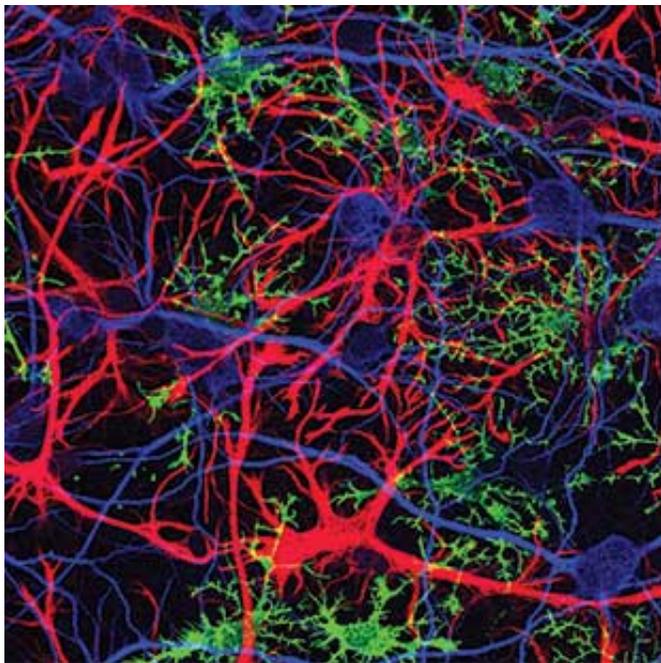


The Scientist » Magazine » Lab Tools

## Into the Limelight

**Glial cells were once considered neurons' supporting actors, but new methods and model organisms are revealing their true importance in brain function.**

By Kate Yandell | October 1, 2015



**GLIAL CELL JUNGLE:** Star-like astrocytes (red) and oligodendrocytes (green) intertwine with neurons (blue) in culture.

JONATHAN COHEN, NATIONAL INSTITUTES OF HEALTH; COURTESY: NATIONAL SCIENCE FOUNDATION

Massachusetts Medical School. "Neurons were the ones that fired signals. . . . Glial cells, when you plug them in, don't do a whole lot."

But attitudes towards glia are finally changing. In recent years, researchers in the field have shown that glia, including astrocytes, oligodendrocytes, and microglia, help form and prune synapses and otherwise actively influence brain structure and

**B**y 1899, nearly 50 years after glia were discovered, Spanish neuroscientist [Santiago Ramón y Cajal](#) recognized that research on these cells was lagging behind studies on their flashier neuronal cousins. "What functional significance may we attribute to the neuroglia?" he wrote in his multivolume book *Texture of the Nervous System of Man and the Vertebrates*. "Unfortunately, in the present state of science it is not possible to answer this important question except through more or less rational conjectures. In the face of this problem, the physiologist is totally disarmed for lack of methods."

Despite making up 50 to 80 percent of the cells of the human brain (estimates vary), glial cells were thought to simply provide structural integrity to the brain and to nourish and mop up after neurons. And it would be the better part of a century before Ramón y Cajal's call for more methods to study glia was answered.

"Glia were not a major point of interest in neuroscience," says [Marc Freeman](#), an investigator with the Howard Hughes Medical Institute (HHMI) who studies fruit fly glia at the University of

**"People are saying neurons are overstudied, and glia**

function. There is also increasing evidence that glial dysfunction can contribute both to neurodegenerative diseases such as Alzheimer's and to neurodevelopmental diseases including autism.

are understudied."

—Ben Barres, Stanford University

"All of a sudden, students are sensing that glia are interesting," says Stanford gliobiologist [Ben Barres](#). "People are saying neurons are overstudied, and glia are understudied."

Here, *The Scientist* profiles an assortment of methods—both adapted from neuronal work and developed specially for glia—that are propelling glial research forward.

## Panning for glia

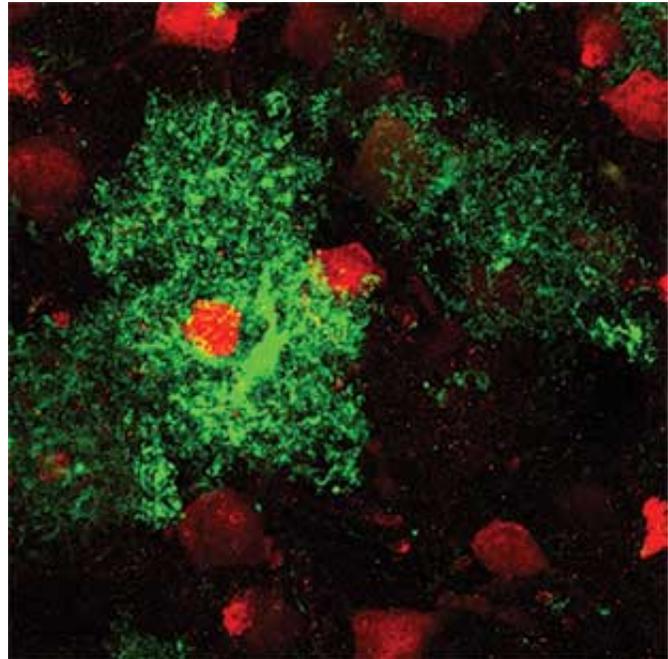
One challenge in studying glia is that the cells have proven difficult to purify and grow in culture. More than two decades ago, Barres began to extract neurons and glia from rodent tissues using a method called immunopanning, in which cells are progressively depleted or harvested from solutions as they bind to selected antibodies stuck to the bottom of petri dishes. Over the years, Barres developed immunopanning antibodies to capture a variety of cells, such as myelin-forming oligodendrocytes, which coat neuronal processes with fatty insulation, as well as astrocytes from the rodent optic nerve. But he and others were unable to purify intact, mature brain astrocytes and maintain them outside the body. Researchers had figured out how to isolate astrocyte precursors from the brains of newborn animals, but the cells lacked normal gene-expression patterns and were flat, not star-shape.

In 2011, Barres and his colleagues finally devised an immunopanning method to extract mature brain astrocytes and developed a protocol to grow the cells in a medium that lacked serum, which contains proteins that are filtered out of blood before it enters the brain. Under these conditions, the astrocytes thrived, retaining their 3-D structure and exhibiting gene-expression patterns characteristic of the cell type in vivo ([Neuron, 71:799-811](#)).

Soon after developing this new immunopanning method, Barres's group demonstrated a new role for murine astrocytes: eating synapses. Astrocytes isolated from mice engulf nerve terminals in culture, and cells missing the phagocytosis-related genes *Megf10* and *Mertk* gobble up fewer neuronal synapses, indicating the genes' importance in synaptic pruning ([Nature, 504:394-400, 2013](#)). "It's been really like taking candy from a baby," says Barres. "As soon as you can purify the cells and culture them, you can start to gene profile them and start to understand what genes are unique, and that gives you clues immediately about their function."

## RNA readouts

Techniques for purifying glia, along with increasingly advanced RNA-sequencing technologies, have aided researchers in ambitious projects to record the transcriptomes of glial cells. Last year, Barres and his colleagues isolated neurons, astrocytes, three types of oligodendrocytes and oligodendrocyte precursors, microglia, and two types of blood vessel-forming cells from mouse brains and published a [database](#) detailing gene expression in the purified cells ([J Neurosci, 34:11929-47, 2014](#)). Barres is working to



STAR STRUCK: The many branches of an astrocyte (green) wrap around neuronal cell bodies (red) and ensheath synapses (not shown) in the cortex of a mouse. Two-way communication between neurons and astrocytes is necessary for normal brain development and function.

BEN BARRES AND LU SUN

publish a similar database of gene expression in human brain cells. "We figured out how to purify human astrocytes from any age: from fetal all the way to 70-year-old human brains," Barres says. "There's just some extraordinary information there."

Glial transcriptomics should help researchers elucidate the roles of different glial subtypes, design better conditional knockout mice, and identify genes that are conserved among mammals, fish, and invertebrate model organisms. And it may not even be necessary to isolate and culture the cells first. [Jim Eberwine](#), a molecular neurobiologist and genomicist at the University of Pennsylvania's Perelman School of Medicine, has developed a new method to sequence RNA from single cells still lodged in their tissue microenvironments (*Nat Methods*, 11:190-96, 2014).

Transcriptome in vivo analysis (TIVA) relies on molecular tags that researchers introduce into live brain tissue. The group can selectively activate the tag in certain cells or regions using light. Once activated, the TIVA tags change conformation, exposing a section that binds the tails of messenger RNA. The researchers then purify and sequence the bound mRNA. (See "Molecular Multitasker," *The Scientist*, April 2014.)

Investigators interested in applying the technique to gliobiology can use microscopy to view how individual astrocyte processes interact with neurons, for instance. Then, using TIVA tags, they can collect RNA from specific astrocyte populations, specific astrocytes, or even from specific astrocyte appendages. "When you homogenize all the cells, they lose their shapes, they lose their interactions," says Eberwine.

Since publishing their original paper, the researchers have gathered transcriptomic data from mouse and human astrocytes.

## Grow your own glia

Some researchers have dispensed with complex methods for isolating glia in their natural context. Instead, they are generating their own glial cells for study. Last May, a Stanford University team led by neuroscientist and stem cell biologist [Sergiu Pasca](#) demonstrated a method for creating spheres of astrocytes and cortical neurons from human induced pluripotent stem cells (*Nat Methods*, 12:671-78, 2015). The neurons in the balls, which reach diameters of 4 to 5 mm, show gene expression patterns that recapitulate neural maturation during human fetal development. "We noticed and were very happy to see that the astrocytes were not flat," says Pasca. "They were these beautiful star-like cells."

The approach allows Pasca and his team to generate such cortical spheroids using cells isolated from people with autism or schizophrenia, which could help researchers study abnormalities in the formation of neuronal connections that are characteristic of these disorders. "There is something unique about the 3-D interaction between the cells. They're very close to each other, and they're interacting much more like they are doing in the brain," says Pasca, who is currently maintaining more than a thousand cortical spheroids from multiple patients in his lab.

## In vivo visions

Researchers are also trying to assess the role of astrocyte signaling in living mice. In 1990, Stanford neuroscientist [Stephen Smith](#) demonstrated that in response to glutamate, astrocytes release calcium in spikes that propagate as waves through networks of astrocytes (*Science*, 247:470-73). "This was a

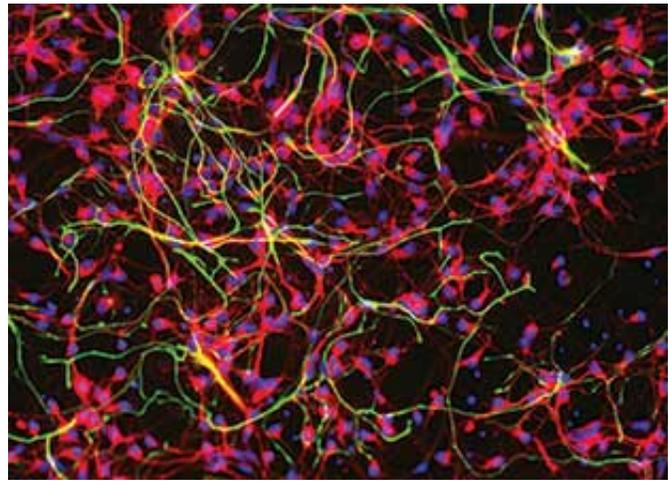


GROW YOUR OWN GLIA: (Top) A 61-day-old human cortical spheroid sits to the right of an intact embryonic mouse brain. At this stage of the spheroid's development, astrocytes are beginning to appear and neurons are forming connections. (Bottom) Glial cells (astrocytes, green) and neurons (red) from a human cortical spheroid that has been enzymatically dissociated and cultured.

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landmark discovery that kind of shook the foundations of neuroscience,” says [Dwight Bergles](#), Smith’s former student, now a neuroscientist at Johns Hopkins University. “People thought that excitability and long-range signaling was the sole province of neurons.”

Since then, scientists have discovered that calcium released by astrocytes can regulate cerebral blood flow, metabolic rates, and structural changes in astrocytes. It can even trigger release of signaling molecules from astrocytes. What’s still unclear is when and why these various glial functions kick in. For this, genetic calcium indicators such as GCaMPs have proven a perfect tool. While GCaMPs were designed to serve as an indirect measure of neuronal firing, they are a direct measure of the calcium activity within cells. “The genetically encoded calcium indicators are just absolutely ideally suited for understanding astrocyte biology,” Bergles says.



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But because calcium indicators were designed for use in neurons, researchers have had to optimize their use for imaging glial activity. For instance, users found that it was difficult to image signaling in astrocytes’ narrow processes, as the GCaMP molecules gathered primarily in the cell body. So Bergles and his colleagues generated mice whose astrocytes tether GCaMP molecules to their cell membranes, making it easier to see when calcium floods the membrane-rich processes.

And, of course, gliobiologists are hard at work generating transgenic mice whose glial cells can be specifically tracked and manipulated. To do this, researchers must identify genes that are uniquely expressed in the subset of glial cells they hope to target, then put a gene for Cre recombinase under the control of the promoters of these glia-specific genes. When this enzyme is expressed, it deletes or inserts genes at sites in the genome that researchers have marked with short identifying sequences.

Researchers have created Cre lines that can be altered specifically in astrocytes, but the lines are not yet perfect. Various progenitor cells are often edited along with astrocytes, Bergles warns. And it is not yet possible to selectively manipulate astrocyte subtypes.

“I think down the line it would be really marvelous for people to develop tools to study heterogeneity among astrocytes [and] heterogeneity among oligodendrocyte progenitor cells,” says the University of Virginia’s [Hui Zong](#), a biologist currently working on developing new tools for overexpressing genes in glial cells. “That will be another big wave of research.”

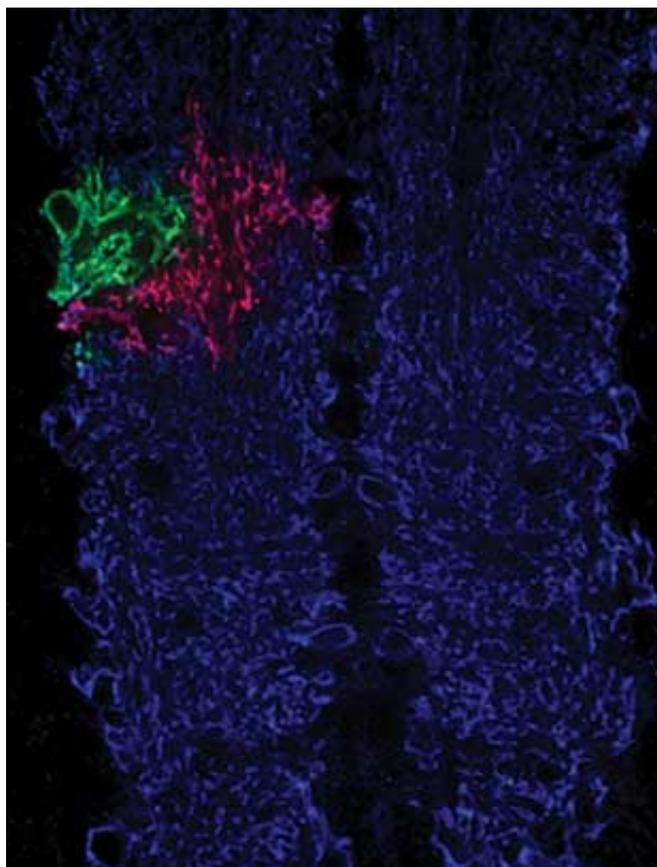
## Exploring other models

Fly biologist Marc Freeman thinks the way to settle some of the basics of gliobiology is to use simpler model organisms. “For whatever reason, the glial field has not embraced invertebrate model organisms like *C. elegans* and *Drosophila* anywhere near to the extent that the people who work on neurons have,” he says.

In the 1990s, some researchers decided that fly glia were too dissimilar from mammalian glia to serve as useful analogs. But after 10 years characterizing subsets of glia, “we are now finally at a point where we have a cell type in the fly that we call an astrocyte,” Freeman says—and these cells are proving to be remarkably similar to mammalian astrocytes. For example, Freeman’s group showed that fly astrocytes engulf synapses, in a publication paralleling Barres’s study showing the same in mice ([Genes Dev, 28:20-33, 2014](#)). “There’s going to be some things that mouse glia do that fly glia don’t,” says Freeman. “But there’s a lot of things that fly glia do that mouse glia do, and I think those things are going to keep us busy for years and years.”

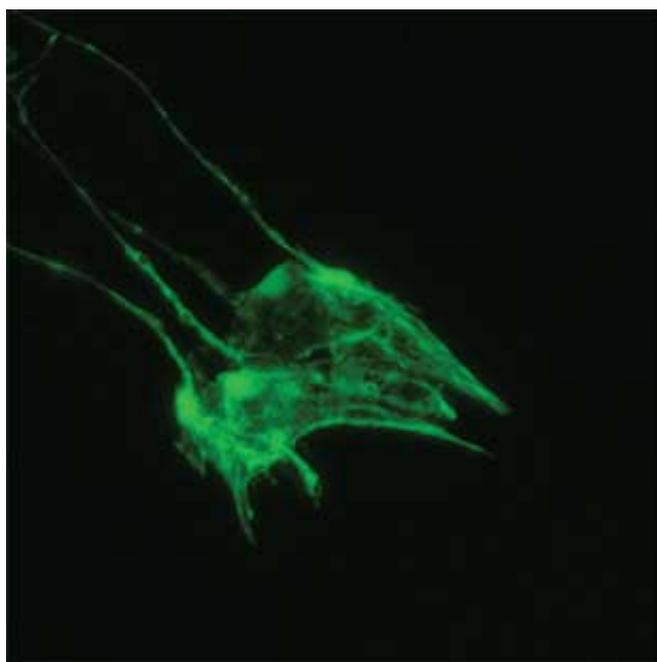
Another organism that might prove beneficial in the study of glial cells is the zebrafish. These animals do not have classical star-shape astrocytes, but their transparent embryos have proven an excellent system for studying how oligodendrocytes myelinate neurons. Developmental neurobiologist [Bruce Appel](#) of the University of Colorado, Denver, for example, made a line of transgenic zebrafish with fluorescently labeled glial cells. “It marked those cells very beautifully,” he says. Earlier this year, Appel and an independent group at the University of Edinburgh used the fish model to show that neuronal signals influence how oligodendrocytes myelinate axons ([Nat Neurosci, 18:683-89, 2015](#); [Nat Neurosci, 18:628-30, 2015](#)).

A third model system that could shed light on glial function is *C. elegans*. The nematode’s glia are less morphologically complex than those of mammals, zebrafish, or flies, but these worms have a unique advantage: researchers can ablate specific glia in the worms without damaging associated neurons. Moreover, the simple nervous system of *C. elegans*—all 302 neurons and 50 glial cells of it—has been comprehensively mapped. Working in *C. elegans*, “we’ve shown there are many levels to the glial influences,” says Rockefeller University’s [Shai Shaham](#). “Glia affect the shapes of neuronal receptive endings [and] the ability to transmit electrical stimuli.”



PRETTY FLY: Two differentially labeled sibling cells (red and green) in a group of *Drosophila* astrocytes (blue) in the brain of a third instar larva. Such labeling shows the size, morphology, and unique placement of each astrocyte in the developing fly brain.

TOBIAS STORK/MARC FREEMAN



WORM POWER: The nematode *C. elegans* is a simple creature with just 50 glia, two of which are shown here in green. These glia have multiple processes reminiscent of those of vertebrate astrocytes and are key to the worms’ development.

GEORGIA RAPTI

“People who are very forward-thinking, they see that worms and flies helped project the field of neuron biology forward by decades, probably, in terms of gene identification and functional characterization,” says Freeman. “If there’s a chance that fly glia could do that [for gliobiology], you have to try, right? And I think it’s going to happen.”

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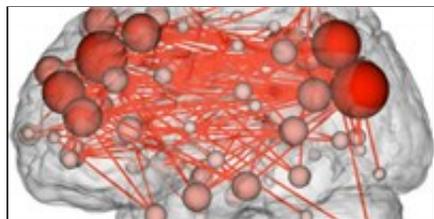
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October 2, 2015

We, and others, have clearly shown that the phenotype of satellite glial cells changes rapidly in the absence of sensory neurons (Tse KH et al (2015) Neuroscience 279:10-22). Within the sensory ganglia, neurons suppress the expression of receptors for inflammatory mediators by satellite glial cells. In the absence of neurons, satellite glial cells become responsive to agents which stimulate CGRP, PGE2 (EP4) and TLR4 receptors. Therefore, it seems probable that studying purified glial cells in the absence of their biological partners may provide misleading information. I look forward to reading about studies using GCaMP-modified glial cells in intact organisms, where neuron-glia interactions are maintained.

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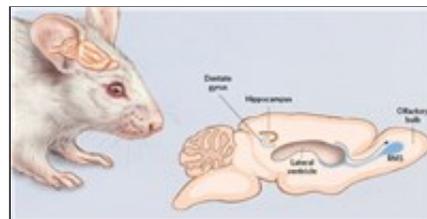
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