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Glia-Neuron Interactions in *Caenorhabditis elegans*

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Abstract

Glia are abundant components of animal nervous systems. Recognized 170 years ago, concerted attempts to understand these cells began only recently. From these investigations glia, once considered passive filler material in the brain, have emerged as active players in neuron development and activity. Glia are essential for nervous system function, and their disruption leads to disease. The nematode *Caenorhabditis elegans* possesses glial types similar to vertebrate glia, based on molecular, morphological, and functional criteria, and has become a powerful model in which to study glia and their neuronal interactions. Facile genetic and transgenic methods in this animal allow the discovery of genes required for glial functions, and effects of glia at single synapses can be monitored by tracking neuron shape, physiology, or animal behavior. Here, we review recent progress in understanding glia-neuron interactions in *C. elegans*. We highlight similarities with glia in other animals, and suggest conserved emerging principles of glial function.

Contents

INTRODUCTION

Nervous systems conform to one of two general configurations: diffuse, with radial symmetry (e.g., in *Cnidaria*), or centralized (e.g., in *Bilateria*). In both types, information is transferred between neurons through chemical and electrical synapses. Although glial cells are not reported in animals with diffuse nerve nets, their presence is a characteristic feature of animals with centralized nervous systems. Thus, glia may have evolved in the *Urbilaterian* ancestor over 550 million years ago (Verkhratsky & Butt 2013). The appearance of glia roughly coincides with the emergence of cephalization, sense organ formation, and interneurons, raising the intriguing possibility that this cell type may have contributed to the rise of complex and adaptive nervous systems. Indeed, in modern-day animals, glia:neuron ratios track, at least to a first approximation, complexity in neural functions, animal mobility, and behaviors, with the human brain sporting about equal numbers of neurons and glia (Goodman et al. 2009, von Bartheld et al. 2016, Zhang & Sejnowski 2000).

In recent years, it has become apparent that glia and neurons monitor and respond to each other's activities as well as to signals from their surroundings. Glia provide neurons with trophic and metabolic support (Corty & Freeman 2013, Hidalgo et al. 2011). They also actively contribute to nervous system development and function, with essential roles in neurogenesis, neurite process specification and guidance, neural circuit assembly, synapse formation and function, and neurodegeneration (Kettenmann & Ransom 1995, Shaham 2015, Zhang & Sejnowski 2000, Zuchero & Barres 2015). Not surprisingly, glial dysfunctions underlie a growing list of nervous system disorders (Allen & Eroglu 2017, Chung et al. 2015, Nedergaard & Verkhratsky 2012). Nonetheless, the molecular characterization of glia-neuron interactions is in its infancy and often challenging to dissect. One factor complicating these studies is that glia in many brain regions also provide trophic and metabolic factors that support neuron survival, which can complicate assessment of their other functions. As the famed neurobiologist Santiago Ramón y Cajal (1899, 1984) noted, physiologists have lacked direct methods to study glia in neural centers. However, this state of affairs has finally begun to change.

One simple approach circumventing this hurdle arose from the observation that while glia of the nematode *Caenorhabditis elegans* share molecular and functional similarities with vertebrate glia, in terms of regulating neural development and function, they are, nonetheless, dispensable for neuron survival (Bacaj et al. 2008, Oikonomou & Shaham 2011, Shaham 2015, Sulston et al. 1983). This happenstance may be due to two properties of this system. First, at least some neuronal cell bodies are directly bathed in nutrient-rich pseudocoelomic fluid, obviating the need for nutritional support from glia. Second, the development of the nervous system in this animal is deterministic, such that cell fate and survival are largely prescribed by cell ancestry and not by cell-cell interactions (Sulston et al. 1983). Additional features make *C. elegans* an exciting setting in which to study glia-neuron interactions, including a powerful molecular genetic toolkit; a transparent cuticle, allowing in vivo microscopy, optogenetics and functional imaging; and cuticular openings, which permit delivery of ligands for chemogenetic studies. Indeed, in this animal, any glia or neuron can be individually visualized and manipulated with temporal precision. The effects of such manipulations can then be tracked at multiple levels, from genes to cells, to circuits, and to animal behavior. The invariant association between individual glia and specific neurons contributes further precision to such studies. Further, the adult neural connectome is mapped in both *C. elegans* sexes, males and hermaphrodites (Doroquez et al. 2014, Jarrell et al. 2012, Varshney et al. 2011, Ward et al. 1975, White et al. 1986), and animals use this structurally (but not behaviorally) invariant neural network for stimulus detection, locomotion, sleep, mating, decision making, memory, and many other behaviors whose specific neural basis can be dissected by manipulating individual cells or genes.

Systematic investigations of *C. elegans* glia-neuron interactions over the past decade and a half have revealed that many aspects of glia-neuron interactions are conserved among species and between the central and peripheral nervous systems. Here we review the current state of the field, noting areas where *C. elegans* can be used to inform our understanding of vertebrate systems and pointing out remaining questions.

C. ELEGANS NERVOUS SYSTEM CELLS

The *C. elegans* hermaphrodite nervous system consists of 302 neurons and 56 glia generated predominantly during embryonic development, and persisting through adulthood. *C. elegans* males have an additional 36 glia and 89 neurons that are born primarily after embryogenesis, some of which control male mating-related behaviors.

Neurons

C. elegans neurons communicate through chemical and electrical junctions using neurotransmitters, neuropeptides, and channels, many of which are conserved across animals (Bargmann 1993, 1998) (**http://www.wormbook.org**). Synaptic communication is mediated through en passant synapses, as in the mammalian postganglionic autonomic nervous system and dentate gyrus (White et al. 1986). Molecular mechanisms regulating *C. elegans* neuronal development and function have been extensively studied, and often parallel mechanisms operating in vertebrates. One notable exception is the lack of voltage-gated sodium channel genes in the *C. elegans* genome, which may also explain why neurons in this animal do not generally fire action potentials. Consistent with this, like many other invertebrates, *C. elegans* does not generate myelin, perhaps because of its small size (adults are 1 mm long), which limits conduction distances. This suggests that control of neuronal spiking is unlikely to be the reason that originally drove glial infiltration of nervous systems.

Glia

In *C. elegans*, specialized glia akin to vertebrate sense organ glia and astrocytes are easily recognized. These same glial cells, as well as other cells of epithelial character, also perform functions or display anatomy reminiscent of vertebrate radial glia, microglia, and ensheathing glia. In adult *C. elegans*, 46 glia are ectodermally derived sense organ glia (80 in males), 4 are dual sense organ and neuropil astroglia, and 6 are mesodermal lineage glia (**Figure 1a**; **Table 1**).
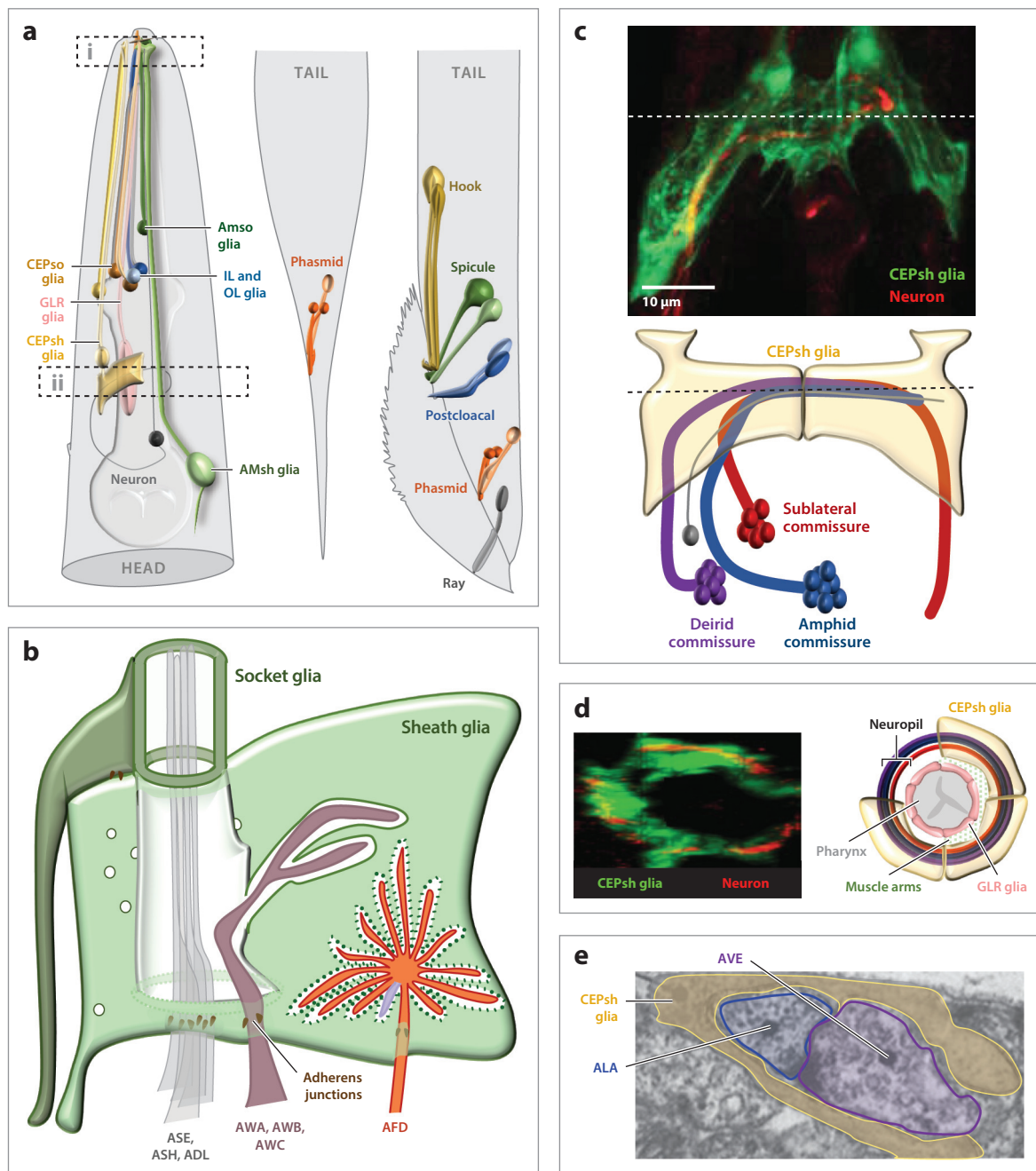
Sense organ glia. *C. elegans* receives information from its surroundings through sensilla, sense organs whose general architecture is conserved across invertebrates and vertebrates (Ward et al. 1975). There are 24 sheath glia, 26 socket glia, and 60 neurons in hermaphrodite sensilla in total. Each sensillum comprises 1–12 sensory neurons and two glia: a sheath glial cell and a socket glial cell (**Figure 1a,b**; **Table 1**). Sensilla glia and neurons are highly polarized cells. Sheath and socket glial processes fasciculate with sensory neuron dendrites. Sheath glia endings either surround proximal aspects of neuronal receptive endings (NREs) or fully encapsulate them, and sheath glia form junctions with partner dendrites at the bases of the ciliated NREs that house receptors for detecting stimuli. For example, the amphid sensillum is the primary sense organ of *C. elegans*, and amphid sheath (AMsh) glia contact 12 sensory NREs, the most of any glia (Perkins et al. 1986). Each AMsh glia surrounds proximal NREs of some neurons (such as ASE) and entirely ensheaths the NREs of some neurons (such as the AFD temperature-sensing neuron) (**Figure 1b**). Socket glia connect the sensillum to the overlying epithelium (hypodermis) through adherens junctions (Ward et al. 1975). They also surround the distal NREs of some neurons and form junctions with sheath glia endings. Sheath and socket glia together form a medial channel through which some NREs project to sample the outside world (**Figure 1b**). Variations on sense organ structures are found in some male-specific sensilla. For example, ray sensilla have only one glial cell, a presumptive sheath-socket hybrid called the structural cell [R(N)st], and male spicule sheath and socket glia are syncytial (Sulston et al. 1980).

Because sensory sensilla exhibit diverse glia-glia and glia-neuron contacts, they are appropriate settings to investigate the molecular and functional nature of such interactions.

Neuropil glia. The nerve ring of *C. elegans* is its main neuropil, or brain, composed of the processes and synapses of over 180 neurons. Two glial cell types contact neuronal processes within the neuropil and delimit its structure: the astrocyte-like CEPsh glia (laterally) and the mesodermally derived GLR glia (medially).

Each of the four CEPsh neuropil glial cells extends an anterior process that contacts NREs of CEP neurons (and also CEM-NREs in males). These glia also project elaborate posterior processes with branches that wrap around and infiltrate between axons of the nerve ring (**Figure 1c,d**). It is possible that the external nerve ring sheath generated by CEPsh glia functions similarly to a blood-brain barrier (Oikonomou & Shaham 2011); however, there is currently no functional evidence to support this idea. The unique association of adult CEPsh glia processes with both sense organs and the main neuropil leads us to speculate that glia may have first evolved in association with sensory NREs, and were subsequently co-opted to ensheath synapses. This hypothesis is consistent with numerous other molecular and anatomical similarities between sensilla and synapses (Shaham 2010).

CEPsh glia resemble vertebrate astrocytes in several aspects. In addition to associating with the *C. elegans* anatomical equivalent of the vertebrate central nervous system, CEPsh glia fine processes abut many synapses (Katz et al. 2019) and ensheath at least one (between ALA and AVE neurons) in classic tripartite configuration (White et al. 1986) (**Figure 1e**). Gene expression studies

Figure 1

(*Continued*)

f

CEPsh glia
Neuron

g Wild type — *dyf-7* mutant

h Wild type — *kcc-3* mutant

AFD-NRE — AFD-NRE

i Wild type

AMsh glia   AWC

Dauer remodeling

AMsh glia   AWC
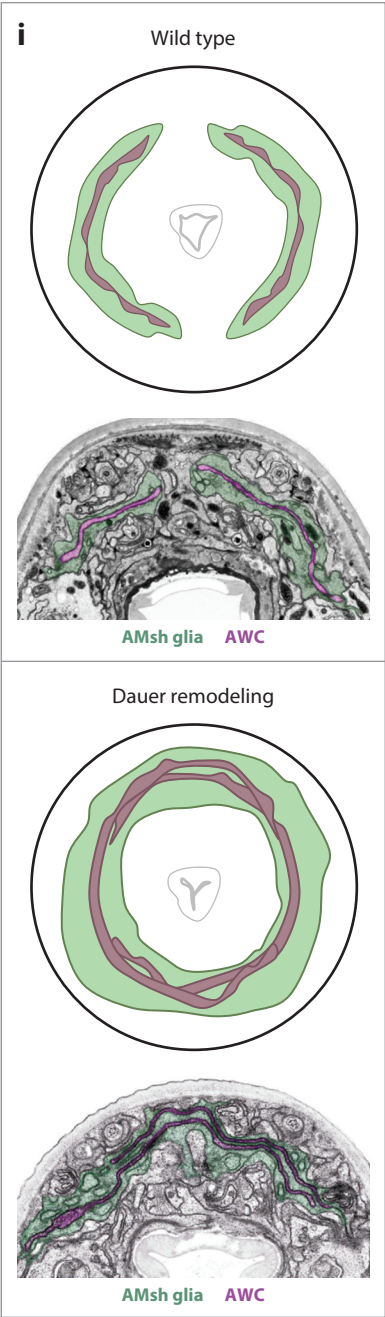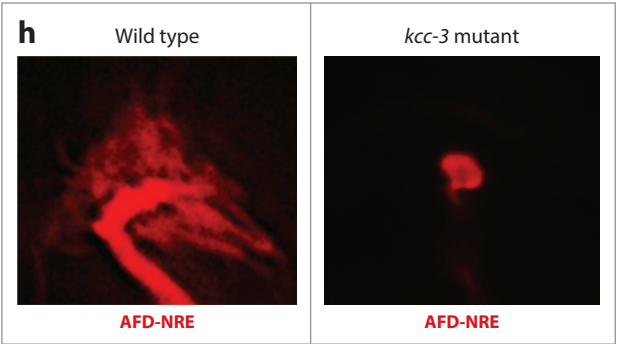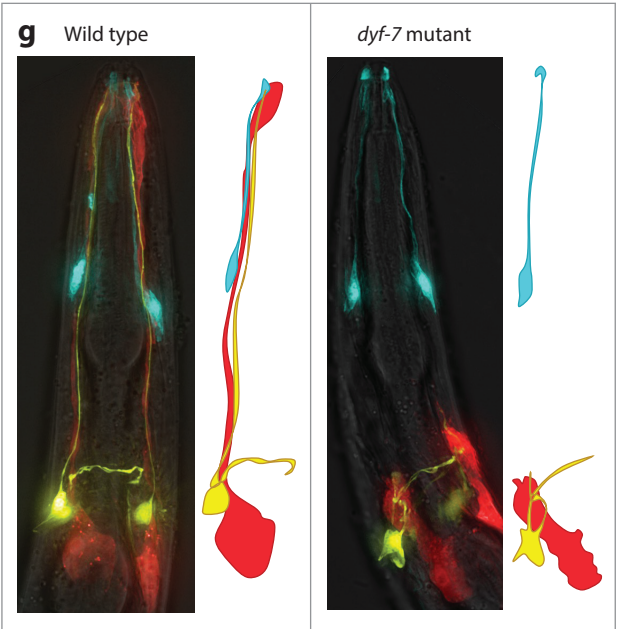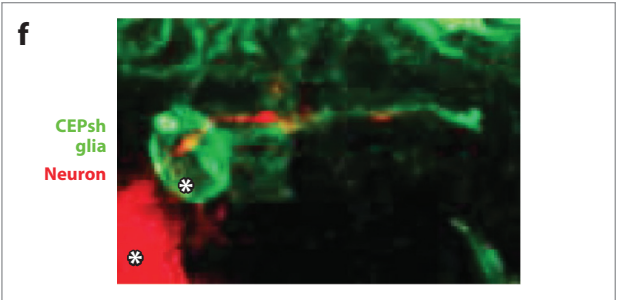
(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

(*a*) A schematic representation of each glia type in the head (*left*), hermaphrodite tail (*middle*), and male tail (*right*). Location of (*i*) magnified amphid sensilla anterior tip depicted in panel *b* and (*ii*) neuropil glia in panel *c*. (*b*) Amphid sensilla schematic showing AMso-AMsh sense organ glia forming a channel lumen, NREs that traverse the glial channel (ASE, ASH, ADL), and embedded NREs (AFD and AWA/B/C neurons). (*c*) Fluorescence micrograph (*top*) of CEPsh neuropil glia processes (*green*) around a single axon (*red*), and a schematic of CEPsh glial processes ensheathing different axon commissures (*bottom*). (*d*) A cross section through the dotted line in panel *c* showing the axon (*red*) within glial processes (*green*) (*left*) and the relative location of neuropil commissures and glia (*right*). (*e*) Electron micrograph showing the CEPsh glia–ALA neuron–AVE neuron tripartite synapse. (*f*) Fluorescence micrograph of CEPsh process (*green*) guiding pioneer axon processes (*red*). Asterisks indicate respective cell bodies. (*g*) Image and diagram of AMsh and dendrite processes in amphid sensilla, which are collapsed in *dyf-7* mutants (*right*) compared to wild-type animals (*left*). (*h*) Image of wild-type AFD-NRE (*left*), which is collapsed in *kcc-3* mutants (*right*). (*i*) Schematics and electron micrographs of AMsh (*green*) and AWC-NRE (*purple*) remodeling in postdauer animals (*bottom*) compared to wild type (*top*). Abbreviations: AMsh, amphid sheath glia; AMso, amphid socket glia; CEPsh, cephalic sheath glia; NRE, neuronal receptive ending. Fluorescence images in panels *c*, *d*, and *f* adapted with permission from Rapti et al. (2017), EM image in panel *e* adapted with permission from White et al. (1986), fluorescence images in panel *g* adapted with permission from Heiman & Shaham (2009), panel *h* adapted with permission from Singhvi et al. (2016), and EM images in panel *i* adapted with permission from Procko et al. (2011).

suggest that astrocytes are the vertebrate brain cells that most resemble CEPsh glia. Importantly, CEPsh glia express a variety of transporters and receptors, including a homolog of the astrocytic glutamate transporter GLT1 (Katz et al. 2019). CEPsh glia processes also tile the neuropil, as do vertebrate astrocytes, forming nonoverlapping adjacent domains that cover the neuropil. In the embryo, however, CEPsh glia have a decidedly different morphology, where they extend bipolar processes that initiate assembly of the nerve ring (see below) (**Figure 1***f*). This developmentally dimorphic function is reminiscent of vertebrate radial glia, which transform into astrocytes after the initial stages of nervous system assembly (Sidman & Rakic 1973). Supporting the idea of possible homology, both radial glia and embryonic CEPsh glia require Pax6 for their differentiation, and both express proteins required for axon guidance (see below) (Heins et al. 2002). VAB-3/Pax6 controls expression of HLH-17, an ortholog of the transcription factor Olig2 (Yoshimura et al. 2008), which is also expressed by a subset of vertebrate astrocytes (Tatsumi et al. 2018).

Mesodermal lineage glia. Cell bodies of the six mesoderm-derived GLR glia lie posterior to the nerve ring, and each sends an anterior projection that flattens into a sheet covering the inside aspect of the nerve ring (**Figure 1***a,d*). More anteriorly, the sheet converges into a thin process that fasciculates with the anteriorly projecting IL neuron dendrites. GLR glia express the myogenic HLH-1/*myoD* transcription factor (Krause et al. 1994) and form gap junctions with head muscles and RME motor neurons, suggesting a role in coordinating neuromuscular junction activity. Whether GLR glia share functions with microglia, the mesoderm-derived glia in vertebrates, remains to be determined.

Glial heterogeneity. In vertebrates, glia of a given type exhibit diverse gene expression and functions in different brain regions (Haim & Rowitch 2016). The same appears to be true in *C. elegans*. Different sense organ sheath glia, for example, express distinct proteins. VAP-1 is expressed only in AMsh glia, while FIG-1 is expressed in both AMsh and PHsh glia (Bacaj et al. 2008). Innexins and DEG/ENaC channels are differentially expressed in sense organ glial subtypes (Altun et al. 2010, Han et al. 2013). A different subset of innexins and the collagen CLE-1 are also differentially expressed between the six mesodermal lineage GLR glia (Ackley et al. 2001, Altun et al. 2010). Lastly, although dorsal CEPsh glia require VAB-3 for their differentiation, ventral CEPsh glia require both VAB-3 and MLS-2/Nkx and differentially express UNC-6/Netrin (see below) (Wadsworth et al. 1996). Indeed, a recent single-cell combinatorial indexing RNA sequencing

Table 1 *Caenorhabditis elegans* glia, associated neurons, and behaviors affected by respective glia-neuron interactions

Organ[a]	Glia	Associated neuron(s)	Number of sensilla	Documented functions of glia-neuron interactions	Animal behavior regulated by glia-neuron interactions
Sensilla glia					
Amphid	AMso, AMsh	Contact AMsh only: AFD, AWA, AWB, AWC AMsh-AMso channel neurons: ASE, ASG, ASH, ASI, ASK, ASJ, ADF, ADL	2 (L/R)	AMsh: NRE shape and plasticity, glial compartment, dendrite outgrowth AMso: adult neurogenesis	Olfaction, gustation, tactile sensation, thermosensation
Phasmid	PHsh, PHso1, PHso2	PHA, PHB, PHC Contact PHso only: PQR	2 (L/R)	PHso: adult neurogenesis	ND
Anterior deirid	ADEsh, ADEso	ADE	2 (L/R)	ND	ND
Posterior deirid	PDEsh, PDEso	PDE	2 (L/R)	ND	ND
Inner labial	ILsh, ILso	IL1, IL2 Contact ILso only: BAG, URX, FLP	6 (L/R, D/V/L)	IL/OL nerve outgrowth, neuropil placement	ND
Outer labial	OL(Q/L)sh, OL(Q/L)so	OLQ or OLL	6 (L/R, D/V/L)	ND	ND
Cephalic	CEPsh, CEPso	CEP (+ CEM in males)	4 (D/V, L/R)	ND	ND
Hook*	HOsh, HOso	HOA, HOB	1	ND	Male mating
Ray*	R(N)st	R(N)A, R(N)B	18 (L/R), N = 1–9	ND	Male mating
Postcloacal*	PCsh, PCso	PCA PCsh only: PCB, PCC	2 (L/R)	ND	Male mating
Spicules*	SPsh syncytium (2 cells) SPso syncytium (4 cells)	SPD, SPV	2 (L/R)	NRE development SPso: extrasynaptic source of dopamine	Male mating
Neuropil glia					
Neuropil	CEPsh	Many neuropil axons	4 (D/V, L/R)	Neuropil assembly, AIY synapse positioning, neurotransmitter clearance	Locomotion during sleep, repetitive behavior, swimming-induced paralysis
Mesodermal-lineage glia					
Mesodermal	GLR	RME, others (?)	6 (D/V/L, L/R)	RME axon specification	ND

[a]Male-specific glia are indicated by an asterisk.

Abbreviations: D, dorsal; L, left (lateral for OLL); ND, not determined; Q, quadrant; R, right; sh, sheath; so, socket; V, ventral.

study examining many *C. elegans* cell types showed that glia-enriched genes cluster in multiple subgroups, indicating different molecular profiles (Cao et al. 2017).

Glial diversity in *C. elegans* is also sexually dimorphic, as in vertebrates (Schwarz & Bilbo 2012). For example, apical endings of CEPsh glia associate with CEM and CEP neurons in males but only with CEP neurons in hermaphrodites. Hermaphrodites employ PHso1 as their primary socket glia of the phasmid sense organ, while males opt for PHso2. AMso glia are neurogenic only in males.

Genetic investigations into glial cell fate specification and heterogeneity are ongoing in several laboratories, including ours, and could uncover underlying principles driving gene expression and functional diversity.

GLIA IN NEURON DEVELOPMENT

C. elegans glia, like vertebrate glia, contribute to neurogenesis, neuron process outgrowth, and synaptogenesis, suggesting that developmental aspects of glia-neuron interactions are evolutionarily conserved and can be usefully probed in this experimental model.

Neurogenesis in Early Development

In embryonic neurogenesis of the vertebrate cortex, neurons derive predominantly from the asymmetric division of neuroepithelial stem cells (Rakic 2003, Taverna et al. 2014). In *C. elegans*, all neurons and glia develop from asymmetric divisions of neuroepithelial precursors. However, unlike radial glia or neuroepithelial stem cells, *C. elegans* neural precursors divide into terminally differentiated daughter cells without self-replenishing (Singhvi et al. 2008, Sulston et al. 1983).

Neurogenesis Later in Development

Vertebrate neurogenesis occurs in discrete neurogenic niches where neurogenic stem cells express glial markers (Falk & Götz 2017). In *C. elegans*, it was recognized 30 years ago that the socket glia of phasmid sensilla in young larvae divide to produce a neuronal lineage precursor and a phasmid socket glia replacement (Hall 1977, Sulston et al. 1980). Recent work suggests that the phenomenon may be more general. Amphid socket (AMso) glia in older male larvae were shown to divide asymmetrically to generate a male-specific MCM interneuron and a cell maintaining AMso glia characteristics (Sammut et al. 2015). Cell-division initiation here is rendered sexually dimorphic through cell-autonomous activities of the sex determination factor *tra-1* (Ci/Gli-like Zinc finger transcription factor). Remarkably, AMso glia retain their polarized morphology and contacts with AMsh glia and the epithelium throughout cell division. This is reminiscent of radial glial cell divisions in vertebrates where the neurogenic stem cell maintains its bipolar morphology and contacts throughout the cell cycle. Thus, *C. elegans* socket glia may present an appropriate setting to study general mechanisms by which glia give rise to neurons.

Glia in Neurite Development

Neurons and glia have elaborate shapes that are often coordinated through glia-neuron communication. This aspect of nervous system development has been studied extensively in *C. elegans*, yielding important insights into early nervous system formation.

Glia in dendrite outgrowth. Following their generation, neurons and glial cells of the anterior sensilla extend short processes that are anchored at the anterior tip of the animal. Soma of both neurons and glia then migrate posteriorly in synchrony, stretching out dendrites and glial processes, respectively. This phenomenon has been termed retrograde extension, and anchoring the extending tips is important to its successful implementation. Sheath glia contribute to anchoring,

possibly through adhesion to socket glia. Ablation of either CEPsh glia in cephalic sensilla or AMsh glia in amphid sensilla results, respectively, in short CEP or amphid neuron dendrites that are unattached to the nose tip (**Figure 1g**) (Heiman & Shaham 2009, Singhal & Shaham 2017, Yoshimura et al. 2008). Neurons also contribute to anchoring. Animals lacking the neuronal zona pellucida domain protein DYF-7 or the nonneuronal zonadhesion domain protein DEX-1 (Heiman & Shaham 2009) exhibit short neuronal amphid sensilla dendrites. DYF-7 likely forms filaments, observed by electron microscopy, that anchor dendrites (Oikonomou et al. 2011).

Curiously, not all sensilla glia and neurons grow through retrograde extension. The URX interoceptive neuron, which fasciculates with OL socket glia, grows its dendrite anteriorly using the MAP3K MAPK-15 (McLachlan et al. 2018). Manipulating kinase activity cell autonomously can lead to increased or decreased dendrite length. As sensilla are prevalent in many animals, discoveries made in *C. elegans* will likely serve as stepping stones to broadly understand the different forms of glia-neuron interactions in sense organ sensilla.

Glia in neuropil formation and axon guidance. How the *C. elegans* nerve ring is assembled has been a long-standing mystery. Recent advances have finally shed light on this spectacular process of self-assembly. Rapti et al. (2017) followed the earliest stages of nerve ring assembly using neuronal and glial reporters. They found that CEPsh glia processes demarcate the embryonic nerve ring ahead of neuronal process infiltration (**Figure 1f**), suggesting key roles for these radial glia–like cells. A bundle of fewer than 10 pioneer neurons of the sublateral commissure follow CEPsh glia processes and are followed by the hierarchical penetration of follower axons. Ablation and mutant studies reveal that this temporal order of assembly reflects a functional hierarchy (Rapti et al. 2017, Yoshimura et al. 2008). In a genetic tour de force that highlights and exploits the unique experimental prowess of *C. elegans*, a mutant defective in two genes, *chin-1* (encoding a Chimaerin/RhoGAP) and *kpc-1* (encoding a Furin pro-protein convertase) was serendipitously identified that blocks follower axon incorporation into the nerve ring. CHIN-1/Chimaerin and KPC-1/Furin act together in both CEPsh glia and pioneer sublateral neurons to control localization, and perhaps trafficking and processing, of axon guidance proteins. It was the isolation of this mutant, which simultaneously blocks multiple guidance pathways, that provided the essential step in cracking open the problem of nerve ring assembly. These studies reveal that *C. elegans* netrin (UNC-6), previously shown to be expressed in CEPsh and ILsh glia (Wadsworth et al. 1996), functions redundantly in CEPsh glia with other proteins to promote pioneer neuron entry into the nerve ring. The contribution of ILsh glia to nerve ring assembly, if any, is not known (Wadsworth et al. 1996). Semaphorin (MAB-20) expressed in CEPsh glia redundantly directs follower axon entry, and flamingo/CELSR (FMI-1) functions from both CEPsh glia and pioneers for follower axon guidance into the nerve ring.

These discoveries recently took on added significance as remarkable similarities in anatomy and molecules were uncovered between *C. elegans* nerve ring assembly and vertebrate neural tube development. In the mouse hindbrain and spinal cord, ventrally located floor plate netrin was long thought to regulate the circumferential guidance of commissural axons (Colamarino & Tessier-Lavigne 1995). Recent work reveals that radial glia end feet are also a major relevant netrin source (Dominici et al. 2017, Varadarajan et al. 2017). Therefore, in both *C. elegans* and vertebrates, a ring-like structure develops with axons extending along the dorsoventral axis before crossing the midline. In both systems, glial membranes demarcating the outer surface of this axonal ring release netrin for midline-directed axon guidance. We anticipate that the panoply of factors now being identified in *C. elegans* nerve ring assembly will yield relatives with similar functions in the mouse. Furthermore, the identification of molecularly defined pioneer neurons in *C. elegans* opens the door to finding neurons of similar status in the wiring of the vertebrate central nervous system.

GLR glia, which line the interior of the larval adult nerve ring, do not appear to have roles in nerve ring assembly (Rapti et al. 2017), but are required to maintain nerve ring placement, as the ablation of GLR cells results in an anteriorly misplaced nerve ring (Shah et al. 2017).

Glia in neurite specification. GLR glia also appear to play roles in neurite specification of RME head motor neurons. By regulating Ca^{2+} flux in RMEs through UNC-7/innexin gap junctions, GLR glia help to specify the RME axon. The loss of gap junction function leads axons to acquire dendritic characteristics by altering microtubule polarity through CDK-5/cyclin-dependent kinase and CLP-4/calpain activity (Meng et al. 2016). In the developing mammalian nervous system, radial glia contact immature migrating neurons through transient gap junctions (Elias & Kriegstein 2008), raising the speculation that this interaction may also drive axon specification.

Glia in Neuron Receptive Ending Development and Plasticity

NREs, such as sensory dendrite endings or postsynaptic dendritic spines, are specialized subcellular domains where neurons receive signals from the environment or from presynaptic neurons, respectively. NREs exhibit dynamic morphological plasticity and are often associated with glia. Vertebrate astrocytes, for example, regulate postsynaptic site stability, plasticity, and pruning (Allen & Eroglu 2017). *C. elegans* sense organ glia exert similar control over sense organ NREs, revealing molecular and functional similarities between these two types of signal-processing structures (Shaham 2010). Studies of *C. elegans* suggest that glia exert lifelong control over NRE shape (Bacaj et al. 2008, Singhvi et al. 2016, Wallace et al. 2016). A single glial cell can differentially regulate distinct NREs (Bacaj et al. 2008, Singhvi et al. 2016), and mechanisms of this differential engagement can be molecularly distinct (Singhvi et al. 2016). Emerging from these studies is a picture of glia as homeostatic control hubs for neuronal structures and functions.

Development and maintenance of sensory neuron receptive ending structure. The ablation of AMsh glia results in structural and functional defects in most NREs of the amphid sense organ, even if performed after the structures have been fully formed (**Figure 1b**) (Bacaj et al. 2008, Singhvi et al. 2016, Wallace et al. 2016). Thus, AMsh glia continuously maintain sensory NRE shape. The conserved transcription factor PROS-1/Prox1 is expressed post-developmentally in vertebrates and in *C. elegans* sense organ glia, and *pros-1* loss phenocopies AMsh glia ablation (Kage-Nakadai et al. 2016, Wallace et al. 2016). Transcriptional profiling reveals that PROS-1 controls expression of much of the AMsh glia secretome, including expression of FIG-1, a thrombospondin-related protein required for the activities of some amphid NREs (Bacaj et al. 2008). Thrombospondin has been implicated in vertebrate synapse formation and maintenance (Christopherson et al. 2005), further supporting a common origin for NREs and postsynaptic sites.

The glial compartment surrounding neuron receptive endings. Across species, glia form isolating compartments around NREs and neuron processes. For example, Schwann cells ensheath sensory NREs in skin Pacinian corpuscles, and the glia-like retinal pigment epithelium surrounds photoreceptor NREs in the retina. In the central nervous system, astrocytic tunnels orient migrating neurons in mice, and glial canals guide decussating commissures in the *Drosophila* antennal lobe (Chen & Hing 2008, Jankovski & Sotelo 1996, Lois et al. 1996).

In *C. elegans*, sense organ glia form a channel through which sensory NREs sample the environment (**Figure 1b**) (Perkins et al. 1986). This setting has been particularly useful in dissecting the size control of such glial compartments. Two sterol-sensing domain proteins,

DAF-6/Patched-related and CHE-14/Dispatched, localize to the luminal portion of the amphid glial channel and control channel size (Perens & Shaham 2005). DAF-6 limits compartment size, while an antagonistic molecular network promotes channel expansion. Specifically, loss of LIT-1/Nemo-like kinase, the sorting nexins SNX-1, SNX-3, and VPS-29, or the IGDB-2/Immunoglobulin-Fibronectin III domain protein in glia restores normal channel size to DAF-6 mutants that exhibit bloated channels (Oikonomou et al. 2011, 2012; Wang et al. 2017). LIT-1 functions with MOM-4/MAP3K and ACT-4/actin and may regulate the actin-polymerizing factor WSP-1/NWASP to remodel actin and lumen shape. IGDB-2/FnIII binds LGC-34, a predicted ion channel, raising the intriguing, yet unproven, possibility that osmotic forces play key roles. Remarkably, localization of DAF-6 and LIT-1 kinase to the channel lumen requires intact NREs (Oikonomou et al. 2011, Perens & Shaham 2005), suggesting ongoing neuron-glia signaling and supporting the hypothesis that glia act as homeostatic regulators in the nervous system.

Synaptogenesis. Vertebrate glia stabilize synapses through thrombospondins, glypicans, Hevin, TGFβ, ephrins, and other factors (Allen & Eroglu 2017, Christopherson et al. 2005, Murai et al. 2003, Ullian et al. 2001). The loss of these secreted glial cues impairs synapse formation and stabilization and correlates with neurodevelopmental disorders (Clarke & Barres 2013). *C. elegans* CEPsh neuropil glia also direct synaptogenesis. Glial UNC-6/Netrin signals through the neuronal UNC-40/DCC receptor not only to guide axons but also to position presynaptic sites of at least the AIY interneuron near postsynaptic sites of the RIA interneuron. Disrupting glial morphology appears to affect synapse formation (Colón-Ramos et al. 2007, Shao et al. 2013). CEPsh glia-epidermis adhesions also impact AIY synapse positioning. The loss of CIMA-1/SLC17 solute transporter increases glia-epidermis adhesion, leading to inappropriate glia-axon contacts and ectopic synapses through EGL-15/FGFR signaling (Shao et al. 2013). Nonetheless, mutants defective in nerve ring formation (see above) (Rapti et al. 2017), where many axons fail to enter this neuropil, are still able to locomote, although with some difficulty, suggesting that gross synaptic connectivity is not destroyed. It is therefore plausible that CEPsh glia primarily direct synapse placement and not formation.

Vertebrate adult-born neurons form synapses and integrate into preexisting circuits with the help of astrocytes (Sultan et al. 2015), although molecular mechanisms of these glia-neuron interactions are unknown. We note that *C. elegans* MCM neurons, generated in late-stage larvae, integrate into preexisting circuits to drive male mating behaviors (Sammut et al. 2015), and MCM axons are likely in proximity to CEPsh glia processes. We suggest that these neurons may present a novel opportunity to investigate post-developmental glia-neuron interactions driving synaptogenesis.

Plasticity of sensory neuron receptive endings. During hormonal surges (e.g., lactation) or dehydration, vertebrate astrocytes retract their processes from associated synapses within the hypothalamus. Although the underlying mechanisms are not known, this remodeling is thought to influence circuit activity (Hatton 1997, Theodosis et al. 2009). In the *C. elegans* amphid sense organ, neurons and glia also remodel under stressful conditions, including crowding, high temperature, and starvation, that drive animals into an alternate developmental stage called dauer (Golden & Riddle 1984).

In dauers, AMsh glia and an embedded neuron, AWC, remodel concomitantly (Golden & Riddle 1984, Procko et al. 2011). The bilateral glia expand their AWC ensheathing domains until these fuse, generating left-right cytoplasmically continuous glia. Fusion, mediated by the fusogen AFF-1, allows bilateral AWC NREs to expand and overlap (**Figure 1i**). As in the hypothalamus, it appears that glial remodeling here is independent of the AWC neuron and likely independently

responsive to hormone or pheromone stimulation. Although the full molecular underpinnings of this process remain unknown, the OTX/OTD transcription factor TTX-1 and the zinc-finger transcription factor ZTF-16, acting in glia, promote expression of the VER-1/receptor tyrosine kinase for remodeling. The purpose of remodeling is not known, although it has been suggested to increase animal sensitivity to favorable environmental conditions.

NEURON-GLIA INTERACTIONS IN NERVOUS SYSTEM FUNCTION

The small cell number and reproducible anatomy of *C. elegans* allow for the direct interrogation of how single synapses or other NREs control animal behavior, including the role of glia at these sites. Thus, consequences of specific glia-neuron interactions for neural functions and animal behaviors can be assessed directly. Studies to date indicate that, as in neural development, principles of glia-neuron interactions driving neural functions and animal behavior may also be evolutionarily conserved across species, and can be dissected in exquisite detail in *C. elegans*.

Glial Transporters and Channels Affecting Neuron Activity

Na^+, K^+, and Cl^- levels are tightly controlled in animals around synapses and within body fluids. Glia in many species and across central/peripheral nervous systems contribute to this meticulous homeostasis (Featherstone 2011, Khakh & Sofroniew 2015, Leiserson et al. 2011, Rusan et al. 2014, Sun et al. 2010). Disrupted astrocyte-dependent extracellular K^+ homeostasis causes severe and broad neurological dysfunctions (Murakami & Kurachi 2016, Tong et al. 2014), demonstrating that maintenance of the ionic milieu is critical for proper nervous system function. More specific effects of ions on neuron activity are also documented. Support cells of the mammalian cochlea, for example, regulate K^+ around hair cells, a requirement for auditory perception. Cochlear support glia also stochastically excite inner hair cells during development through the Ca^{2+}-activated Cl^- channel TMEM16A/anoctamin-1/ANO1 (Wang et al. 2015), a process perhaps involved in hair cell differentiation and tuning.

Studies of *C. elegans* glia suggest that the control of ionic milieu around NREs is a conserved function for glia and reveal some of the mechanisms at play and their effects on behavior. The *C. elegans* AMsh glia K^+/Cl^- cation-chloride cotransporter (CCC) KCC-3 is localized to glial membranes surrounding NREs of the AFD thermosensory neuron (Singhvi et al. 2016). *kcc-3* mutants show progressive defects in NRE shape maintenance. A surprising mechanism has been uncovered for these effects: Extracellular Cl^- ions, whose levels are likely affected by KCC-3, bind to an AFD-specific receptor-guanylyl cyclase (rGC), GCY-8, and inhibit its activity. Altered cGMP levels, determined by GCY-8 activity, along with the phosphodiesterases PDE-1 and PDE-5 then tune AFD-NRE shape through the actin-polymerizing factor WSP-1/NWASP. Unexpectedly, NRE shape modulation is independent of neuronal activity (Singhvi et al. 2016). *kcc-3* mutants also display progressive thermotaxis behavior defects, which correlate with these shape changes and an inability to sustain stimulus-induced Ca^{2+} influx into the AFD neuron, presumably caused by depleted extracellular K^+ (Yoshida et al. 2016). Lesions in human and mouse KCC-3 are associated with peripheral neuropathies, epilepsy, and familial autism through unknown mechanisms (Kahle & Delpire 2015, Medina et al. 2014). The retinal pigment epithelium and Müller glia express related transporters whose perturbation causes retinal degeneration and vision defects through unknown mechanisms (Gallemore et al. 1997, Payne et al. 2003, Wimmers et al. 2007). Thus, *C. elegans* studies may point the way towards a mechanistic understanding of these phenomena.

The *C. elegans* AMsh glia DEG/ENaC channel ACD-1 regulates the ability of these animals to avoid acidic surroundings and track attractants including lysine, Na^+ ion, and isoamyl alcohol

(Wang et al. 2012). Unlike glial KCC-3, ACD-1 does not impact sensory NRE shape but can occasionally impact axon outgrowth (Wang et al. 2012). ACD-1 activity in AMsh glia depends on intracellular acidification regulated by CHL-1/ClC-2 channels (Grant et al. 2015). Mammalian glia express both DEG/ENaC and ClC channels. Although the functions of the former are not entirely clear, the latter are implicated in regulating synaptic transmission (Rinke et al. 2010).

The DEG/ENaC channels DELM-1 and DELM-2 are expressed in OL and IL socket glia and regulate the *C. elegans* touch responses and foraging behaviors mediated by OL and IL neurons (Han et al. 2013). Like ACD-1, they do not regulate NRE shape but control neuronal excitability through TRPA-1/TRP channels.

Glial Uptake and Release of Neurotransmitters

Astrocytes express transporters that regulate neurotransmitter levels and clearance (Nedergaard & Verkhratsky 2012), and *C. elegans* glia appear to have similar functions, and in some cases, their contributions to animal behavior have been explored.

CEPsh neuropil glia ensheath glutamatergic synapses and express GLT-1, a conserved glutamate transporter (Katz et al. 2019, Mano et al. 2007). *glt-1* mutants, like CEPsh glia-ablated animals, exhibit repetitive bouts of reversal locomotion generated by a glutamate-dependent autocrine presynaptic feedback loop and mediated by the presynaptic glutamate receptor MGL-2/mGluR5 (Katz et al. 2018, 2019). In the mouse, both GLT-1 and mGluR5 are implicated in repetitive behavior (grooming), and modifiers are implicated in human neurodevelopmental disorders, including autism and epilepsy (Haroon et al. 2016). It is thus plausible that the underlying physiology uncovered in *C. elegans* may parallel that driving this type of aberrant mammalian behavior.

CEPsh glia–expressed HLH-17/Olig2 has been proposed to impact dopamine-dependent *C. elegans* behaviors, consistent with the expression of putative dopamine receptors and transporters in these cells (Felton & Johnson 2014, Katz et al. 2019). However, molecular mechanisms underlying this association await inquiry.

Interestingly, in males, SP socket glia themselves express CAT-2, a rate-limiting dopamine biosynthetic enzyme, suggesting that these glia act as dopamine sources. Consistent with this, these glia do not express the DA reuptake transporter DAT-1, and dopamine in these glia is required for male mating behaviors (Leboeuf et al. 2014, Lints & Emmons 1999).

GABA immune reactivity is detected in *C. elegans* GLR glia, and GABA accumulation in these glia depends on the SNF-11 GABA transporter (Gendrel et al. 2016). However, the role of GLR glia in *C. elegans* GABAergic transmission is currently not known.

Although the functional understanding of neurotransmitter accumulation and release by *C. elegans* glia is currently rudimentary, the powerful tools for manipulating neurotransmitter function and imaging and manipulating Ca^{2+} levels in this animal, in both glia and neurons, are likely to aid in uncovering conserved roles for these events in the context of specific animal behaviors.

Vertebrate glia exhibit Ca^{2+} transients that correlate with the release of neurotransmitter (Agarwal et al. 2017, Rousse & Robitaille 2006, Schummers et al. 2008), and Ca^{2+} imaging studies reveal dynamic subcellular micro-domain transients in both glial cell bodies and processes (Khakh & McCarthy 2015). A recent study suggests a role for glial Ca^{2+} transients in the mouse striatum in regulating repetitive grooming behaviors (Yu et al. 2018). Ca^{2+} signals have also been detected in *C. elegans* glia. AMsh glia reduce intracellular Ca^{2+} upon odor stimulation with isoamyl alcohol and elevate Ca^{2+} upon tactile stimulation (Ding et al. 2015, Wang et al. 2012). Ca^{2+} transients are also detected in the male SPso during mating (Leboeuf et al. 2014). As in vertebrates, the meaning of glial Ca^{2+} changes remains obscure in *C. elegans*.

Glia-Neuron Interactions in Sleep Control

Ramón y Cajal (1895) proposed that glia modulate sleep (García-Marín et al. 2007). Multiple lines of evidence, including measurements of glial Ca^{2+} activity, neurotransmitter regulation, and correlation with cortical slow oscillations, now support this hypothesis (Halassa & Haydon 2010; Pelluru et al. 2016; Poskanzer & Yuste 2016; Whalley 2013, 2017). Nonetheless, the underlying molecular and neuronal mechanisms remain unclear.

C. elegans also sleep, and in vivo whole-brain Ca^{2+} imaging reveals a global quiescent brain state similar to mammalian sleep (Nichols et al. 2017), with the notable exception of the ALA neuron and a handful of others. The ALA interneuron has been previously implicated in sleep control (Nath et al. 2016), and CEPsh neuropil glia act on the ALA circuit to regulate sleep (Katz et al. 2018). Under wakefulness, CEPsh glia block an inhibitory synapse between ALA and a key postsynaptic interneuron, AVE, which regulates animal locomotion. Ablation of CEPsh glia results in narcoleptic-like locomotory pausing and extension of normal sleep periods. Remarkably, glial removal does not appear to silence AVE. Rather, inputs into AVE become uncoupled from the output of this neuron, and this depends on the presynaptic ALA neuron. The molecular basis of this uncoupling is not understood, and the ALA neurotransmitter responsible for the effects has not been identified. These studies represent the first detailed analysis correlating glia-neuron interaction and the activity of a specific synapse with sleep. Future studies are likely to reveal additional principles controlling this important process.

Glia in Aging and Neurodegeneration

Glial dysfunction is increasingly appreciated as a contributor to neurodegeneration and cognitive decline (Kaminsky et al. 2016, Parpura et al. 2016), although, again, the mechanisms are not clear. Many investigations in *C. elegans* support this idea and shed light on the relevant molecular mechanisms. The glial neuropeptide RGBA-1 (regulatory gene for behavioral aging 1) regulates organismal aging and progressive decline in mating behaviors. It acts through the NPR-28 neuropeptide receptor in serotonergic and dopaminergic neurons, causing SIR-2.1-dependent activation of mitochondrial unfolded protein response pathways (Yin et al. 2017). Further, the creatine kinase ARGK-1 is expressed in glia, among other cells, and regulates stress resistance and animal life span (McQuary et al. 2016). Glia have also been studied in *C. elegans* models of Parkinson's disease. Loss of SWIP-10/metallo-lactamase causes glutamate-dependent hyperexcitability of, and increased dopamine release from, dopaminergic neurons (Gibson et al. 2018, Hardaway et al. 2015, Verkhratsky et al. 2014). These studies together suggest a role for glia in organismal aging and neurodegeneration in *C. elegans*.

CONCLUSION

Glia influence nearly every aspect of neural circuit development and function in health and in disease (Allen & Eroglu 2017, Zuchero & Barres 2015). Although neurons have been investigated in detail, glial roles in neural functions remain largely enigmatic, and studies at molecular resolution are only now beginning to emerge. Understanding the mechanisms by which glia interact with neurons is therefore an exciting frontier in molecular neuroscience.

After over a decade, *C. elegans* glia research is now coming of age as a powerful experimental platform for understanding glia-neuron interactions in mechanistic detail. The studies summarized here exemplify the facile manipulation and molecular resolution of this system, as well as the utility of its invariant developmental lineages and glia-neuron contacts in overcoming technical challenges of studying glia-neuron interactions in other systems. Importantly, these studies

highlight aspects of glia-neuron interactions that are evolutionarily conserved in molecular detail. Studies of glia-neuron interactions in *C. elegans* are therefore beginning to provide important conceptual contributions to the field, and we believe that more is yet to come.

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LITERATURE CITED

Ackley BD, Crew JR, Elamaa H, Pihlajaniemi T, Kuo CJ, Kramer JM. 2001. The NC1/endostatin domain of *Caenorhabditis elegans* type XVIII collagen affects cell migration and axon guidance. *J. Cell Biol.* 152(6):1219–32

Agarwal A, Wu PH, Hughes EG, Fukaya M, Tischfield MA, et al. 2017. Transient opening of the mitochondrial permeability transition pore induces microdomain calcium transients in astrocyte processes. *Neuron* 93(3):587–605.e7

Allen NJ, Eroglu C. 2017. Cell biology of astrocyte-synapse interactions. *Neuron* 96(3):697–708

Altun ZF, Chen B, Wang Z, Hall DH. 2010. High resolution map of *Caenorhabditis elegans* gap junction proteins. *Dev. Dyn.* 238(8):1936–50

Bacaj T, Tevlin M, Lu Y, Shaham S. 2008. Glia are essential for sensory organ function in *C. elegans. Science* 322(5902):744–47

Bargmann CI. 1993. Genetic and cellular analysis of behavior in *C. elegans. Annu. Rev. Neurosci.* 16:47–71

Bargmann CI. 1998. Neurobiology of the *Caenorhabditis elegans* genome. *Science* 282(5396):2028–33

Cao J, Packer JS, Ramani V, Cusanovich DA, Huynh C, et al. 2017. Comprehensive single-cell transcriptional profiling of a multicellular organism. *Science* 357:661–67

Chen W, Hing H. 2008. The L1-CAM, Neuroglian, functions in glial cells for *Drosophila* antennal lobe development. *Dev. Neurobiol.* 68:1029–45

Christopherson KS, Ullian EM, Stokes CCA, Mullowney CE, Hell JW, et al. 2005. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell* 120(3):421–33

Chung W-S, Welsh CA, Barres BA, Stevens B. 2015. Do glia drive synaptic and cognitive impairment in disease? *Nat. Neurosci.* 18(11):1539–45

Clarke LE, Barres BA. 2013. Emerging roles of astrocytes in neural circuit development. *Nat. Rev. Neurosci.* 14(5):311–21

Colamarino SA, Tessier-Lavigne M. 1995. The role of the floor plate in axon guidance. *Annu. Rev. Neurosci.* 18:497–529

Colón-Ramos DA, Margeta MA, Shen K. 2007. Glia promote local synaptogenesis through UNC-6 (netrin) signaling in *C. elegans. Science* 318(5847):103–6

Corty MM, Freeman MR. 2013. Architects in neural circuit design: Glia control neuron numbers and connectivity. *J. Cell Biol.* 203:395

Ding G, Zou W, Zhang H, Xue Y, Cai Y, et al. 2015. In vivo tactile stimulation-evoked responses in *Caenorhabditis elegans* amphid sheath glia. *PLOS ONE* 10(2):e0117114

Dominici C, Moreno-Bravo JA, Puiggros SR, Rappeneau Q, Rama N, et al. 2017. Floor-plate-derived netrin-1 is dispensable for commissural axon guidance. *Nature* 545(7654):350–54

Doroquez DB, Berciu C, Anderson JR, Sengupta P, Nicastro D. 2014. A high-resolution morphological and ultrastructural map of anterior sensory cilia and glia in *Caenorhabditis elegans. eLife* 3:e01948

Elias LAB, Kriegstein AR. 2008. Gap junctions: multifaceted regulators of embryonic cortical development. *Trends Neurosci.* 31(5):243–50

Falk S, Götz M. 2017. Glial control of neurogenesis. *Curr. Opin. Neurobiol.* 47:188–95

Featherstone DE. 2011. Glial solute carrier transporters in *Drosophila* and mice. *Glia* 59(9):1351–63

Felton CM, Johnson CM. 2014. Dopamine signaling in *C. elegans* is mediated in part by HLH-17-dependent regulation of extracellular dopamine levels. *G3* 4:1081–89

Gallemore RP, Hughes BA, Miller SS. 1997. Retinal pigment epithelial transport mechanisms and their contributions to the electroretinogram. *Prog. Retin. Eye Res.* 16(4):509–66

García-Marín V, García-López P, Freire M. 2007. Cajal's contributions to glia research. *Trends Neurosci.* 30(9):479–87

Gendrel M, Atlas EG, Hobert O. 2016. A cellular and regulatory map of the GABAergic nervous system of *C. elegans. eLife* 5:e17686

Gibson CL, Balbona JT, Niedzwiecki A, Rodriguez P, Nguyen KCQ, et al. 2018. Glial loss of the metallo β-lactamase domain containing protein, SWIP-10, induces age- and glutamate-signaling dependent, dopamine neuron degeneration. *PLOS Genet.* 14:e1007269

Golden JW, Riddle DL. 1984. The *Caenorhabditis elegans* dauer larva: developmental effects of pheromone, food, and temperature. *Dev. Biol.* 102(2):368–78

Goodman M, Sterner KN, Islam M, Uddin M, Sherwood CC, et al. 2009. Phylogenomic analyses reveal convergent patterns of adaptive evolution in elephant and human ancestries. *PNAS* 106(49):20824–29

Grant J, Matthewman C, Bianchi L. 2015. A novel mechanism of pH buffering in *C. elegans* glia: bicarbonate transport via the voltage-gated ClC Cl⁻ channel CLH-1. *J. Neurosci.* 35(50):16377–97

Haim LB, Rowitch D. 2016. Functional diversity of astrocytes in neural circuit regulation. *Nat. Rev. Neurosci.* 18(1):31–41

Halassa MM, Haydon PG. 2010. Integrated brain circuits: Astrocytic networks modulate neuronal activity and behavior. *Annu. Rev. Physiol.* 72:335–55

Hall DH. 1977. *The posterior nervous system of the nematode Caenorhabditis elegans*. PhD Diss., Calif. Inst. Technol., Pasadena, CA

Han L, Wang Y, Sangaletti R, D'Urso G, Lu Y, et al. 2013. Two novel DEG/ENaC channel subunits expressed in glia are needed for nose-touch sensitivity in *Caenorhabditis elegans. J. Neurosci.* 33(3):936–49

Hardaway JA, Sturgeon SM, Snarrenberg CL, Li Z, Xu XZS, et al. 2015. Glial expression of the *Caenorhabditis elegans* gene *swip-10* supports glutamate dependent control of extrasynaptic dopamine signaling. *J. Neurosci.* 35(25):9409–23

Haroon E, Miller AH, Sanacora G. 2016. Inflammation, glutamate, and glia: a trio of trouble in mood disorders. *Neuropsychopharmacology* 42(1):193–215

Hatton GI. 1997. Function-related plasticity. *Annu. Rev. Neurosci.* 20:375–97

Heiman MG, Shaham S. 2009. DEX-1 and DYF-7 establish sensory dendrite length by anchoring dendritic tips during cell migration. *Cell* 137(2):344–55

Heins N, Malatesta P, Cecconi F, Nakafuku M, Tucker KL, et al. 2002. Glial cells generate neurons: the role of the transcription factor Pax6. *Nat. Neurosci.* 5(4):308–15

Hidalgo A, Kato K, Sutcliffe B, Mcilroy G, Bishop S, Alahmed S. 2011. Trophic neuron-glia interactions and cell number adjustments in the fruit fly. *Glia* 59(9):1296–303

Jankovski A, Sotelo C. 1996. Subventricular zone-olfactory bulb migratory pathway in the adult mouse: cellular composition and specificity as determined by heterochronic and heterotopic transplantation. *J. Comp. Neurol.* 371(3):376–96

Jarrell TA, Wang Y, Bloniarz AE, Brittin CA, Xu M, et al. 2012. The connectome of a decision-making neural network. *Science* 337(6093):437–44

Kage-Nakadai E, Ohta A, Ujisawa T, Sun S, Nishikawa Y, et al. 2016. *Caenorhabditis elegans* homologue of Prox1/prospero is expressed in the glia and is required for sensory behavior and cold tolerance. *Genes Cells* 21:936–48

Kahle KT, Delpire E. 2015. Kinase-KCC2 coupling: Cl⁻ rheostasis, disease susceptibility, therapeutic target. *J. Neurophysiol.* 115:8–18

Kaminsky N, Bihari O, Kanner S, Barzilai A. 2016. Connecting malfunctioning glial cells and brain degenerative disorders. *Genom. Proteom. Bioinform.* 14(3):155–65

Katz M, Corson F, Iwanir S, Biron D, Shaham S, et al. 2018. Glia modulate a neuronal circuit for locomotion suppression during sleep in *C. elegans. Cell Rep.* 22(10):2575–83

Katz M, Corson F, Keil W, Singhal A, Bae A, et al. 2019. Glutamate spillover in *C. elegans* triggers repetitive behavior through presynaptic activation of MGL-2/mGluR5. *Nat. Commun.* 10:1882

Kettenmann H, Ransom BR, eds. 1995. *Neuroglia*. Oxford, UK: Oxford Univ. Press

Khakh BS, McCarthy KD. 2015. Astrocyte calcium signaling: from observations to functions and the challenges therein. *Cold Spring Harb. Perspect. Biol.* 7(4):a020404

Khakh BS, Sofroniew MV. 2015. Diversity of astrocyte functions and phenotypes in neural circuits. *Nat. Neurosci.* 18(7):942–52

Krause M, Harrison SW, Xu S, Chen L, Fire A. 1994. Elements regulating cell- and stage-specific expression of the *C. elegans* MyoD family homolog *hlh-1. Dev. Biol.* 166:133–48

Leboeuf B, Correa P, Jee C, García LR. 2014. *Caenorhabditis elegans* male sensory-motor neurons and dopaminergic support cells couple ejaculation and post-ejaculatory behaviors. *eLife* 3:e02938

Leiserson WM, Forbush B, Keshishian H. 2011. *Drosophila* glia use a conserved cotransporter mechanism to regulate extracellular volume. *Glia* 59(2):320–32

Lints R, Emmons SW. 1999. Patterning of dopaminergic neurotransmitter identity among *Caenorhabditis elegans* ray sensory neurons by a TGFbeta family signaling pathway and a Hox gene. *Development* 126:5819–31

Lois C, García-Verdugo J-M, Alvarez-Buylla A. 1996. Chain migration of neuronal precursors. *Science* 271(5251):978–81

Mano I, Straud S, Driscoll M. 2007. *Caenorhabditis elegans* glutamate transporters influence synaptic function and behavior at sites distant from the synapse. *J. Biol. Chem.* 282(47):34412–19

McLachlan IG, Beets I, de Bono M, Heiman MG. 2018. A neuronal MAP kinase constrains growth of a *Caenorhabditis elegans* sensory dendrite throughout the life of the organism. *PLOS Genet.* 14:e1007435

McQuary PR, Liao CY, Chang JT, Kumsta C, She X, et al. 2016. *C. elegans* S6K mutants require a creatine-kinase-like effector for lifespan extension. *Cell Rep.* 14(9):2059–67

Medina I, Friedel P, Rivera C, Kahle KT, Kourdougli N, et al. 2014. Current view on the functional regulation of the neuronal K^+-Cl^- cotransporter KCC2. *Front. Cell. Neurosci.* 8:27

Meng L, Zhang A, Jin Y, Yan D. 2016. Regulation of neuronal axon specification by glia-neuron gap junctions in *C. elegans. eLife* 5:e19510

Murai KK, Nguyen LN, Irie F, Yamaguchi Y, Pasquale EB. 2003. Control of hippocampal dendritic spine morphology through ephrin-A3/EphA4 signaling. *Nat. Neurosci.* 6(2):153–60

Murakami S, Kurachi Y. 2016. Mechanisms of astrocytic K^+ clearance and swelling under high extracellular K^+ concentrations. *J. Physiol. Sci.* 66(2):127–42

Nath RD, Chow ES, Wang H, Schwarz EM, Sternberg PW. 2016. *C. elegans* stress-induced sleep emerges from the collective action of multiple neuropeptides. *Curr. Biol.* 26(18):2446–55

Nedergaard M, Verkhratsky A. 2012. Artifact versus reality—how astrocytes contribute to synaptic events. *Glia* 60(7):1013–23

Nichols ALA, Eichler T, Latham R, Zimmer M. 2017. A global brain state underlies *C. elegans* sleep behavior. *Science* 356(6344):eaam6851

Oikonomou G, Perens EA, Lu Y, Shaham S. 2012. Some, but not all, retromer components promote morphogenesis of *C. elegans* sensory compartments. *Dev. Biol.* 362(1):42–49

Oikonomou G, Perens EA, Lu Y, Watanabe S, Jorgensen EM, Shaham S. 2011. Opposing activities of LIT-1/NLK and DAF-6/patched-related direct sensory compartment morphogenesis in *C. elegans. PLOS Biol.* 9(8):e1001121

Oikonomou G, Shaham S. 2011. The glia of *Caenorhabditis elegans. Glia* 59(9):1253–63

Parpura V, Zorec R, Verkhratsky A. 2016. Astrocytes in physiological aging and Alzheimer's disease. *Neuroscience* 323:170–82

Payne JA, Rivera C, Voipio J, Kaila K. 2003. Cation-chloride co-transporters in neuronal communication, development and trauma. *Trends Neurosci.* 26:199–206

Pelluru D, Konadhode RR, Bhat NR, Shiromani PJ. 2016. Optogenetic stimulation of astrocytes in the posterior hypothalamus increases sleep at night in C57BL/6J mice. *Eur. J. Neurosci.* 43:1298–306

Perens EA, Shaham S. 2005. *C. elegans daf-6* encodes a patched-related protein required for lumen formation. *Dev. Cell.* 8(6):893–906

Perkins LA, Hedgecock EM, Thomson JN, Culotti JG. 1986. Mutant sensory cilia in the nematode *Caenorhabditis elegans*. *Dev. Biol.* 117(2):456–87

Poskanzer KE, Yuste R. 2016. Astrocytes regulate cortical state switching in vivo. *PNAS* 113(19):2675–84

Procko C, Lu Y, Shaham S. 2011. Glia delimit shape changes of sensory neuron receptive endings in *C. elegans*. *Development* 138(7):1371–81

Rakic P. 2003. Elusive radial glial cells: historical and evolutionary perspective. *Glia* 43(1):19–32

Ramón y Cajal S. 1895. Algunas conjeturas sobre el mecanismo anatómico de la ideación, asociación y atención. *Rev. Med. Cirug. Pract.* 36:497–508

Ramón y Cajal S. 1899. *Textura del Sistema Nervioso del Hombre y Delos Vertebrados: Estudios Sobre el Plan Estructural y Composición Histológica de los Centros Nerviosos Adicionados de Consideraciones Fisiológicas Fundadas en los Nuevos Descubrimientos*. Madrid: Imprenta Libr. Nicolás Moya

Ramón y Cajal S. 1984. *The Neuron and the Glial Cell*, transl. J de la Torre, WC Gibson. Springfield, IL: Charles C. Thomas

Rapti G, Li C, Shan A, Lu Y, Shaham S. 2017. Glia initiate brain assembly through noncanonical Chimaerin-Furin axon guidance in *C. elegans*. *Nat. Neurosci.* 20(10):1350–60

Rinke I, Artmann J, Stein V. 2010. ClC-2 voltage-gated channels constitute part of the background conductance and assist chloride extrusion. *J. Neurosci.* 30(13):4776–86

Rousse I, Robitaille R. 2006. Calcium signaling in Schwann cells at synaptic and extra-synaptic sites: active glial modulation of neuronal activity. *Glia* 54(14):691–99

Rusan ZM, Kingsford OA, Tanouye MA. 2014. Modeling glial contributions to seizures and epileptogenesis: cation-chloride cotransporters in *Drosophila melanogaster*. *PLOS ONE* 9(6):e101117

Sammut M, Cook SJ, Nguyen KCQ, Felton T, Hall DH, et al. 2015. Glia-derived neurons are required for sex-specific learning in *C. elegans*. *Nature* 526(7573):385–90

Schummers J, Yu H, Sur M. 2008. Tuned responses of astrocytes and their influence on hemodynamic signals in the visual cortex. *Science* 320:1638–44

Schwarz JM, Bilbo SD. 2012. Hormones and behavior sex, glia, and development: interactions in health and disease. *Horm. Behav.* 62(3):243–53

Shah PK, Santella A, Jacobo A, Siletti K, Hudspeth AJ, et al. 2017. An *in toto* approach to dissecting cellular interactions in complex tissues. *Dev. Cell* 43(4):530–40.e4

Shaham S. 2010. Chemosensory organs as models of neuronal synapses. *Nat. Rev. Neurosci.* 11(3):212–17

Shaham S. 2015. Glial development and function in the nervous system of *Caenorhabditis elegans*. *Cold Spring Harb. Perspect. Biol.* 7(4):a020578

Shao Z, Watanabe S, Christensen R, Jorgensen EM, Colón-Ramos DA. 2013. Synapse location during growth depends on glia location. *Cell* 154(2):337–50

Sidman RL, Rakic P. 1973. Neuronal migration, with special reference to developing human brain: a review. *Brain Res.* 62(1):1–35

Singhal A, Shaham S. 2017. Infrared laser-induced gene expression for tracking development and function of single *C. elegans* embryonic neurons. *Nat. Commun.* 8:14100

Singhvi A, Frank CA, Garriga G. 2008. The T-box gene *tbx-2*, the homeobox gene *egl-5*, and the asymmetric cell division gene *ham-1* specify neural fate in the HSN/PHB lineage. *Genetics* 179(2):887–98

Singhvi A, Liu B, Friedman CJ, Fong J, Lu Y, et al. 2016. A glial K/Cl transporter controls neuronal receptive ending shape by chloride inhibition of an rGC. *Cell* 165(4):936–48

Sulston JE, Albertson D, Thomson JN. 1980. The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. *Dev. Biol.* 78:542–76

Sulston JE, Schierenberg E, White JG, Thomson JN. 1983. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 100(1):64–119

Sultan S, Li L, Moss J, Petrelli F, Casse F, et al. 2015. Synaptic integration of adult-born hippocampal neurons is locally controlled by astrocytes. *Neuron* 88:957–72

Sun YT, Lin TS, Tzeng SF, Delpire E, Shen MR. 2010. Deficiency of electroneutral K$^+$-Cl$^-$ cotransporter 3 causes a disruption in impulse propagation along peripheral nerves. *Glia* 58(13):1544–52

Tatsumi K, Isonishi A, Yamasaki M, Kawabe Y, Morita-Takemura S, et al. 2018. Olig2-lineage astrocytes: a distinct subtype of astrocytes that differs from GFAP astrocytes. *Front. Neuroanat.* 12:8

Taverna E, Götz M, Huttner WB. 2014. The cell biology of neurogenesis: toward an understanding of the development and evolution of the neocortex. *Annu. Rev. Cell Dev. Biol.* 30:465–502

Theodosis DT, Poulain DA, Oliet SHR. 2009. Activity-dependent structural and functional plasticity of astrocyte-neuron interactions. *Physiol. Rev.* 88:983–1008

Tong X, Ao Y, Faas GC, Nwaobi SE, Xu J, et al. 2014. Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in Huntington's disease model mice. *Nat. Neurosci.* 17(5):694–703

Ullian EM, Sapperstein SK, Christopherson KS, Barres BA. 2001. Control of synapse number by glia. *Science* 291(5504):657–61

Varadarajan SG, Kong JH, Phan KD, Kao TJ, Panaitof SC, et al. 2017. Netrin1 produced by neural progenitors, not floor plate cells, is required for axon guidance in the spinal cord. *Neuron* 94(4):790–99.e3

Varshney LR, Chen BL, Paniagua E, Hall DH, Chklovskii DB. 2011. Structural properties of the *Caenorhabditis elegans* neuronal network. *PLOS Comput. Biol.* 7(2):e1001066

Verkhratsky A, Butt AM. 2013. Neuroglia: definition, classification, evolution, numbers, development. In *Glial Physiology and Pathophysiology*, pp. 73–104. Chichester, UK: Wiley-Blackwell

Verkhratsky A, Parpura V, Pekna M, Pekny M, Sofroniew M. 2014. Glia in the pathogenesis of neurodegenerative diseases. *Biochem. Soc. Trans.* 42(5):1291–301

von Bartheld CS, Bahney J, Herculano-Houzel S. 2016. The search for true numbers of neurons and glial cells in the human brain: a review of 150 years of cell counting. *J. Comp. Neurol.* 524(18):3865–95

Wadsworth WG, Bhatt H, Hedgecock EM. 1996. Neuroglia and pioneer neurons express UNC-6 to provide global and local netrin cues for guiding migrations in *C. elegans*. *Neuron* 16:35–46

Wallace SW, Singhvi A, Liang Y, Lu Y, Shaham S. 2016. PROS-1/prospero is a major regulator of the glia-specific secretome controlling sensory-neuron shape and function in *C. elegans*. *Cell Rep.* 15(3):550–62

Wang HC, Lin C, Ellis-Davies G, Bergles DE, Wang HC, et al. 2015. Spontaneous activity of cochlear hair cells triggered by fluid secretion mechanism in adjacent support cells. *Cell* 163:1348–59

Wang W, Perens EA, Oikonomou G, Wallace SW, Lu Y, Shaham S. 2017. IGDB-2, an Ig/FNIII protein, binds the ion channel LGC-34 and controls sensory compartment morphogenesis in *C. elegans*. *Dev. Biol.* 430(1):105–12

Wang Y, Urso GD, Bianchi L. 2012. Knockout of glial channel ACD-1 exacerbates sensory deficits in a *C. elegans* mutant by regulating calcium levels of sensory neurons. *J. Neurophysiol.* 107:148–58

Ward S, Thomson N, White JG, Brenner S. 1975. Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. *J. Comp. Neurol.* 160(3):313–37

Whalley K. 2013. Astroglial regulation of sleep homeostasis. *Curr. Opin. Neurobiol.* 23(5):812–18

Whalley K. 2017. Astrocytes keep time. *Nat. Rev. Neurosci.* 18:264

White JG, Southgate E, Thomson JN, Brenner S. 1986. The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 314(1165):1–340

Wimmers S, Karl MO, Strauss O. 2007. Ion channels in the RPE. *Prog. Retin. Eye Res.* 26:263–301

Yin JA, Gao G, Liu XJ, Hao ZQ, Li K, et al. 2017. Genetic variation in glia-neuron signaling modulates ageing rate. *Nature* 551(7679):198–203

Yoshida A, Nakano S, Suzuki T. 2016. A glial K$^+$/Cl$^-$ cotransporter modifies temperature-evoked dynamics in *Caenorhabditis elegans* sensory neurons. *Genes Brain Behav.* 15:429–40

Yoshimura S, Murray JI, Lu Y, Waterston RH, Shaham S. 2008. *mls-2* and *vab-3* control glia development, *hlh-17*/Olig expression and glia-dependent neurite extension in *C. elegans*. *Development* 135(13):2263–75

Yu X, Taylor AMW, Nagai J, Golshani P, Evans CJ, et al. 2018. Reducing astrocyte calcium signaling in vivo alters striatal microcircuits and causes repetitive behavior. *Neuron* 99:1170–87.e9

Zhang K, Sejnowski TJ. 2000. A universal scaling law between gray matter and white matter of cerebral cortex. *PNAS* 97(10):5621–26

Zuchero JB, Barres BA. 2015. Glia in mammalian development and disease. *Development* 142(22):3805–9