionized by single-cell transcriptome sequencing and spatial transcriptomics, revealing that cortical astrocytes exhibit layering distinct from the traditional neuronal laminae, aligning instead with the synaptic layers in the adult brain [64, 70]. Crucially,

adult astrocyte identity is largely determined by local micro-environment and/or neuronal activity. Genetic manipulations that disrupt cortical layering (as in the Reeler mouse model) lead to aberrant astrocyte layering [70], whereas secreted factors such as glutamate [77] and Sonic Hedgehog [67] significantly influence astrocyte molecular identity. These findings strongly suggest that neurons serve as an “instructional” scaffold, that “sculpts” astrocytes to support the formation, maintenance and function of specific synapses within distinct microcircuits, fundamental units of information processing in the adult CNS. This phenomenon may explain the differences in astrocyte morphology [67] and physiology [64] across cortical layers, as well as differences in functional outputs, given that astrocytes regulate synaptic transmission in both a column- and layer-specific manner [78]. Interestingly, the deletion of μ Crystallin from a specific population of striatal astrocytes resulted in profound effects, with mutant mice displaying abnormal perseverative behavior, potentially linking astrocyte molecular heterogeneity to neuropsychiatric disorders, including autism and obsessive-compulsive disorder [79].

Another question concerns the level at which diversity arises within astrocyte populations (Figure 3). Since astrocytes in the rodent cortex occupy nonoverlapping spatial positions, each astrocyte likely contacts tens if not hundreds of thousands of synapses of mixed types. Astrocyte-neuron interactions at these synapses are mediated through specialized molecular machineries, such as neurexin-neurologin interactions at excitatory synapses [80] and NRCAM1 at inhibitory synapses [81]. This observation suggests that individual astrocyte processes are specialized nanomachines that interact with synapses either at their tips or en passant along their length [82]. It is plausible to speculate that an individual process might be segregated into multiple domains, each involved in interacting with different synapse types. Although still in early developmental stages, proximity biotinylation techniques show great promise in dissecting such subcellular heterogeneity [83].

As the structural and molecular diversity of astrocytes becomes more apparent, so does their integrative role in shaping developing circuits. Astrocytes actively regulate numerous developmental processes, including synapse formation, neurotransmitter recycling [84], and neuronal metabolic support [85] during early developmental stages. Novel signaling pathways have been identified to mediate these developmental roles, some of which will be discussed in the next section.

The Wnt/ β -catenin signaling pathway is a fundamental regulator of brain development, and multiple high-confidence risk genes in this pathway are linked to neurodevelopmental disorders, including autism spectrum disorder (ASD) [86]. Among these, *CTNNB1* (encoding β -catenin) and *TCF7L2* stand out, exhibiting roles that vary by cell type and developmental stage. Canonical Wnt signaling involves β -catenin translocation to the nucleus, where it interacts with TCF/LEF proteins to regulate gene expression [87]. *TCF7L2*, encoding a key Wnt effector, is involved in brain development, influencing radial glia proliferation [88], oligodendrocyte maturation [89, 90], and neuronal specification [91]. In both human and murine brains, *TCF7L2* is expressed in neuroglia, and in astrocytes in particular, during postnatal maturation. Despite extensive research, the role of

TCF7L2 in astrocyte maturation and its impact on neuronal circuitry and behavior remains unclear, highlighting a crucial gap in understanding the molecular underpinnings of ASD and other psychiatric disorders.

TCF7L2 is highly expressed in human and rodent astrocytes during brain development but diminishes with age, indicating a role of the canonical Wnt/ β -catenin signaling in postnatal astrocyte maturation [92]. Conditional knockout demonstrated that astrocyte-specific deletion of *Tcf7l2* led to impaired astrocyte maturation, abnormal gene expression, reduced gap junction coupling, and defective astrocyte tiling. These molecular and morphological alterations are associated with aberrant astrocytic support, including synaptic development and function.

Conditional knockout of *Tcf7l2* in astrocytes led to increased excitatory synapse density, strength, and activity in the cortical layer five pyramidal neurons. Electrophysiological recordings revealed enhanced excitatory postsynaptic currents, suggesting that neurotransmission is regulated by astrocytes in a *Tcf7l2*-dependent manner [93]. Behavioral tests on conditional *Tcf7l2* KO mice demonstrated hyper-sociability, characterized by heightened social preference in both naturalistic and standardized tests, such as the three-chamber apparatus and scent-based assays. Deletion of *Tcf7l2* selectively impacted social behaviors without affecting learning and memory. Increased sociability was observed in both male and female mice, indicating a nonsex-specific role for *Tcf7l2*. These results highlight the dual importance of astrocytic gene expression in shaping both synaptic architecture and behavior. Collectively, these findings establish *Tcf7l2* as a critical astrocytic regulator of synaptic networks, with significant implications for understanding its involvement in neurodevelopmental disorders such as ASD.

In summary, astrocyte diversity emerges as a multi-scale phenomenon rooted in early-established molecular identities that are tightly linked to functional specialization across circuits and synapses. This diversity is generated through the combined action of developmental lineage programs, local environmental cues, and activity-dependent signaling pathways. Understanding how these mechanisms interact will be essential for elucidating astrocyte contributions to brain function and dysfunction. Key questions remain, including how subcellular specialization is established and maintained, how it is modified in response to local circuit activity across different levels, and how aging and disease influence these processes [94].

4 | Astrocyte Physiology: From Metabolism to Neural Circuits and Behavior

4.1 | The Astrocyte–Neuron Lactate Shuttle

Beyond their developmental regulation, astrocytes are essential supporters of brain homeostasis. This begins at the metabolic level: the brain's disproportionately high energy demands require precise and continuous glial regulation, and astrocytes are uniquely positioned to bridge glucose supply with neuronal energy consumption, being at the interface between vasculature and synapses, and having the enzymatic and structural machinery to both store and rapidly mobilize

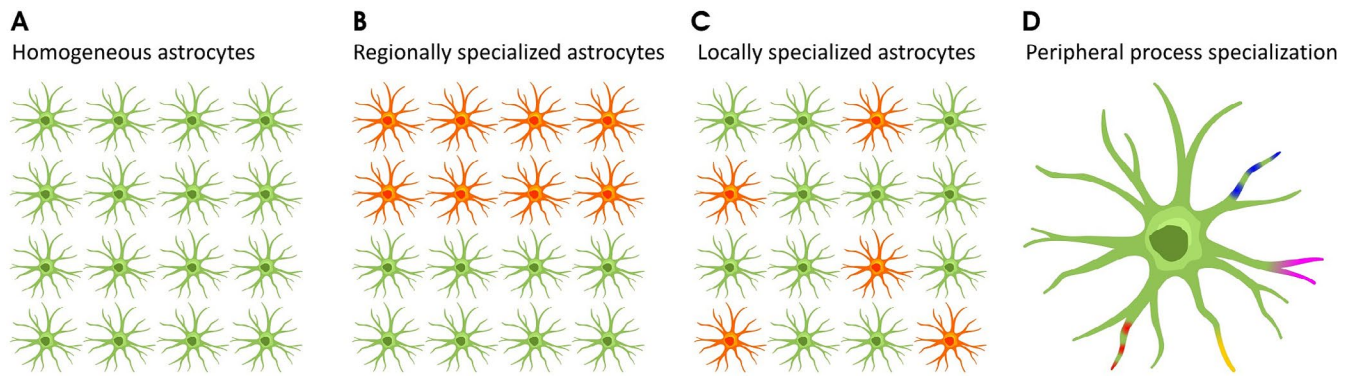


FIGURE 3 | Possible levels of astrocyte heterogeneity in the mammalian brain. A number of different levels for astrocyte heterogeneity have been proposed including (A) homogeneous (identical) astrocytes throughout the brain, (B) heterogeneity between brain regions, (C) heterogeneity within brain regions and (D) heterogeneity at the level of individual astrocyte peripheral processes (colored segments). Each process may contain several specialized sites that act as independent nanomachines, either making the same or different types of synaptic connections. Modified from Holt, *Essays Biochem*, 2023.

energy substrates. Neuronal activity and long-term memory formation are accompanied by a substantial increase in extracellular L-lactate [95–97]. The astrocyte-to-neuron lactate shuttle (ANLS) hypothesis, first proposed in 1994, posited that astrocytes serve as the primary producers of lactate, which is then transferred to neurons to meet their energy demands during activation [98]. This mechanistic metabolic coupling is based on a combination of complementary properties: astrocytes preferentially express the low-affinity, high-capacity lactate dehydrogenase isoform LDH5 (which favors lactate production) while neurons are enriched in LDH1, which favors lactate oxidation [85, 99–103]; astrocytes also maintain a high NADH/NAD⁺ ratio that thermodynamically favors glycolysis [104]; and their uniquely large glycogen stores [105, 106], concentrated within peri-synaptic processes [107], enable rapid local mobilization of glucose equivalents precisely where neuronal demand is highest (reviewed by Brooks [108]). While ANLS model has attracted considerable experimental reinforcement, it has also been criticized [109], and an alternative view has gained traction in recent years: neurons may preferentially consume glucose and subsequently release lactate back to astrocytes, effectively reversing the direction of the shuttle under certain conditions [110–112]. The directionality of metabolic coupling has direct implications for how energy availability is regulated during sustained activity, and for which cell type becomes vulnerable under conditions of metabolic stress. Adding further complexity, all major brain cell types are now recognized as potential contributors to the extracellular lactate pool [113–119]. This challenges the exclusively astrocyte-centric interpretation of ANLS and points toward a more dynamic, context-dependent model of cerebral lactate metabolism in which the direction and magnitude of inter-cellular lactate flux may shift depending on activity state, region, and metabolic conditions.

Besides its role as an energy substrate, lactate appears to function as a bona fide extracellular signaling molecule, a distinction of functional importance, as it implies that astrocyte-derived lactate does not simply fuel neurons passively but actively modulates their activity. Stimulation of astrocytes with extracellular L-lactate elicits secondary messenger responses reminiscent of those induced by noradrenaline [120, 121], and inhibition of

adenylate cyclase (AC) reduced stimulation-dependent cAMP and lactate elevations, raising the possibility of receptor-mediated lactate signaling. The hydroxycarboxylic acid receptor HCA1 (formerly GPR81) has been implicated in this process. HCA1 is a Gi-coupled receptor that suppresses neuronal activity through the AC–cAMP–PKA pathway, providing a feedback mechanism by which rising extracellular lactate could dampen neuronal excitability during high metabolic demand. Conversely, in astrocytes, HCA1 activation elevates intracellular cAMP and lactate levels, suggesting a cell-type-specific signal amplification role [122, 123]. The persistence of these effects in HCA1-knockout astrocytes [121], however, indicates that additional, as yet unidentified receptors contribute to this signaling loop, and that astrocytic lactate sensing is likely mediated by a multi-receptor system rather than a single molecular target.

A bioinformatic screen for alternative lactate-binding receptors identified GPR27, a member of the super-conserved receptor expressed in the brain (SREB) family, as essential for stimulus-induced increases in cytosolic lactate. GPR27 is of particular interest because, unlike HCA1, it appears to act on intracellular rather than extracellular lactate dynamics, suggesting it may serve a distinct role in regulating astrocyte-intrinsic lactate homeostasis rather than mediating responses to the extracellular lactate pool. The involvement of GPR27 was validated by CRISPR-Cas9-mediated knockout in 3T3 cells, and two surrogate agonists (compounds 8535 and 8535n [124] were shown to significantly increase intracellular lactate levels in rat cortical astrocytes using FRET-based imaging [124, 125]). GPR27 has also been proposed to modulate ERK and Akt signaling pathways [126], both of which intersect with glycolytic regulation, positioning this receptor as a candidate mediator of astrocyte-intrinsic lactate homeostasis.

Lactate signaling extends to the neuromodulatory system as well. Extracellular L-lactate triggers noradrenaline release from locus coeruleus projections in a concentration-dependent manner, an effect that appears to involve K_{ATP} channel inhibition downstream of imported lactate, and that may be partly independent of direct lactate uptake through a cAMP-mediated, receptor-dependent mechanism [127]. This provides a mechanistic link between local metabolic state and global neuromodulatory tone:

as astrocytic lactate release increases during periods of high synaptic activity, rising extracellular lactate could simultaneously signal energy availability to neurons and recruit noradrenergic arousal circuits. Another possible mechanism of depolarization involves K_{ATP} channel inhibition downstream of imported lactate, as it was proposed for orexin neurons in the hypothalamus [128, 129]. Together, these observations recast astrocytic lactate not merely as a metabolic byproduct, but as an active, multi-target signal coordinating energy status with neuronal excitability and neuromodulatory tone.

4.2 | Mitochondrial Dynamics and Metabolic Flexibility in Astrocytes

The view on astrocytes as primarily glycolytic cells has been substantially revised [130]. Astrocytes express genes associated with a broad range of mitochondrial metabolic processes—including oxidative phosphorylation (OXPHOS), glutamine oxidation, the tricarboxylic acid (TCA) cycle, lipid synthesis, and fatty acid β -oxidation (FAO)—and functional studies confirm that they can actively deploy these pathways [131, 132]. This metabolic flexibility allows astrocytes to adapt to changes in energy demands and to respond to the metabolic needs of neighboring neurons. Notably, several studies demonstrated that astrocytes oxidize fatty acids through the FAO pathway [133–137], with a particularly important role in detoxifying lipids derived from stressed neurons [134, 136]. Knockout of a rate-limiting FAO enzyme specifically in astrocytes impaired both working and spatial memory in adult mice [138], underscoring the behavioral relevance of astrocytic mitochondrial function.

Mitochondria in astrocytes are not static organelles but participate in highly dynamic remodeling through fusion, fission, and membrane restructuring that are tightly coupled to cellular function. Inhibition of mitochondrial network formation significantly impairs astrocyte maturation and synaptogenesis during early postnatal brain development, in a manner dependent on glutamatergic synaptic activity [139]. Consistent with this, deletion of Dynamin-related protein 1 (Drp1), a key effector of mitochondrial fission, in astrocytes adversely affects their development across the postnatal maturation period [140]. These findings collectively position mitochondrial dynamics as a critical regulator of astrocyte identity, synaptic capacity, and ultimately, brain circuit assembly. Future work integrating transcriptomic profiling with functional metabolic assays will be essential to resolve how mitochondrial state shapes astrocytic function across physiological and pathological contexts.

4.3 | Astrocytic Ca^{2+} Signaling and Functional Diversity

Astrocytic Ca^{2+} signaling is a fundamental feature of glial physiology, and extensive research over recent decades has demonstrated its complex initiation, propagation, and downstream consequences at cellular, network, and behavioral levels [141]. Unlike neurons, which use action potentials as their primary signaling method, astrocytes rely on intracellular ion (Ca^{2+} , Na^+ , Cl^- , and K^+) transients as their principal mode of

excitability, enabling them to integrate synaptic inputs and communicate across cellular networks without generating electrical spikes. Vertebrate astrocytes display highly elaborate morphologies characterized by fine protrusions known as leaflets that pervade the neuropil; this structural complexity enables them to sense synaptic activity and modulate neural circuits in a spatially precise and temporally dynamic manner. These Ca^{2+} signals are not uniform: they range from highly localized microdomains in individual leaflets, where astrocyte processes contact synaptic terminals, to large somatic waves that can propagate across astrocytic networks via gap junctions. This spatial hierarchy allows astrocytes to encode information at multiple scales simultaneously, responding to activity at a single synapse while also coordinating responses across broader tissue domains. A central challenge in this field has been the accurate quantification of Ca^{2+} signals in these submicron compartments, which are difficult to resolve with conventional imaging techniques due to their small volume and therefore their weak fluorescence signal.

Recent methodological advances have substantially improved the resolution and interpretability of astrocytic Ca^{2+} measurements. Early approaches relied on fluorescent Ca^{2+} indicators such as fura-2, fluo-3, fluo-4, and Oregon Green 488 BAPTA-1 (OGB-1), which enabled observation of complex and asynchronous Ca^{2+} signaling within and between astrocytes in vitro, in situ, and in vivo (reviewed in [142]). However, these methods are limited by incomplete loading, signal instability, and a bias toward somatic signals, restricting their ability to resolve fine astrocytic processes. The development of genetically encoded Ca^{2+} indicators (GECIs), including FRET-based sensors such as Cameleons [143] and single-wavelength indicators of the GCaMP family [144], has enabled stable, cell-type-specific labeling of astrocytes and improved visualization of Ca^{2+} dynamics across their processes [145]. GCaMP indicators are particularly well suited to detecting fast, dynamic Ca^{2+} transients driven by IP_3 -mediated ER release or store-operated Ca^{2+} entry (the two dominant pathways for astrocytic Ca^{2+} elevation) but are less appropriate for absolute quantification. In contrast, ratiometric approaches, based on fluorescence emission ratios, allow more reliable estimation of cytosolic or even mitochondrial Ca^{2+} levels [146, 147]. Furthermore, membrane-targeted GECIs enhance detection of subcellular Ca^{2+} signals by increasing sensitivity to events in fine astrocytic compartments, revealing more Ca^{2+} transients than bulk-loaded dyes. The distinction between these signal types matters functionally: localized leaflet Ca^{2+} transients have been linked to the regulation of gliotransmitter release and synaptic strength at individual synapses, whereas somatic Ca^{2+} elevations are more commonly associated with astrocyte-wide responses to neuromodulators such as noradrenaline and acetylcholine, suggesting that spatial compartmentalization of Ca^{2+} signals allows astrocytes to decode distinct upstream inputs and generate distinct downstream outputs.

Building on these advances, analytical and imaging approaches have further improved the detection of fine-scale Ca^{2+} events. The Multi-Threshold-based Event Detection (MTED) algorithm incorporates signal strengths to enhance the quantitative analysis of Ca^{2+} events across compartments [148]. Complementing this, a ratiometric Ca^{2+} biosensor designed specifically for

astrocytes was developed by covalently linking the genetically encoded indicator GCaMP6s to the bright red fluorescent protein tdTomato [149]. This construct enables accurate measurement of relative basal Ca^{2+} levels across individual astrocytic compartments and brain regions, revealing significant differences in resting $[\text{Ca}^{2+}]_i$ under both physiological and pathological conditions. When combined with machine learning-based denoising and volume fraction analysis—a morphological strategy capable of capturing fine astrocytic architecture beyond the optical diffraction limit—these tools collectively provide unprecedented resolution of astrocytic Ca^{2+} dynamics, opening new avenues to investigate how compartment-specific signals translate into circuit-level and behavioral outcomes.

Given its central role in astrocyte physiology, the tight regulation of astroglial Ca^{2+} signaling is critical for maintaining homeostasis, and its disruption has emerged as a common feature across multiple neurodegenerative diseases (reviewed in [150]). Downstream of Ca^{2+} elevations, astrocytes can release gliotransmitters including glutamate, ATP, and D-serine, modulating synaptic transmission and plasticity, as well as triggering cytoskeletal remodeling, adjusting metabolic output, and altering gene expression, illustrating that Ca^{2+} functions as a convergence point linking upstream neural activity to a broad repertoire of astrocyte effector responses. However, it has been noted that other ions, most notably Na^+ and Cl^- also act as signaling molecules that define and regulate multiple astrocytic functions [151, 152]. These ionic signals interact with Ca^{2+} dynamics in ways that are still being defined: for example, Na^+ influx through glutamate transporters can influence mitochondrial Ca^{2+} uptake and metabolic activity, while Cl^- fluxes can modulate membrane potential and thereby the driving force for Ca^{2+} entry, emphasizing that Ca^{2+} signaling in astrocytes must be understood within a broader ionic context rather than in isolation [151, 152].

4.4 | Astrocyte Roles in Neural Circuits and Cortical Plasticity

Distinct astrocyte subtypes integrate into specific local circuits and activate distinct intracellular signaling programs, contributing to the remarkable functional heterogeneity of this cell class. Chemogenetic approaches—particularly the use of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs)—have proven especially powerful in dissecting how astrocyte-specific activation shapes Ca^{2+} dynamics, downstream signaling, and astrocyte–neuron communication (reviewed in [153]). Astrocytes respond differentially to G_i -DREADD-mediated activation even within a single brain region, underscoring the complexity of their signaling repertoire. Combining the DREADD approach with astrocyte subtype-specific promoters thus offers a promising strategy for precise, time- and region-resolved manipulation of astrocyte function in both healthy and disease contexts.

The visual system has emerged as a particularly informative model for probing astrocyte contributions to both developmental and adult neuroplasticity [153, 154]. Following monocular enucleation (ME) in adult mice—a manipulation that causes an immediate loss of neuronal activity in defined regions of the

visual cortex—a specific increase in Aldh1l1-expressing astrocytes is detected in the visual cortex [155, 156]. Neuronal activity levels recover over approximately 7 weeks post-ME [156]. During this recovery period, the sign of DREADD-mediated astrocyte activation determines its effect on neural restoration: G_q -DREADD activation compromises neuronal recovery, whereas G_i -DREADD activation enhances it [155]. These contrasting outcomes reflect the activation of distinct intracellular signaling cascades within astrocytes and provide direct evidence that astrocyte activity can either facilitate or impede adult cortical plasticity, depending on the receptor pathways engaged.

Goal-directed actions (GDAs)—voluntary behaviors guided by learned action–outcome contingencies—depend on the integrity and plasticity of circuits spanning the cerebral cortex, basal ganglia, and thalamus [65, 153, 157–159]. These circuits require ongoing synapse formation and reshaping to support the cognitive flexibility that allows individuals to adapt effort allocation when contingencies change [155, 156]. Recent evidence positions astrocytes as active participants in this process, acting through secreted synaptogenic signals that regulate the structural remodeling of excitatory circuits.

A central molecular axis involves the astrocyte-secreted family of thrombospondins (TSPs) and their neuronal receptor $\alpha 2\delta$ –1 [160–162]. The $\alpha 2\delta$ –1 receptor—originally identified as a subunit of voltage-gated Ca^{2+} channels but subsequently shown to interact with multiple synaptic and synaptogenic proteins including TSPs—is essential for the formation and maturation of intracortical excitatory synapses during development [163] and remains highly expressed in adult mouse cortex [36]. Its synaptogenic function operates via Rac-1 signaling within cortical neuron dendrites [30] and is independent of its role in Ca^{2+} channel trafficking [162, 164].

During operant training, new excitatory, but not inhibitory, synapses are formed onto anterior cingulate cortex (ACC) neurons projecting to the dorsomedial striatum (DMS). These training-induced synapses are not required for learning the action–outcome relationship per se but are necessary for adaptively adjusting effort allocation. $\alpha 2\delta$ –1 mediates this training-induced excitatory synaptogenesis: in $\alpha 2\delta$ –1 knockout animal, synaptogenesis is diminished and mice exert significantly more effort to obtain the same reward [165]. Conversely, constitutive knockout of TSP1 and TSP2 does not impair training-induced excitatory synaptogenesis but reduces inhibitory synapse density, leading to a net increase in excitation of ACC $\rightarrow$ DMS neurons and consequently to reduced effort exertion [166]. Consistent with this interpretation, optogenetic excitation of ACC $\rightarrow$ DMS neurons is sufficient to reduce effort, whereas their inhibition increases it [165]. These findings establish a direct mechanistic link between astrocyte-secreted signals, structural synaptic remodeling, and the allocation of cognitive and motor effort—placing astrocytes at the heart of adaptive circuit function.

4.5 | Astrocytes and Behavior: Regulation of Food Intake and Homeostatic Networks

Food intake is primarily orchestrated by the hypothalamus and the brainstem dorsal vagal complex (DVC) [167, 168]. Astrocytes

residing in these regions integrate circulating hormonal and nutritional signals to modulate the activity of feeding-related neural circuits [169]. In the mediobasal hypothalamus (MBH), hormonal signaling through astrocytes is considered essential for organizing the neural circuits controlling energy balance [170, 171]. In response to ghrelin, released during fasting, MBH astrocytes undergo structural changes that modulate synaptic plasticity and regulate the activity of agouti-related peptide (AgRP)-expressing neurons [6] which are critical drivers of food-seeking and consummatory behaviors [172, 173]. Although hypothalamic astrocytes express ghrelin receptors and respond directly to ghrelin [9], their activation in this context is primarily driven by GABAergic signaling from ghrelin-excited AgRP neurons [174]. Astrocytes thus operate as embedded sensors within hunger-regulating circuits rather than as primary detectors of peripheral hormonal signals. In addition, astrocyte-derived transmitters such as prostaglandins further modulate AgRP neuron activity and feeding responses [174, 175], although some discrepancies across experimental conditions remain [176].

Astrocytes in the MBH are also implicated in modulating the activity of pro-opiomelanocortin (POMC)-expressing neurons, which attenuate food intake [172, 177]. Postprandial hyperglycemia induces retraction of astrocytic processes, with a concurrent increase in POMC neuron firing activity [178], though this response is not observed following a fat-rich meal, which also induces transient hyperglycemia [178], suggesting that dietary composition shapes the nature of astrocytic responses. Furthermore, astrocyte-secreted acyl-CoA-binding protein (ACBP)/endozepines may mediate satiety through direct action on POMC neurons [179, 180], and selective expression of ACBP in MBH astrocytes is sufficient to prevent diet-induced hyperphagia [179].

The brainstem DVC harbors astrocytes capable of integrating diverse nutritional and hormonal energy-related cues [181]. For example, astrocytic metabolism in this region appears necessary for glucagon-like peptide-1 (GLP-1) to effectively attenuate appetite [182]. Chemogenetic activation of DVC astrocytes reduces food intake and activates neurons in the lateral parabrachial nucleus (IPBN) [183], consistent with the known role of the DVC-IPBN connectivity in appetite suppression [184, 185]. Activation of astrocytes in the IPBN itself similarly reduces feeding and blunts ghrelin-induced hyperphagia [186]. The consistent anorexigenic effects of astrocyte activation in both the DVC and IPBN likely reflect the predominance of satiety-promoting neurons in these regions [167], in contrast to the opposing neuronal populations in the hypothalamus, where astrocytes must integrate competing hunger- and satiety-promoting signals [168].

Taken together, these findings establish astrocytes as active regulators of feeding behavior, operating at multiple levels of the central feeding circuitry—from peptidergic hypothalamic networks to brainstem satiety relays. Their capacity to sense, integrate, and transmit metabolic signals positions them as key nodes in the neural architecture governing energy homeostasis, with emerging implications for understanding feeding disorders and the mechanisms through which dietary state influences brain function.

5 | Astrocytes in Pathophysiology

In pathological conditions, astrocytes exhibit extensive morphological, molecular, and functional alterations that significantly shape disease initiation and progression [187, 188]. In response to diverse insults—including protein aggregates, excitotoxicity, neuroinflammation, and ischemia—astrocytes may lose supportive and functional functions, gain neurotoxic properties, or actively participate in tissue remodeling. Understanding these context-specific astrocytic responses is crucial to deciphering their dualistic roles in neurodegenerative diseases, stroke, and other CNS pathologies, and to identifying new targets for therapeutic intervention.

5.1 | Astroglia in Aging

Aging is the major risk factor for neurodegenerative diseases, and yet aging as such is a physiological process associated with a functional decline of all organs and systems. The brain, surprisingly, withstands aging better than other organs; indeed mental capacity continues to rise well into middle age, whereas physical capabilities decline earlier [189]. Physiological aging of the brain is not accompanied by prominent cell loss and represents constant adaptive remodeling which sustains cognitive abilities even into advanced years [190, 191]. This adaptive remodeling, achieved through neuroplasticity is very much defined by the performance of neuroglia, which through regulation of brain maintenance, resilience and brain compensation defines the cognitive reserve, which translates into cognitive longevity [192]. Neuroglia experience larger age-dependent changes when compared to neurons [193]. In particular, transcriptomic analyses revealed substantial changes in glial genes, whereas changes in neuronal transcripts were much milder [194–196]. Oligodendrocytes suffer the most [197], and the white matter is reduced by ~10% in old brains, while gray matter shrinks only by ~3% [198]; with total brain length of myelinated axons decreasing from 176 000 km at the age of 20 to 97 200 km at the age of 80 in males, and from 149 000 km at the age of 20 to 82 000 km at the age of 80 in females [199]. Microglia in the aged human brain undergoes dystrophic changes with loss of neuroprotective functions [200], astrocytic functions in the old brain are declining too.

Overall number and density of astrocytes does not change with age in rodents, marmosets and humans [201–203]. Expression of GFAP is generally believed to increase with age, although the data remain contradictory [204]. Golgi staining of astrocytes did not reveal age-dependent changes [205], whereas immunostaining with other markers such as glutamine synthetase and protein S100B showed complex region-specific alterations [206]. Detailed analysis of astrocytic morphology with using cell perfusion with uorescent dyes and confocal microscopy revealed widespread atrophy of astrocytes in hippocampus and cortex; astrocytic territorial domains were reduced; arborization complexity was simplified and leaflets retracted thus affecting synaptic coverage, which translated in the impaired synaptic plasticity [207–209]. Astrocytic atrophy was paralleled with loss of function (asthenia), with all major homeostatic functions of astrocytes, including,

glutamate clearance, K⁺ buffering, metabolism of neurotransmitters, water transport, cholesterol synthesis and many more are declining with advanced age. In summary the degree of glial decline defines the brain resilience to age-dependent diseases, while glial paralysis facilitates the development of neurodegeneration [193, 204, 210].

5.2 | Astrocytes in Hormone-Related Disorders: A Focus on Mood

Astrocytes are increasingly recognized as key mediators of hormonal signaling in the brain, with emerging evidence linking astrocyte dysfunction to a range of hormone-related psychiatric conditions. Astrocytes possess an intrinsic capacity to sense and respond to chronic hormonal stress, potentially contributing to differential vulnerability in mood disorders. Chronic exposure to the stress hormone cortisol induces distinct transcriptomic changes in human induced Pluripotent Stem Cell (hiPSC)-derived human astrocytes, with differential regulation of genes involved in GPCR ligand binding, ion transport, synaptic signaling, and neurotransmitter receptor expression (e.g., serotonin receptor HTR1B and glutamate receptors GRM1/GRM8) compared with acute exposure. This indicates that prolonged glucocorticoid exposure reprograms astrocyte gene networks relevant to mood and stress responses [211].

Thyroid hormone is another crucial regulator for CNS function that interacts closely with astrocyte biology and mood-related behavior. Primary astrocytes isolated from postnatal mouse cerebral cortex express key thyroid hormone receptors as well as thyroid hormone transporters and deiodinases, and treatment with tri-iodothyronine in vitro alters the expression of hundreds of genes including those involved in transcriptional regulatory pathways, suggesting that thyroid hormone directly regulates astrocyte gene networks [212]. Supporting these findings, mouse models with astrocyte-specific disruption of the enzyme type-2 deiodinase, which converts thyroxine to active T3 in astrocytes, exhibit anxiety- and depressive-like behaviors alongside altered expression of hippocampal genes implicated in mood regulation, linking impaired astrocyte thyroid hormone signaling with mood-related phenotypes [213].

Animal studies also have provided compelling mechanistic insights into the role of astrocytes in hormone-sensitive disorders such as postpartum depression. Postpartum depression is triggered in part by the rapid withdrawal of reproductive hormones following delivery, which disrupts neuroactive steroid signaling and renders mood-regulating brain circuits vulnerable. For instance, downregulation of lipocalin-type prostaglandin D₂ synthase protein signaling in the central amygdala leads to Src phosphorylation inhibition, which in turn induces astrocyte atrophy and depressive-like behaviors in postpartum female mice, implicating structural and signaling defects in astrocytes as causal contributors to hormone withdrawal-linked mood changes [214]. These findings suggest that preserving astrocyte morphological integrity through the L-PGDS/PGD₂/Src signaling axis may represent a novel therapeutic strategy for postpartum depression and related hormone withdrawal-associated mood disorders.

Taken together, these findings position astrocytes as active and hormonally sensitive cellular mediators in brain circuits governing mood. Expanding in vitro hormone-sensitivity paradigms to include variable estrogen, progesterone, and neurosteroid regimens alongside cortisol exposure could reveal whether astrocytes exhibit pathological hormonal sensitivity that predisposes individuals to affective dysregulation. Multimodal approaches combining transcriptomics, epigenomics, and functional assays in human astrocytes will be essential for identifying biomarkers and therapeutic targets specific to reproductive hormone-linked mood disorders.

5.3 | Neurodevelopmental Disorders: A Focus on Rett Syndrome

Corroborating the role of astrocytes as active regulators of brain development, synaptogenesis, synaptic pruning, neurotransmitter homeostasis, and synaptic circuits maturation, as extensively discussed in the previous sections, astrocyte malfunction has been implicated in various early-onset neurodevelopmental disorders (NDDs) [215, 216]. Astrocyte maturation and morphology are altered in NDDs. Astrocyte density is increased in Down syndrome [217] whereas astrocyte reactivity, characterized by hypertrophy, hyperproliferation and higher GFAP expression have been reported in ASD and Fragile X syndrome [218, 219]. In contrast, astrocytic atrophy, defined as a reduction in astrocytic branching and domain extension, is commonly found in Rett syndrome [220], leading to decrease in synapse coverage by astrocytic leaflets, neuronal support and overall brain homeostasis. Structural defects are paralleled by defective Ca²⁺ dynamic and gliotransmission in NDDs, ultimately causing a disruption of neuronal connectivity and plasticity [221]. Furthermore, impaired astrocyte-mediated regulation of extracellular glutamate uptake and potassium buffering contribute to excitatory/inhibitory imbalance, while abnormal inflammatory responses exacerbate network instability [221]. In NDD with identified genetic lesions, such as Rett syndrome, Fragile X syndrome and Angelman syndrome, astrocyte malfunction exerts profound noncell autonomous effects on neurons, underscoring the importance of a better understanding of astrocyte-neuron crosstalk for future therapeutic strategies [218].

Rett syndrome is a rare and devastating neurodevelopmental disorder being the most common genetic cause of severe intellectual disability in girls. Mutations in the X-linked *MECP2* gene, which encodes the epigenetic regulator methyl-CpG-binding protein 2 (MeCP2), are responsible for over 95% of classical RTT cases [222]. Neurological defects are the primary manifestations of RTT, with neurons exhibiting both structural and functional abnormalities [223, 224]. Among the various affected cellular compartments, synapses are particularly vulnerable, supporting the notion of RTT as a synaptopathy [225]. However, neuronal alterations in RTT are not solely attributable to *MECP2* mutations within neurons themselves; they may arise from malfunction in other cell types, especially astrocytes, which influence neurons through noncell-autonomous mechanisms.

Indeed, in vitro studies have shown that *Mecp2*-mutant astrocytes provide insufficient support for neurons, negatively affecting dendritic growth, synapse formation, and synaptic function

[215, 226–228]. Strikingly, the selective re-expression of *Mecp2* in astrocytes within an otherwise *Mecp2*-null mouse model is sufficient to restore dendritic complexity and adequate expression of the vesicular glutamate transporter vGlut1, accompanied by improvements in locomotor and anxiety-related behaviors, corrected respiratory defects, as well as significantly extended lifespan [229].

The detrimental effects of MeCP2-deficient astrocytes on neuronal function and synapses result either from the release of synaptotoxic factors and/or the absence of synaptogenic molecules. Therefore, identifying these aberrantly secreted signals may offer new avenues for pharmacological intervention in RTT. Previous studies exploring the proteomic and metabolomic profiles of RTT astrocyte-conditioned medium (ACM) identified differentially expressed molecules and pathways that partially explain the observed neuronal impairments [230–232]. However, these studies analyzed astrocytes in monoculture, failing to capture their dynamic interactions with neurons and the whole brain parenchyma, interactions known to strongly influence the phenotype and function of both cell types [233].

A transwell-based co-culture system, which allows molecular exchange without direct cell contact, was developed to assess how *Mecp2*-KO astrocytes affect WT neurons. It appeared that KO astrocytes aberrantly secrete high amounts of interleukin-6 (IL-6), impairing synapse formation and function. Notably, this IL-6 overproduction occurred only when KO astrocytes were co-cultured with WT neurons and was absent in monocultures or in cultures with KO neurons—underscoring the importance of neuron-astrocyte communication in regulating cytokine release [226]. This result is particularly relevant in RTT, where X-chromosome inactivation creates cellular mosaicism comprising both WT and mutant *MeCP2*-expressing cells. Particularly in Rett syndrome, disrupted astrocyte-neuron interactions are central to the disease pathogenesis.

5.4 | Astrocytes and Aberrant in Ion and Water Homeostasis: Molecular Insights From Studying Rare Genetic Diseases

As illustrated throughout this review, astrocytes exert crucial homeostatic role that supports brain function, with their dysfunction implicated in a wide range of neurological diseases. Yet disentangling their primary contribution from secondary reactive changes is often challenging: do astrocytes just react to injuries to maintain/restore homeostasis, or can they also contribute to driving the pathology? Genetic astrocytopathies—rare monogenic diseases in which astrocytes are the primary affected cell type—offer much clearer mechanistic insight by revealing which molecular pathways are indispensable for astrocyte function and, by extension, for normal brain physiology [234, 235]. They also demonstrate that secondary neuronal and oligodendrocyte involvement can follow as a downstream consequence.

Alexander Disease (AxD) is the prototypical genetic astrocytopathy. It is caused by dominant mutations in the gene encoding GFAP, the major intermediate filament of astrocytes. These mutations lead to abnormal GFAP aggregation, formation of Rosenthal fibers, and profound astrocytic stress responses (for

review see Ref. [236]). Because white matter is particularly affected, AxD is classified as a leukodystrophy, although this label should not obscure the fact that gray matter pathology is also present. Oligodendrocytes and neurons are secondarily affected, demonstrating that astrocytic dysfunction alone can trigger a devastating neurological disorder.

Beyond AxD, additional genetic astrocytopathies caused by mutations in connexins, transporters, ion channels, and structural molecules expressed by astrocytes were identified [237]. Many of these disorders are likewise classified as leukodystrophies because of their striking white matter changes on MRI, even though underlying gray matter involvement is common. A recurrent theme in this group of diseases is the disruption of brain ion and water homeostasis, highlighting the central role of astrocytes in this process.

Ion fluxes through the cell membrane drive the electrical activity of neurons. This mechanism inevitably moves osmotically obliged water, and ion and water homeostasis are therefore inseparably linked in brain physiology. Astrocytes not only stabilize the extracellular ionic environment and prevent potentially lethal brain swelling [238], they also shape large-scale brain fluid circulation. Their specialized endfeet form a crucial border of the perivascular pathways involved in cerebrospinal fluid circulation and are central to the glymphatic hypothesis, a proposed system for macroscopic brain fluid dynamics to clear metabolic waste from the brain [239]. Endfeet are enriched with aquaporin-4 (AQP4) water channels, $K_{ir}4.1$ potassium channels, chloride channels, adhesion molecules such as GlialCAM and MLC1, and structural complexes like the dystrophin-associated glycoprotein complex that maintain endfoot architecture. Together, these proteins enable astrocytes to buffer ionic shifts, maintain tissue stability, and link cellular processes to the brain's global fluid circulation.

Distinguishing clearly between changes in total AQP4 protein expression and changes in its subcellular localization when discussing AQP4 in relation to disease, brain water homeostasis, or clearance is not trivial, as these are not equivalent and carry distinct functional consequences. It is the polarized enrichment of AQP4 at perivascular astrocytic endfeet, not total protein abundance, that most directly governs transcellular water exchange at the blood–brain interface and supports glymphatic function. AQP4 mislocalization away from endfeet can arise secondary to neuroinflammation, mitochondrial stress, blood–brain barrier dysfunction, or extracellular matrix remodeling, and has been documented across conditions including epilepsy [240, 241], Alzheimer's disease [242], traumatic brain injury [243], and cerebrovascular disease [244]. Critically, such mislocalization can occur without any equivalent change in total protein abundance, meaning that assays measuring only bulk AQP4 expression may miss a functionally significant pathological shift. This has been demonstrated in primary human cortical astrocytes and extended to hypoxia-driven calmodulin-dependent AQP4 relocalization, where inhibiting this mechanism abrogated CNS oedema in vivo ([245, 246], reviewed in [244]). In addition to this, glymphatic clearance should avoid conflating transcellular water movement with paracellular tracer flow, as these are distinct processes. Classical fluorescent and gadolinium tracers reflect paracellular routes that AQP4 cannot directly

mediate, whereas H_2^{17}O MRI captures AQP4-dependent transcellular water exchange; AQP4 inhibition reduced parenchymal water delivery by ~80% using this approach [247], an effect gadolinium studies would underestimate. Both modalities are AQP4-sensitive but through distinct mechanisms, and conclusions about AQP4's contribution to clearance should specify which process is being measured and by which technique [244, 248, 249].

Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a genetic astrocytopathy, which highlights the consequence of disrupted astrocytic ion and water homeostasis. It is caused by genetic defects that damage the endfoot proteins MLC1 or GLIALCAM, resulting in impaired astrocytic volume regulation and chronic white matter swelling. Clinically, MLC is characterized by motor and mild cognitive impairment and seizures [250].

Recent discoveries expanded the genetic landscape of MLC and revealed additional components of the endfoot homeostatic complex. Recessive pathogenic *AQP4* variants that fully disrupt aquaporin-4 water channel function were recently shown to cause a remitting form of MLC [251], offering the first insight into the consequences of aquaporin-4 deficiency in humans. Similarly, newly identified *GPRC5B* variants in rare MLC patients have introduced a completely new player to brain fluid dynamics: the orphan G protein-coupled receptor GPRC5B [251]. Although its function in the brain was previously unknown, GPRC5B interacts with MLC1 and GlialCAM [252], suggesting that it helps orchestrate signaling pathways crucial for astrocytic volume regulation and fluid homeostasis. Since GPCRs are highly druggable, GPRC5B may even represent a therapeutic entry point for modulating brain fluid flow and treating brain edema.

Other leukodystrophies further illustrate how defects in astrocytic ion and water regulation can produce diverse white matter pathologies. For instance, *CLCN2*-related leukoencephalopathy, caused by mutations in the ClC-2 chloride channel, leads to white matter vacuolization without the chronic edema seen in MLC [253]; a surprising finding given the physical and functional interaction of ClC-2 with GlialCAM [254, 255]. Simultaneously, white matter diseases might also result from distinct mechanisms involving genetic damage to Kir4.1 potassium channels—causing a disruption in potassium homeostasis—or connexins, which would impair panglial syncytial coupling [237, 256]. Collectively, these disorders show that while astrocytic ion and water regulation is a shared theme, its disruption leads to variable disease manifestations shaped by the specific molecular players involved.

Studying such rare diseases is crucial for the development of urgently needed therapies. Yet the insights extend far beyond rare disorders. Brain edema is a common and life-threatening feature of stroke, traumatic brain injury, and tumors [257], and glymphatic dysfunction is implicated in a growing number of brain diseases, including AD and PD [258, 259]. Intriguingly, recent work in multiple sclerosis suggests that the autoimmune attack in this common neuroinflammatory condition may not be directed at myelin, but rather target endfoot proteins involved in astrocytic ion and water homeostasis [260–262], suggesting that disrupted astrocyte homeostasis is a primary driver of disease

[263]. Studying rare genetic astrocytopathies therefore can help unveil fundamental principles by which astrocytes maintain brain homeostasis, but also deepen our understanding of common neurological conditions.

5.5 | Reactive Astrogliosis

Astrocyte reactivity accompanies many disorders of the CNS and it often influences disease outcomes. While historically described as a uniform response to injury, it is now clear that astrocyte reactivity is highly heterogeneous, context-specific, and dynamically regulated [187]. Reactive astrocytes exhibit transcriptional and morphological changes that can range from hypertrophy and proliferation to the release of neurotoxic or neuroprotective factors, depending on the type, severity, and chronicity of the insult [187, 188]. In early studies, reactivity was primarily characterized by GFAP upregulation and cellular hypertrophy; however, advances in single-cell and spatial transcriptomics have highlighted the complexity of reactive states and their diverse molecular signatures. Indeed, distinct subtypes of reactive astrocytes have now been described in several neuropathological contexts, including neurodevelopmental disorders, neurodegenerative diseases, trauma, and infections.

Astrocytes are capable of rapidly detecting a wide range of pathological stimuli and mounting a specific response, a process known as reactive astrogliosis. This phenomenon, recognized in 1920s [264] was observed in various pathological conditions, including neurodegenerative diseases, stroke, brain tumors, and trauma. Reactive astrocytes undergo morphological reorganization associated with major transcriptional remodeling and potential changes in their multiple functions. Some genes induced in reactive astrocytes, such as GFAP, are commonly upregulated across many pathological contexts and are thus used as markers of reactivity, albeit being imperfect. Depending on the context, other genes may also be downregulated. Ultimately, the impact on astrocyte physiology itself, and secondarily on neighboring cells, is manifold. These changes may involve loss or enhancement of normal astrocyte functions (e.g., neurotransmitter uptake, metabolite detoxification, energy substrate supply), as well as the gain of new functions (e.g., secretion of bioactive or neurotoxic molecules).

Reactive remodeling is controlled by complex signaling pathways, including Ca^{2+} signaling, intermediate protein phosphorylation, second messenger induction, and finally activation of transcription factors or epigenetic regulators that induce long-lasting changes in astrocytes. The JAK-STAT3 and NF- κB pathways are some of the best characterized signaling pathways controlling astrocyte states, yet their dynamics and potential crosstalk remain incompletely understood [265]. Moreover, the upstream signals that activate these pathways are often poorly defined, especially in complex multifactorial diseases, where several cell types may be dysfunctional and release bioactive molecules. In contrast to the rapid kinetics of Ca^{2+} changes, transcription factor-based signaling pathways regulate gene expression over longer time scales—ranging from hours to days, or even years in chronic diseases. Astrocytes therefore quickly respond to pathological cues and often maintain their reactive state until this homeostasis is restored [266, 267].

This section provides an overview of astrocyte reactivity in pathology, focusing on the molecular and functional changes that define reactive states and on their impact on disease progression and recovery. Building on the general features of astrocyte reactivity outlined above, we next consider disease-specific manifestations, with particular emphasis on Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic Lateral Sclerosis (ALS), and neurotrauma.

5.6 | Astrocytes in Alzheimer's Disease

Alzheimer's disease (AD) is the predominant form of dementia, affecting approximately one in nine individuals over the age of 65 [268]. The global incidence of AD is rising, which leads to increased financial burdens for medical and social services and family caregivers. Early-stage alterations in mood and social behavior, anxiety and sleep disturbances are among the clinical features commonly observed in AD patients, together with progressive memory loss and emergence of more severe neuropsychiatric symptoms [269]. Histologically, individuals with AD exhibit accumulations of amyloid- β ($A\beta$) plaques and neurofibrillary Tau tangles in the brain, in addition to pathological evidence of neuronal cell death, synapse loss, neuroinflammation, vascular alterations and metabolism changes [270]. Whilst there have been recent advances in antibody therapies that target and sequester $A\beta$ to slow cognitive decline in patients, the effect size is relatively small [271]. There is therefore a need to better understand the cellular mechanisms of AD to refine therapeutic strategies and identify novel targets for intervention.

Astrocytes are emerging as central modulators of brain energy metabolism, adapting their metabolic profile in response to neurodegenerative insults. These changes are particularly evident in AD, where astrocytic glycolysis and associated metabolic shifts contribute to both neuroprotective and neurotoxic outcomes. As key metabolic regulators in the brain, astrocytes play a crucial role in sensing the organism's energetic state. These glial cells monitor and integrate signals related to cellular energy demands and relay this information to support the brain's adaptive energy management strategies. Under diseased conditions, the reactive states of astrocytes are accompanied by metabolic and functional adaptations, including changes in glycolytic and lipid metabolism, which are critical for meeting increased energy demands and mediating roles in inflammation, neuroprotection, or neurotoxicity [272, 273].

Reactive astrocytes enhance glycolysis to meet the elevated energy requirements associated with injury response, a phenomenon observed during the early stages of AD [85]. This metabolic shift drives the production of L-lactate, which serves as an energy substrate for neighboring neurons and supports their survival under pathological conditions [109, 274]. Recent FDG-PET studies in humans identified hypermetabolic states—reflected by increased glucose utilization—even in individuals with minimal amyloid burden, up to 25 years before the clinical onset of AD [275, 276]. Similarly, hypermetabolic brain states were reported in several transgenic mouse models of AD [277, 278], preceding the later-stage decline in glucose consumption. Moreover, astrocytes derived from human induced pluripotent

stem cells (hiPSCs) carrying familial AD mutations exhibit enhanced glycolysis and oxidative glucose metabolism [279].

However, excessive glycolytic activity can also exacerbate inflammation, driving heightened astrocyte activation and contributing to neuroinflammatory responses in certain brain disorders [280, 281]. Reducing astrocytic glycolytic capacity by GLUT1 ablation improves both peripheral energy homeostasis and cognitive performance [282]. Conversely, some studies have reported a reduction in astrocytic glycolysis upon exposure to proinflammatory signals [283]. This reduction, associated with decreased glycogen and lactate levels, may represent a neuroprotective adaptation, although this mechanism has thus far been observed primarily in primary cultures [284]. Over the course of disease progression, reactive astrocytes may therefore transition from a hypermetabolic to a hypometabolic state, marked by reduced glycolysis, diminished glycogen stores, and decreased lactate production. This shift may serve to limit the inflammatory cascade and protect the surrounding tissue. Overall, this dynamic switch from hyper- to hypometabolism illustrates the dual, context-dependent roles of astrocytes in balancing neuroprotection and neurotoxicity throughout disease evolution.

In parallel with their metabolic remodeling, astrocytes in AD are profoundly affected by disruptions in intracellular signaling pathways, particularly those involving Ca^{2+} homeostasis and redox balance. Increasing evidence suggests that astrocytes undergo functional impairments triggered by amyloid-beta species—especially soluble oligomers. Understanding how $A\beta$ disrupts resting astrocytic Ca^{2+} dynamics is therefore essential to elucidate their contribution to neuronal dysfunction and disease progression in AD.

$A\beta$ plaques have long been associated with astroglial dysfunction and disease progression; however, soluble $A\beta$ is now widely recognized as the primary neurotoxic species [285]. In astrocytes, these oligomers induce marked disturbances in Ca^{2+} homeostasis. In the APP/PS1 transgenic mouse model of cerebral β -amyloidosis, harboring mutations in the amyloid precursor protein APP and presenilin-1 PS1, astrocytes exhibit globally elevated resting cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) [286]. Similarly, in vivo application of naturally secreted $A\beta$ to the healthy brain of wild type mice caused widespread increases in resting $[Ca^{2+}]_i$ all astrocytic compartments, including soma, processes and endfeet [287]. Notably, these alterations occur independently of astrocyte proximity to $A\beta$ plaques, indicating that soluble $A\beta$ drives early and spatially diffuse astrocyte Ca^{2+} dysregulation. Together, these findings support a model in which soluble $A\beta$, rather than plaque deposition, initiates astrocytic dysfunction through widespread perturbation of Ca^{2+} signaling.

Besides alterations in basal $[Ca^{2+}]_i$ levels, the dysregulation of astrocytic Ca^{2+} dynamics is observed in AD models, following complex and often divergent trajectories depending on the disease stage, brain region, and proximity to pathological features. Astrocytic Ca^{2+} abnormalities can manifest as hyperactivity, hypoactivity, or spatial reorganization, each with distinct consequences for astrocyte function and circuit dynamics. Understanding these patterns is crucial for elucidating the

contribution of astrocytes to synaptic vulnerability and disease progression.

Many studies report that astrocytic Ca^{2+} signaling is altered early in AD, often showing a trend toward increased spontaneous activity reminiscent of the neuronal hyperexcitability. For example, in APPPS1 transgenic mice, a higher proportion of active astrocytes and the emergence of long-distance intercellular Ca^{2+} waves have been observed in the somatosensory cortex, linked to overactivation of purinergic receptor [286, 288]. These findings are consistent with soluble A β driving widespread, spatially diffuse Ca^{2+} elevations in astrocytes, independent of plaque proximity [287], as mentioned before.

However, this hyperactivity is often accompanied by functional impairments. Despite spontaneous hyperactivity, cortical astrocytes in APPPS1 mice displayed reduced and slower responses to sensory stimulation [289]. In the same mouse model, reduced amplitude of astrocytic Ca^{2+} signals—especially at the microdomain level—was associated with slow-wave sleep disturbances [290]. Optogenetic activation of astrocytes at slow-wave frequency restored network activity and improved memory function. Early reduction in spontaneous activity in astrocytes was detected in the cingulate cortex of APP^{NL-F} mice [291], and early impairments in both spontaneous and evoked signals were observed in astrocytes from the somatosensory cortex of B6.152H (PS2APP) mice [292]. In both mouse models, signal deficits were linked to reduced Ca^{2+} mobilization from the endoplasmic reticulum (ER), though by distinct mechanisms. In APP^{NL-F} astrocytes, decreased expression of InsP3R2 was implicated in diminished ER Ca^{2+} release [291], whereas in PS2APP mice downregulation of the ER Ca^{2+} sensor STIM1 led to decreased ER Ca^{2+} content, most likely via reduced store-operated Ca^{2+} entry [292]. Remarkably, restoring astrocytic Ca^{2+} signaling, either by chemogenetic activation or selective overexpression of STIM1, reversed network hyperactivity in APP^{NL-F} mice and rescued plasticity deficits in PS2APP mice, reinforcing the idea that reduced astrocytic signaling can significantly impact brain performance in AD. In line with these deficits, astrocyte Ca^{2+} alterations also disrupt neurovascular coupling: in vivo two-photon imaging in awake AD mice shows that astrocytic endfoot Ca^{2+} signals become uncoupled from adjacent arteriole dilations—exhibiting delayed onset, reduced amplitude, and impaired synchrony—highlighting an early breakdown in astrocyte–vascular communication [291].

Taken together, the available evidence argues against a simplistic or unidirectional model of astrocytic Ca^{2+} dysregulation in AD. Robust data support both Ca^{2+} hyperactivity and hypoactivity, depending on disease stage, brain region, and model. It is increasingly clear that astrocyte dysfunction evolves dynamically throughout disease progression, with both excessive and insufficient signaling potentially harming brain function [293]. Studying astrocytes across different genetic models, brain areas, and stages of pathology is thus essential to fully understand this diversity. Factors such as disease-specific mutations, local circuit interactions, and inflammatory states are likely to shape astrocytic responses and must be carefully considered when designing targeted therapeutic strategies.

Aberrant Ca^{2+} signaling in astrocytes has emerged as a central contributor to the network dysfunction in AD, and its consequences extend well beyond intracellular signaling. Astrocytes are deeply integrated into the structural and functional organization of neural circuits, particularly through their intimate relationships with synapses. Alterations in astrocytic Ca^{2+} dynamics can disrupt these interactions, potentially affecting synapse formation, maintenance, and elimination. This raises important questions about whether astrocyte dysfunction in AD might also drive synaptic vulnerability—either directly through aberrant secretory activity or indirectly via changes in morphology and territory coverage [294].

In the TauP301S mouse, which models the aggregations of hyperphosphorylated tau protein as seen in AD patients, astrocytes contribute to synapse loss via activation of the classical complement cascade [295]. Interestingly, astrocyte lysosomes in the TauP301S model were found to be more enriched with excitatory synapse proteins, whilst microglia were more enriched with inhibitory synapse markers, suggesting divergent glial mechanisms that contribute to the vulnerability of different types of synapses. In an alternative study, astrocytic expression of the Atypical Chemokine Receptor 3 (Ackr3) recognizes the phosphatidylethanolamine-bound chemokine, Cxcl12, at excitatory terminals, leading to astrocytic engulfment of synapses in the 5XFAD model of AD [296]. Importantly, these chemokine receptors could be targeted to reduce synapse engulfment in the AD mouse model, suggesting astrocyte–synapse engulfment mechanisms could represent therapeutic targets.

Astrocytes participate in the removal of A β from the brain parenchyma [297, 298]. However, unlike microglia, astrocytes tend to accumulate rather than degrade ingested A β [299]. The resulting intracellular load of A β impacts endo-lysosomal function and exerts toxic effects on the astrocytes themselves [300–302]. Consequently, astrocyte exposure to A β triggers not only astrogliosis but also apoptotic cell death, as documented in experimental models of AD and in the brains of AD patients [268, 303]. Furthermore, exposure to A β induces the release of pro-inflammatory factors from astrocytes [304, 305] which may exacerbate neuroinflammation, neuronal death, and the progression of A β and tau pathologies.

The significance of the astrocytic response to A β for AD pathogenesis is further supported by a recent study in which astrocytes were rendered hypersensitive to A β through the removal of the protective sorting receptor SorCS2 [300]. These SorCS2-deficient astrocytes exhibited increased uptake and intracellular accumulation of A β , leading to enhanced reactivity, defects in lysosomal acidification, and apoptotic cell death. In murine AD models, this astrocytic hypersensitivity resulted in heightened inflammation and massive amyloid and tau pathologies. The underlying mechanism involved the APP-processing enzyme δ secretase, which was found to be elevated in neurons as a result of crosstalk with A β -stressed astrocytes.

5.7 | Astrocytes in Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder, primarily affecting voluntary movements

and significantly reducing quality of life [306, 307]. In PD, dopaminergic (DA) neurons of the substantia nigra (SN), which project to the dorsal striatum, are especially vulnerable to degeneration. Neurodegeneration is thought to begin at striatal synapses and then progress retrogradely toward the neuronal soma in the SN [308], ultimately leading to DA neuron death. The progressive loss of these neurons underlies the hallmark motor symptoms of PD, including tremors and rigidity and as the disease advances, additional faculties become affected, giving rise to additional clinical features such as cognitive impairment and dementia [306, 307]. A defining pathological feature of PD is the presence of Lewy bodies, primarily composed of aggregated α -synuclein (α -Syn), in affected brain regions [309]. Alongside neuronal degeneration, widespread inflammation and reactive gliosis are also prominent [310]. However, the precise mechanisms linking α -Syn aggregation, neuronal death, and glial involvement remain poorly understood. Among the various cellular and molecular contributors to PD pathology, astrocyte dysfunction has emerged as a key factor in both disease onset and progression [311, 312].

Mounting evidence suggests that astrocytes contribute to DAergic neurodegeneration by losing their homeostatic and neuroprotective functions, including neurotransmitter recycling, synaptic maintenance, blood–brain barrier regulation, and antioxidant activity to support the high energy demands of neurons [313]. iPSC-derived astrocytes from PD patients carrying the LRRK2(G2019S) mutation exhibit atrophic morphology and reduced branching compared to age-matched astrocytes from healthy donors, suggesting impaired capacity to support neuronal metabolism [313]. These findings were corroborated in post-mortem human brain tissues (Braak stages II/III), where astrocytes in the basal ganglia and subthalamic nucleus showed similar morphological alterations following GFAP immunostaining [313].

To further characterize astrocytic malfunction in PD, mitochondrial function and Ca^{2+} homeostasis in iPSC-derived astrocytes from PD patients with the LRRK2(G2019S) mutation have been investigated [313, 314]. It is now recognized that the spacing between endoplasmic reticulum (ER) and mitochondria, referred to as mitochondria-ER contact sites (MERCS), is essential for efficient Ca^{2+} transfer between these organelles. Using a novel class of genetically encoded sensors (SPLICS), it was observed disrupted ER-mitochondria interactions in PD astrocytes, including altered MERCS distance (normally 40–50 nm), along with impaired mitochondrial bioenergetics [314]. Importantly, these structural alterations were accompanied by impaired ER-mitochondrial Ca^{2+} transfer and defective mitochondrial Ca^{2+} dynamics, indicating that disrupted organelle coupling directly compromises Ca^{2+} -dependent metabolic regulation. Restoring ER-mitochondria coupling at optimal distances rescues Ca^{2+} flux and mitochondrial function, highlighting the causal role of MERCS disruption in astrocyte dysfunction [314]. Taken together, these data indicate that the astrocytic deficits including reduced homeostatic support, elevated oxidative stress, and impaired neuroprotective capacity may contribute to dopaminergic neuron vulnerability.

In PD, astrocytes normally mitigate excitotoxicity by maintaining glutamate homeostasis via EAAT2 transporters [315].

However, pathogenic variants such as LRRK2 G2019S impair the trafficking and surface expression of EAAT2 [316], resulting in reduced glutamate uptake, increased extracellular glutamate spillover, neuronal damage, and exacerbated inflammation [316]. Beyond glutamate regulation, the phagocytic roles of both astrocytes and microglia are increasingly recognized as pivotal for clearing pathological protein aggregates, such as the α -synuclein fibrils seen in PD [296, 317]. The LRRK2 G2019S variant impairs astrocyte-mediated uptake and degradation of α -synuclein, in part through disruptions in annexin A2-dependent endocytic pathways [318]. In addition, α -synuclein accumulation has been shown to disrupt ER-mitochondria interactions and Ca^{2+} signaling, suggesting that impaired proteostasis and organelle crosstalk may converge to exacerbate astrocyte dysfunction [319, 320]. Notably, many endo-lysosomal proteins—including those with PD-linked variants—are highly expressed in glial cells, suggesting that lysosomal dysfunction may underlie defective clearance mechanisms [317]. Enhancing or restoring the clearance capacity of these glial cells could offer promising strategies to slow or halt the neurodegenerative cascade in PD, AD, and related disorders.

Emerging evidence highlights the importance of astrocyte-derived extracellular vesicles (EVs) in PD. These vesicles comprise a heterogeneous population of lipid-bound nanocarriers secreted by cells under both physiological and pathological conditions [321, 322]. They transport a variety of bioactive cargoes, including DNA, RNA, metabolites and proteins, and are increasingly recognized as mediators of glia-to-neuron communication [323]. Initially identified as a potential contributor of α -Syn spread in the brain, astrocyte-derived EVs may also exert neuroprotective effects in PD [324]. Under neurotoxic conditions induced in vitro in DAergic-like SH-SY5Y neurons by 1-methyl-4-phenylpyridinium (MPP+), EVs isolated from cultured primary astrocytes were able to counteract mitochondrial complex I inhibition and improve ATP production, suggesting the potential neuroprotective properties of astrocyte-derived EVs in PD-related mechanisms [325]. Interestingly, primary astrocytes cultured from the ventral midbrain produced a greater content of EVs and exerted a more neuroprotective effect on cultured neurons, indicating a source for regional specific astrocyte-dependent neuroprotective mechanisms [325]. Therefore, astrocyte-derived EVs add a further layer of complexity to the communication between the glial compartment and neurons, which may play a fundamental role in the intricate pathogenesis of PD.

5.8 | Astrocytes in Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease marked by the progressive degeneration of both upper and lower motor neurons [326–328]. Clinically, ALS manifests as adult-onset muscle weakness and atrophy, with frontotemporal dementia occurring in 10%–15% of patients [329, 330]. The median survival time following symptom onset is approximately 3–5 years [331]. ALS can be classified into familial (fALS) and sporadic (sALS) forms. More than 30 genes have been implicated in ALS, with the most common mutations found in genes encoding C9orf72, TDP43, FUS, and SOD1. This genetic heterogeneity, in addition to several proposed environmental factors, suggests

that multiple, intersecting cellular and molecular mechanisms contribute to disease onset and progression [332–336]. The following section explores how astrocytes contribute to ALS pathophysiology through metabolic and inflammatory changes and altered synaptic interactions, highlighting both regional heterogeneity and disease-stage-specific alterations in their function and reactivity.

ALS astrocytes exhibit inflammatory signatures [337] have been shown to induce neuronal loss of function in human iPSC-derived models of the disease [338]. These findings imply that astrocytes may be active participants in driving or exacerbating ALS progression. One of the first clinical indications of astrocyte pathology in ALS was the observed loss of the glutamate transporter GLT1 from the spinal cord [339]. This loss of glutamate regulation by astrocytes was thought to contribute to glutamate-mediated excitotoxicity and subsequently neuronal death. However, approaches in mouse models to increase GLT1 levels have failed to show therapeutic potential [340], indicating that other mechanisms drive the pathology of ALS.

Distinct metabolic and structural abnormalities have been identified in astrocytes isolated from neonatal (P2) SOD1^{G93A} ALS mice [341, 342]. Astrocytes obtained from both the motor cortex and spinal cord exhibit ER enlargement and disrupted ER-mitochondria contact sites, leading to elevated oxidative stress

compared to controls. Given the critical role of these sites in Ca²⁺ exchange, such alterations are likely to impair organelle Ca²⁺ crosstalk and contribute to mitochondrial dysfunction. Notably, these alterations were more pronounced in motor cortex astrocytes, suggesting a region-specific vulnerability. In contrast, spinal cord astrocytes mount a more effective antioxidant response, maintaining mitochondrial respiration and exhibiting greater resistance to oxidative stress. This resilience is likely mediated by activation of the ER-localized pentose phosphate pathway (ER-PPP), as evidenced by increased catalytic activity of hexose-6-phosphate dehydrogenase and enhanced (18)F-fluorodeoxyglucose uptake. This metabolic shift mirrors findings from PET imaging studies in symptomatic ALS patients [342], suggesting that ER-PPP activation may serve as a biomarker of disease progression.

The phenotypic changes acquired by astrocytes during ALS progression remain an area of active investigation. A recent study showed that astrocytes isolated from the spinal cords of late symptomatic (P120) SOD1^{G93A} mice exhibited alterations in intracellular Ca²⁺ dynamics and disrupted energy metabolism, specifically a reduced ATP synthesis-to-oxygen consumption ratio [343, 344]. P120 SOD1^{G93A} astrocytes also displayed excessive release of pro-inflammatory cytokines (IL-1 β , TNF- α , IL-6) and glutamate—factors known to exert neurotoxic effects. Importantly, co-culture experiments demonstrated that these

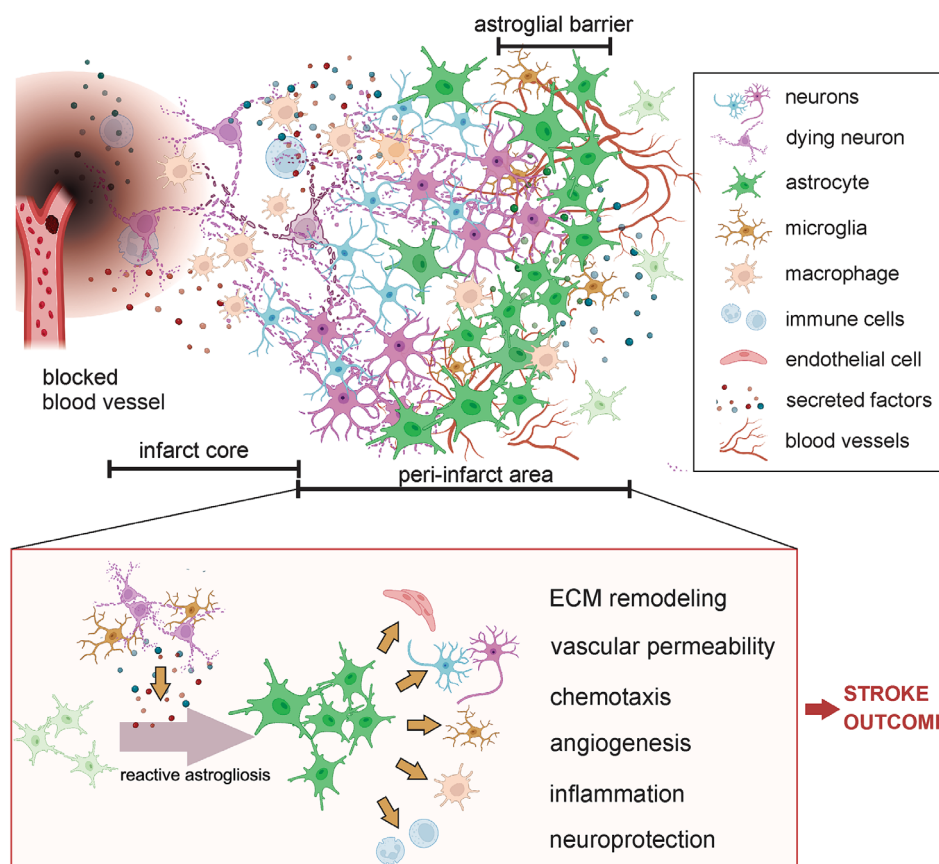


FIGURE 4 | Astrocytes in ischemic stroke. Following ischemic injury, neurons die, and an inflammatory response develops. Factors released by damaged neurons and activated microglia and macrophages induce astrocyte responses. Activated astrocytes proliferate, undergo hypertrophy, and form a barrier surrounding the lesioned tissue. At the same time, they exhibit high secretory activity, releasing factors that shape the brain microenvironment and influence the phenotypes of neighboring cells. Consequently, astrocyte secretory activity plays a key role in modulating post-stroke recovery.

astrocytes significantly accelerated motor neuron death [344]. Notably, in vivo and ex vivo studies indicate that ALS astrocytes display increased Ca^{2+} microdomains driven by mitochondrial dynamics and transient opening of the mitochondrial permeability transition pore (mPTP), rather than global Ca^{2+} elevation or enhanced ER Ca^{2+} release [345].

Together, these findings underscore the central role of astrocytes in shaping the pathological environment in ALS and support the concept that they acquire a disease- and region-specific toxic phenotype. Nonetheless, rescuing the pathological phenotype of P120 spinal cord astrocytes reduces their neurotoxicity in both murine and human in vitro models of ALS [344, 346]. These results strengthen the rationale for targeting astrocytes as a therapeutic strategy to mitigate disease progression and improve outcomes in ALS.

Prior to motor neuron loss in ALS, synaptic changes in the central and peripheral nervous system are thought to contribute to early-stage hyperactivity—suggestive of excitotoxic mechanisms [347]. Synaptic pathology in ALS appears to occur concurrently with astrocytic pathology [338, 348, 349].

Within spinal motor circuits, astrocytes modulate synaptic activity through bidirectional signaling mechanisms; astrocytes provide inhibitory purinergic feedback to neurons in response to neuronal glutamate-mediated activation of astrocytic mGluR5 receptors [348, 350]. Notably, several components of this signaling pathway have been independently reported to undergo alterations in ALS [350, 351], suggesting that disrupted astrocyte-neuron communication at synapses may represent a critical pathological feature of the disease.

A selective loss of multipartite excitatory synapses—that is, glutamatergic synapses that were contacted by the perisynaptic astrocytic leaflet marker, Ezrin—was reported in the lumbar spinal cord of $\text{SOD1}^{\text{G93A}}$ ALS mice as well as in human post-mortem spinal cord tissue from ALS patients [352]. This structural degeneration was corroborated by transcriptomic analyses from both $\text{SOD1}^{\text{G93A}}$ mice and ALS patient tissue, revealing gene expression changes related to neuron–glia communication [349]. Together, these data support the hypothesis that tripartite synapses are an early and specific target of ALS pathology.

To investigate whether this synapse loss was driven by either intrinsic astrocytic or neuronal factors, co-culture experiments were performed using spinal neurons and astrocytes derived from neonatal wild-type and $\text{SOD1}^{\text{G93A}}$ mice. Surprisingly, these in vitro assays did not reveal synaptic differences across genotype combinations [353], contrasting with the synaptic deficits observed in vivo. This discrepancy may reflect the developmental timing of synaptic degeneration, which could emerge only later in disease progression.

Synaptic alterations were studied in an inducible model of sporadic ALS (TDP-43 Δ NLS), in which neurons—but not neuroglia—display cytoplasmic mislocalization of the RNA-binding protein TDP-43, a hallmark of ALS pathology [354]. In this model, it has been observed substantial changes in excitatory and cholinergic synapses within the spinal cord, but no corresponding astrocytic pathology or selective loss of tripartite synapses [355].

These findings suggest that synaptic degeneration may primarily result from neuron-intrinsic mechanisms, and that astrocytic contributions may depend on cell-autonomous dysfunction.

These findings point to a complex and context-dependent relationship between astrocytes and synaptic integrity in ALS. While synaptic degeneration may be neuron-driven in some models, astrocyte-mediated mechanisms likely contribute in others. Ultimately, a deeper understanding of basic tripartite synapse biology and its contributions to the maintenance of neural circuits may help identify the mechanisms of its vulnerability in ALS and thus guide the development of more targeted and effective therapies.

5.9 | Astrocytes in Neurotrauma

Astrocytes, as part of the brain's defense system, respond to a wide range of injuries that induce focal brain damage, including mechanical (traumatic brain injury), ischemic damage (ischemic stroke), or autoimmune (Figure 4). In response to these insults, astrocytes become reactive and erect a perilesional border around the injured site [356, 357], where they form a glial barrier. This barrier physically separates the lesioned area from the surrounding healthy tissue, exerting protective effects by limiting the spread of damage, but also incurring detrimental effects by impeding tissue regeneration [188].

In addition to forming this barrier, astrocytes play key roles in coordinating the inflammatory response, clearing cellular debris by phagocytosis, remodeling the extracellular matrix (ECM), and regulating angiogenesis. These reparative and immune-related functions are largely mediated by a diverse repertoire of biologically active molecules secreted into the interstitial fluid of the brain. While microglia and infiltrating macrophages are known for their rapid cytokine and chemokine release, astrocytes emerge as pivotal regulators that fine-tune inflammatory signaling and orchestrate tissue remodeling during the subacute and chronic phases following injury [267, 358–360].

These processes have been extensively studied in the context of ischemic stroke [361–363]. Ischemia and injury-associated cues released from damaged neurons and reactive microglia instigate reactive astrogliosis (Figure 1). Reactive remodeling leads to astrocyte proliferation and hypertrophy, accompanied by upregulation of characteristic markers such as GFAP [364]. One key inducer of astrocyte activation is transforming growth factor-beta ($\text{TGF}\beta$), which is markedly elevated in post-ischemic brain regions [363]. $\text{TGF}\beta$ drives GFAP expression, cellular hypertrophy, and the enhancement of astrocytic secretory activity [365, 366].

Given the prolonged reactivity and robust secretory profile of astrocytes after stroke [357, 367, 368], these cells are increasingly recognized as central signaling hubs that shape the post-ischemic brain environment and modulate the behavior of surrounding cell types (Figure 1). Astrocytes play critical roles in supporting post-stroke angiogenesis [228, 363], highlighting their regenerative potential. However, this same secretory activity can become maladaptive—excessive pro-inflammatory signaling and deposition of ECM components can create a

nonpermissive environment for axonal regeneration and hinder functional recovery.

Therefore, a promising therapeutic strategy may lie in precise modulation of astrocytic secretory functions to enhance regeneration while limiting detrimental effects. Yet, the intracellular mechanisms that govern astrocyte secretion remain poorly characterized. For example, the identification of the sorting receptor SorCS2 as a regulator of endostatin release suggests that astrocytes may possess specialized trafficking and sorting machinery to control their secretome [363]. Of note, SorCS2 expression is induced in the reactive astrocytes following ischemic stroke, which points to the dynamic adjustment of the astrocytic intracellular sorting machinery upon activation. Further research is essential to elucidate the components and regulation of this machinery, which could open new avenues for promoting neurorepair in the aftermath of stroke.

6 | Cellular Models for Studying Astrocyte Malfunction

Over the past decades, the study of astrocyte biology has been advanced by a range of experimental *in vitro* models, each with distinct advantages and limitations. Traditional two-dimensional (2D) primary cultures, often relying on serum-supplemented media [369], remain widely used but yield immature astrocytes with transcriptomic profiles that differ substantially from their *in vivo* counterparts [370, 371]. To better approximate the mature, quiescent phenotype, serum-free protocols supplemented with growth factors such as fibroblast growth factor 2 (FGF2) and epidermal growth factor (EGF) have been developed; astrocytes cultured under these conditions more closely resemble mature cells *in vivo* [372]. Chemically defined serum-free media also promote *in vivo*-like homeostatic ion channel expression in primary astrocytes [373]. *Ex vivo* preparations, such as brain slices, have additionally provided insight into neuron-astroglia crosstalk in both physiological and pathological settings [374].

Several two- and three-dimensional iPSC-based differentiation protocols have been developed to generate functional human astrocytes, ranging from long-term NPC-based approaches to rapid one-step methods and 3D spheroid systems, with the resulting cells demonstrating canonical astrocyte properties including glutamate uptake, Ca^{2+} signaling, and synaptic support [375–379].

Disease-specific iPSC-derived astrocytes have revealed how astrocytes contribute to pathology across multiple neurological conditions, including Alzheimer's disease [338, 380–384], amyotrophic lateral sclerosis [338], and Huntington's disease [381].

In addition, in a humanized tricellular transwell model comprising human brain microvascular endothelial cells, pericytes, and astrocytes, photobiomodulation restored BBB integrity following hypoxic injury by attenuating endothelial thrombo-inflammatory signaling while simultaneously reducing oxidative stress across all three cell types, demonstrating coordinated astrocyte participation in multicellular repair responses [385]. Complementing this, astrocyte enrichment of

microfluidics-generated 3D cortical constructs enhanced neuronal maturation and viability *in vitro*, and following implantation into mouse brains reduced lesion size, increased axonal growth, and improved vascular coupling within the graft [386]. Together, these studies illustrate how astrocytes function as instructive contributors to neural repair and how human-relevant platforms can bridge fundamental astrocyte biology to regenerative and therapeutic applications.

Together, these complementary models reflect a progressive refinement in astrocyte biology research, with iPSC-derived astrocytes proving particularly powerful in revealing patient-specific, cell-intrinsic disease mechanisms. While limitations such as epigenetic age resetting and lack of vascularization remain, ongoing advances in aging paradigms and assembloid systems are expected to further improve their physiological relevance and utility for therapeutic development.

7 | Conclusion

The picture of astrocytes that emerges from this review is one of remarkable evolutionary conservation, cellular sophistication, and functional versatility. From the primordial glia of flatworms and astrocyte-like cells of *Drosophila* to the morphologically elaborate, synapse-enwrapping protoplasmic astrocytes of the human neocortex, a continuous thread of homeostatic innovation runs through metazoan evolution. In mammals, and especially in primates, this trajectory peaks in a cell type whose sheer structural complexity, thousands of fine perisynaptic leaflets contacting hundreds of thousands of synapses per cell, positions it as an indispensable partner to neurons at every level of brain organization. The sections of this review have traced how those interactions are established during development, through lineage-specific programs and local instructional cues from neurons; how they are maintained in the adult brain through metabolic coupling, Ca^{2+} signaling, gliotransmission, and dynamic synaptic remodeling; and how they break down in the spectrum of disorders from neurodevelopmental conditions and hormonal dysregulation to neurodegeneration, genetic astrocytopathies, and acute injury. A unifying theme throughout is heterogeneity: astrocytes are not a monolithic population but rather a constellation of molecularly distinct subtypes shaped by region, layer, developmental origin, and circuit context. Moreover, it is increasingly clear that this diversity is functionally consequential rather than merely descriptive. Equally important is the growing recognition that many disease processes once attributed solely to neurons are, in part, driven or amplified by intrinsic astrocyte dysfunction, a realization with direct therapeutic implications. Looking ahead, integrating single-cell multi-omics, spatial transcriptomics, advanced imaging, and functional *in vivo* tools will be essential to decode the full repertoire of astrocyte states across health and disease. Astrocytes are one of the central players in brain biology, and harnessing their biology represents one of the most promising frontiers in neuroscience.

Disclaimer

This review originates from the Astrocyte Cafe, an in-person meeting held in Trieste in 2024, where astrocyte researchers gathered to discuss current advances in astrocyte biology across a range of topics. The presenters at

this meeting subsequently collaborated to produce the present review article, which synthesizes and expands upon the themes discussed during the conference. The authors wish to emphasize that this is not intended as a comprehensive review of the astrocyte research field. Rather, it reflects the specific topics that were presented and debated at the meeting, shaped primarily by the research backgrounds and expertise of the participating scientists. The authors recognize that many important and valuable contributions to astrocyte research exist beyond what is presented here, and explicitly acknowledge that the absence of certain topics reflects the focus of the meeting rather than a judgment on their scientific merit.

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Conflicts of Interest

The authors declare no conflicts of interest.

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References

1. A. Verkhratsky and A. M. Butt, *Neuroglia: Function and Pathology* (Elsevier, 2023).
2. A. Verkhratsky and M. Nedergaard, "The Homeostatic Astroglia Emerges From Evolutionary Specialization of Neural Cells," *Philosophical Transactions of the Royal Society, B: Biological Sciences* 371, no. 1700 (2016): 20150428, <https://doi.org/10.1098/rstb.2015.0428>.
3. A. Pękowska, A. Verkhratsky, and C. Falcone, "Evolution of Neuroglia: From Worm to Man," *Handbook of Clinical Neurology* 209 (2025): 7–26, <https://doi.org/10.1016/B978-0-443-19104-6.00004-8>.
4. G. Oikonomou and S. Shaham, "The Glia of *Caenorhabditis elegans*," *Glia* 59, no. 9 (2011): 1253–1263, <https://doi.org/10.1002/glia.21084>.
5. G. Oikonomou and S. Shaham, "On the Morphogenesis of Glial Compartments in the Sensory Organs of *Caenorhabditis elegans*," *Worm* 1, no. 1 (2012): 51–55, <https://doi.org/10.4161/worm.19343>.
6. R. F. Stout, A. Verkhratsky, and V. Parpura, "*Caenorhabditis elegans* Glia Modulate Neuronal Activity and Behavior," *Frontiers in Cellular Neuroscience* 8 (2014): 67, <https://doi.org/10.3389/fncel.2014.00067>.
7. S. Ray and A. Singhvi, "Charging up the Periphery: Glial Ionic Regulation in Sensory Perception," *Frontiers in Cell and Developmental Biology* 9 (2021): 687732, <https://doi.org/10.3389/fcell.2021.687732>.
8. S. C. Sukhdeo and M. V. Sukhdeo, "Mesenchyme Cells in *Fasciola hepatica* (Platyhelminthes): Primitive Glia?," *Tissue & Cell* 26, no. 1 (1994): 123–131, [https://doi.org/10.1016/0040-8166\(94\)90088-4](https://doi.org/10.1016/0040-8166(94)90088-4).

9. I. E. Wang, S. W. Lapan, M. L. Scimone, T. R. Clandinin, and P. W. Reddien, "Hedgehog Signaling Regulates Gene Expression in Planarian Glia," *eLife* 5 (2016): e16996, <https://doi.org/10.7554/eLife.16996>.
10. M. Csoknya, V. Dénes, and M. Wilhelm, "Glial Cells in the Central Nervous System of Earthworm, *Eisenia fetida*," *Acta Biologica Hungarica* 63, no. S1 (2012): 114–128, <https://doi.org/10.1556/ABiol.63.2012.Suppl.1.11>.
11. J. W. Deitmer, C. R. Rose, T. Munsch, et al., "Leech Giant Glial Cell: Functional Role in a Simple Nervous System," *Glia* 28, no. 3 (1999): 175–182.
12. F. Le Marrec-Croq, F. Drago, J. Vizioli, P.-E. Sautière, and C. Lefebvre, "The Leech Nervous System: A Valuable Model to Study the Microglia Involvement in Regenerative Processes," *Clinical and Developmental Immunology* 2013 (2013): 274019, <https://doi.org/10.1155/2013/274019>.
13. T. Munsch and J. W. Deitmer, "Calcium Transients in Identified Leech Glial Cells In Situ Evoked by High Potassium Concentrations and 5-Hydroxytryptamine," *Journal of Experimental Biology* 167 (1992): 251–265, <https://doi.org/10.1242/jeb.167.1.251>.
14. J. G. Nicholls and S. W. Kuffler, "Extracellular Space as A Pathway for Exchange Between Blood and Neurons in the Central Nervous System of the Leech: Ionic Composition of Glial Cells and Neurons," *Journal of Neurophysiology* 27 (1964): 645–671, <https://doi.org/10.1152/jn.1964.27.4.645>.
15. A. J. Saubermann, C. M. Castiglia, and M. C. Foster, "Preferential Uptake of Rubidium From Extracellular Space by Glial Cells Compared to Neurons in Leech Ganglia," *Brain Research* 577, no. 1 (1992): 64–72, [https://doi.org/10.1016/0006-8993\(92\)90538-k](https://doi.org/10.1016/0006-8993(92)90538-k).
16. S. W. Kuffler and D. D. Potter, "Glia in the Leech Central Nervous System: Physiological Properties and Neuron-Glia Relationship," *Journal of Neurophysiology* 27 (1964): 290–320, <https://doi.org/10.1152/jn.1964.27.2.290>.
17. S. D. Carlson and R. L. S. Marie, "Structure and Function of Insect Glia," *Annual Review of Entomology* 35 (1990): 597–621, <https://doi.org/10.1146/annurev.en.35.010190.003121>.
18. M. A. Charlton-Perkins, M. Friedrich, and T. A. Cook, "Semper's Cells in the Insect Compound Eye: Insights Into Ocular Form and Function," *Developmental Biology* 479 (2021): 126–138, <https://doi.org/10.1016/j.ydbio.2021.07.015>.
19. M. A. Charlton-Perkins, E. D. Sendler, E. K. Buschbeck, and T. A. Cook, "Multifunctional Glial Support by Semper Cells in the Drosophila Retina," *PLoS Genetics* 13, no. 5 (2017): e1006782, <https://doi.org/10.1371/journal.pgen.1006782>.
20. V. Hartenstein, "Morphological Diversity and Development of Glia in Drosophila," *Glia* 59, no. 9 (2011): 1237–1252, <https://doi.org/10.1002/glia.21162>.
21. R. Kottmeier, J. Bittern, A. Schoofs, et al., "Wrapping Glia Regulates Neuronal Signaling Speed and Precision in the Peripheral Nervous System of Drosophila," *Nature Communications* 11 (2020): 4491, <https://doi.org/10.1038/s41467-020-18291-1>.
22. I. Lago-Baldaia, M. Cooper, A. Seroka, et al., "A Drosophila Glial Cell Atlas Reveals a Mismatch Between Transcriptional and Morphological Diversity," *PLoS Biology* 21, no. 10 (2023): e3002328, <https://doi.org/10.1371/journal.pbio.3002328>.
23. T. Matzat, F. Sieglitz, R. Kottmeier, F. Babatz, D. Engelen, and C. Klämbt, "Axonal Wrapping in the Drosophila PNS Is Controlled by Glia-Derived Neuregulin Homolog Vein," *Development* 142, no. 7 (2015): 1336–1345, <https://doi.org/10.1242/dev.116616>.
24. S. Prelic, V. Pal Mahadevan, V. Venkateswaran, S. Lavista-Llanos, B. S. Hansson, and D. Wicher, "Functional Interaction Between Drosophila Olfactory Sensory Neurons and Their Support Cells,"

- Frontiers in Cellular Neuroscience* 15 (2022): 789086, <https://doi.org/10.3389/fncel.2021.789086>.
25. S. Rathore, A. Stahl, J. B. Benoit, and E. K. Buschbeck, "Exploring the Molecular Makeup of Support Cells in Insect Camera Eyes," *bioRxiv: The Preprint Server for Biology* (2023), 2023.07.19.549729, <https://doi.org/10.1101/2023.07.19.549729>.
 26. G. Hoyle, "Glial Cells of an Insect Ganglion," *Journal of Comparative Neurology* 246, no. 1 (1986): 85–103, <https://doi.org/10.1002/cne.902460106>.
 27. T. Stork, A. Sheehan, O. E. Tasdemir-Yilmaz, and M. R. Freeman, "Neuron-Glia Interactions Through the Heartless FGF Receptor Signaling Pathway Mediate Morphogenesis of Drosophila Astrocytes," *Neuron* 83, no. 2 (2014): 388–403, <https://doi.org/10.1016/j.neuron.2014.06.026>.
 28. M. R. Freeman, "Drosophila Central Nervous System Glia," *Cold Spring Harbor Perspectives in Biology* 7, no. 11 (2015): a020552, <https://doi.org/10.1101/cshperspect.a020552>.
 29. K. M. Markey, J. C. Saunders, J. Smuts, C. R. von Reyn, and A. D. R. Garcia, "Astrocyte Development—More Questions Than Answers," *Frontiers in Cell and Developmental Biology* 11 (2023): 1063843, <https://doi.org/10.3389/fcell.2023.1063843>.
 30. S. D. Ackerman, N. A. Perez-Catalan, M. R. Freeman, and C. Q. Doe, "Astrocytes Close a Motor Circuit Critical Period," *Nature* 592, no. 7854 (2021): 414–420, <https://doi.org/10.1038/s41586-021-03441-2>.
 31. E. Peco, S. Davla, D. Camp, S. M. Stacey, M. Landgraf, and D. J. van Meyel, "Drosophila Astrocytes Cover Specific Territories of the CNS Neuropil and Are Instructed to Differentiate by Prospero, a Key Effector of Notch," *Development* 143, no. 7 (2016): 1170–1181, <https://doi.org/10.1242/dev.133165>.
 32. N. Parinejad, E. Peco, T. Ferreira, S. M. Stacey, and D. J. van Meyel, "Disruption of an EAAT-Mediated Chloride Channel in a Drosophila Model of Ataxia," *Journal of Neuroscience* 36, no. 29 (2016): 7640–7647, <https://doi.org/10.1523/JNEUROSCI.0197-16.2016>.
 33. A. K. Muthukumar, T. Stork, and M. R. Freeman, "Activity-Dependent Regulation of Astrocyte GAT Levels During Synaptogenesis," *Nature Neuroscience* 17, no. 10 (2014): 1340–1350, <https://doi.org/10.1038/nn.3791>.
 34. T. Rival, L. Soustelle, C. Strambi, M.-T. Besson, M. Iché, and S. Birman, "Decreasing Glutamate Buffering Capacity Triggers Oxidative Stress and Neuropil Degeneration in the Drosophila Brain," *Current Biology* 14, no. 7 (2004): 599–605, <https://doi.org/10.1016/j.cub.2004.03.039>.
 35. W. W. Liu and R. I. Wilson, "Glutamate Is an Inhibitory Neurotransmitter in the Drosophila Olfactory System," *Proceedings of the National Academy of Sciences of the United States of America* 110, no. 25 (2013): 10294–10299, <https://doi.org/10.1073/pnas.1220560110>.
 36. O. E. Tasdemir-Yilmaz and M. R. Freeman, "Astrocytes Engage Unique Molecular Programs to Engulf Pruned Neuronal Debris From Distinct Subsets of Neurons," *Genes & Development* 28, no. 1 (2014): 20–33, <https://doi.org/10.1101/gad.229518.113>.
 37. Q. Ren, T. Awasaki, Y.-C. Wang, Y.-F. Huang, and T. Lee, "Lineage-Guided Notch-Dependent Gliogenesis by Drosophila Multi-Potent Progenitors," *Development* 145, no. 11 (2018): dev160127, <https://doi.org/10.1242/dev.160127>.
 38. J. J. Omoto, P. Yogi, and V. Hartenstein, "Origin and Development of Neuropil Glia of the Drosophila Larval and Adult Brain: Two Distinct Glial Populations Derived From Separate Progenitors," *Developmental Biology* 404, no. 2 (2015): 2–20, <https://doi.org/10.1016/j.ydbio.2015.03.004>.
 39. F. Pinto-Teixeira, N. Konstantinides, and C. Desplan, "Programmed Cell Death Acts at Different Stages of Drosophila Neurodevelopment to Shape the Central Nervous System," *FEBS Letters* 590, no. 15 (2016): 2435–2453, <https://doi.org/10.1002/1873-3468.12298>.
 40. K. Kato, M. Orihara-Ono, and T. Awasaki, "Multiple Lineages Enable Robust Development of the Neuropil-Glia Architecture in Adult Drosophila," *Development* 147, no. 5 (2020): dev184085, <https://doi.org/10.1242/dev.184085>.
 41. Y. Iwasaki, T. Hosoya, H. Takebayashi, Y. Ogawa, Y. Hotta, and K. Ikenaka, "The Potential to Induce Glial Differentiation Is Conserved Between Drosophila and Mammalian Glial Cells Missing Genes," *Development* 130, no. 24 (2003): 6027–6035, <https://doi.org/10.1242/dev.00822>.
 42. J. Schreiber, E. Sock, and M. Wegner, "The Regulator of Early Gliogenesis Glial Cells Missing Is a Transcription Factor With a Novel Type of DNA-Binding Domain," *Proceedings of the National Academy of Sciences of the United States of America* 94, no. 9 (1997): 4739–4744, <https://doi.org/10.1073/pnas.94.9.4739>.
 43. V. S. Mashanov, O. R. Zueva, and J. E. Garcia-Arraras, "Organization of Glial Cells in the Adult Sea Cucumber Central Nervous System," *Glia* 58, no. 13 (2010): 1581–1593, <https://doi.org/10.1002/glia.21031>.
 44. V. Mashanov and O. Zueva, "Radial Glia in Echinoderms," *Developmental Neurobiology* 79, no. 5 (2019): 396–405, <https://doi.org/10.1002/dneu.22659>.
 45. N. Jurisch-Yaksi, E. Yaksi, and C. Kizil, "Radial Glia in the Zebrafish Brain: Functional, Structural, and Physiological Comparison With the Mammalian Glia," *Glia* 68, no. 12 (2020): 2451–2470, <https://doi.org/10.1002/glia.23849>.
 46. F. J. Alenghat, Q. J. Baca, N. T. Rubin, et al., "Macrophages Require Skap2 and Sirpα for Integrin-Stimulated Cytoskeletal Rearrangement," *Journal of Cell Science* 125, no. 22 (2012): 5535–5545, <https://doi.org/10.1242/jcs.111260>.
 47. C. Ari and M. Kálmán, "Evolutionary Changes of Astroglia in Elasmobranchii Comparing to Amniotes: A Study Based on Three Immunohistochemical Markers (GFAP, S-100, and Glutamine Synthetase)," *Brain, Behavior and Evolution* 71, no. 4 (2008): 305–324, <https://doi.org/10.1159/000129654>.
 48. C. Yanes, M. Monzon-Mayor, M. S. Ghandour, J. de Barry, and G. Gombos, "Radial Glia and Astrocytes in Developing and Adult Telencephalon of the Lizard Gallotia Galloti as Revealed by Immunohistochemistry With Anti-GFAP and Anti-Vimentin Antibodies," *Journal of Comparative Neurology* 295, no. 4 (1990): 559–568, <https://doi.org/10.1002/cne.902950405>.
 49. C. Falcone, E. L. McBride, W. D. Hopkins, et al., "Redefining Varicose Projection Astrocytes in Primates," *Glia* 70, no. 1 (2021): 145–154, <https://doi.org/10.1002/glia.24093>.
 50. C. Falcone, M. Wolf-Ochoa, S. Amina, et al., "Cortical Interlaminar Astrocytes Across the Therian Mammal Radiation," *Journal of Comparative Neurology* 527, no. 10 (2019): 1654–1674, <https://doi.org/10.1002/cne.24605>.
 51. N. A. Oberheim, T. Takano, X. Han, et al., "Uniquely Hominid Features of Adult Human Astrocytes," *Journal of Neuroscience* 29, no. 10 (2009): 3276–3287, <https://doi.org/10.1523/JNEUROSCI.4707-08.2009>.
 52. G. Pistorio and C. Falcone, "Beyond Rodents and Primates: Uncovering Cortical Astrocyte Diversity Across Mammals," *Brain, Behavior and Evolution* 100, no. 4 (2025): 263–280, <https://doi.org/10.1159/000546178>.
 53. A. Verkhratsky and M. Nedergaard, "Physiology of Astroglia," *Physiological Reviews* 98, no. 1 (2018): 239–389, <https://doi.org/10.1152/physrev.00042.2016>.
 54. L. Lambeth and M. J. Blunt, "Electron-Microscopic Study of Monotreme Neuroglia," *Cells, Tissues, Organs* 93, no. 1 (1975): 115–125, <https://doi.org/10.1159/000144502>.

55. J. S. King, "A Comparative Investigation of Neuroglia in Representative Vertebrates: A Silver Carbonate Study," *Journal of Morphology* 119, no. 4 (1966): 435–465, <https://doi.org/10.1002/jmor.1051190405>.
56. A. Bairati and G. Tripoli, "Ricerche morfologiche ed istochimiche sulla glia del nevrasso di vertebrati," *Zeitschrift für Zellforschung und Mikroskopische Anatomie* 39, no. 4 (1954): 392–413, <https://doi.org/10.1007/BF00343889>.
57. P. Contu, "Ricerche sulla glioarchitettonica dei rettili (cheloni, sauri ed ofidi)" (1953), 58, 295–320.
58. M. López-Hidalgo, W. B. Hoover, and J. Schummers, "Spatial Organization of Astrocytes in Ferret Visual Cortex," *Journal of Comparative Neurology* 524, no. 17 (2016): 3561–3576, <https://doi.org/10.1002/cne.24015>.
59. K. Ciuba, A. Piotrowska, D. Chaudhury, et al., "Molecular Signature of Primate Astrocytes Reveals Pathways and Regulatory Changes Contributing to Human Brain Evolution," *Cell Stem Cell* 32, no. 3 (2025): 426–444.e14, <https://doi.org/10.1016/j.stem.2024.12.011>.
60. C. Ciani, G. Pistorio, S. Giancola, et al., "Varicose-Projection Astrocytes: Conserved reactive cells in brain pathology," *Science Advances* 12, no. 29 (2026): eady8204, <https://www.science.org/doi/10.1126/sciadv.ady8204>.
61. D. H. Rowitch and A. R. Kriegstein, "Developmental Genetics of Vertebrate Glial-Cell Specification," *Nature* 468, no. 7321 (2010): 214–222, <https://doi.org/10.1038/nature09611>.
62. S. Clavreul, L. Abdeladim, E. Hernández-Garzón, et al., "Cortical Astrocytes Develop in a Plastic Manner at Both Clonal and Cellular Levels," *Nature Communications* 10, no. 1 (2019): 4884, <https://doi.org/10.1038/s41467-019-12791-5>.
63. S. Clavreul, L. Dumas, and K. Loulier, "Astrocyte Development in the Cerebral Cortex: Complexity of Their Origin, Genesis, and Maturation," *Frontiers in Neuroscience* 16 (2022): 916055, <https://doi.org/10.3389/fnins.2022.916055>.
64. M. Y. Batiuk, A. Martirosyan, J. Wahis, et al., "Identification of Region-Specific Astrocyte Subtypes at Single Cell Resolution," *Nature Communications* 11, no. 1 (2020): 1, <https://doi.org/10.1038/s41467-019-14198-8>.
65. H. Chai, B. Diaz-Castro, E. Shigetomi, et al., "Neural Circuit-Specialized Astrocytes: Transcriptomic, Proteomic, Morphological, and Functional Evidence," *Neuron* 95, no. 3 (2017): 531–549.e9, <https://doi.org/10.1016/j.neuron.2017.06.029>.
66. F. Endo, A. Kasai, J. S. Soto, et al., "Molecular Basis of Astrocyte Diversity and Morphology Across the CNS in Health and Disease," *Science* 378, no. 6619 (2022): eadc9020, <https://doi.org/10.1126/science.adc9020>.
67. W. T. Farmer, T. Abrahamsson, S. Chierzi, et al., "Neurons Diversify Astrocytes in the Adult Brain Through Sonic Hedgehog Signaling," *Science* 351, no. 6275 (2016): 849–854, <https://doi.org/10.1126/science.aab3103>.
68. Á. Herrero-Navarro, L. Puche-Aroca, V. Moreno-Juan, et al., "Astrocytes and Neurons Share Region-Specific Transcriptional Signatures That Confer Regional Identity to Neuronal Reprogramming," *Science Advances* 7, no. 15 (2021): eabe8978, <https://doi.org/10.1126/sciadv.abe8978>.
69. N. Mattugini, R. Bocchi, V. Scheuss, et al., "Inducing Different Neuronal Subtypes From Astrocytes in the Injured Mouse Cerebral Cortex," *Neuron* 103, no. 6 (2019): 1086–1095.e5, <https://doi.org/10.1016/j.neuron.2019.08.009>.
70. O. A. Bayraktar, T. Bartels, S. Holmqvist, et al., "Astrocyte Layers in the Mammalian Cerebral Cortex Revealed by a Single-Cell in Situ Transcriptomic Map," *Nature Neuroscience* 23, no. 4 (2020): 500–509, <https://doi.org/10.1038/s41593-020-0602-1>.
71. D. Lanjakornsiripan, B.-J. Pior, D. Kawaguchi, et al., "Layer-Specific Morphological and Molecular Differences in Neocortical Astrocytes and Their Dependence on Neuronal Layers," *Nature Communications* 9, no. 1 (2018): 1, <https://doi.org/10.1038/s41467-018-03940-3>.
72. J. Zhou, I. Vitali, S. Roig-Puiggros, et al., "Dual Lineage Origins Contribute to Neocortical Astrocyte Diversity," *Nature Communications* 16, no. 1 (2025): 6992, <https://doi.org/10.1038/s41467-025-61829-4>.
73. R. Bocchi, M. Thorwirth, T. Simon-Ebert, et al., "Astrocyte Heterogeneity Reveals Region-Specific Astrogenesis in the White Matter," *Nature Neuroscience* 28, no. 3 (2025): 457–469, <https://doi.org/10.1038/s41593-025-01878-6>.
74. J. Enriquez, L. Q. Rio, R. Blazeski, et al., "Differing Strategies Despite Shared Lineages of Motor Neurons and Glia to Achieve Robust Development of an Adult Neuropil in *Drosophila*," *Neuron* 97, no. 3 (2018): 538–554.e5, <https://doi.org/10.1016/j.neuron.2018.01.007>.
75. J. García-Marqués and L. López-Mascaraque, "Clonal Identity Determines Astrocyte Cortical Heterogeneity," *Cerebral Cortex* 23, no. 6 (2013): 1463–1472, <https://doi.org/10.1093/cercor/bhs134>.
76. R. C. Bandler, I. Vitali, R. N. Delgado, et al., "Single-Cell Delineation of Lineage and Genetic Identity in the Mouse Brain," *Nature* 601, no. 7893 (2022): 404–409, <https://doi.org/10.1038/s41586-021-04237-0>.
77. I. Farhy-Tselnicker, M. M. Boisvert, H. Liu, et al., "Activity-Dependent Modulation of Synapse-Regulating Genes in Astrocytes," *eLife* 10 (2021): e70514, <https://doi.org/10.7554/eLife.70514>.
78. A. M. Baraibar, L. Belisle, G. Marsicano, et al., "Spatial Organization of Neuron–Astrocyte Interactions in the Somatosensory Cortex," *Cerebral Cortex* 33, no. 8 (2023): 4498–4511, <https://doi.org/10.1093/cercor/bhac357>.
79. M. Ollivier, J. S. Soto, K. E. Linker, et al., "Crym-Positive Striatal Astrocytes Gate Perseverative Behaviour," *Nature* 627, no. 8003 (2024): 358–366, <https://doi.org/10.1038/s41586-024-07138-0>.
80. J. A. Stogsdill, J. Ramirez, D. Liu, et al., "Astrocytic Neuroligins Control Astrocyte Morphogenesis and Synaptogenesis," *Nature* 551, no. 7679 (2017): 192–197, <https://doi.org/10.1038/nature24638>.
81. T. Takano, J. T. Wallace, K. T. Baldwin, et al., "Chemico-Genetic Discovery of Astrocytic Control of Inhibition in Vivo," *Nature* 588, no. 7837 (2020): 296–302, <https://doi.org/10.1038/s41586-020-2926-0>.
82. C. K. Salmon, T. A. Syed, J. B. Kacerovsky, et al., "Organizing Principles of Astrocytic Nanoarchitecture in the Mouse Cerebral Cortex," *Current Biology* 33, no. 5 (2023): 957–972.e5, <https://doi.org/10.1016/j.cub.2023.01.043>.
83. J. S. Soto, Y. Jami-Alahmadi, J. Chacon, et al., "Astrocyte-Neuron Subproteomes and Obsessive-Compulsive Disorder Mechanisms," *Nature* 616, no. 7958 (2023): 764–773, <https://doi.org/10.1038/s41586-023-05927-7>.
84. N. J. Allen and D. A. Lyons, "Glia as Architects of Central Nervous System Formation and Function," *Science* 362, no. 6411 (2018): 181–185, <https://doi.org/10.1126/science.aat0473>.
85. P. J. Magistretti and I. Allaman, "Lactate in the Brain: From Metabolic End-Product to Signalling Molecule," *Nature Reviews. Neuroscience* 19, no. 4 (2018): 235–249, <https://doi.org/10.1038/nrn.2018.19>.
86. D. Liu, T. T. L. Nguyen, H. Gao, et al., "TCF7L2 lncRNA: A Link Between Bipolar Disorder and Body Mass Index Through Glucocorticoid Signaling," *Molecular Psychiatry* 26, no. 12 (2021): 7454–7464, <https://doi.org/10.1038/s41380-021-01274-z>.
87. J. Bem, N. Brožko, C. Chakraborty, et al., "Wnt/ β -Catenin Signaling in Brain Development and Mental Disorders: Keeping TCF7L2 in Mind," *FEBS Letters* 593, no. 13 (2019): 1654–1674, <https://doi.org/10.1002/1873-3468.13502>.

88. O. Chodelkova, J. Masek, V. Korinek, Z. Kozmik, and O. Machon, "Tcf7L2 Is Essential for Neurogenesis in the Developing Mouse Neocortex," *Neural Development* 13, no. 1 (2018): 8, <https://doi.org/10.1186/s13064-018-0107-8>.
89. E. Hammond, J. Lang, Y. Maeda, et al., "The Wnt Effector Transcription Factor 7-Like 2 Positively Regulates Oligodendrocyte Differentiation in a Manner Independent of Wnt/ β -Catenin Signaling," *Journal of Neuroscience* 35, no. 12 (2015): 5007–5022, <https://doi.org/10.1523/JNEUROSCI.4787-14.2015>.
90. C. Zhao, Y. Deng, L. Liu, et al., "Dual Regulatory Switch Through Interactions of Tcf7l2/Tcf4 With Stage-Specific Partners Propels Oligodendroglial Maturation," *Nature Communications* 7 (2016): 10883, <https://doi.org/10.1038/ncomms10883>.
91. M. A. Lipiec, J. Bem, K. Koziński, et al., "TCF7L2 Regulates Postmitotic Differentiation Programmes and Excitability Patterns in the Thalamus," *Development* 147, no. 16 (2020): dev190181, <https://doi.org/10.1242/dev.190181>.
92. L. M. Szewczyk, M. A. Lipiec, E. Liszewska, et al., "Astrocytic β -Catenin Signaling via TCF7L2 Regulates Synapse Development and Social Behavior," *Molecular Psychiatry* 29, no. 1 (2024): 57–73, <https://doi.org/10.1038/s41380-023-02281-y>.
93. M. Popek, K. Goryca, D. Adamska, et al., "Astrocytic TCF7L2 Impacts Brain Osmoregulation and Restricts Neuronal Excitability," *Glia* 74, no. 2 (2026): e70103, <https://doi.org/10.1002/glia.70103>.
94. M. G. Holt, "Astrocyte Heterogeneity and Interactions With Local Neural Circuits," *Essays in Biochemistry* 67, no. 1 (2023): 93–106, <https://doi.org/10.1042/EBC20220136>.
95. Y. Hu and G. S. Wilson, "A Temporary Local Energy Pool Coupled to Neuronal Activity: Fluctuations of Extracellular Lactate Levels in Rat Brain Monitored With Rapid-Response Enzyme-Based Sensor," *Journal of Neurochemistry* 69, no. 4 (1997): 1484–1490, <https://doi.org/10.1046/j.1471-4159.1997.69041484.x>.
96. J. Prichard, D. Rothman, E. Novotny, et al., "Lactate Rise Detected by ¹H NMR in Human Visual Cortex During Physiologic Stimulation," *Proceedings of the National Academy of Sciences of the United States of America* 88, no. 13 (1991): 5829–5831, <https://doi.org/10.1073/pnas.88.13.5829>.
97. I. K. Suzuki, D. Gacquer, R. Van Heurck, et al., "Human-Specific NOTCH2NL Genes Expand Cortical Neurogenesis Through Delta/Notch Regulation," *Cell* 173, no. 6 (2018): 1370–1384.e16, <https://doi.org/10.1016/j.cell.2018.03.067>.
98. L. Pellerin and P. J. Magistretti, "Glutamate Uptake Into Astrocytes Stimulates Aerobic Glycolysis: A Mechanism Coupling Neuronal Activity to Glucose Utilization," *Proceedings of the National Academy of Sciences of the United States of America* 91, no. 22 (1994): 10625–10629, <https://doi.org/10.1073/pnas.91.22.10625>.
99. P. G. Bittar, Y. Charnay, L. Pellerin, C. Bouras, and P. J. Magistretti, "Selective Distribution of Lactate Dehydrogenase Isoenzymes in Neurons and Astrocytes of Human Brain," *Journal of Cerebral Blood Flow & Metabolism* 16, no. 6 (1996): 1079–1089, <https://doi.org/10.1097/00004647-199611000-00001>.
100. L. A. Demetrius and D. K. Simon, "The Inverse Association of cancer and Alzheimer's: A Bioenergetic Mechanism," *Journal of the Royal Society Interface* 10, no. 82 (2013): 20130006, <https://doi.org/10.1098/rsif.2013.0006>.
101. J. D. Laughton, P. Bittar, Y. Charnay, et al., "Metabolic Compartmentalization in the Human Cortex and hippocampus: Evidence for a Cell- and Region-Specific Localization of Lactate Dehydrogenase 5 and Pyruvate Dehydrogenase," *BMC Neuroscience* 8 (2007): 35, <https://doi.org/10.1186/1471-2202-8-35>.
102. B. Quistorff and N. Grunnet, "The Isoenzyme Pattern of LDH Does Not Play a Physiological Role; Except Perhaps During Fast Transitions in Energy Metabolism," *Aging (Albany NY)* 3, no. 5 (2011): 457–460, <https://doi.org/10.18632/aging.100329>.
103. Y. Zhang, K. Chen, S. A. Sloan, et al., "An RNA-Sequencing Transcriptome and Splicing Database of Glia, Neurons, and Vascular Cells of the Cerebral Cortex," *Journal of Neuroscience* 34, no. 36 (2014): 11929–11947, <https://doi.org/10.1523/JNEUROSCI.1860-14.2014>.
104. R. Mongeon, V. Venkatachalam, and G. Yellen, "Cytosolic NADH-NAD(+) Redox Visualized in Brain Slices by Two-Photon Fluorescence Lifetime Biosensor Imaging," *Antioxidants & Redox Signaling* 25, no. 10 (2016): 553–563, <https://doi.org/10.1089/ars.2015.6593>.
105. A. M. Cataldo and R. D. Broadwell, "Cytochemical Identification of Cerebral Glycogen and Glucose-6-Phosphatase Activity Under Normal and Experimental Conditions. II. Choroid Plexus and Ependymal Epithelia, Endothelia and Pericytes," *Journal of Neurocytology* 15, no. 4 (1986): 511–524, <https://doi.org/10.1007/BF01611733>.
106. M. Z. M. Ibrahim, *Glycogen and Its Related Enzymes of Metabolism in the Central Nervous System* (Springer, 1975), <https://doi.org/10.1007/978-3-642-86875-7>.
107. Y. Oe, O. Baba, H. Ashida, K. C. Nakamura, and H. Hirase, "Glycogen Distribution in the Microwave-Fixed Mouse Brain Reveals Heterogeneous Astrocytic Patterns," *Glia* 64, no. 9 (2016): 1532–1545, <https://doi.org/10.1002/glia.23020>.
108. G. A. Brooks, "Cell–Cell and Intracellular Lactate Shuttles," *Journal of Physiology* 587, no. 23 (2009): 5591–5600, <https://doi.org/10.1113/jphysiol.2009.178350>.
109. G. A. Dienel, "Lack of Appropriate Stoichiometry: Strong Evidence Against an Energetically Important Astrocyte–Neuron Lactate Shuttle in Brain," *Journal of Neuroscience Research* 95, no. 11 (2017): 2103–2125, <https://doi.org/10.1002/jnr.24015>.
110. M. DiNuzzo, S. Mangia, B. Maraviglia, and F. Giove, "Changes in Glucose Uptake Rather Than Lactate Shuttle Take Center Stage in Subservient Neuroenergetics: Evidence From Mathematical Modeling," *Journal of Cerebral Blood Flow and Metabolism* 30, no. 3 (2010): 586–602, <https://doi.org/10.1038/jcbfm.2009.232>.
111. S. Mangia, I. A. Simpson, S. J. Vannucci, and A. Carruthers, "The in Vivo Neuron-To-Astrocyte Lactate Shuttle in Human Brain: Evidence From Modeling of Measured Lactate Levels During Visual Stimulation," *Journal of Neurochemistry* 109, no. Suppl 1 (2009): 55–62, <https://doi.org/10.1111/j.1471-4159.2009.06003.x>.
112. I. A. Simpson, A. Carruthers, and S. J. Vannucci, "Supply and Demand in Cerebral Energy Metabolism: The Role of Nutrient Transporters," *Journal of Cerebral Blood Flow and Metabolism* 27, no. 11 (2007): 1766–1791, <https://doi.org/10.1038/sj.jcbfm.9600521>.
113. S. Baltan, "Can Lactate Serve as an Energy Substrate for Axons in Good Times and in Bad, in Sickness and in Health?," *Metabolic Brain Disease* 30, no. 1 (2015): 25–30, <https://doi.org/10.1007/s11011-014-9595-3>.
114. C. M. Díaz-García, R. Mongeon, C. Lahmann, D. Koveal, H. Zucker, and G. Yellen, "Neuronal Stimulation Triggers Neuronal Glycolysis and Not Lactate Uptake," *Cell Metabolism* 26, no. 2 (2017): 361–374.e4, <https://doi.org/10.1016/j.cmet.2017.06.021>.
115. U. Fünfschilling, L. M. Supplie, D. Mahad, et al., "Glycolytic Oligodendrocytes Maintain Myelin and Long-Term Axonal Integrity," *Nature* 485, no. 7399 (2012): 517–521, <https://doi.org/10.1038/nature11007>.
116. Y. Lee, B. M. Morrison, Y. Li, et al., "Oligodendroglia Metabolically Support Axons and Contribute to Neurodegeneration," *Nature* 487, no. 7408 (2012): 443–448, <https://doi.org/10.1038/nature11314>.
117. S. Mason, "Lactate Shuttles in Neuroenergetics-Homeostasis, Allostasis and Beyond," *Frontiers in Neuroscience* 11 (2017): 43, <https://doi.org/10.3389/fnins.2017.00043>.

118. Y. Sawada, A. Konno, J. Nagaoka, and H. Hirai, "Inflammation-Induced Reversible Switch of the Neuron-Specific Enolase Promoter From Purkinje Neurons to Bergmann Glia," *Scientific Reports* 6 (2016): 27758, <https://doi.org/10.1038/srep27758>.
119. E. W. Westi, R. B. Carlsen, J. V. Andersen, et al., "Metabolic Flexibility of Microglia: Energy Substrate Utilization and Impact on Neuronal Metabolism," *Journal of Neurochemistry* 169, no. 11 (2025): e70304, <https://doi.org/10.1111/jnc.70304>.
120. A. Horvat, R. Zorec, and N. Vardjan, "Adrenergic Stimulation of Single Rat Astrocytes Results in Distinct Temporal Changes in Intracellular Ca(2+) and cAMP-Dependent PKA Responses," *Cell Calcium* 59, no. 4 (2016): 156–163, <https://doi.org/10.1016/j.ceca.2016.01.002>.
121. N. Vardjan, H. H. Chowdhury, A. Horvat, et al., "Enhancement of Astroglial Aerobic Glycolysis by Extracellular Lactate-Mediated Increase in cAMP," *Frontiers in Molecular Neuroscience* 11 (2018): 148, <https://doi.org/10.3389/fnmol.2018.00148>.
122. H. d. C. Abrantes, M. Briquet, C. Schmuziger, et al., "The Lactate Receptor HCAR1 Modulates Neuronal Network Activity Through the Activation of Gα and Gβγ Subunits," *Journal of Neuroscience* 39, no. 23 (2019): 4422–4433, <https://doi.org/10.1523/JNEUROSCI.2092-18.2019>.
123. L. Bozzo, J. Puyal, and J.-Y. Chatton, "Lactate Modulates the Activity of Primary Cortical Neurons Through a Receptor-Mediated Pathway," *PLoS One* 8, no. 8 (2013): e71721, <https://doi.org/10.1371/journal.pone.0071721>.
124. N. Dupuis, C. Laschet, D. Franssen, et al., "Activation of the Orphan G Protein–Coupled Receptor GPR27 by Surrogate Ligands Promotes β-Arrestin 2 Recruitment," *Molecular Pharmacology* 91, no. 6 (2017): 595–608, <https://doi.org/10.1124/mol.116.107714>.
125. D. Dolanc, T. M. Zorec, Z. Smole, et al., "The Activation of GPR27 Increases Cytosolic L-Lactate in 3T3 Embryonic Cells and Astrocytes," *Cells* 11, no. 6 (2022): 1009, <https://doi.org/10.3390/cells11061009>.
126. M. S. Hossain, K. Mineno, and T. Katafuchi, "Neuronal Orphan G-Protein Coupled Receptor Proteins Mediate Plasmalogens-Induced Activation of ERK and Akt Signaling," *PLoS One* 11, no. 3 (2016): e0150846, <https://doi.org/10.1371/journal.pone.0150846>.
127. F. Tang, S. Lane, A. Korsak, et al., "Lactate-Mediated Glia-Neuronal Signalling in the Mammalian Brain," *Nature Communications* 5 (2014): 3284, <https://doi.org/10.1038/ncomms4284>.
128. J. Clasadonte, E. Scemes, Z. Wang, D. Boison, and P. G. Haydon, "Connexin 43-Mediated Astroglial Metabolic Networks Contribute to the Regulation of the Sleep-Wake Cycle," *Neuron* 95, no. 6 (2017): 1365–1380.e5, <https://doi.org/10.1016/j.neuron.2017.08.022>.
129. M. P. Parsons and M. Hirasawa, "ATP-Sensitive Potassium Channel-Mediated Lactate Effect on Orexin Neurons: Implications for Brain Energetics During Arousal," *Journal of Neuroscience* 30, no. 24 (2010): 8061–8070, <https://doi.org/10.1523/JNEUROSCI.5741-09.2010>.
130. A. Verkhratsky and A. Semyanov, "The Great Astroglial Metabolic Revolution: Mitochondria Fuel Astrocyte Homeostatic Support and Neuroprotection," *Cell Calcium* 104 (2022): 102583, <https://doi.org/10.1016/j.ceca.2022.102583>.
131. T. Düking, L. Spieth, S. A. Berghoff, et al., "Ketogenic Diet Uncovers Differential Metabolic Plasticity of Brain Cells," *Science Advances* 8, no. 37 (2022): eabo7639, <https://doi.org/10.1126/sciadv.abo7639>.
132. C. Fecher, L. Trovò, S. A. Müller, et al., "Cell-Type-Specific Profiling of Brain Mitochondria Reveals Functional and Molecular Diversity," *Nature Neuroscience* 22, no. 10 (2019): 1731–1742, <https://doi.org/10.1038/s41593-019-0479-z>.
133. C. Blázquez, C. Sánchez, G. Velasco, and M. Guzmán, "Role of Carnitine Palmitoyltransferase I in the Control of Ketogenesis in Primary Cultures of Rat Astrocytes," *Journal of Neurochemistry* 71, no. 4 (1998): 1597–1606, <https://doi.org/10.1046/j.1471-4159.1998.71041597.x>.
134. M. S. Ioannou, J. Jackson, S.-H. Sheu, et al., "Neuron-Astrocyte Metabolic Coupling Protects Against Activity-Induced Fatty Acid Toxicity," *Cell* 177, no. 6 (2019): 1522–1535.e14, <https://doi.org/10.1016/j.cell.2019.04.001>.
135. Y. Mi, G. Qi, F. Vitali, et al., "Loss of Fatty Acid Degradation by Astrocytic Mitochondria Triggers Neuroinflammation and Neurodegeneration," *Nature Metabolism* 5, no. 3 (2023): 445–465, <https://doi.org/10.1038/s42255-023-00756-4>.
136. G. Qi, Y. Mi, X. Shi, H. Gu, R. D. Brinton, and F. Yin, "ApoE4 Impairs Neuron-Astrocyte Coupling of Fatty Acid Metabolism," *Cell Reports* 34, no. 1 (2021): 108572, <https://doi.org/10.1016/j.celrep.2020.108572>.
137. K. Timper, A. Del Río-Martín, A. L. Cremer, et al., "GLP-1 Receptor Signaling in Astrocytes Regulates Fatty Acid Oxidation, Mitochondrial Integrity, and Function," *Cell Metabolism* 31, no. 6 (2020): 1189–1205.e13, <https://doi.org/10.1016/j.cmet.2020.05.001>.
138. B. Morant-Ferrando, D. Jimenez-Blasco, P. Alonso-Batan, et al., "Fatty Acid Oxidation Organizes Mitochondrial Supercomplexes to Sustain Astrocytic ROS and Cognition," *Nature Metabolism* 5, no. 8 (2023): 1290–1302, <https://doi.org/10.1038/s42255-023-00835-6>.
139. T. Zehnder, F. Petrelli, J. Romanos, et al., "Mitochondrial Biogenesis in Developing Astrocytes Regulates Astrocyte Maturation and Synapse Formation," *Cell Reports* 35, no. 2 (2021): 108952, <https://doi.org/10.1016/j.celrep.2021.108952>.
140. M. P. Rodriguez Salazar, S. Kolanukuduru, V. Ramirez, et al., "Mitochondrial Fission Controls Astrocyte Morphogenesis and Organization in the Cortex," *Journal of Cell Biology* 224, no. 10 (2025): e202410130, <https://doi.org/10.1083/jcb.202410130>.
141. D. Lim, A. Semyanov, A. Genazzani, and A. Verkhratsky, "Calcium signaling in neuroglia," *International Review of Cell and Molecular Biology* 362 (2021): 1–53, <https://doi.org/10.1016/bs.ircmb.2021.01.003>.
142. P. Kelly, E. Hudry, S. S. Hou, and B. J. Bacskaï, "In Vivo Two Photon Imaging of Astrocytic Structure and Function in Alzheimer's Disease," *Frontiers in Aging Neuroscience* 10 (2018): 219, <https://doi.org/10.3389/fnagi.2018.00219>.
143. T. Nagai, S. Yamada, T. Tominaga, M. Ichikawa, and A. Miyawaki, "Expanded Dynamic Range of Fluorescent Indicators for Ca(2+) by Circularly Permuted Yellow Fluorescent Proteins," *Proceedings of the National Academy of Sciences of the United States of America* 101, no. 29 (2004): 10554–10559, <https://doi.org/10.1073/pnas.0400417101>.
144. C. Lohr, A. Beiersdorfer, T. Fischer, et al., "Using Genetically Encoded Calcium Indicators to Study Astrocyte Physiology: A Field Guide," *Frontiers in Cellular Neuroscience* 15 (2021): 690147, <https://doi.org/10.3389/fncel.2021.690147>.
145. E. Shigetomi, E. A. Bushong, M. D. Haustein, et al., "Imaging Calcium Microdomains Within Entire Astrocyte Territories and Endfeet With GCaMPs Expressed Using Adeno-Associated Viruses," *Journal of General Physiology* 141, no. 5 (2013): 633–647, <https://doi.org/10.1085/jgp.201210949>.
146. V. Pérez Koldenkova and T. Nagai, "Genetically Encoded ca(2+) Indicators: Properties and Evaluation," *Biochimica et Biophysica Acta* 1833, no. 7 (2013): 1787–1797, <https://doi.org/10.1016/j.bbamcr.2013.01.011>.
147. N. Zhang, Z. Zhang, I. Ozden, and S. Ding, "Imaging Mitochondrial Ca2+ Uptake in Astrocytes and Neurons Using Genetically Encoded Ca2+ Indicators (GECIs)," *Journal of Visualized Experiments* 179 (2022), <https://doi.org/10.3791/62917>.
148. F. E. Müller, V. Cherkas, G. Stopper, et al., "Elucidating Regulators of Astrocytic Ca2+ Signaling via Multi-Threshold Event Detection

- (MTED),” *Glia* 69, no. 12 (2021): 2798–2811, <https://doi.org/10.1002/glia.24070>.
149. F. E. Müller, F. Ivanov, A.-C. Studt, et al., “LPS-Induced Inflammation Differentially Affects Endogenous Ca²⁺ Activity in Mouse and Human iPSC-Derived Astrocytes,” *Molecular Medicine* 32, no. 1 (2026): 52, <https://doi.org/10.1186/s10020-026-01450-3>.
 150. M. V. Sanchez-Mico, M. Calvo-Rodriguez, and B. J. Bacskaï, “Role of Dysregulated Calcium Homeostasis in Astrocytes in Neurodegenerative Disorders,” *Nature Reviews Neuroscience* 27 (2026): 377–394, <https://doi.org/10.1038/s41583-026-01032-6>.
 151. C. R. Rose and A. Verkhratsky, “Sodium Homeostasis and Signalling: The Core and the Hub of Astrocyte Function,” *Cell Calcium* 117 (2024): 102817, <https://doi.org/10.1016/j.ceca.2023.102817>.
 152. V. Untiet, F. R. M. Beinlich, P. Kusk, et al., “Astrocytic Chloride Is Brain State Dependent and Modulates Inhibitory Neurotransmission in Mice,” *Nature Communications* 14, no. 1 (2023): 1871, <https://doi.org/10.1038/s41467-023-37433-9>.
 153. M. J. Pereira, R. Ayana, M. G. Holt, and L. Arckens, “Chemogenetic Manipulation of Astrocyte Activity at the Synapse—A Gateway to Manage Brain Disease,” *Frontiers in Cell and Developmental Biology* 11 (2023): 1193130, <https://doi.org/10.3389/fcell.2023.1193130>.
 154. J. Wahis, M. Hennes, L. Arckens, and M. G. Holt, “Star Power: The Emerging Role of Astrocytes as Neuronal Partners During Cortical Plasticity,” *Current Opinion in Neurobiology* 67 (2021): 174–182, <https://doi.org/10.1016/j.conb.2020.12.001>.
 155. M. Hennes, N. Lombaert, J. Wahis, C. Van den Haute, M. G. Holt, and L. Arckens, “Astrocytes Shape the Plastic Response of Adult Cortical Neurons to Vision Loss,” *Glia* 68, no. 10 (2020): 2102–2118, <https://doi.org/10.1002/glia.23830>.
 156. L. Van Brussel, A. Gerits, and L. Arckens, “Evidence for Cross-Modal Plasticity in Adult Mouse Visual Cortex Following Monocular Enucleation,” *Cerebral Cortex* 21, no. 9 (2011): 2133–2146, <https://doi.org/10.1093/cercor/bhq286>.
 157. B. S. Khakh and B. Deneen, “The Emerging Nature of Astrocyte Diversity,” *Annual Review of Neuroscience* 42 (2019): 187–207, <https://doi.org/10.1146/annurev-neuro-070918-050443>.
 158. J. Nagai, X. Yu, T. Papouin, et al., “Behaviorally Consequential Astrocytic Regulation of Neural Circuits,” *Neuron* 109, no. 4 (2021): 576–596, <https://doi.org/10.1016/j.neuron.2020.12.008>.
 159. F. Pestana, G. Edwards-Faret, T. G. Belgard, A. Martirosyan, and M. G. Holt, “No Longer Underappreciated: The Emerging Concept of Astrocyte Heterogeneity in Neuroscience,” *Brain Sciences* 10, no. 3 (2020): 168, <https://doi.org/10.3390/brainsci10030168>.
 160. K. S. Christopherson, E. M. Ullian, C. C. A. Stokes, et al., “Thrombospondins Are Astrocyte-Secreted Proteins That Promote CNS Synaptogenesis,” *Cell* 120, no. 3 (2005): 421–433, <https://doi.org/10.1016/j.cell.2004.12.020>.
 161. C. Eroglu, “The Role of Astrocyte-Secreted Matricellular Proteins in Central Nervous System Development and Function,” *Journal of Cell Communication and Signaling* 3, no. 3–4 (2009): 167–176, <https://doi.org/10.1007/s12079-009-0078-y>.
 162. Ç. Eroglu, N. J. Allen, M. W. Susman, et al., “The Gabapentin Receptor $\alpha 2\delta$ -1 Is the Neuronal Thrombospondin Receptor Responsible for Excitatory CNS Synaptogenesis,” *Cell* 139, no. 2 (2009): 380–392, <https://doi.org/10.1016/j.cell.2009.09.025>.
 163. W. C. Risher, N. Kim, S. Koh, et al., “Thrombospondin Receptor $\alpha 2\delta$ -1 Promotes Synaptogenesis and Spinogenesis via Postsynaptic Rac1,” *Journal of Cell Biology* 217, no. 10 (2018): 3747–3765, <https://doi.org/10.1083/jcb.201802057>.
 164. P. T. Kurshan, A. Oztan, and T. L. Schwarz, “Presynaptic $\alpha 2\delta$ -3 Is Required for Synaptic Morphogenesis Independent of Its Ca²⁺—Channel Functions,” *Nature Neuroscience* 12, no. 11 (2009): 1415–1423, <https://doi.org/10.1038/nn.2417>.
 165. F. P. Ulloa Severino, O. O. Lawal, K. Sakers, et al., “Training-Induced Circuit-Specific Excitatory Synaptogenesis in Mice Is Required for Effort Control,” *Nature Communications* 14, no. 1 (2023): 5522, <https://doi.org/10.1038/s41467-023-41078-z>.
 166. O. O. Lawal, F. P. U. Severino, S. Wang, et al., “Astrocytic Thrombospondins 1 and 2 Are Required for Cortical Synapse Development Controlling Instrumental Performance,” *bioRxiv: The Preprint Server for Biology* (2024), 2024.03.01.582935, <https://doi.org/10.1101/2024.03.01.582935>.
 167. W. Cheng, D. Gordian, M. Q. Ludwig, T. H. Pers, R. J. Seeley, and M. G. Myers, “Hindbrain Circuits in the Control of Eating Behaviour and Energy Balance,” *Nature Metabolism* 4, no. 7 (2022): 826–835, <https://doi.org/10.1038/s42255-022-00606-9>.
 168. K. Timper and J. C. Brüning, “Hypothalamic Circuits Regulating Appetite and Energy Homeostasis: Pathways to Obesity,” *Disease Models & Mechanisms* 10, no. 6 (2017): 679–689, <https://doi.org/10.1242/dmm.026609>.
 169. C. García-Cáceres, E. Balland, V. Prevot, et al., “Role of Astrocytes, Microglia, and Tanycytes in Brain Control of Systemic Metabolism,” *Nature Neuroscience* 22, no. 1 (2019): 7–14, <https://doi.org/10.1038/s41593-018-0286-y>.
 170. C. García-Cáceres, C. Quarta, L. Varela, et al., “Astrocytic Insulin Signaling Couples Brain Glucose Uptake With Nutrient Availability,” *Cell* 166, no. 4 (2016): 867–880, <https://doi.org/10.1016/j.cell.2016.07.028>.
 171. J. G. Kim, S. Suyama, M. Koch, et al., “Leptin Signaling in Astrocytes Regulates Hypothalamic Neuronal Circuits and Feeding,” *Nature Neuroscience* 17, no. 7 (2014): 908–910, <https://doi.org/10.1038/nn.3725>.
 172. Y. Aponte, D. Atasoy, and S. M. Sternson, “AGRP Neurons Are Sufficient to Orchestrate Feeding Behavior Rapidly and Without Training,” *Nature Neuroscience* 14, no. 3 (2011): 351–355, <https://doi.org/10.1038/nn.2739>.
 173. J. N. Betley, S. Xu, Z. F. H. Cao, et al., “Neurons for Hunger and Thirst Transmit a Negative-Valence Teaching Signal,” *Nature* 521, no. 7551 (2015): 180–185, <https://doi.org/10.1038/nature14416>.
 174. L. Varela, B. Stutz, J. E. Song, et al., “Hunger-Promoting AgRP Neurons Trigger an Astrocyte-Mediated Feed-Forward Autoactivation Loop in Mice,” *Journal of Clinical Investigation* 131, no. 10 (2021): e144239, <https://doi.org/10.1172/JCI144239>.
 175. N. Chen, H. Sugihara, J. Kim, et al., “Direct Modulation of GFAP-Expressing Glia in the Arcuate Nucleus Bi-Directionally Regulates Feeding,” *eLife* 5 (2016): e18716, <https://doi.org/10.7554/eLife.18716>.
 176. L. Yang, Y. Qi, and Y. Yang, “Astrocytes Control Food Intake by Inhibiting AGRP Neuron Activity via Adenosine A1 Receptors,” *Cell Reports* 11, no. 5 (2015): 798–807, <https://doi.org/10.1016/j.celrep.2015.04.002>.
 177. C. Zhan, J. Zhou, Q. Feng, et al., “Acute and Long-Term Suppression of Feeding Behavior by POMC Neurons in the Brainstem and Hypothalamus, Respectively,” *Journal of Neuroscience* 33, no. 8 (2013): 3624–3632, <https://doi.org/10.1523/JNEUROSCI.2742-12.2013>.
 178. D. Nuzzaci, C. Cansell, F. Liénard, et al., “Postprandial Hyperglycemia Stimulates Neuroglial Plasticity in Hypothalamic POMC Neurons After a Balanced Meal,” *Cell Reports* 30, no. 9 (2020): 3067–3078.e5, <https://doi.org/10.1016/j.celrep.2020.02.029>.
 179. K. Bouyakdan, H. Martin, F. Liénard, et al., “The Gliotransmitter ACBP Controls Feeding and Energy Homeostasis via the Melanocortin System,” *Journal of Clinical Investigation* 129, no. 6 (2019): 2417–2430, <https://doi.org/10.1172/JCI123454>.

180. F. Guillebaud, M. Duquenne, M. Djelloul, et al., "Glial Endozepines Reverse High-Fat Diet-Induced Obesity by Enhancing Hypothalamic Response to Peripheral Leptin," *Molecular Neurobiology* 57, no. 8 (2020): 3307–3333, <https://doi.org/10.1007/s12035-020-01944-z>.
181. J.-D. Troadec, S. Gaigé, M. Barbot, B. Lebrun, R. Barbouche, and A. Abysique, "Glial Modulation of Energy Balance: The Dorsal Vagal Complex Is No Exception," *International Journal of Molecular Sciences* 23, no. 2 (2022): 960, <https://doi.org/10.3390/ijms23020960>.
182. D. J. Reiner, E. G. Mietlicki-Baase, L. E. McGrath, et al., "Astrocytes Regulate GLP-1 Receptor-Mediated Effects on Energy Balance," *Journal of Neuroscience* 36, no. 12 (2016): 3531–3540, <https://doi.org/10.1523/JNEUROSCI.3579-15.2016>.
183. A. J. MacDonald, F. E. Holmes, C. Beall, A. E. Pickering, and K. L. J. Ellacott, "Regulation of Food Intake by Astrocytes in the Brainstem Dorsal Vagal Complex," *Glia* 68, no. 6 (2020): 1241–1254, <https://doi.org/10.1002/glia.23774>.
184. W. Cheng, I. Gonzalez, W. Pan, et al., "Calcitonin Receptor Neurons in the Mouse Nucleus Tractus Solitarius Control Energy Balance via the Non-Aversive Suppression of Feeding," *Cell Metabolism* 31, no. 2 (2020): 301–312.e5, <https://doi.org/10.1016/j.cmet.2019.12.012>.
185. C. W. Roman, V. A. Derkach, and R. D. Palmiter, "Genetically and Functionally Defined NTS to PBN Brain Circuits Mediating Anorexia," *Nature Communications* 7 (2016): 11905, <https://doi.org/10.1038/ncomms11905>.
186. D. Mishra, J. E. Richard, I. Maric, et al., "Lateral Parabrachial Nucleus Astrocytes Control Food Intake," *Frontiers in Endocrinology* 15 (2024): 1389589, <https://doi.org/10.3389/fendo.2024.1389589>.
187. A. Verkhratsky, A. Butt, B. Li, et al., "Astrocytes in Human Central Nervous System Diseases: A Frontier for New Therapies," *Signal Transduction and Targeted Therapy* 8, no. 1 (2023): 396, <https://doi.org/10.1038/s41392-023-01628-9>.
188. A. Verkhratsky, C. J. Lee, H. Chun, et al., "Curing the Brain: In Search for New Astrocyte-Specific Therapies," *Experimental & Molecular Medicine* 58, no. 4 (2026): 1086–1127, <https://doi.org/10.1038/s12276-026-01712-4>.
189. A. Semyanov and A. Verkhratsky, "Neuroglia in aging," *Handbook of Clinical Neurology* 209 (2025): 49–67, <https://doi.org/10.1016/B978-0-443-19104-6.00002-4>.
190. E. C. Toescu and A. Verkhratsky, "The Importance of Being Subtle: Small Changes in Calcium Homeostasis Control Cognitive Decline in Normal Aging," *Aging Cell* 6, no. 3 (2007): 267–273, <https://doi.org/10.1111/j.1474-9726.2007.00296.x>.
191. A. Verkhratsky, M. P. Mattson, and E. C. Toescu, "Aging in the Mind," *Trends in Neurosciences* 27, no. 10 (2004): 577–578, <https://doi.org/10.1016/j.tins.2004.07.008>.
192. A. Verkhratsky and R. Zorec, "Neuroglia in Cognitive Reserve," *Molecular Psychiatry* 29, no. 12 (2024): 3962–3967, <https://doi.org/10.1038/s41380-024-02644-z>.
193. A. Verkhratsky and A. Semyanov, "Astrocytes in Ageing," *Sub-Cellular Biochemistry* 103 (2023): 253–277, https://doi.org/10.1007/978-3-031-26576-1_11.
194. L. Soreq, UK Brain Expression Consortium, North American Brain Expression Consortium, J. Rose, et al., "Major Shifts in Glial Regional Identity Are a Transcriptional Hallmark of Human Brain Aging," *Cell Reports* 18, no. 2 (2017): 557–570, <https://doi.org/10.1016/j.celrep.2016.12.011>.
195. M. Verkerke, E. M. Hol, and J. Middeldorp, "Physiological and Pathological Ageing of Astrocytes in the Human Brain," *Neurochemical Research* 46, no. 10 (2021): 2662–2675, <https://doi.org/10.1007/s11064-021-03256-7>.
196. W. Wruck and J. Adjaye, "Meta-Analysis of Human Prefrontal Cortex Reveals Activation of GFAP and Decline of Synaptic Transmission in the Aging Brain," *Acta Neuropathologica Communications* 8, no. 1 (2020): 26, <https://doi.org/10.1186/s40478-020-00907-8>.
197. A. M. Butt, J. Niu, C. Yi, and A. Verkhratsky, "Physiology of Oligodendroglia," *Physiological Reviews* 106, no. 3 (2026): 1837–1911, <https://doi.org/10.1152/physrev.00023.2025>.
198. H. Haug and R. Eggers, "Morphometry of the Human Cortex Cerebri and Corpus Striatum During Aging," *Neurobiology of Aging* 12, no. 4 (1991): 336–338, Discussion 352–355, [https://doi.org/10.1016/0197-4580\(91\)90013-a](https://doi.org/10.1016/0197-4580(91)90013-a).
199. L. Marner, J. R. Nyengaard, Y. Tang, and B. Pakkenberg, "Marked Loss of Myelinated Nerve Fibers in the Human Brain With Age," *Journal of Comparative Neurology* 462, no. 2 (2003): 144–152, <https://doi.org/10.1002/cne.10714>.
200. W. J. Streit, N. W. Sammons, A. J. Kuhns, and D. L. Sparks, "Dystrophic Microglia in the Aging Human Brain," *Glia* 45, no. 2 (2004): 208–212, <https://doi.org/10.1002/glia.10319>.
201. M. Olabarria, H. N. Noristani, A. Verkhratsky, and J. J. Rodríguez, "Concomitant Astroglial Atrophy and Astrogliosis in a Triple Transgenic Animal Model of Alzheimer's Disease," *Glia* 58, no. 7 (2010): 831–838, <https://doi.org/10.1002/glia.20967>.
202. B. Pakkenberg and H. J. Gundersen, "Neocortical Neuron Number in Humans: Effect of Sex and Age," *Journal of Comparative Neurology* 384, no. 2 (1997): 312–320.
203. J. D. Rodriguez-Callejas, M. Irene-Fierro, S. Aguilar-Navarro, E. Fuchs, and C. Perez-Cruz, "Astroglial Atrophy Associates With Loss of Nuclear S100A10 in the Hippocampus of Aged Male Tree Shrews," *bioRxiv: The Preprint Server for Biology* (2024), 2024.07.04.602113, <https://doi.org/10.1101/2024.07.04.602113>.
204. A. Verkhratsky, M. Augusto-Oliveira, A. Pivoriūnas, A. Popov, A. Brazhe, and A. Semyanov, "Astroglial Asthenia and Loss of Function, Rather Than Reactivity, Contribute to the Ageing of the Brain," *Pflügers Archiv - European Journal of Physiology* 473, no. 5 (2021): 753–774, <https://doi.org/10.1007/s00424-020-02465-3>.
205. A. J. Castiglioni, M. E. Legare, D. L. Busbee, and E. Tiffany-Castiglioni, "Morphological Changes in Astrocytes of Aging Mice Fed Normal or Caloric Restricted Diets," *Age* 14, no. 4 (1991): 102–106, <https://doi.org/10.1007/BF02435015>.
206. J. J. Rodríguez, C.-Y. Yeh, S. Terzieva, M. Olabarria, M. Kulijewicz-Nawrot, and A. Verkhratsky, "Complex and Region-Specific Changes in Astroglial Markers in the Aging Brain," *Neurobiology of Aging* 35, no. 1 (2014): 15–23, <https://doi.org/10.1016/j.neurobiolaging.2013.07.002>.
207. A. Popov, A. Brazhe, P. Denisov, et al., "Astrocyte Dystrophy in Ageing Brain Parallels Impaired Synaptic Plasticity," *Aging Cell* 20, no. 3 (2021): e13334, <https://doi.org/10.1111/acer.13334>.
208. A. Popov, N. Brazhe, K. Morozova, et al., "Mitochondrial Malfunction and Atrophy of Astrocytes in the Aged Human Cerebral Cortex," *Nature Communications* 14, no. 1 (2023): 8380, <https://doi.org/10.1038/s41467-023-44192-0>.
209. A. Verkhratsky and A. Semyanov, "Decline and Fall of Aging Astrocytes: The Human Perspective," *Neural Regeneration Research* 20, no. 6 (2025): 1713–1714, <https://doi.org/10.4103/NRR.NRR-D-24-00418>.
210. A. Verkhratsky, J. J. Rodrigues, A. Pivoriūnas, R. Zorec, and A. Semyanov, "Astroglial Atrophy in Alzheimer's Disease," *Pflügers Archiv - European Journal of Physiology* 471, no. 10 (2019): 1247–1261, <https://doi.org/10.1007/s00424-019-02310-2>.
211. K. J. Heard, M. N. Shokhirev, C. Becronis, et al., "Chronic Cortisol Differentially Impacts Stem Cell-Derived Astrocytes From Major Depressive Disorder Patients," *Translational Psychiatry* 11, no. 1 (2021): 608, <https://doi.org/10.1038/s41398-021-01733-9>.
212. B. Morte, P. Gil-Ibáñez, and J. Bernal, "Regulation of Gene Expression by Thyroid Hormone in Primary Astrocytes: Factors

- Influencing the Genomic Response,” *Endocrinology* 159, no. 5 (2018): 2083–2092, <https://doi.org/10.1210/en.2017-03084>.
213. B. M. L. C. Bocco, J. P. Werneck-de-Castro, K. C. Oliveira, et al., “Type 2 Deiodinase Disruption in Astrocytes Results in Anxiety-Depressive-Like Behavior in Male Mice,” *Endocrinology* 157, no. 9 (2016): 3682–3695, <https://doi.org/10.1210/en.2016-1272>.
214. Z. Sheng, Q. Liu, Y. Song, et al., “Astrocyte Atrophy Induced by L-PGDS/PGD2/Src Signaling Dysfunction in the Central Amygdala Mediates Postpartum Depression,” *Journal of Affective Disorders* 359 (2024): 241–252, <https://doi.org/10.1016/j.jad.2024.05.083>.
215. A. V. Molofsky, R. Krencik, R. Krenick, et al., “Astrocytes and Disease: A Neurodevelopmental Perspective,” *Genes & Development* 26, no. 9 (2012): 891–907, <https://doi.org/10.1101/gad.188326.112>.
216. S. A. Sloan and B. A. Barres, “Mechanisms of Astrocyte Development and Their Contributions to Neurodevelopmental Disorders,” *Current Opinion in Neurobiology* 27 (2014): 75–81, <https://doi.org/10.1016/j.conb.2014.03.005>.
217. B. Uguagliati and M. Grilli, “Astrocytic Alterations and Dysfunction in Down Syndrome: Focus on Neurogenesis, Synaptogenesis, and Neural Circuits Formation,” *Cells* 13, no. 24 (2024): 2037, <https://doi.org/10.3390/cells13242037>.
218. K. Talvio and M. L. Castrén, “Astrocytes in Fragile X Syndrome,” *Frontiers in Cellular Neuroscience* 17 (2023): 1322541, <https://doi.org/10.3389/fncel.2023.1322541>.
219. F. Zeidán-Chuliá, A. B. Salmina, N. A. Malinovskaya, M. Noda, A. Verkhatsky, and J. C. F. Moreira, “The Glial Perspective of Autism Spectrum Disorders,” *Neuroscience and Biobehavioral Reviews* 38 (2014): 160–172, <https://doi.org/10.1016/j.neubiorev.2013.11.008>.
220. E. Albizzati, E. Florio, F. Miramondi, I. Sormonta, N. Landsberger, and A. Frasca, “Identification of Region-Specific Cytoskeletal and Molecular Alterations in Astrocytes of Mecp2 Deficient Animals,” *Frontiers in Neuroscience* 16 (2022): 823060, <https://doi.org/10.3389/fnins.2022.823060>.
221. N. Cresto, L.-E. Pillet, P. Billuart, and N. Rouach, “Do Astrocytes Play a Role in Intellectual Disabilities?,” *Trends in Neurosciences* 42, no. 8 (2019): 518–527, <https://doi.org/10.1016/j.tins.2019.05.011>.
222. A. G. Lopes, S. K. Loganathan, and J. Caliaiperumal, “Rett Syndrome and the Role of MECP2: Signaling to Clinical Trials,” *Brain Sciences* 14, no. 2 (2024): 120, <https://doi.org/10.3390/brainsci14020120>.
223. G. Calfa, J. J. Hablitz, and L. Pozzo-Miller, “Network Hyperexcitability in Hippocampal Slices From Mecp2 Mutant Mice Revealed by Voltage-Sensitive Dye Imaging,” *Journal of Neurophysiology* 105, no. 4 (2011): 1768–1784, <https://doi.org/10.1152/jn.00800.2010>.
224. C. A. Chapleau, G. D. Calfa, M. C. Lane, et al., “Dendritic Spine Pathologies in Hippocampal Pyramidal Neurons From Rett Syndrome Brain and After Expression of Rett-Associated MECP2 Mutations,” *Neurobiology of Disease* 35, no. 2 (2009): 219–233, <https://doi.org/10.1016/j.nbd.2009.05.001>.
225. E. M. Boggio, G. Lonetti, T. Pizzorusso, and M. Giustetto, “Synaptic Determinants of Rett Syndrome,” *Frontiers in Synaptic Neuroscience* 2 (2010): 28, <https://doi.org/10.3389/fnsyn.2010.00028>.
226. E. Albizzati, M. Breccia, E. Florio, et al., “Mecp2 Knock-Out Astrocytes Affect Synaptogenesis by Interleukin 6 Dependent Mechanisms,” *iScience* 27, no. 3 (2024): 109296, <https://doi.org/10.1016/j.isci.2024.109296>.
227. N. Ballas, D. T. Lioy, C. Grunseich, and G. Mandel, “Non-Cell Autonomous Influence of MeCP2-Deficient Glia on Neuronal Dendritic Morphology,” *Nature Neuroscience* 12, no. 3 (2009): 311–317, <https://doi.org/10.1038/nn.2275>.
228. M. R. Williamson, C. J. A. Fuertes, A. K. Dunn, M. R. Drew, and T. A. Jones, “Reactive Astrocytes Facilitate Vascular Repair and Remodeling After Stroke,” *Cell Reports* 35, no. 4 (2021): 109048, <https://doi.org/10.1016/j.celrep.2021.109048>.
229. D. T. Lioy, S. K. Garg, C. E. Monaghan, et al., “A Role for Glia in the Progression of Rett’s Syndrome,” *Nature* 475, no. 7357 (2011): 497–500, <https://doi.org/10.1038/nature10214>.
230. A. L. M. Caldwell, L. Sancho, J. Deng, et al., “Aberrant Astrocyte Protein Secretion Contributes to Altered Neuronal Development in Multiple Models of Neurodevelopmental Disorders,” *Nature Neuroscience* 25, no. 9 (2022): 1163–1178, <https://doi.org/10.1038/s41593-022-01150-1>.
231. Y. Ehinger, V. Matagne, V. Cunin, et al., “Analysis of Astroglial Secretomic Profile in the Mecp2-Deficient Male Mouse Model of Rett Syndrome,” *International Journal of Molecular Sciences* 22, no. 9 (2021): 4316, <https://doi.org/10.3390/ijms22094316>.
232. T. Sun and R. F. Hevner, “Growth and Folding of the Mammalian Cerebral Cortex: From Molecules to Malformations,” *Nature Reviews. Neuroscience* 15, no. 4 (2014): 217–232, <https://doi.org/10.1038/nrn3707>.
233. P. Hasel, O. Dando, Z. Jiwaji, et al., “Neurons and Neuronal Activity Control Gene Expression in Astrocytes to Regulate Their Development and Metabolism,” *Nature Communications* 8 (2017): 15132, <https://doi.org/10.1038/ncomms15132>.
234. D. M. de Waard and M. Bugiani, “Astrocyte–Oligodendrocyte–Microglia Crosstalk in Astrocytopathies,” *Frontiers in Cellular Neuroscience* 14 (2020): 608073, <https://doi.org/10.3389/fncel.2020.608073>.
235. A. Lanciotti, M. S. Brignone, P. Macioce, S. Visentin, and E. Ambrosini, “Human iPSC-Derived Astrocytes: A Powerful Tool to Study Primary Astrocyte Dysfunction in the Pathogenesis of Rare Leukodystrophies,” *International Journal of Molecular Sciences* 23, no. 1 (2022): 274, <https://doi.org/10.3390/ijms23010274>.
236. M. Brenner, J. E. Goldman, R. A. Quinlan, and A. Messing, “Alexander Disease: A Genetic Disorder of Astrocytes,” in *Astrocytes in (Patho)physiology of the Nervous System*, ed. P. G. Haydon and V. Parpura (Springer US, 2009), 591–648, https://doi.org/10.1007/978-0-387-79492-1_24.
237. R. Min and M. S. van der Knaap, “Genetic Defects Disrupting Glial Ion and Water Homeostasis in the Brain,” *Brain Pathology* 28, no. 3 (2018): 372–387, <https://doi.org/10.1111/bpa.12602>.
238. M. Simard and M. Nedergaard, “The Neurobiology of Glia in the Context of Water and Ion Homeostasis,” *Neuroscience* 129, no. 4 (2004): 877–896, <https://doi.org/10.1016/j.neuroscience.2004.09.053>.
239. L. M. Hablitz and M. Nedergaard, “The Glymphatic System: A Novel Component of Fundamental Neurobiology,” *Journal of Neuroscience* 41, no. 37 (2021): 7698–7711, <https://doi.org/10.1523/JNEUROSCI.0619-21.2021>.
240. S. Alvestad, J. Hammer, E. H. Hoddevik, et al., “Mislocalization of AQP4 Precedes Chronic Seizures in the Kainate Model of Temporal Lobe Epilepsy,” *Epilepsy Research* 105, no. 1–2 (2013): 30–41, <https://doi.org/10.1016/j.eplepsyres.2013.01.006>.
241. J. I. Szu and D. K. Binder, “Mechanisms Underlying Aquaporin-4 Subcellular Mislocalization in Epilepsy,” *Frontiers in Cellular Neuroscience* 16 (2022): 900588, <https://doi.org/10.3389/fncel.2022.900588>.
242. D. M. Zeppenfeld, M. Simon, J. D. Haswell, et al., “Association of Perivascular Localization of Aquaporin-4 With Cognition and Alzheimer Disease in Aging Brains,” *JAMA Neurology* 74, no. 1 (2017): 91–99, <https://doi.org/10.1001/jamaneurol.2016.4370>.
243. M. Braun, M. Sevaio, S. A. Keil, et al., “Macroscopic Changes in Aquaporin-4 Underlie Blast Traumatic Brain Injury-Related Impairment in Glymphatic Function,” *Brain* 147, no. 6 (2024): 2214–2229, <https://doi.org/10.1093/brain/awae065>.
244. M. M. Salman, P. Kitchen, A. Halsey, et al., “Emerging Roles for Dynamic Aquaporin-4 Subcellular Relocalization in CNS Water

- Homeostasis,” *Brain: A Journal of Neurology* 145, no. 1 (2022): 64–75, <https://doi.org/10.1093/brain/awab311>.
245. P. Kitchen, M. M. Salman, A. M. Halsey, et al., “Targeting Aquaporin-4 Subcellular Localization to Treat Central Nervous System Edema,” *Cell* 181, no. 4 (2020): 784–799.e19, <https://doi.org/10.1016/j.cell.2020.03.037>.
246. M. M. Salman, P. Kitchen, M. N. Woodroffe, et al., “Hypothermia Increases Aquaporin 4 (AQP4) Plasma Membrane Abundance in Human Primary Cortical Astrocytes via a Calcium/Transient Receptor Potential Vanilloid 4 (TRPV4)- and Calmodulin-Mediated Mechanism,” *European Journal of Neuroscience* 46, no. 9 (2017): 2542–2547, <https://doi.org/10.1111/ejn.13723>.
247. M. S. Alshuhri, L. Gallagher, L. M. Work, and W. M. Holmes, “Direct Imaging of Glymphatic Transport Using H217O MRI,” *JCI Insight* 6, no. 10 (2021): e141159, <https://doi.org/10.1172/jci.insight.141159>.
248. S. B. Hladky and M. A. Barrand, “The Glymphatic Hypothesis: The Theory and the Evidence,” *Fluids and Barriers of the CNS* 19, no. 1 (2022): 9, <https://doi.org/10.1186/s12987-021-00282-z>.
249. H. Mestre, Y. Mori, and M. Nedergaard, “The Brain’s Glymphatic System: Current Controversies,” *Trends in Neurosciences* 43, no. 7 (2020): 458–466, <https://doi.org/10.1016/j.tins.2020.04.003>.
250. M. S. van der Knaap, I. Boor, and R. Estévez, “Megalencephalic Leukoencephalopathy With Subcortical Cysts: Chronic White Matter Oedema due to a Defect in Brain Ion and Water Homeostasis,” *Lancet. Neurology* 11, no. 11 (2012): 973–985, [https://doi.org/10.1016/S1474-4422\(12\)70192-8](https://doi.org/10.1016/S1474-4422(12)70192-8).
251. E. M. J. Passchier, S. Kerst, E. Brouwers, et al., “Aquaporin-4 and GPRC5B: Old and New Players in Controlling Brain Oedema,” *Brain* 146, no. 8 (2023): 3444–3454, <https://doi.org/10.1093/brain/awad146>.
252. M. Alonso-Gardón, X. Elorza-Vidal, A. Castellanos, et al., “Identification of the GlialCAM Interactome: The G Protein-Coupled Receptors GPRC5B and GPR37L1 Modulate Megalencephalic Leukoencephalopathy Proteins,” *Human Molecular Genetics* 30, no. 17 (2021): 1649–1665, <https://doi.org/10.1093/hmg/ddab155>.
253. C. Depienne, M. Bugiani, C. Dupuits, et al., “Brain White Matter Oedema due to CIC-2 Chloride Channel Deficiency: An Observational Analytical Study,” *Lancet. Neurology* 12, no. 7 (2013): 659–668, [https://doi.org/10.1016/S1474-4422\(13\)70053-X](https://doi.org/10.1016/S1474-4422(13)70053-X).
254. M. B. Hoegg-Beiler, S. Sirisi, I. J. Orozco, et al., “Disrupting MLC1 and GlialCAM and CIC-2 Interactions in Leukodystrophy Entails Glial Chloride Channel Dysfunction,” *Nature Communications* 5 (2014): 3475, <https://doi.org/10.1038/ncomms4475>.
255. E. Jeworutzki, T. López-Hernández, X. Capdevila-Nortes, et al., “GlialCAM, a Protein Defective in a Leukodystrophy, Serves as a CIC-2 Cl[−] Channel Auxiliary Subunit,” *Neuron* 73, no. 5 (2012): 951–961, <https://doi.org/10.1016/j.neuron.2011.12.039>.
256. D. Bockenbauer, S. Feather, H. C. Stanescu, et al., “Epilepsy, Ataxia, Sensorineural Deafness, Tubulopathy, and KCNJ10 Mutations,” *New England Journal of Medicine* 360, no. 19 (2009): 1960–1970, <https://doi.org/10.1056/NEJMoa0810276>.
257. J. A. Stokum, V. Gerzanich, and J. M. Simard, “Molecular Pathophysiology of Cerebral Edema,” *Journal of Cerebral Blood Flow and Metabolism* 36, no. 3 (2016): 513–538, <https://doi.org/10.1177/0271678X15617172>.
258. B. A. Plog and M. Nedergaard, “The Glymphatic System in Central Nervous System Health and Disease: Past, Present, and Future,” *Annual Review of Pathology: Mechanisms of Disease* 13 (2018): 379–394, <https://doi.org/10.1146/annurev-pathol-051217-111018>.
259. M. K. Rasmussen, H. Mestre, and M. Nedergaard, “The Glymphatic Pathway in Neurological Disorders,” *Lancet Neurology* 17, no. 11 (2018): 1016–1024, [https://doi.org/10.1016/S1474-4422\(18\)30318-1](https://doi.org/10.1016/S1474-4422(18)30318-1).
260. J. R. Dahl, A. Weier, C. Winter, et al., “Modulator of VRAC Current 1 Is a Potential Target Antigen in Multiple Sclerosis,” *Neurology(R) Neuroimmunology & Neuroinflammation* 12, no. 2 (2025): e200374, <https://doi.org/10.1212/NXI.0000000000200374>.
261. T. V. Lanz, R. C. Brewer, P. P. Ho, et al., “Clonally Expanded B Cells in Multiple Sclerosis Bind EBV EBNA1 and GlialCAM,” *Nature* 603, no. 7900 (2022): 321–327, <https://doi.org/10.1038/s41586-022-04432-7>.
262. R. Srivastava, M. Aslam, S. R. Kalluri, et al., “Potassium Channel KIR4.1 as an Immune Target in Multiple Sclerosis,” *New England Journal of Medicine* 367, no. 2 (2012): 115–123, <https://doi.org/10.1056/NEJMoa1110740>.
263. M. S. van der Knaap and R. Min, “Multiple Sclerosis: An Immune Attack on Astrocyte-Mediated Ion and Water Homeostasis,” *Nature Reviews. Neurology* 21, no. 5 (2025): 283–289, <https://doi.org/10.1038/s41582-025-01081-y>.
264. W. P. Pío del Río-Hortega, “Cerebral Cicatrix: The Reaction of Neuroglia and Microglia to Brain Wounds,” *Bulletin of the Johns Hopkins Hospital* 41 (1927): 278–303.
265. K. Ceyzeriat, L. Abjean, M.-A. C. Sauvage, L. B. Haim, and C. Escartin, “The Complex STATES of Astrocyte Reactivity: How Are They Controlled by the JAK–STAT3 Pathway?,” *Neuroscience* 330 (2016): 205–218, <https://doi.org/10.1016/j.neuroscience.2016.05.043>.
266. C. Escartin, E. Galea, A. Lakatos, et al., “Reactive Astrocyte Nomenclature, Definitions, and Future Directions,” *Nature Neuroscience* 24, no. 3 (2021): 312–325, <https://doi.org/10.1038/s41593-020-00783-4>.
267. M. V. Sofroniew, “Astrocyte Reactivity: Subtypes, States, and Functions in CNS Innate Immunity,” *Trends in Immunology* 41, no. 9 (2020): 758–770, <https://doi.org/10.1016/j.it.2020.07.004>.
268. H. W. Querfurth and F. M. LaFerla, “Alzheimer’s Disease,” *New England Journal of Medicine* 362, no. 4 (2010): 329–344, <https://doi.org/10.1056/NEJMr0909142>.
269. J. Cummings, “New Approaches to Symptomatic Treatments for Alzheimer’s Disease,” *Molecular Neurodegeneration* 16, no. 1 (2021): 2, <https://doi.org/10.1186/s13024-021-00424-9>.
270. D. S. Knopman, H. Amieva, R. C. Petersen, et al., “Alzheimer Disease,” *Nature Reviews. Disease Primers* 7, no. 1 (2021): 33, <https://doi.org/10.1038/s41572-021-00269-y>.
271. R. Tonegawa-Kuji, Y. Hou, B. Hu, et al., “Efficacy and Safety of Passive Immunotherapies Targeting Amyloid beta in Alzheimer’s Disease: A Systematic Review and meta-Analysis,” *PLoS Medicine* 22, no. 3 (2025): e1004568, <https://doi.org/10.1371/journal.pmed.1004568>.
272. M. Morita, H. Ikeshima-Kataoka, M. Kreft, N. Vardjan, R. Zorec, and M. Noda, “Metabolic Plasticity of Astrocytes and Aging of the Brain,” *International Journal of Molecular Sciences* 20, no. 4 (2019): 941, <https://doi.org/10.3390/ijms20040941>.
273. X.-Y. Xiong, Y. Tang, and Q.-W. Yang, “Metabolic Changes Favor the Activity and Heterogeneity of Reactive Astrocytes,” *Trends in Endocrinology and Metabolism* 33, no. 6 (2022): 390–400, <https://doi.org/10.1016/j.tem.2022.03.001>.
274. G. A. Dienel, “Brain Glucose Metabolism: Integration of Energetics With Function,” *Physiological Reviews* 99, no. 1 (2019): 949–1045, <https://doi.org/10.1152/physrev.00062.2017>.
275. A. Ashraf, Z. Fan, D. J. Brooks, and P. Edison, “Cortical Hypermetabolism in MCI Subjects: A Compensatory Mechanism?,” *European Journal of Nuclear Medicine and Molecular Imaging* 42, no. 3 (2015): 447–458, <https://doi.org/10.1007/s00259-014-2919-z>.
276. B. A. Gordon, T. M. Blazey, Y. Su, et al., “Spatial Patterns of Neuroimaging Biomarker Change in Individuals From Families With Autosomal Dominant Alzheimer’s Disease: A Longitudinal Study,” *Lancet Neurology* 17, no. 3 (2018): 241–250, [https://doi.org/10.1016/S1474-4422\(18\)30028-0](https://doi.org/10.1016/S1474-4422(18)30028-0).

277. F. Luo, N. R. Rustay, U. Ebert, et al., "Characterization of 7- and 19-Month-Old Tg2576 Mice Using Multimodal in Vivo Imaging: Limitations as a Translatable Model of Alzheimer's Disease," *Neurobiology of Aging* 33, no. 5 (2012): 933–944, <https://doi.org/10.1016/j.neurobiolaging.2010.08.005>.
278. S. Rojas, J. R. Herance, J. D. Gispert, et al., "In Vivo Evaluation of Amyloid Deposition and Brain Glucose Metabolism of 5XFAD Mice Using Positron Emission Tomography," *Neurobiology of Aging* 34, no. 7 (2013): 1790–1798, <https://doi.org/10.1016/j.neurobiolaging.2012.12.027>.
279. C. Salcedo, V. Pozo Garcia, B. García-Adán, et al., "Increased Glucose Metabolism and Impaired Glutamate Transport in Human Astrocytes Are Potential Early Triggers of Abnormal Extracellular Glutamate Accumulation in hiPSC-Derived Models of Alzheimer's Disease," *Journal of Neurochemistry* 168, no. 5 (2024): 822–840, <https://doi.org/10.1111/jnc.16014>.
280. D. Pamies, C. Sartori, D. Schwartz, et al., "Neuroinflammatory Response to TNF α and IL1 β Cytokines Is Accompanied by an Increase in Glycolysis in Human Astrocytes in Vitro," *International Journal of Molecular Sciences* 22, no. 8 (2021): 4065, <https://doi.org/10.3390/ijms22084065>.
281. J. L. Robb, N. A. Hammad, P. G. Weightman Potter, J. K. Chilton, C. Beall, and K. L. J. Ellacott, "The Metabolic Response to Inflammation in Astrocytes Is Regulated by Nuclear Factor-Kappa B Signaling," *Glia* 68, no. 11 (2020): 2246–2263, <https://doi.org/10.1002/glia.23835>.
282. C. G. Ardanaz, A. de la Cruz, M. Elizalde-Horcada, et al., "GLUT1 Ablation in Astrocytes Paradoxically Improves Central and Peripheral Glucose Metabolism via Enhanced Insulin-Stimulated ATP Release," *bioRxiv: The Preprint Server for Biology* (2022), 2022.10.06.511112, <https://doi.org/10.1101/2022.10.06.511112>.
283. M. Gavillet, I. Allaman, and P. J. Magistretti, "Modulation of Astrocytic Metabolic Phenotype by Proinflammatory Cytokines," *Glia* 56, no. 9 (2008): 975–989, <https://doi.org/10.1002/glia.20671>.
284. C. Vége, L. Pellerin, R. Dantzer, and P. J. Magistretti, "Long-Term Modulation of Glucose Utilization by IL-1 Alpha and TNF-Alpha in Astrocytes: Na⁺ Pump Activity as a Potential Target via Distinct Signaling Mechanisms," *Glia* 39, no. 1 (2002): 10–18, <https://doi.org/10.1002/glia.10080>.
285. K. L. Viola and W. L. Klein, "Amyloid β Oligomers in Alzheimer's Disease Pathogenesis, Treatment, and Diagnosis," *Acta Neuropathologica* 129, no. 2 (2015): 183–206, <https://doi.org/10.1007/s00401-015-1386-3>.
286. K. V. Kuchibhotla, C. R. Lattarulo, B. T. Hyman, and B. J. Bacskai, "Synchronous Hyperactivity and Intercellular Calcium Waves in Astrocytes in Alzheimer Mice," *Science* 323, no. 5918 (2009): 1211–1215, <https://doi.org/10.1126/science.1169096>.
287. P. Kelly, M. V. Sanchez-Mico, S. S. Hou, et al., "Neuronally Derived Soluble A β Evokes Cell-Wide Astrocytic Calcium Dysregulation in Absence of Amyloid Plaques In Vivo," *Journal of Neuroscience* 43, no. 26 (2023): 4926–4940, <https://doi.org/10.1523/JNEUROSCI.1988-22.2023>.
288. A. Delekate, M. Fuchtemeier, T. Schumacher, C. Ulbrich, M. Foddias, and G. C. Petzold, "Metabotropic P2Y₁ Receptor Signalling Mediates Astrocytic Hyperactivity In Vivo in an Alzheimer's Disease Mouse Model," *Nature Communications* 5 (2014): 5422, <https://doi.org/10.1038/ncomms6422>.
289. J. Lines, A. M. Baraibar, C. Fang, et al., "Astrocyte-Neuronal Network Interplay Is Disrupted in Alzheimer's Disease Mice," *Glia* 70, no. 2 (2022): 368–378, <https://doi.org/10.1002/glia.24112>.
290. Y. F. Lee, A. N. Russ, Q. Zhao, et al., "Optogenetic Targeting of Astrocytes Restores Slow Brain Rhythm Function and Slows Alzheimer's Disease Pathology," *Scientific Reports* 13 (2023): 13075, <https://doi.org/10.1038/s41598-023-40402-3>.
291. D. Shah, W. Gsell, J. Wahis, et al., "Astrocyte Calcium Dysfunction Causes Early Network Hyperactivity in Alzheimer's Disease," *Cell Reports* 40, no. 8 (2022): 111280, <https://doi.org/10.1016/j.celrep.2022.111280>.
292. A. Lia, G. Sansevero, A. Chiavegato, et al., "Rescue of Astrocyte Activity by the Calcium Sensor STIM1 Restores Long-Term Synaptic Plasticity in Female Mice Modelling Alzheimer's Disease," *Nature Communications* 14, no. 1 (2023): 1590, <https://doi.org/10.1038/s41467-023-37240-2>.
293. A. Di Spiezio and M. Zonta, "Astrocyte Ca²⁺ Dysregulation in Alzheimer's Disease Mouse Models: Revisiting the Dogma of Hyperactivity," *Biomolecules* 16, no. 3 (2026): 404, <https://doi.org/10.3390/biom16030404>.
294. L. A. Hulshof, D. van Nuijs, E. M. Hol, and J. Middeldorp, "The Role of Astrocytes in Synapse Loss in Alzheimer's Disease: A Systematic Review," *Frontiers in Cellular Neuroscience* 16 (2022): 899251, <https://doi.org/10.3389/fncel.2022.899251>.
295. B. Dejanovic, T. Wu, M.-C. Tsai, et al., "Complement C1q-Dependent Excitatory and Inhibitory Synapse Elimination by Astrocytes and Microglia in Alzheimer's Disease Mouse Models," *Nature Aging* 2, no. 9 (2022): 837–850, <https://doi.org/10.1038/s43587-022-00281-1>.
296. V. Giusti, G. Kaur, E. Giusto, and L. Civiero, "Brain Clearance of Protein Aggregates: A Close-Up on Astrocytes," *Molecular Neurodegeneration* 19, no. 1 (2024): 5, <https://doi.org/10.1186/s13024-024-00703-1>.
297. H. Funato, M. Yoshimura, T. Yamazaki, et al., "Astrocytes Containing Amyloid beta-Protein (A β)-Positive Granules Are Associated With A β 40-Positive Diffuse Plaques in the Aged Human Brain," *American Journal of Pathology* 152, no. 4 (1998): 983–992.
298. R. G. Nagele, M. R. D'Andrea, H. Lee, V. Venkataraman, and H.-Y. Wang, "Astrocytes Accumulate A β 42 and Give Rise to Astrocytic Amyloid Plaques in Alzheimer Disease Brains," *Brain Research* 971, no. 2 (2003): 197–209, [https://doi.org/10.1016/S0006-8993\(03\)02361-8](https://doi.org/10.1016/S0006-8993(03)02361-8).
299. E. Konstantinidis, A. Dakhel, C. Beretta, and A. Erlandsson, "Long-Term Effects of Amyloid-beta Deposits in Human iPSC-Derived Astrocytes," *Molecular and Cellular Neurosciences* 125 (2023): 103839, <https://doi.org/10.1016/j.mcn.2023.103839>.
300. V. Schmidt, E. Ziemska, T. Obrebski, et al., "Astrocytes Distress Triggers Brain Pathology Through Induction of δ Secretase in a Murine Model of Alzheimer's Disease," *Nature Communications* 16, no. 1 (2025): 9653, <https://doi.org/10.1038/s41467-025-65536-y>.
301. S. Sölvander, E. Nikitidou, R. Brolin, et al., "Accumulation of Amyloid- β by Astrocytes Result in Enlarged Endosomes and Microvesicle-Induced Apoptosis of Neurons," *Molecular Neurodegeneration* 11, no. 1 (2016): 38, <https://doi.org/10.1186/s13024-016-0098-z>.
302. M. Zysk, C. Beretta, L. Naia, et al., "Amyloid- β Accumulation in Human Astrocytes Induces Mitochondrial Disruption and Changed Energy Metabolism," *Journal of Neuroinflammation* 20, no. 1 (2023): 43, <https://doi.org/10.1186/s12974-023-02722-z>.
303. P. Saha and S. C. Biswas, "Amyloid- β Induced Astrocytosis and Astrocyte Death: Implication of FoxO3a-Bim-caspase3 Death Signaling," *Molecular and Cellular Neurosciences* 68 (2015): 203–211, <https://doi.org/10.1016/j.mcn.2015.08.002>.
304. C. J. Garwood, A. M. Pooler, J. Atherton, D. P. Hanger, and W. Noble, "Astrocytes Are Important Mediators of A β -Induced Neurotoxicity and Tau Phosphorylation in Primary Culture," *Cell Death & Disease* 2, no. 6 (2011): e167, <https://doi.org/10.1038/cddis.2011.50>.
305. V. Ozenci, M. Kouwenhoven, N. Teleshova, M. Pashenkov, S. Fredrikson, and H. Link, "Multiple Sclerosis: Pro- and Anti-Inflammatory Cytokines and Metalloproteinases Are Affected Differentially by Treatment With IFN-beta," *Journal of Neuroimmunology* 108, no. 1–2 (2000): 236–243, [https://doi.org/10.1016/S0165-5728\(00\)00281-2](https://doi.org/10.1016/S0165-5728(00)00281-2).

306. B. R. Bloem, M. S. Okun, and C. Klein, "Parkinson's Disease," *Lancet* 397, no. 10291 (2021): 2284–2303, [https://doi.org/10.1016/S0140-6736\(21\)00218-X](https://doi.org/10.1016/S0140-6736(21)00218-X).
307. C. M. Tanner and J. L. Ostrem, "Parkinson's Disease," *New England Journal of Medicine* 391, no. 5 (2024): 442–452, <https://doi.org/10.1056/NEJMr2401857>.
308. P. Tagliaferro and R. E. Burke, "Retrograde Axonal Degeneration in Parkinson Disease," *Journal of Parkinson's Disease* 6, no. 1 (2016): 1–15, <https://doi.org/10.3233/JPD-150769>.
309. H. Park, T.-I. Kam, V. L. Dawson, and T. M. Dawson, " α -Synuclein Pathology as a Target in Neurodegenerative Diseases," *Nature Reviews. Neurology* 21, no. 1 (2025): 32–47, <https://doi.org/10.1038/s41582-024-01043-w>.
310. G. Arena, K. Sharma, G. Agyeah, R. Krüger, A. Grünwald, and J. C. Fitzgerald, "Neurodegeneration and Neuroinflammation in Parkinson's Disease: A Self-Sustained Loop," *Current Neurology and Neuroscience Reports* 22, no. 8 (2022): 427–440, <https://doi.org/10.1007/s11910-022-01207-5>.
311. H. D. E. Booth, W. D. Hirst, and R. Wade-Martins, "The Role of Astrocyte Dysfunction in Parkinson's Disease Pathogenesis," *Trends in Neurosciences* 40, no. 6 (2017): 358–370, <https://doi.org/10.1016/j.tins.2017.04.001>.
312. R. Garcia, S. Zarate, and R. Srinivasan, "The Role of Astrocytes in Parkinson's Disease: Astrocytes in Parkinson's Disease," *Advances in Neurobiology* 39 (2024): 319–343, https://doi.org/10.1007/978-3-031-64839-7_13.
313. P. Ramos-Gonzalez, S. Mato, J. C. Chara, A. Verkhatsky, C. Matute, and F. Cavaliere, "Astrocytic Atrophy as a Pathological Feature of Parkinson's Disease With LRRK2 Mutation," *npj Parkinson's Disease* 7, no. 1 (2021): 31, <https://doi.org/10.1038/s41531-021-00175-w>.
314. G. Dematteis, L. Tapella, C. Casali, et al., "ER-Mitochondria Distance Is a Critical Parameter for Efficient Mitochondrial Ca²⁺ Uptake and Oxidative Metabolism," *Communications Biology* 7 (2024): 1294, <https://doi.org/10.1038/s42003-024-06933-9>.
315. L. Iovino, M. E. Tremblay, and L. Civiero, "Glutamate-Induced Excitotoxicity in Parkinson's Disease: The Role of Glial Cells," *Journal of Pharmacological Sciences* 144, no. 3 (2020): 151–164, <https://doi.org/10.1016/j.jphs.2020.07.011>.
316. L. Iovino, V. Giusti, F. Pischedda, et al., "Trafficking of the Glutamate Transporter Is Impaired in LRRK2-Related Parkinson's Disease," *Acta Neuropathologica* 144, no. 1 (2022): 81–106, <https://doi.org/10.1007/s00401-022-02437-0>.
317. M.-E. Tremblay, M. R. Cookson, and L. Civiero, "Glial Phagocytic Clearance in Parkinson's Disease," *Molecular Neurodegeneration* 14, no. 1 (2019): 16, <https://doi.org/10.1186/s13024-019-0314-8>.
318. L. Streubel-Gallasch, V. Giusti, M. Sandre, et al., "Parkinson's Disease-Associated LRRK2 Interferes With Astrocyte-Mediated Alpha-Synuclein Clearance," *Molecular Neurobiology* 58, no. 7 (2021): 3119–3140, <https://doi.org/10.1007/s12035-021-02327-8>.
319. V. Lindström, G. Gustafsson, L. H. Sanders, et al., "Extensive Uptake of α -Synuclein Oligomers in Astrocytes Results in Sustained Intracellular Deposits and Mitochondrial Damage," *Molecular and Cellular Neurosciences* 82 (2017): 143–156, <https://doi.org/10.1016/j.mcn.2017.04.009>.
320. S. Paillusson, P. Gomez-Suaga, R. Stoica, et al., " α -Synuclein Binds to the ER-Mitochondria Tethering Protein VAPB to Disrupt Ca²⁺ Homeostasis and Mitochondrial ATP Production," *Acta Neuropathologica* 134, no. 1 (2017): 129–149, <https://doi.org/10.1007/s00401-017-1704-z>.
321. G. van Niel, G. D'Angelo, and G. Raposo, "Shedding Light on the Cell Biology of Extracellular Vesicles," *Nature Reviews. Molecular Cell Biology* 19, no. 4 (2018): 213–228, <https://doi.org/10.1038/nrm.2017.125>.
322. J. A. Welsh, D. C. I. Goberdhan, L. O'Driscoll, et al., "Minimal Information for Studies of Extracellular Vesicles (MISEV2023): From Basic to Advanced Approaches," *Journal of Extracellular Vesicles* 13, no. 2 (2024): e12404, <https://doi.org/10.1002/jev2.12404>.
323. E. Kim, J. Camacho, Z. Combs, et al., "Preliminary Findings Suggest the Number and Volume of Supragranular and Infragranular Pyramidal Neurons Are Similar in the Anterior Superior Temporal Area of Control Subjects and Subjects With Autism," *Neuroscience Letters* 589 (2015): 98–103, <https://doi.org/10.1016/j.neulet.2015.01.021>.
324. B. Marchetti, L. Leggio, F. L'Episcopo, et al., "Glia-Derived Extracellular Vesicles in Parkinson's Disease," *Journal of Clinical Medicine* 9, no. 6 (2020): 1941, <https://doi.org/10.3390/jcm9061941>.
325. L. Leggio, F. L'Episcopo, A. Magri, et al., "Small Extracellular Vesicles Secreted by Nigrostriatal Astrocytes Rescue Cell Death and Preserve Mitochondrial Function in Parkinson's Disease," *Advanced Healthcare Materials* 11, no. 20 (2022): e2201203, <https://doi.org/10.1002/adhm.202201203>.
326. R. H. Brown, "Amyotrophic Lateral Sclerosis: Recent Insights From Genetics and Transgenic Mice," *Cell* 80, no. 5 (1995): 687–692, [https://doi.org/10.1016/0092-8674\(95\)90346-1](https://doi.org/10.1016/0092-8674(95)90346-1).
327. R. H. Brown and A. Al-Chalabi, "Amyotrophic Lateral Sclerosis," *New England Journal of Medicine* 377, no. 2 (2017): 162–172, <https://doi.org/10.1056/NEJMr1603471>.
328. S. Dhasmana, A. Dhasmana, A. S. Narula, M. Jaggi, M. M. Yallapu, and S. C. Chauhan, "The Panoramic View of Amyotrophic Lateral Sclerosis: A Fatal Intricate Neurological Disorder," *Life Sciences* 288 (2022): 120156, <https://doi.org/10.1016/j.lfs.2021.120156>.
329. J. Phukan, N. P. Pender, and O. Hardiman, "Cognitive Impairment in Amyotrophic Lateral Sclerosis," *Lancet. Neurology* 6, no. 11 (2007): 994–1003, [https://doi.org/10.1016/S1474-4422\(07\)70265-X](https://doi.org/10.1016/S1474-4422(07)70265-X).
330. M. A. van Es, O. Hardiman, A. Chio, et al., "Amyotrophic Lateral Sclerosis," *Lancet* 390, no. 10107 (2017): 2084–2098, [https://doi.org/10.1016/S0140-6736\(17\)31287-4](https://doi.org/10.1016/S0140-6736(17)31287-4).
331. A. Eisen, "Amyotrophic Lateral Sclerosis: A 40-Year Personal Perspective," *Journal of Clinical Neuroscience* 16, no. 4 (2009): 505–512, <https://doi.org/10.1016/j.jocn.2008.07.072>.
332. D. W. Cleveland, L. I. Bruijn, P. C. Wong, et al., "Mechanisms of Selective Motor Neuron Death in Transgenic Mouse Models of Motor Neuron Disease," *Neurology* 47, no. 4 Suppl 2 (1996): S54–S61, Discussion S61–62., https://doi.org/10.1212/wnl.47.4_suppl_2.54s.
333. A. E. King, A. Woodhouse, M. T. K. Kirkcaldie, and J. C. Vickers, "Excitotoxicity in ALS: Overstimulation, or Overreaction?," *Experimental Neurology* 275, no. Pt 1 (2016): 162–171, <https://doi.org/10.1016/j.expneurol.2015.09.019>.
334. R. Mejzini, L. L. Flynn, I. L. Pitout, S. Fletcher, S. D. Wilton, and P. A. Akkari, "ALS Genetics, Mechanisms, and Therapeutics: Where Are We Now?," *Frontiers in Neuroscience* 13 (2019): 1310, <https://doi.org/10.3389/fnins.2019.01310>.
335. W. Tan, P. Pasinelli, and D. Trotti, "Role of Mitochondria in Mutant SOD1 Linked Amyotrophic Lateral Sclerosis," *Biochimica et Biophysica Acta* 1842, no. 8 (2014): 1295–1301, <https://doi.org/10.1016/j.bbadis.2014.02.009>.
336. L. Van Den Bosch, P. Van Damme, E. Bogaert, and W. Robberecht, "The Role of Excitotoxicity in the Pathogenesis of Amyotrophic Lateral Sclerosis," *Biochimica et Biophysica Acta* 1762, no. 11–12 (2006): 1068–1082, <https://doi.org/10.1016/j.bbadis.2006.05.002>.
337. A. Lakatos, "State-of-Art Modelling of Inflammatory Astrocyte-Synapse Interactions in Injury and Amyotrophic Lateral Sclerosis," *Neural Regeneration Research* 12, no. 1 (2017): 75–76, <https://doi.org/10.4103/1673-5374.198977>.
338. C. Zhao, A.-C. Devlin, A. K. Chouhan, et al., "Mutant C9orf72 Human iPSC-Derived Astrocytes Cause Non-Cell Autonomous Motor

- Neuron Pathophysiology,” *Glia* 68, no. 5 (2020): 1046–1064, <https://doi.org/10.1002/glia.23761>.
339. J. D. Rothstein, L. Martin, A. I. Levey, et al., “Localization of Neuronal and Glial Glutamate Transporters,” *Neuron* 13, no. 3 (1994): 713–725, [https://doi.org/10.1016/0896-6273\(94\)90038-8](https://doi.org/10.1016/0896-6273(94)90038-8).
340. K. Li, T. J. Hala, S. Seetharam, D. J. Poulsen, M. C. Wright, and A. C. Lepore, “GLT1 Overexpression in SOD1(G93A) Mouse Cervical Spinal Cord Does Not Preserve Diaphragm Function or Extend Disease,” *Neurobiology of Disease* 78 (2015): 12–23, <https://doi.org/10.1016/j.nbd.2015.03.010>.
341. M. E. Gurney, H. Pu, A. Y. Chiu, et al., “Motor Neuron Degeneration in Mice That Express a Human Cu, Zn Superoxide Dismutase Mutation,” *Science* 264, no. 5166 (1994): 1772–1775, <https://doi.org/10.1126/science.8209258>.
342. C. Marini, V. Cossu, M. Kumar, et al., “The Role of Endoplasmic Reticulum in the Differential Endurance Against Redox Stress in Cortical and Spinal Astrocytes From the Newborn SOD1G93A Mouse Model of Amyotrophic Lateral Sclerosis,” *Antioxidants* 10, no. 9 (2021): 1392, <https://doi.org/10.3390/antiox10091392>.
343. R. P. Norante, C. Peggion, D. Rossi, et al., “ALS-Associated SOD1(G93A) Decreases SERCA Pump Levels and Increases Store-Operated Ca²⁺ Entry in Primary Spinal Cord Astrocytes From a Transgenic Mouse Model,” *International Journal of Molecular Sciences* 20, no. 20 (2019): 5151, <https://doi.org/10.3390/ijms20205151>.
344. C. Torazza, F. Provenzano, E. Gallia, et al., “Genetic Downregulation of the Metabotropic Glutamate Receptor Type 5 Dampens the Reactive and Neurotoxic Phenotype of Adult ALS Astrocytes,” *Cells* 12, no. 15 (2023): 1952, <https://doi.org/10.3390/cells12151952>.
345. A. Agarwal, P.-H. Wu, E. G. Hughes, et al., “Transient Opening of the Mitochondrial Permeability Transition Pore Induces Microdomain Calcium Transients in Astrocyte Processes,” *Neuron* 93, no. 3 (2017): 587–605.e7, <https://doi.org/10.1016/j.neuron.2016.12.034>.
346. F. Provenzano, S. Nyberg, D. Giunti, et al., “Micro-RNAs Shuttled by Extracellular Vesicles Secreted From Mesenchymal Stem Cells Dampen Astrocyte Pathological Activation and Support Neuroprotection in In-Vitro Models of ALS,” *Cells* 11, no. 23 (2022): 3923, <https://doi.org/10.3390/cells11233923>.
347. M. J. Fogarty, “Amyotrophic Lateral Sclerosis as a Synaptopathy,” *Neural Regeneration Research* 14, no. 2 (2019): 189–192, <https://doi.org/10.4103/1673-5374.244782>.
348. M. J. Broadhead and G. B. Miles, “Bi-Directional Communication Between Neurons and Astrocytes Modulates Spinal Motor Circuits,” *Frontiers in Cellular Neuroscience* 14 (2020): 30, <https://doi.org/10.3389/fncel.2020.00030>.
349. M. MacLean, R. López-Díez, C. Vasquez, P. F. Gugger, and A. M. Schmidt, “Neuronal-Glial Communication Perturbations in Murine SOD1G93A Spinal Cord,” *Communications Biology* 5, no. 1 (2022): 177, <https://doi.org/10.1038/s42003-022-03128-y>.
350. M. J. Broadhead and G. B. Miles, “A Common Role for Astrocytes in Rhythmic Behaviours?,” *Progress in Neurobiology* 202 (2021): 102052, <https://doi.org/10.1016/j.pneurobio.2021.102052>.
351. M. Cieślak, K. Roszek, and M. Wujak, “Purinergic Implication in Amyotrophic Lateral Sclerosis-From Pathological Mechanisms to Therapeutic Perspectives,” *Purinergic Signalling* 15, no. 1 (2019): 1–15, <https://doi.org/10.1007/s11302-018-9633-4>.
352. M. J. Broadhead, C. Bonthron, J. Waddington, et al., “Selective Vulnerability of Tripartite Synapses in Amyotrophic Lateral Sclerosis,” *Acta Neuropathologica* 143, no. 4 (2022): 471–486, <https://doi.org/10.1007/s00401-022-02412-9>.
353. C. Bonthron, S. Burley, M. J. Broadhead, et al., “Excitatory to Inhibitory Synaptic Ratios Are Unchanged at Presymptomatic Stages in Multiple Models of ALS,” *PLoS One* 19, no. 8 (2024): e0306423, <https://doi.org/10.1371/journal.pone.0306423>.
354. I. R. A. Mackenzie, E. H. Bigio, P. G. Ince, et al., “Pathological TDP-43 Distinguishes Sporadic Amyotrophic Lateral Sclerosis From Amyotrophic Lateral Sclerosis With SOD1 Mutations,” *Annals of Neurology* 61, no. 5 (2007): 427–434, <https://doi.org/10.1002/ana.21147>.
355. A. Ayvazian-Hancock, E. Butler, C. F. Meehan, G. B. Miles, and M. J. Broadhead, “Synaptopathy in the TDP-43 Δ NLS Mouse Model of Sporadic Amyotrophic Lateral Sclerosis,” *European Journal of Neuroscience* 62, no. 10 (2025): e70320, <https://doi.org/10.1111/ejn.70320>.
356. M. B. Cieri and A. J. Ramos, “Astrocytes, Reactive Astrogliosis, and Glial Scar Formation in Traumatic Brain Injury,” *Neural Regeneration Research* 20, no. 4 (2025): 973–989, <https://doi.org/10.4103/NRR.NRR-D-23-02091>.
357. N. R. Sims and W. P. Yew, “Reactive Astrogliosis in Stroke: Contributions of Astrocytes to Recovery of Neurological Function,” *Neurochemistry International* 107 (2017): 88–103, <https://doi.org/10.1016/j.neuint.2016.12.016>.
358. Q. M. Alhadidi, G. A. Bahader, O. Arvola, P. Kitchen, Z. A. Shah, and M. M. Salman, “Astrocytes in Functional Recovery Following Central Nervous System Injuries,” *Journal of Physiology* 602, no. 13 (2024): 3069–3096, <https://doi.org/10.1113/JP284197>.
359. J. R. Faulkner, J. E. Herrmann, M. J. Woo, K. E. Tansey, N. B. Doan, and M. V. Sofroniew, “Reactive Astrocytes Protect Tissue and Preserve Function After Spinal Cord Injury,” *Journal of Neuroscience* 24, no. 9 (2004): 2143–2155, <https://doi.org/10.1523/JNEUROSCI.3547-03.2004>.
360. M. Linnerbauer and V. Rothhammer, “Protective Functions of Reactive Astrocytes Following Central Nervous System Insult,” *Frontiers in Immunology* 11 (2020): 573256.
361. G. R. Choudhury and S. Ding, “Reactive Astrocytes and Therapeutic Potential in Focal Ischemic Stroke,” *Neurobiology of Disease* 85 (2016): 234–244, <https://doi.org/10.1016/j.nbd.2015.05.003>.
362. L. Li, J. Zhou, L. Han, et al., “The Specific Role of Reactive Astrocytes in Stroke,” *Frontiers in Cellular Neuroscience* 16 (2022): 850866, <https://doi.org/10.3389/fncel.2022.850866>.
363. A. R. Malik, J. Lips, M. Gorniak-Walas, et al., “SorCS2 Facilitates Release of Endostatin From Astrocytes and Controls Post-Stroke Angiogenesis,” *Glia* 68, no. 6 (2020): 1304–1316, <https://doi.org/10.1002/glia.23778>.
364. C. Escartin, O. Guillemaud, and M.-A. Carrillo-de Sauvage, “Questions and (Some) Answers on Reactive Astrocytes,” *Glia* 67, no. 12 (2019): 2221–2247, <https://doi.org/10.1002/glia.23687>.
365. J. J. Bae, Y.-Y. Xiang, A. Martinez-Canabal, P. W. Frankland, B. B. Yang, and W.-Y. Lu, “Increased Transforming Growth Factor- β 1 Modulates Glutamate Receptor Expression in the hippocampus,” *International Journal of Physiology, Pathophysiology and Pharmacology* 3, no. 1 (2011): 9–20.
366. P. Yu, H. Wang, Y. Katagiri, and H. M. Geller, “An in Vitro Model of Reactive Astrogliosis and Its Effect on Neuronal Growth,” *Methods in Molecular Biology* 814 (2012): 327–340, https://doi.org/10.1007/978-1-61779-452-0_21.
367. J. Enes, M. Haburčák, S. Sona, et al., “Satellite Glial Cells Modulate Cholinergic Transmission Between Sympathetic Neurons,” *PLoS One* 15, no. 2 (2020): e0218643, <https://doi.org/10.1371/journal.pone.0218643>.
368. V. G. Hernandez, K. J. Lechtenberg, T. C. Peterson, et al., “Translatome Analysis Reveals Microglia and Astrocytes To Be Distinct Regulators of Inflammation in the Hyperacute and Acute Phases After Stroke,” *Glia* 71, no. 8 (2023): 1960–1984, <https://doi.org/10.1002/glia.24377>.

369. K. D. McCarthy and J. de Vellis, "Preparation of Separate Astroglial and Oligodendroglial Cell Cultures From Rat Cerebral Tissue," *Journal of Cell Biology* 85, no. 3 (1980): 890–902, <https://doi.org/10.1083/jcb.85.3.890>.
370. L. C. Foo, N. J. Allen, E. A. Bushong, et al., "Development of a Method for the Purification and Culture of Rodent Astrocytes," *Neuron* 71, no. 5 (2011): 799–811, <https://doi.org/10.1016/j.neuron.2011.07.022>.
371. S. A. Liddelow and B. A. Barres, "Reactive Astrocytes: Production, Function, and Therapeutic Potential," *Immunity* 46, no. 6 (2017): 957–967, <https://doi.org/10.1016/j.immuni.2017.06.006>.
372. J. Prah, A. Winters, K. Chaudhari, J. Hersh, R. Liu, and S.-H. Yang, "A Novel Serum Free Primary Astrocyte Culture Method That Mimic Quiescent Astrocyte Phenotype," *Journal of Neuroscience Methods* 320 (2019): 50–63, <https://doi.org/10.1016/j.jneumeth.2019.03.013>.
373. F. Formaggio, M. Fazzina, R. Estévez, M. Caprini, and S. Ferroni, "Dynamic Expression of Homeostatic Ion Channels in Differentiated Cortical Astrocytes in Vitro," *Pflügers Archiv - European Journal of Physiology* 474, no. 2 (2022): 243–260, <https://doi.org/10.1007/s00424-021-02627-x>.
374. G. Perea and A. Araque, "Properties of Synaptically Evoked Astrocyte Calcium Signal Reveal Synaptic Information Processing by Astrocytes," *Journal of Neuroscience* 25, no. 9 (2005): 2192–2203, <https://doi.org/10.1523/JNEUROSCI.3965-04.2005>.
375. R. Krencik and S.-C. Zhang, "Directed Differentiation of Functional Astroglial Subtypes From Human Pluripotent Stem Cells," *Nature Protocols* 6, no. 11 (2011): 1710–1717, <https://doi.org/10.1038/nprot.2011.405>.
376. B. Lendemeijer, M. Unkel, H. Smeenk, et al., "Human Pluripotent Stem Cell-Derived Astrocyte Functionality Compares Favorably With Primary Rat Astrocytes," *eNeuro* 11, no. 9 (2024): ENEURO.0148-24.2024, <https://doi.org/10.1523/ENEURO.0148-24.2024>.
377. S. A. Sloan, S. Darmanis, N. Huber, et al., "Human Astrocyte Maturation Captured in 3D Cerebral Cortical Spheroids Derived From Pluripotent Stem Cells," *Neuron* 95, no. 4 (2017): 779–790.e6, <https://doi.org/10.1016/j.neuron.2017.07.035>.
378. C. E. Strong, J. Zhang, M. Carrasco, et al., "Functional Brain Region-Specific Neural Spheroids for Modeling Neurological Diseases and Therapeutics Screening," *Communications Biology* 6, no. 1 (2023): 1211, <https://doi.org/10.1038/s42003-023-05582-8>.
379. J. Tcw, M. Wang, A. A. Pimenova, et al., "An Efficient Platform for Astrocyte Differentiation From Human Induced Pluripotent Stem Cells," *Stem Cell Reports* 9, no. 2 (2017): 600–614, <https://doi.org/10.1016/j.stemcr.2017.06.018>.
380. A. di Domenico, G. Carola, C. Calatayud, et al., "Patient-Specific iPSC-Derived Astrocytes Contribute to Non-Cell-Autonomous Neurodegeneration in Parkinson's Disease," *Stem Cell Reports* 12, no. 2 (2019): 213–229, <https://doi.org/10.1016/j.stemcr.2018.12.011>.
381. V. J. García, D. J. Rushton, C. M. Tom, et al., "Huntington's Disease Patient-Derived Astrocytes Display Electrophysiological Impairments and Reduced Neuronal Support," *Frontiers in Neuroscience* 13 (2019): 669, <https://doi.org/10.3389/fnins.2019.00669>.
382. V. C. Jones, R. Atkinson-Dell, A. Verkhatsky, and L. Mohamet, "Aberrant iPSC-Derived Human Astrocytes in Alzheimer's Disease," *Cell Death & Disease* 8, no. 3 (2017): e2696, <https://doi.org/10.1038/cddis.2017.89>.
383. M. Oksanen, A. J. Petersen, N. Naumenko, et al., "PSEN1 Mutant iPSC-Derived Model Reveals Severe Astrocyte Pathology in Alzheimer's Disease," *Stem Cell Reports* 9, no. 6 (2017): 1885–1897, <https://doi.org/10.1016/j.stemcr.2017.10.016>.
384. M. Pons-Espinal, L. Blasco-Agell, I. Fernandez-Carasa, et al., "Blocking IL-6 Signaling Prevents Astrocyte-Induced Neurodegeneration in an iPSC-Based Model of Parkinson's Disease," *JCI Insight* 9, no. 3 (2024): e163359, <https://doi.org/10.1172/jci.insight.163359>.
385. M. Domocos, D. E. Bragin, N. C. Shanbhag, L. Schlotterose, and M. M. Salman, "Photobiomodulation restores blood-brain barrier integrity after hypoxia via endothelial von Willebrand factor modulation in a humanised tricellular transwell model," *Journal of Physiology* 606, no. 14 (2026): 6080–6103, <https://doi.org/10.1113/JP291064>.
386. E. M. Cruz, L. C. Soares, G. Greene, et al., "Astrocyte Enrichment of 3D Cortical Constructs Enhances Brain Repair," *Advanced Science* 13, no. 20 (2026): e07423, <https://doi.org/10.1002/adv.202507423>.