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### Authors

Poskanzer, Kira E  
Molofsky, Anna V

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# Dynamism of an Astrocyte In Vivo: Perspectives on Identity and Function

Kira E. Poskanzer<sup>1,3,\*</sup> and Anna V. Molofsky<sup>2,4,\*</sup>

<sup>1</sup>Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143; email: kira.poskanzer@ucsf.edu

<sup>2</sup>Weill Institute for Neurosciences, University of California, San Francisco, California 94143; email: anna.molofsky@ucsf.edu

<sup>3</sup>Kavli Institute for Fundamental Neuroscience, University of California, San Francisco, California 94143

<sup>4</sup>Department of Psychiatry, University of California, San Francisco, California 94143

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\*These authors contributed equally to this article

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astrocyte, glia, neural circuits, physiology, identity, morphology, evolution

## Abstract

Astrocytes are an abundant and evolutionarily conserved central nervous system cell type. Despite decades of evidence that astrocytes are integral to neural circuit function, it seems as though astrocytic and neuronal biology continue to advance in parallel to each other, to the detriment of both. Recent advances in molecular biology and optical imaging are being applied to astrocytes in new and exciting ways but without fully considering their unique biology. From this perspective, we explore the reasons that astrocytes remain enigmatic, arguing that their responses to neuronal and environmental cues shape form and function in dynamic ways. Here, we provide a roadmap for future experiments to explore the nature of astrocytes in situ.



## INTRODUCTION

Astrocytes—the most abundant type of non-neuronal cells in the brain—have received increased attention as researchers have developed and applied more tools to unravel their roles in the central nervous system (CNS). Mammalian astrocytes begin to be produced during mid-embryogenesis and are therefore poised to contribute to neural circuit development and function through much of the life span. During development, astrocytes are a key source of trophic molecules that promote neuronal excitatory synapse formation (1–3). In adulthood, astrocytes provide neuronal circuits with trophic and metabolic support, regulate cerebrovascular tone, and play key roles in circuit-level brain state transitions, such as those that occur between sleep and wake (4–6). Despite these findings, it seems as though progress in astrocyte biology has continued in parallel with the rest of neuroscience but excluded from working models of a unified theory of brain function.

A major hurdle in current astrocyte research is the dynamic nature of the astrocyte itself at both the molecular and physiological levels. We posit that this both distinguishes their function in neural circuits and inhibits our current understanding of their varied roles in the nervous system. To illustrate this idea, we refer to *Dynamism of a Dog on a Leash*, a painting by the futurist Giacomo Balla from 1912 (**Figure 1**). In this work, Balla presents the moving parts of a dog and the woman walking it down the street, but we do not get a clear picture of either: They only exist in motion. In the same way, we argue that astrocytes are a type of brain cell in constant flux and are defined by their responses to the neural circuits they support.

In this review, we discuss the literature on astrocyte molecular identity and function, with particular attention to blind spots—areas that we have ignored or in which we applied a biased lens to the defining parameter. We then provide thoughts on how we might modify these parameters to guide future studies. Rather than reiterating the many excellent recent reviews on astrocytes, we focus on new conceptual and methodological approaches to examine the intricacies of astrocyte



**Figure 1**

*Dynamism of a Dog on a Leash* by Giacomo Balla from 1912. This painting provides a graphic analogy for astrocytes in a state of constant flux. These cells are often defined by their responses to the particular neural circuit in which they exist. Reproduced with permission from Albright-Knox Art Gallery.



**Figure 2**

Cellular identity as a balance between cell-autonomous cues and cell-extrinsic cues. In contrast to neurons, few transcription factors that define the astrocytes as a unique lineage have been identified. This raises the question of whether astrocyte molecular identity is primarily state dependent and thus responsive to changes in their local environment.

identity and function. We focus on protoplasmic (or synapse-associated) astrocytes and their emerging roles in neural circuit development and function, with particular attention to *in vivo* data. Finally, although much of this review depends on findings from rodent astrocytes, we conclude with a discussion of how the conservation of astrocytes across model organisms reflects their essential roles within the CNS.

## THE SHIFTING MOLECULAR IDENTITY OF ASTROCYTES

### Astrocyte Lineage Determination

Are astrocytes a distinct cell lineage? Although all cells are molecularly defined by a balance of cell-autonomous and cell-extrinsic cues, we propose that cell-extrinsic cues play a larger role in astrocyte identity and function relative to other brain cell types (**Figure 2**). As previously reviewed (7, 8), neural stem cells generate neurons first, followed by glia, and proglial transcription regulators including the Notch pathway act at least in part by repressing neuronal fate. From this generic gliogenic pool emerge the two major macroglial cell types in the brain: astrocytes and oligodendrocytes. Difficulties in defining astrocyte lineage have often been attributed to the relative lack of markers to positively identify astrocytes and their intermediate progenitors and to the fact that most astrocyte markers are also expressed in neural stem cells. In addition, although there is strong evidence that astrocytes are developmentally patterned (9, 10), it is not clear that these patterning transcription factors generate stable molecular and functional diversity in astrocytes, as they clearly do in neurons (11, 12). Efforts to understand the specification of mammalian astrocytes from neural stem cells have identified cues that make a cell competent to become an astrocyte, but they have so far found no evidence of a cell-intrinsic program that instructively directs progenitors toward an astrocyte fate.

In the many studies on glial fate determination, no single transcription factor has emerged as a unique positive regulator of astrocyte fate, in striking contrast to oligodendrocytes, their nearest relatives (13, 14). For example, several recent studies have used proglial transcription factors to identify regulatory cascades that determine whether oligodendrocytes or astrocytes are produced. Sox9 is a gliogenic transcription factor (15) that regulates induction of nuclear factor IA (NFIA) (16), a transcription factor necessary and sufficient for gliogenesis in the embryonic spinal cord (17). NFIA promotes astrogenesis via interactions with Sox9 and by antagonizing the oligodendrocyte-specific transcription factor Sox10 (18). However, although Sox9 is expressed in many astrocytes (19), it is required for both astrocyte and oligodendrocyte generation (15), perhaps via maintenance of multipotent neural progenitors (20). Epigenetic modifications can also alter astrocyte-oligodendrocyte fate choice, such as the histone deacetylase Hdac3, which promotes oligodendrocyte fate commitment and concurrently prevents astrogenesis by repressing the transcription factor Stat3 (21).

With no evidence of any cell-autonomous factors that uniquely regulate astrocyte identity, we are left with only the proastrogenic Stat3, a latent (i.e., signal-dependent) transcription factor (22) better known as a regulator of astrocyte reactivity (23). Although this review focuses on physiological roles of astrocytes, there is a vast literature on the transition from resting to reactive astrocyte under conditions of stress, injury, or neurodegeneration (24). Reactive astrocytes, induced under pathological conditions—typically hypertrophy—proliferate and upregulate markers, including glial fibrillary acidic protein (GFAP) and vimentin. Recent research has begun to molecularly dissect this response, identifying strikingly different gene expression profiles of reactive astrocytes induced by diverse injuries, such as hypoxia-ischemia or endotoxemia (25), and identifying functional differences between physiologic and reactive astrocytes (26). The next step is the recognition of astrocyte reactivity along a spectrum of normal to pathological, wherein changes in circuit function, circuit rearrangements, and circuit stress all may engage a type of astrocyte reaction that can be molecularly and functionally characterized.

Although it is possible that the astrocyte-defining transcription factors in mammals are yet to be discovered, this absence of evidence itself may be the message. Astrocyte identity may indeed be state dependent, which is a function of its context in the brain: unperturbed versus reactive, cortical versus subcortical, etc. Unlike neurons, which are highly methylated, the glial methylome resembles the fetal methylome throughout life, suggesting the possibility of dynamic transcriptional flexibility (27). State-dependent transcription factors such as Stat3 and NF- $\kappa$ B are typically broadly expressed across cell types and often respond to more than one upstream signaling pathway. How multiple inputs can converge on one regulatory pathway while still generating diverse cellular responses is unknown. However, detailed studies of NF- $\kappa$ B signaling in response to two distinct input signals reveal distinct temporal patterns of protein phosphorylation in response to each and reveal dynamic decision making when faced with simultaneous inputs, suggesting that these pathways are more than simple on/off switches (28). It seems likely that astrocytes respond to multiple upstream cues that dynamically affect gene expression and cellular function. Understanding this in the context of astrocytes *in vivo* will require a better understanding of the types of environmental cues that astrocytes can sense and how sensing these cues may translate to functional diversity, as discussed below.

### Astrocyte Molecular Dynamism in Response to Cell Extrinsic Cues

What molecular cues might drive astrocyte functional diversity? Astrocytes in different brain regions have been shown to coordinate with region-specific neuronal functions, such as pH sensing in respiratory areas (29), indicating that their location within the nervous system or proximity to certain types of neurons may determine their functional roles. In the ventral spinal cord, developing astrocytes secrete the guidance cue Sema3a to promote motor neuron survival and synaptogenesis (30). Cortical astrocytes can be segregated based on cell surface markers into distinct populations that vary in their synaptogenic potential (31). Using different tools, these case reports from different CNS regions suggest that astrocytes can be highly specialized to support subtypes of neurons. Might this heterogeneity be in response to the neurons themselves? One elegant example is suggested in a study of the role of the well-known embryonic patterning factor Sonic hedgehog (Shh) in diversifying two molecularly and morphologically distinct subtypes of cerebellar astrocytes: vellate astrocytes and Bergmann glia. The authors showed that Shh continues to be expressed by cerebellar Purkinje neurons into adulthood and helps to promote the molecular and functional identity of the adjacent Bergman glia as distinct from vellate astrocytes (32). These data suggest that one well-described developmental patterning pathway operates postnatally as a neuronal signal that regulates astrocyte identity.



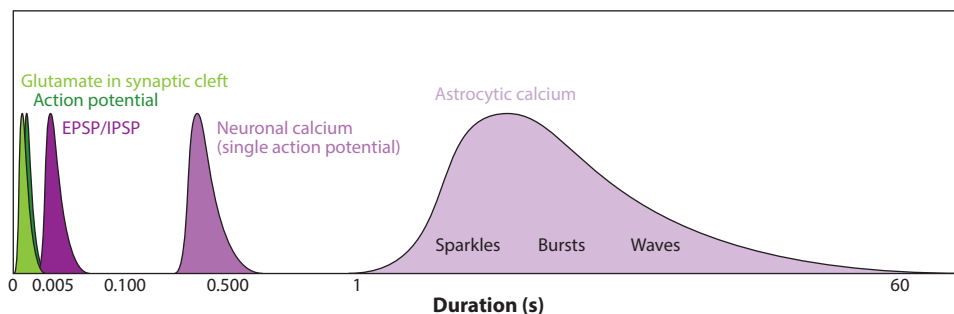
Although gene expression data indicate that astrocytes express receptors for a wide variety of neurotransmitters, hormones, patterning factors, and immune cues (33), there are few to no data on protein levels or on the downstream molecular or functional implications of the loss of such signaling. Several research groups have proposed that neuronal activity could regulate myelination by oligodendrocytes in the CNS, though this has been contentious (34). Could neuronal activity also regulate astrocyte molecular identity or function? The answer to this question is unknown, but if so, this communication would presumably be mediated by molecular cues released from neurons in an activity-dependent manner. Astrocytes express most neurotransmitter receptors, including metabotropic glutamate receptors [particularly mGluR5 developmentally and mGluR2/3 in adulthood (35)], GABA receptors (36), and adrenergic receptors (37). Diminished glutamatergic signaling with global VGluT1 deficiency leads to a modest decrease in astrocyte size, implicating glutamatergic signaling in their functional maturation (38). How activation of these receptors may in turn activate downstream signaling pathways to regulate astrocyte identity or function is unknown. What other molecular pathways might modulate astrocyte physiology? In the setting of injury, astrocytes clearly respond to cytokine signaling from microglia and other sources (26), raising the question of whether similar immune cues could play a physiologic role. Given that protoplasmic astrocytes contact blood vessels as part of the glia limitans, circulating factors or blood-derived cues also have the potential to regulate astrocyte function. Exploring these questions may lead to a different understanding of astrocyte identity—one that is defined by their functional adaptations to a dynamic environment.

### PHYSIOLOGICAL DYNAMISM

Astrocytes play increasingly appreciated roles in population-level neural circuit functions, including arousal-state and sleep-wake-state transitions (4, 6, 39–41), whereas synapse-specific functions previously described for astrocytes, such as the vesicular release of neurotransmitter via a SNARE-dependent mechanism (gliotransmission), remain controversial (42). The very fact that a potentially fundamental function of this cell is currently debated points to the lack of a basic understanding of how this cell functions in information flow in neural circuits. Like any other cell type integrated in a larger tissue structure, astrocytes are not limited to one particular role. Their importance in extracellular neurotransmitter clearance and potassium ( $K^+$ ) homeostasis, for example, is not contentious. However, the challenge for the future, when considering their *raison d'être*, will be to address how all of their physiological roles fit together, when and where each function is deployed, and how can they be experimentally dissected. The overarching question will be how potentially disparate functions are integrated and integral to neural circuit computation over time and space. Below, we discuss recent developments that have begun to address these physiological concerns.

Modern fluorescent imaging tools, although largely developed to visualize and manipulate neuronal activity, have revealed rich calcium ( $Ca^{2+}$ ) signals in astrocytes and extracellular glutamate signals (among others), thus greatly increasing our ability to interrogate astrocyte function in intact neural circuits. However, it is important to bear in mind that much of the astrocytic information being obtained with these tools (43, 44) is circumscribed by the context in which the tools were developed: to study neurons, a different cell type, whose fundamental function in neural circuits is comparatively clear. Neurons integrate input from other neurons and output (via action potential-triggered neurotransmitter release) to downstream neurons. Optical techniques applied to neurons interrogate various steps in this sequence, either by monitoring or manipulating their activities. For astrocytes, the correspondence of optical imaging data to biological function is still being discovered. Technically and conceptually, these tools are thus carrying out very different





**Figure 3**

Schematic of the wide range of time scales for various basic neuronal (action potential, EPSP, IPSP, and calcium change in response to action potential using genetically encoded sensors) and astrocytic calcium events. Astrocyte calcium excitability tends to be slower than many neuronal events. Abbreviations: EPSP, excitatory post-synaptic potential; IPSP, inhibitory post-synaptic potential.

scientific discovery functions in neurons and astrocytes. We argue that this situation is one exemplified by the illustration used at the beginning of this review (**Figure 1**), wherein the time and place at which the astrocyte is observed optically may impart incomplete or potentially misleading information about astrocytic function.

Perhaps the most salient difference between neuronal and astrocytic activity in neural circuits stems from the fact that neurons are electrically excitable, whereas astrocytes are largely electrophysiologically silent, although they exhibit spatiotemporally rich intracellular  $\text{Ca}^{2+}$  activity. A corollary to this observation is that neuronal and astrocytic activities occur at vastly different timescales, with the many observed types of astrocyte  $\text{Ca}^{2+}$  activity lasting up to orders of magnitude longer, thus spanning a much wider temporal window than neuronal action potentials or postsynaptic events (**Figure 3**). It has been suggested that the differing timescales of observed astrocytic  $\text{Ca}^{2+}$  events likely reflect widely varying cellular functions that have yet to be uncovered. In this framework, astrocytic  $\text{Ca}^{2+}$  would relay qualitatively different signals than neuronal somatic  $\text{Ca}^{2+}$  does in mature neural circuits, where changes in the amplitude and duration of fluorescence [measured either by fluorescent dyes or genetically encoded indicators (43, 45)] largely reflect differences in neuronal firing frequency. In the case of astrocytic  $\text{Ca}^{2+}$ , rather than indicating a change in degree of activity, differences in duration of events might signify differing responses to extracellular or intracellular molecular events. However, these hypotheses remain largely untested, as astrocyte biologists are continuing to disambiguate the circuit signals driving each particular astrocytic  $\text{Ca}^{2+}$  event observed and the downstream events that astrocytic  $\text{Ca}^{2+}$  signals precipitate at any given place and time. Because many excellent recent reviews discuss the state of the field of astrocytic  $\text{Ca}^{2+}$  signaling (46, 47), we focus here on gaps in our understanding of astrocyte activity dynamics and potential avenues for future progress.

Spatially, investigations of astrocyte activity have tended to fall into two distinct groups: those that examine the dynamics of a single cell and/or its subcellular compartments (48, 49) and those that take a larger view of the astrocytic population in a particular brain structure (4, 6, 39, 40). Both perspectives are critical to understand how astrocytes work, and although it remains technically difficult to examine both cell- and population-level resolutions simultaneously, we believe that tools to allow this kind of integrated analysis will be necessary to answer outstanding physiology questions. However, astrocyte researchers can be hindered in these endeavors—particularly on the analysis side—by the great spatiotemporal heterogeneity of astrocyte signals, both within and among astrocytes. To make progress on overcoming these analysis hurdles, astrocyte-specific

analytical techniques will need to move away from region-of-interest (ROI)-based analyses, which assume a fixed size of the compartment to analyze (6, 49–53). Although ROI-based approaches are sufficient for most neuronal (whether cell soma or dendritic) imaging analyses, they cannot account for the heterogeneity of astrocyte signals. For example, a small-amplitude, short-duration event in an astrocyte may overlap spatially, at a later time point, with a large-amplitude, longer event. In addition, many observed astrocytic events are propagative, so static ROI-based analyses are insufficient. We propose that a satisfying computational solution to these problems would take into account these dynamics, potentially applying mathematical models that have not been developed with neurons in mind (54). Furthermore, we posit that analytical tools built around these astrocyte-specific constraints may address current research discrepancies in this field and allow researchers to link cellular- and circuit-level physiology in meaningful ways.

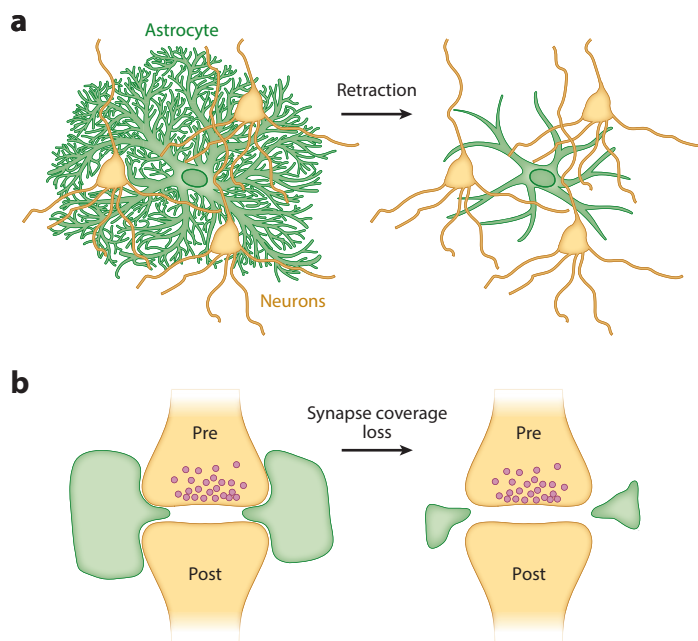
Finally, although neurons and astrocytes are two distinct cell types, they are intimately connected in neural circuits and communicate within and between themselves as part of ongoing neuronal physiology. Thus, disentangling their functions is a challenging technical and conceptual problem that will require simultaneous imaging of both in vivo. In the past, astrocyte physiology at their fine branches could be assessed only in the context of simultaneous neuronal electrophysiological recordings. However, with the introduction of high signal-to-noise sensors that are excitable outside of the green spectrum (55), there are opportunities to assess fine-scale astrocyte and neuronal activity simultaneously and at high spatial resolution (56–58). In addition, advances in in vivo two-photon imaging techniques in three dimensions, as well as in deep brain structures (59), should expand the reach of fluorescent and analytical tools beyond the most commonly studied brain structures, nuclei, and layers. Applying these tools to neural circuits throughout the nervous system will allow researchers to assess interactions of astrocytes and neurons on a moment-to-moment basis and to dissect the interplay of these two cell types across time and cytoarchitectural space.

## MORPHOLOGICAL DYNAMISM

The physiological and molecular dynamism of astrocytes discussed in the previous sections are potentially the cause or consequence of another type of cellular dynamics: morphological. The first suggestion that astrocytic branches might be motile likely came from Cajal, who hypothesized that astrocytes invade the synaptic cleft during sleep and separate the pre- and postsynaptic neurons to halt synaptic transmission, while retracting during wake (60). Although there is no evidence that this occurs and that sleep is not characterized by a lack of synaptic transmission, some recent clues suggest that morphological dynamics of astrocytes may underlie differences between sleep and wake. First, the Nedergaard group (5) has demonstrated that there is a dramatic shift in the perivascular movement of cerebrospinal fluid through the brain parenchyma between sleep and wake and that this difference is correlated with a change in extracellular space—one that may be mediated by astrocytes. Although not shown directly, it is likely that astrocyte branches may move during the course of these large-scale, tissue-wide expansions and retractions over sleep and wake (5). Second, ribosome profiling of astrocytes demonstrated that many more genes were uniquely expressed in wake compared to sleep, and these wakefulness astrocyte-specific genes included some with the potential to mediate branch elongation: *Trio*, *Synj2*, and *Gem* (61). Furthermore, ultrastructural evidence indicates that sleep deprivation is accompanied by increased astrocytic coverage of dendritic spines, demonstrating that astrocytes have the potential to be motile over the time course of days. It is worth noting that, when evaluating electron microscopy data, that a recent paper found an almost twofold difference in astrocytic coverage of synapses in tissues prepared via cryofixation (~34%) when compared to traditional chemical fixation methods (~62%) (62). These







**Figure 4**

Schematic of the different possible types of morphological changes, including (a) full-scale, whole-cell branch retraction and (b) subtler changes in the astrocytic coverage of synapses. Pre- and post- refer to the pre- and post-synaptic cells. Both large-scale retraction (a) and smaller-scale synaptic coverage changes (b) can affect neuronal signaling, but these have not yet been explored in many brain regions.

data suggest that the tissue shrinkage that occurs with chemical fixation may be driving astrocytic branches into synapses and may not reflect the *in vivo* morphology of these synapses. In addition, they should encourage a comparison between these fixation methods for future ultrastructural studies of astrocytes.

A dramatic example of morphological dynamism documented in astrocytes has been observed in the hypothalamus and in the supraoptic and paraventricular nuclei (summarized in 63). Compared to the subtler changes in synaptic coverage discussed above, the astrocytes in these regions—identified by GFAP labeling—fully retract their branches during lactation, parturition, and dehydration on a rapid timescale, within hours of the onset of these processes that stimulate oxytocin and/or vasopressin secretion (**Figure 4a**). These morphological changes are reversible, with the speed of reversal negatively correlated with duration of the stimulation. For example, astrocytes in the supraoptic nucleus revert back to their basal morphology within one month if rats have lactated, but this reversion takes two months if the animals have undergone two consecutive gestation and lactation periods. The morphological astrocytic dynamics exhibited in this circuit have enabled elegant work in which neuronal activity in the presence and absence of astrocyte branches can be quantified, yielding a greater understanding of astrocytes' roles in volume transmission, neurotransmitter uptake, and synaptic plasticity (64, 65).

Whether the effects on neurons due to the types of changes observed in these hypothalamic nuclei can be generalized to astrocytes in other areas remains unclear. In addition, it is unknown whether the morphological changes observed in these hypothalamic astrocytes represent an active (astrocyte-driven) or a passive (neuron-driven) process from the perspective of the astrocyte. Simultaneous two-color imaging of astrocyte processes and dendritic spines in hippocampus has

shown that astrocytes can extend and retract their fine processes at a rapid timescale (on the order of minutes) to engage and disengage with dendritic spines (66) (**Figure 4b**). This astrocytic motility is more dynamic than that of the apposed spines (66), suggesting the possibility of an astrocyte-intrinsic motility. More recent work, in which astrocyte and neuronal components were again tracked using two-color fluorescence and accompanied by astrocytic manipulations, observed extensive structural plasticity in the astrocytic processes. These dynamics were shown to be causally related to increased spine stability and astrocytic spine coverage (67).

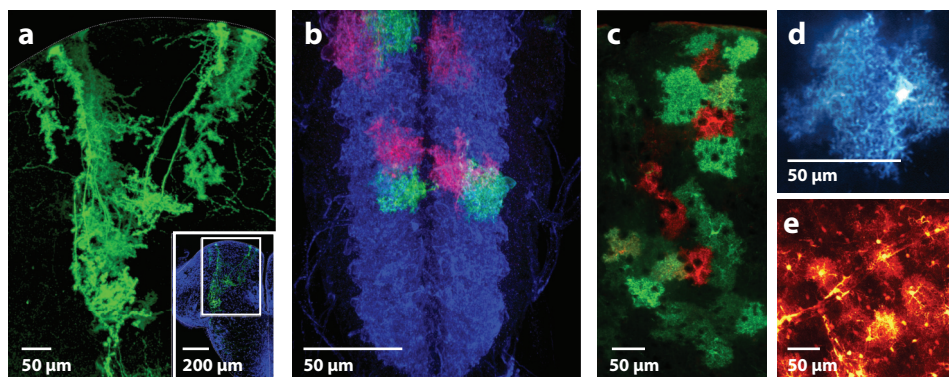
Related work addresses the larger concept that fine-scale changes in astrocyte morphology can drive synaptic physiological differences. One of the connexin proteins [connexin 30 (Cx30)] that forms gap junctions between astrocytes can influence synaptic strength via the degree of astrocytic invasion into the synaptic space (68). Because these conclusions were drawn from fixed brain sections of wild-type and Cx30 knockout mice rather than live imaging experiments, the timescale of these effects remains unclear, but a significant change in astrocyte branch length and complexity is evident. Together with the aforementioned work, these data strongly implicate astrocytes as active players in morphological dynamics at the synapse. However, not all evidence points toward synaptic stability as an outcome of astrocyte-based motility. Indeed, astrocyte motility may play an important role in synapse elimination, as based on evidence showing that astrocytes engulf synapses wholesale—pre- and postsynaptic compartments together—in a neuronal activity-dependent manner (69). Besides raising fascinating questions about the function of astrocytes in neural circuits, this research also reinforces the notion that the motility of astrocytes at synapses is a critical component of their physiological functions.

## ASTROCYTES IN AN EVOLUTIONARY CONTEXT

Astrocyte biology is no different from the rest of neuroscience in its reliance on rodent models (70), and this bias is reflected in almost all the aforementioned studies. How might perspectives from different model organisms contribute to our understanding of astrocyte biology? Many excellent recent reviews have addressed how astrocytes differ across model organisms (71–73). Here, we focus instead on conserved features that can inform our understanding of astrocyte function. Given the vagaries of glial lineage determination (discussed in Physiological Dynamism), perhaps it is not surprising that glial specification is not conserved across species. The gene *glial cells missing* (*gcm*), which is necessary and sufficient for gliogenesis in *Drosophila melanogaster* (74, 75), is not expressed in the mammalian brain and does not affect gliogenesis (76), nor is it obviously homologous with mechanisms of glial specification in other organisms such as *Caenorhabditis elegans* (77).

A focus on conserved function—rather than lineage—of astrocytes across species has been more informative. Glial diversity is more limited in lower organisms, in which the central and peripheral nervous systems are not as fully diverged. In some species, including *C. elegans*, a defined number of glia (~45 versus 302 neurons) play roles in positioning and guidance of neurons (78, 79); these roles are echoed in mammals (30). *C. elegans* glia also share roles in ensheathing and providing trophic support for neuronal dendrites (77, 78). However, these glia share few of the morphologic features of mammalian astrocytes, and it is not clear to what extent the molecular pathways mediating these effects may be conserved across species. Several species, including birds (80) and zebrafish (81), are not thought to have astrocytes at all but instead have postnatal persistence of a radial glial-like cell that spans the pial to ventricular surface. In the mature zebrafish, these cells develop fine processes strikingly similar to mammalian protoplasmic astrocytes (see **Figure 5**). Similar to neurons, mammalian protoplasmic astrocytes carry out local translation of mRNA in their fine processes (82), raising the question of whether astrocytic fine processes may act as semiautonomous subdomains when they interact with synapses or neural circuits. In such a scenario, zebrafish radial





**Figure 5**

Astrocytes across model organisms display extensive elaboration of fine branches. (a) Zebrafish radial glia in an adult dorsal telencephalon sparse labeled with a membrane-bound fluorescent reporter. Image courtesy of Marion Coolen and Laure Bally-Cuif, Institut Pasteur, Paris. (b) Third instar larval astrocytes in *Drosophila* ventral nerve cord: Astrocytes are blue and sparse labeled in red and green using twin-spot MARCM (mosaic analysis with a repressible cell marker). Image courtesy of Tobias Stork and Marc Freeman, Vollum Institute, Portland, Oregon. (c) Adult mouse cortical astrocytes tiling across cortical layers and sparse labeled in red and green using a confetti:hGFAPcre construct. Image by Kimberly Hoi and Anna Molofsky. (d) Single juvenile murine cortical astrocyte labeled with a membrane-bound fluorescent protein. Image by Kira Poskanzer. (e) Partially penetrant EAAT2-tdTomato BAC transgenic cortical astrocytes indicating fine process labeling and astrocyte-vascular contacts (98). Image by Michelle Cahill and Kira Poskanzer.

glia could have conserved functional roles with mammalian protoplasmic astrocytes with respect to synaptic and vascular interactions.

*Drosophila* larval astrocytes are elegant models for the synapse-ensheathing morphology of mammalian protoplasmic astrocytes (Figure 5b). Their branches project into a dense neuropil rich in synapses but devoid of neuronal cell bodies, providing a unique opportunity to study their synapse-supporting functions. Their association with synapses is a morphologic specialization that requires the fibroblast growth factor (FGF) receptor *heartless* (83), reminiscent of the role of *Fgfr3* in mammalian gliogenesis (84). *Drosophila* astrocytes modulate dopaminergic neuronal signaling (56) and mammalian astrocytes also involved in dopaminergic signaling (85). *Drosophila* astrocytes regulate excitatory/inhibitory balance via the GABA transporter, whose developmental expression is in turn regulated by neuronal activity (86). Mammalian astrocytes express *Gat1* and *Gat3* and regulate diffusion of GABA away from synaptic sites (87, 88); the roles of mammalian astrocytes in inhibitory synapse development and the maturation of astrocyte functional properties are major issues being addressed in the field. A developmentally distinct population of ensheathing astrocytes dependent on Notch signaling (89) reside outside the neuropil and provide neuronal support as well as engulfing degenerating axons via the engulfment receptor *draper* (90), whose mammalian analog *Megf10* regulates synapse engulfment by astrocytes (69). These examples illustrate multiple conserved molecular pathways from flies to mammals.

Other morphologic features of rodent astrocytes are variably conserved across species, raising the question of whether these differences are functionally relevant. Rodent and fly astrocytes tile in discrete domains (Figure 5b,c), in contrast to some domain overlap in human astrocytes (91). When examined with membrane-tagged labels, astrocytes in most species have extensive synapse-contacting fine processes (Figure 5) and interact closely with vasculature (Figure 5e). However, several groups have described specialized processes in primate astrocytes, some of which span

and possibly connect different cortical layers, as in the so-called interlaminar astrocytes (92). This could confer entirely new primate-specific properties in astrocytes. Likewise, human astrocytes are bigger than rodent and other primate astrocytes (91) and may support higher cognitive function (93). These morphological differences suggest that the extent of astrocyte-synaptic connectivity could vary among species, buffering neurotransmitters and modulating neuronal synchrony in unique and possibly primate-specific ways. Exploring these questions is largely impossible in intact primate brains. However, studies of the numerous gene expression differences between human and mouse astrocytes (33) begin to hint at molecular pathways that could be explored in emerging organoid cultures (94, 95) and could yield insights into primate-specific astrocyte properties.

## CONCLUSION

In summary, the explosion of molecular and optical tools in neuroscience is poised to change our understanding of how astrocytes participate in neural function, if we allow it to. As with any data set, biological complexity can be obliterated by the analysis parameters applied to the question. For example, the discovery of transcription factors required for circadian rhythms (96) and the description of transcript accumulation and decay over the course of a 24-h cycle (97) were only possible when performing experiments with the defining parameter in mind: time of day. As we argue in this review, the neuron-centric assumption of a fixed lineage may obscure the amazing capacity of astrocytes to react to an enormous range of extracellular cues. Likewise, being married to a strict input-output functional relationship akin to neuronal integration can obscure potential subcellular functional units in astrocytes. Conversely, investigating astrocytic involvement in state switching over a long timescale has opened up areas of inquiry previously believed to be solely in the domain of neurons. Much remains to be learned about how astrocytes respond to their environment, how they communicate with each other over space and time, and the link between their molecular identity and physiology. We hope that a directed focus on the uniqueness of the astrocyte will shed light on these questions and many others.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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