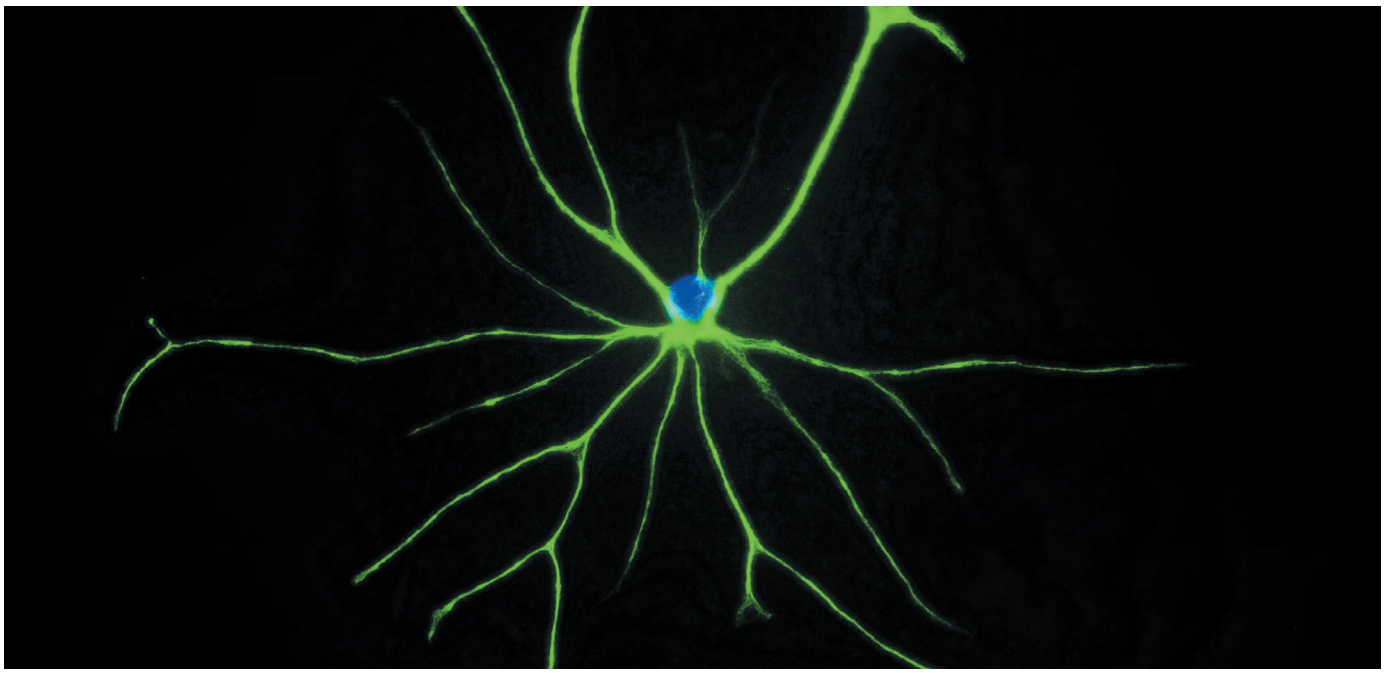
TECHNOLOGY FEATURE

TAPPING INTO THE BRAIN'S STAR POWER

No longer just 'brain glue', astrocytes are coming to the fore as a broadening toolset reveals the cells' complexity and diversity.

RACHEL KIM



An astrocyte from a rat pup that was investigated using a technique known as immunopanning.

BY ESTHER LANDHUIS

In the mid-1850s, German anatomist Rudolf Virchow and others examining brains under the microscope noticed mysterious structures filling the space around and between the neurons that held their interest. Virchow dubbed these structures *nervenkitt*, literally 'nerve-glue', and translated as 'neuroglia'.

Today, researchers know that the *nervenkitt* are mostly astrocytes, representing 20–40% of all the cells in a mammalian brain. Yet for decades, the roles of these plentiful cells remained as mysterious as when Virchow first espied the structures.

Unlike neurons, astrocytes are electrically quiet, so their activity goes undetected by conventional electrophysiology methods. They're also astoundingly complex: a single astrocyte can connect to tens of thousands of neurons.

That's why "there hasn't been a set of tools with which we can probe these cells selectively and reliably throughout the brain", says Baljit Khakh, a neuroscientist at the University of California, Los Angeles (UCLA).

But Khakh and a growing number of researchers worldwide are starting to move past the field's neuron-centric focus to take a closer look at astrocytes. They are developing technologies to classify the cells into distinct subtypes with diverse roles and uncover how astrocytes support and shape neural circuits. The tools might even help researchers to engineer approaches for treating brain diseases.

SPOKES AND SNOWFLAKES

When stained with antibodies and viewed under a microscope, astrocytes resemble bicycle wheels — a big central cell body with half a dozen or so thick spokes radiating out, says Shane Liddelow, a neuroscientist at New York University. But when individual cells are injected with fluorescent dye, more structures become visible. These 'processes' branch off into ever-finer structures, like a 3D snowflake, Khakh says. The snowflakes wrap around every synapse in the brain, and some of the fine processes have bulb-like ends that surround blood vessels, forming a protective zone between brain tissue and vasculature.

Glossed over as mere support cells for more than a century, astrocytes actually have crucial roles in the brain. Researchers are probing these diverse functions with methods for sorting and growing the cells, as well as gene-delivery tools and optical techniques that track how the cells behave in circuits. These technologies are showing that astrocytes adjust their metabolic activities to control neurotransmitter levels, regulate extracellular potassium ions to influence thresholds for nerve-cell firing, and release molecules that promote the formation and pruning back of synapses. Astrocytes are "the major brain homeostat", says Khakh.

For Benjamin Deneen, early clues to that diversity emerged about a decade ago, while he was a postdoc at the California Institute of Technology (Caltech) in Pasadena. He and his co-workers were analysing spinal-cord ▶

tissue from chicks and mice, and found that astrocytes exhibit spatial patterning[1]. That is, regional combinations of DNA-binding regulatory proteins called transcription factors determine when and which astrocyte subtypes are produced.

That got Deneen wondering about heterogeneity in the brain. But, unlike in the spinal cord, where patterning is well defined, each brain area seems to be subject to different rules.

Deneen, who now heads a lab at Baylor College of Medicine in Houston, Texas, turned to fluorescence-activated cell sorting (FACS). Using the technique in mice that had been genetically modified to express green fluorescent protein specifically in astrocytes, his team was able to subdivide the cells into five distinct but overlapping groups on the basis of their expression of three cell-surface proteins[2]. The researchers then used RNA sequencing to identify molecular signatures for each subpopulation.

In a separate study, Khakh and his colleagues isolated astrocytes from distinct brain circuits in the striatum and hippocampus of mice from the same strain. They used methods such as calcium imaging, mass spectrometry, immunohistochemistry, electron microscopy and RNA sequencing to probe cellular function, morphology and molecular features[3].

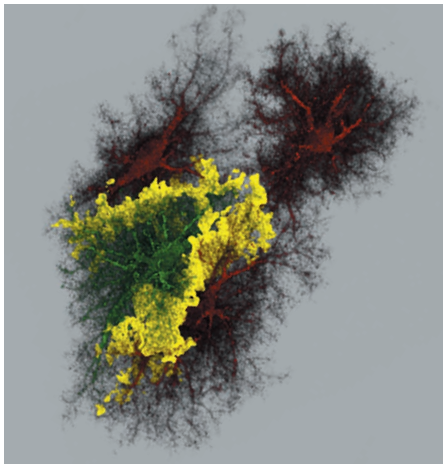
"Astrocytes in different parts of the brain are not the same," Khakh says. For example, in the hippocampus, they nestle closer to excitatory neural synapses and respond more robustly to the neurotransmitter glutamate than do those in the striatum. "This challenges the notion of the 'glue' because it suggests that astrocytes may be brain-area — or even neural-circuit — specific," says Khakh.

For its part, Deneen's group not only collected molecular data from FACS-isolated astrocyte subsets, but also screened for the presence of those cells in mouse models of brain cancer. The team identified populations that correlated with tumour progression and onset of seizures. "These populations could be used as an entry point for understanding diverse or malignant astrocyte identities and properties in disease," says Deneen.

PANNING FOR ASTROCYTES

The fastest, easiest method to grow astrocytes in cell culture was published in 1980 and has been a stalwart of research[4]. "Essentially, you mush up the brain, wait a couple of weeks, grow the cells in serum, shake off the top cells, and the ones left are astrocytes," says Liddelow. The protocol is fast and cheap, requires no special equipment, and produces hundreds of millions of cells.

But there are caveats to this 'MD astrocyte' method — named after its developers, Ken McCarthy at the University of North Carolina School of Medicine at Chapel Hill, and Jean de Vellis at UCLA. One is that when astrocytes grow in serum, they can start behaving quite differently from in the physiological resting state. Plus, Liddelow says, the method generates a fair number of precursor cells, which could be problematic depending on what researchers want to do with the cultures.



Astrocytes have a distinctive 3D branching shape.

In 2011, a protocol known as immunopanning emerged from Ben Barres' lab at Stanford University in California[5]. The procedure first removes unwanted cells through a series of incubations on plates coated with antibodies to antigens that are present on the surfaces of those cells, and then isolates the astrocytes using antibodies that recognize a specific protein marker, known as integrin β-5. "We get serum-free, mature cells in a dish, and less than 1% contamination," says Liddelow, who uses the technique in his lab .

"Astrocytes in different parts of the brain are not the same."

Immunopanning requires about 6 hours hands-on time, compared with 2–3 hours for the MD protocol. But immunopanned cells can be used immediately, whereas the MD method requires another 3–4 weeks. Still, immunopanning is not always the best choice, Liddelow says. Its yield is about an order of magnitude lower than for the MD procedure. It is also more expensive because it uses purified growth factors rather than serum.

Liddelow and his colleagues have used immunopanned cells to identify two astrocyte subpopulations[6]. A1 astrocytes lose their ability to promote synapse formation, whereas A2 astrocytes upregulate neurotrophic factors. Consistent with those features, Liddelow's team found an abundance of A1 astrocytes in Alzheimer's, Huntington's and Parkinson's diseases, and in amyotrophic lateral sclerosis and multiple sclerosis, suggesting that A1 astrocytes contribute to cell death in neurodegenerative disorders.

LAB-GROWN ASTROCYTES

For Henrik Ahlenius, a group leader at Lund University in Sweden, astrocytes provide a handle for modelling Alzheimer's, frontotemporal dementia and other age-related diseases. But his research requires human astrocytes, which are larger and have more synapses than do mouse astrocytes. They are also harder to obtain.

"If you want to study disease progression or mechanisms in chronic brain diseases, it's very tricky to get a hold of the tissue," Ahlenius says. In the rare instances in which his team obtains cells from biopsies or post-mortem tissue, the samples are often hard to work with because they come from end-stage disease. In 2015, researchers in Italy showed how mouse astrocytes could be generated in the lab by culturing fibroblasts with three transcription factors — a process called direct conversion[7]. Ahlenius and his colleagues have since tested those molecules in human embryonic stem cells and found that two of them — NFIB alone or with SOX9 — yielded astrocytes with more than 90% purity in just one week, says Ahlenius[8].

These cells behave just like purified human astrocytes: they can take up and release glutamate; respond to growth factors and support synapses and express similar genes. They can also be used to model Alexander disease, a rare disorder of the nervous system. Ahlenius' team reported its method on *Nature*'s *Protocol Exchange*[8].

STELLAR GENE DELIVERY

To understand how astrocytes behave in both health and disease, researchers need tools with which to introduce genes reliably and specifically. To that end, Viviana Gradinaru's lab at Caltech has engineered vectors based on adeno-associated viruses (AAVs) that can deliver genes to the mouse brain after intravenous injection. These vectors allow researchers to dial up or down the number of cells that get labelled while maintaining high expression[9].

Ultimately, scientists want to study how astrocytes interact with neurons. Earlier this year, Khakh's team described a neuron–astrocyte proximity assay that could determine which of the tens of thousands of nearby synapses the astrocytes actually connected to[10]. "Without knowing what [a cell] can listen to, you're kind of looking around in the dark trying to determine what it might do in that setting," Khakh says.

The assay uses a technique called Förster resonance energy transfer, or FRET, to detect physical contacts between astrocytes and neurons that express fluorescent proteins on their surface. Using this method, and his lab's custom AAVs, Khakh's team examined how astrocytes wire with neural inputs in several diseases. In a mouse model of stroke, astrocytes labelled with these proteins seemed to expand their reach, whereas in animals modelling Huntington's disease, astrocytes retracted their processes and interacted with fewer synapses. Plasmid constructs and viral vectors from both Gradinaru and Khakh are available through the global non-profit plasmid repository, Addgene.

CIRCUIT DESIGN

Kira Poskanzer, a neuroscientist at the University of California, San Francisco, studies yet another aspect of astrocyte biology: how the

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cells respond to external signals. "One big push in our lab is to figure out which astrocytes are listening to which neurotransmitters," she says.

Poskanzer collaborates with analytical chemist Roberto Etchenique at the University of Buenos Aires, to design 'caged' compounds, in which neurotransmitters bind reversibly to chemical groups that restrict their interactions with other molecules in the cell. The compounds are light-sensitive, and will release the caged molecule when zapped with light of a particular wavelength.

The researchers have so far generated photo-activatable forms of the neurotransmitters glutamate, GABA and serotonin[11], and are developing a caged form of noradrenaline. "We're trying to build up an arsenal of tools so that we can 'play god' with the circuit," Poskanzer says — that is, photo-activate various neurotransmitters and watch how astrocytes respond.

Meanwhile, to probe what happens when astrocytes are silenced, Khakh's team has developed a method that uses a molecular pump to move calcium ions from the inside of the cell to the outside and thereby dampen astrocyte calcium signals in the mouse brain[12]. And his team is working with Loren Looger at Howard Hughes Medical Institute's Janelia Research Campus in Ashburn, Virginia, to develop sensors for ATP and other molecules released by astrocytes.

Such tools should help researchers to further tease apart the biology of the *nervenkitt*, and of the interconnected brain as a whole. The technologies are providing an "unparalleled appreciation of the richness and dynamics in how astrocytes contribute to the function of the brain as an organ", Khakh says. ■

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NEUROSCIENCE

Web service makes big data available to neuroscientists

NeuroData allows researchers to explore terabytes of brain images in multiple formats.

BY JEFFREY M. PERKEL

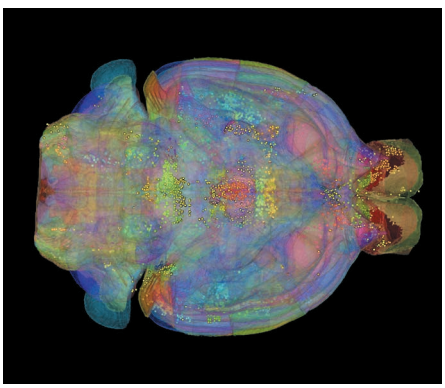
Randal Burns recalls that the brain-science community was "abuzz" in 2011. Burns, a computer scientist at Johns Hopkins University in Baltimore, Maryland, was focusing on astrophysics and fluid dynamics data management at the time. But he was intrigued when Joshua Vogelstein, a neuroscientist and colleague at Johns Hopkins, told him that the first large-scale neural-connectivity data sets had just been collected and asked for his help to present them online.

"It was the first time that you had data of that quality, at that resolution and scale, where you had the sense that you could build a neural map of an interesting portion of the brain," says Burns.

Vogelstein worked with Burns to build a system that would make those data — 20 trillion voxels' worth — available to the larger neuroscience community. The team has now generalized the software to support different classes of imaging data and describes the system this week (J. T. *Vogelstein et al. Nature Meth.* **15**, 846-847; 2018).

NeuroData is a free, cloud-based collection of web services that supports large-scale neuroimaging data, from electron microscopy to magnetic resonance imaging and fluorescence photomicrographs.

Key to its functionality, Vogelstein says, is the spatial database bossDB, which allows researchers to retrieve images of any section of the brain, at any resolution, and in several standard formats. Users can then explore those data using a tool known as Neuroglancer. As they navigate the images, the URL changes to reflect their specific view, allowing them to share particular visualizations with their colleagues. "These links become a core part of the way in which we communicate and pass data back and forth to one another," says Forrest Collman, a neuroscientist at the Allen Institute for Brain Science in Seattle, Washington, and a co-author of the paper.



J. T. VOGELSTEIN *ET AL.*

Neuroimaging data sets contain vast amounts of information.

But data browsing "is not where the science happens", Burns says. "The science happens by doing a statistical analysis of the images." To that end, NeuroData also includes tools for machine learning, image analysis and 3D volume rendering.

More than 100 public and private data sets have been deposited, Vogelstein says. Two of those came from Nelson Spruston, a neuroscientist at the Howard Hughes Medical Institute Janelia Research Campus in Ashburn, Virginia, who assembled 2D images into 3D brain reconstructions that are several terabytes in size. Because it can take weeks to upload that much data, Spruston's team actually gave the information to NeuroData on a physical hard drive — an anachronistic, if practical solution.

Erik Bloss, a neuroscientist in Spruston's laboratory, compiled those data sets to study neural synapses in the hippocampus. But researchers could mine the same data to, for instance, quantify the branching of neural arbors or the density of neural spines, Spruston says. "There's all kinds of information in those data sets that in principle other people might be interested in," he says. ■

CORRECTION

The Toolbox article 'AI tames the scientific literature' (*Nature* **561**, 273–274; 2018) erroneously referred to the CORE repository by its old name, Connecting Repositories.