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Astrocyte Morphology Is Controlled by Neuron-Derived FGF

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The highly ramified processes of astrocytes enable cellular interactions and extracellular homeostasis. In this issue of *Neuron*, Stork et al. (2014) report that extension and elaboration of astrocyte processes in *Drosophila* is controlled by the release of FGF by neurons.

Astrocytes are among the most structurally complex cells in the nervous system. Although they are compact—covering less than 100 μm^3 in the mouse CNS—their processes weave their way through the neuropil, seeming to fill all the remaining extracellular space as if poured into an intricate mold. In many regions, their processes are so fine, flattening to form thin lamellae with negligible cytoplasm, that accurate representations of their structure can only be obtained through serial reconstruction of electron micrographs (Grosche et al., 1999) or though high-voltage electron microscopy (Kosaka and Hama, 1986). This extraordinary complexity enables each astrocyte to interact with many synapses, blood vessels, and nodes of Ranvier in their vicinity, and establish an interconnected network through extensive gap junctional coupling, interactions that are presumably crucial to their homeostatic and neuromodulatory functions. How astrocytes achieve such morphological complexity has remained a mystery. In this issue of *Neuron*, Stork et al. (2014) exploit the genetic accessibility of *Drosophila* to uncover a key signaling pathway responsible for controlling the elaboration of astrocyte processes into the synapse-rich region of the neuropil.

Drosophila astrocytes constitute one of two classes of neuropil glia (astrocytes and ensheathing glia) in the fly larval nerve cord. They are derived from embryonic neural stem cells (longitudinal glioblasts) and organized in a stereotyped fashion along each hemisegment, with three astrocytes positioned dorsomedially, two dorsolaterally, and one ventrally. During development, the six immature astrocytes position themselves on the dorsal nerve cord and extend their processes along the surface of the neuropil, while one astrocyte migrates ventrally. Here, Stork et al. (2014) used the MARCM approach (Lee and Luo, 2001) to achieve sparse labeling of astrocytes, revealing that their main processes infiltrate the synaptic neuropil and branch into a dense ramified network, forming nonoverlapping territories similar to the tiling behavior exhibited by mammalian astrocytes (Bushong et al., 2002). Astrocytes in fly are morphologically distinct from enseathing glia, which wrap major structures of the brain and cover the surface of the neuropil but do not closely associate with synapses. Genetic ablation of subsets of astrocytes resulted in expansion of the territory of the remaining cells, suggesting that potent self-repulsive interactions normally limit their size (Figure 1). The elaboration of astrocyte processes in the mammalian CNS helps limit functional interactions among neighboring synapses, by increasing diffusional distance for neurotransmitters and by allowing astrocytes to position neurotransmitter transporters near sites of release (Bergles et al., 1999). Stork et al. (2014) find that *Drosophila* astrocytes express the GABA transporter GAT, and RNAi-based gene knockdown of GAT specifically in astrocytes resulted in severe behavioral deficits during the larval stage, such as uncoordinated movements and reduced crawling speed. These deficits in motor function persisted in adult flies, suggesting that astrocytes play a critical role in controlling normal motor function in adults via GABA clearance.

Although astrocyte processes were in proximity to synapses in the fly CNS, electron microscopic analysis showed that their processes do not contact all synapses and do not wrap individual synapses, in contrast to the close association between astrocytes and synapses in mammals (Grosche et al., 1999; Ventura and Harris, 1999). Astrocyte processes in the fly were distant from synapses, with an average synapse-to-astrocyte distance of about 1 μm, providing coverage of only 5% of the neuropil, and the extent of astrocyte coverage was not positively correlated with synapse density, suggesting that the fly CNS may have evolved additional adaptations to reduce synaptic cross-talk. Nevertheless, by expressing a glutamate sensor (iGluSnFR) in astrocytes, Stork et al. (2014) show that astrocytes are capable of detecting synaptic glutamate release. Indeed, previous studies indicate that astrocytes in both mammals and *Drosophila* express glutamate transporters (Rival et al., 2006) and play an important role in clearing synaptic glutamate (Bergles et al., 1999; Stacey et al., 2010), pointing to a key conservation of function between these cells in neurotransmitter clearance.

How is the elaboration of astrocyte processes within the neuropil controlled? To address this question, Stork et al. (2014) focused on fibroblast growth factor (FGF) signaling, as the FGF receptor *heartless* (*Htl*) is expressed by astrocytes during early development. In htl^{AB42} null mutant flies, all six astrocytes successfully positioned themselves on the dorsal surface of the neuropil, but their processes failed to infiltrate this region. Furthermore, astrocytes lacking *htl* were markedly smaller and their processes less elaborate. Also, the ventral astrocyte

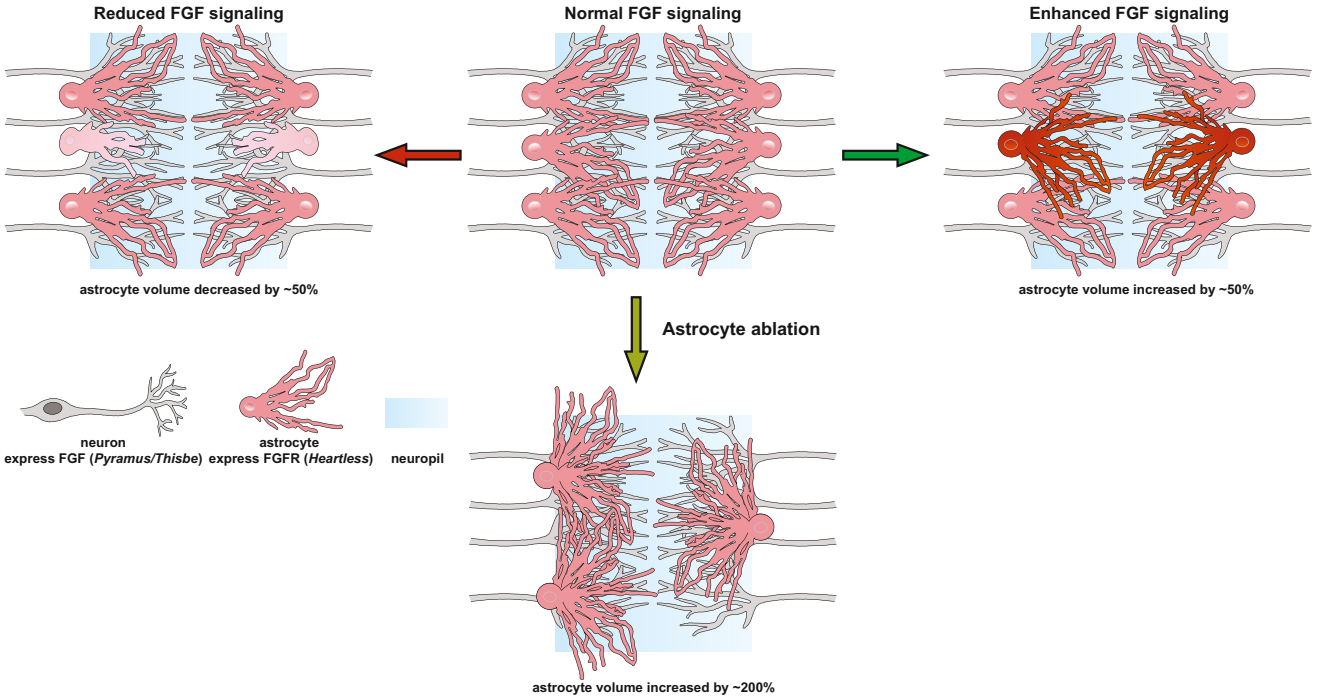

Figure 1. FGFR Activation in *Drosophila* Astrocytes Encourages the Infiltration and Elaboration of Their Processes into the Synapse-rich Neuropil

consistently failed to migrate to its correct position in the absence of FGF signaling. Reexpression of Htl specifically in astrocytes in htl^{AB42} null mutant flies restored these size and infiltration deficits, while overexpression of constitutively active *htl* in astrocytes increased the area occupied by individual astrocytes above that seen in controls (Figure 1). This genetic tour de force indicates that cell-autonomous FGFR signaling in astrocytes modulates elaboration of their processes within the neuropil.

There are two FGFR ligands in flies, Pyramus (*Pyr*, FGF-8-like2) and Thisbe (*Ths*, FGF-8-like1), which are most closely related to the FGF8/17/18 subfamily that controls heart and limb development in vertebrates. Stork et al. (2014) show that fly mutants lacking both *htl* ligands phenocopy htl^{AB42} null mutant flies; however, *ths* single mutants showed a clear but weak infiltration and migration phenotype, while *pyr* mutants exhibited an even milder phenotype, indicating that these ligands have some functionally redundancy, with Ths being the dominant ligand. What is the source of FGF in the fly CNS? Selective panneuronal reexpression of Pyr or Ths, in flies lacking both Pyr and Ths, restored process infiltration deficits, but not the ventral astrocyte migration, and when Ths was reexpressed in a single neuron in a Pyr and Ths double mutant background, only astrocytes surrounding the neurites of the Ths+ neuron showed robust infiltration of their processes. However, these FGFR ligands need not be released directly onto the astrocyte, as ectopic expression of Pyr or Ths by subperineurial and nerve root glia partially rescued the mutant phenotype, causing only minor differences in the location of cell soma and localization of the processes. Using similar knockdown strategies, Stork et al. (2014) show that the heparan sulfate proteoglycan Syndecan plays a modulatory role in FGFR signaling, most likely by concentrating FGFs in the neuropil region, allowing these ligands to accumulate near their receptors even when released at a distance.

Recent studies suggest that there is close conservation between mammalian and *Drosophila* astrocytes in this developmental regulation of cell structure, as viral expression of a dominant-negative FGFR3 receptor in astrocytes in vivo also reduced the size and morphological complexity of their processes, while expression of constitutively active FGFR3 enhanced their size and complexity (Kang et al., 2014). Astrocyte morphology has become more elaborate with increased brain size and complexity of the nervous system. For example, it has been estimated that each astrocyte in the mouse brain contacts approximately 100,000 synapses (Bushong et al., 2002), while astrocytes in the human brain occupy a 27-fold larger volume and can contact up to two million synapses (Oberheim et al., 2006). It is not yet known whether enhanced FGFR signaling accounts for this remarkable expansion of astrocyte size in the human CNS.

Together, these studies reinforce the conclusion that many functional similarities exist between astrocytes in flies and mammals. Indeed, astrocytes in flies and mammals express the engulfment receptor Draper/Megf10 and play a role in reorganization of the developing CNS by removing neuronal processes and synapses (Chung et al., 2013). However, it is likely that not all astrocytic functions are well conserved across invertebrates and vertebrates. For example, in the adult CNS, fly astrocytes do not show any detectable changes in morphology after

axonal injury and do not contribute to the clearance of degenerated axons (Doherty et al., 2009). This is in contrast to mammalian astrocytes, which respond to injury by increasing expression of GFAP, exhibiting hypertrophy, and contributing to the formation of glial scars. These differences reflect independent specialization of glia in different species. In particular, mouse astrocytes have been broadly classified as fibrous and protoplasmic, while human astrocytes as interlaminar, protoplasmic, polarized, and fibrous, and evidence for further regional specification of mammalian astrocytes is emerging (Molofsky et al., 2014). The studies reported here involved exclusive analysis of astrocytes in the larval nerve cord; much less is known about the properties and potential diversification of astrocytes in the *Drosophila* brain.

Astrocytes are thought to participate in a multitude of crucial events in the CNS, from neurotransmitter and ion homeostasis to vascular control and tissue repair. And yet, a detailed understanding of how they accomplish such diverse tasks has remained elusive. Unfortunately, astrocytes maintained in vitro exhibit properties distinct from their counterparts in the intact CNS (Cahoy et al., 2008), and their complex structure presents significant challenges for localizing proteins of interest and monitoring physiological changes at sites of interaction with other cells. Although the development of new transgenic mouse lines has increased our ability to manipulate astrocytes in vivo, specificity remains a problem due to the similar genes expressed by radial glial cells and astrocytes. Moreover, the need to generate mice that carry multiple transgenes slows the pace of discovery and places constraints on what manipulations can be performed. As these studies by Stork et al. (2014) demonstrate, the identification of a glial cell in *Drosophila*, which exhibits many key features of astrocytes in the mammalian CNS, has the potential to rapidly expand our knowledge of the functions performed by these cells under different physiological and pathological conditions and uncover the molecular underpinnings of their diverse behaviors.

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Slow Neuromodulation Mediated by ATP P2X Receptors

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ATP-gated P2X receptors are widely expressed in the nervous system, but their physiological roles are not fully understood. New insights from Pougnet et al. (2014) in this issue of *Neuron* show that postsynaptic P2X receptors may be activated by ATP released from astrocytes and function to downregulate synaptic AMPA receptors in hippocampal neurons.

Over 40 years ago, Geoffrey Burnstock proposed the existence of purinergic nerves that released ATP (Burnstock, 1972). Although initially met with considerable skepticism, there is now overwhelming evidence that ATP is widely used as a signaling molecule in the body, including in the brain (Khakh and Burnstock, 2009). ATP functions as a