DEEP DIVE

Portrait of a research field: astrocytes in autism

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Animation by Dave Whyte

Star-shaped cells with mysterious properties: Astrocytes may sound like something out of science fiction, but they are the most abundant type of non-neuronal cell in the brain — and a growing focus for autism researchers over the past 15 years or so.

“Only in recent decades we’ve started to appreciate the role of astrocytes in the nervous system and, in autism research, this followed the same trend,” says **Dilek Colak**, assistant professor of neuroscience at Weill Cornell Medicine of Cornell University in New York City.

The cells are known mainly for supporting roles: helping to maintain **synapses**, contributing to brain metabolism and helping the organ fight infection. Thanks to research on mouse models, human stem cells and post-mortem brains, however, it’s now clear that astrocytes play starring parts in autism and other neurodevelopmental conditions.

“They’re definitely emerging as key players in autism,” says **Cagla Eroglu**, associate professor of cell biology and neurobiology at Duke University in Durham, North Carolina, who studies chemicals astrocytes secrete during development.

Starry cells:

In 1895, the Hungarian neurohistologist Mihály Lenhossék, aka Michael von Lenhossék, coined the term “astrocytes,” meaning “star cells” in Greek, to describe the many-limbed structures he and others saw in human brain tissue under a microscope.

Though plentiful, astrocytes escaped early attention from neuroscientists, who concentrated

instead on neurons. “Historically, obviously, because neurons are electrically excitable cells,” they were easier to detect and record, says **Yongjie Yang**, professor of neuroscience at Tufts University in Boston, Massachusetts. Non-neuronal cells, known as glia, were considered secondary.

Over time, it became apparent that astrocytes help maintain homeostasis in the brain. Their appendages, called processes, have “end-feet” that plaster themselves around blood vessels to prevent molecules from passing through, forming part of the blood-brain barrier. Astrocytes also help scaffold neurons; clean up excess **neurotransmitters**, ions and reactive oxygen species; produce glycogen to feed neurons; and, if there’s an injury in the brain, migrate to the site and surround it with a protective scar-like tissue.

Growth guides: Astrocytes (cytoskeletons shown in magenta and membranes shown in cyan) tell synapses when and how to form, and prune unnecessary ones.

Courtesy of Isabel Salas and Nicola Allen

Scientists long thought of astrocytes as “silent,” Yang says, but now appreciate that they communicate — by changing calcium ion levels via surface ion channels in conjunction with organelles — in response to stimuli.

Astrocytes talk in other ways, too: They, or the chemicals they produce, must be present to guide neuronal growth, telling synapses **when and how to form**, according to a 2005 **study**, and pruning unnecessary ones. Throughout this process, “[astrocytes] are releasing different signals, matching the time of development,” says **Nicola Allen**, associate professor of molecular neurobiology at the Salk Institute in La Jolla, California.

Related papers:

- Thrombospondins are astrocyte-secreted proteins that **promote CNS synaptogenesis** (2005)
- Astrocyte-endothelial interactions at the **blood-brain barrier** (2006)
- The indispensable roles of microglia and astrocytes during **brain development** (2016)

First clues:

Astrocytes’ essential contribution to brain development led some researchers, including **Nurit Ballas**, research professor at Stony Brook University in New York, to wonder whether the cells

were involved in autism. In 2009, some research claimed that only neurons expressed the gene **MECP2**, which is implicated in the autism-linked **Rett Syndrome**. But to Ballas, “it kind of didn’t make much sense why glia would not express MECP2 at all,” she says.

The gene is indeed expressed in glia of mice, including astrocytes, Ballas and her colleagues soon showed. In fact, wild-type neurons grown in culture dishes with astrocytes from mice lacking MECP2 developed fewer, shorter and less elaborate dendrites. On the other hand, culturing neurons from MECP2 mice with wild-type astrocytes allowed dendrites to grow normally. Reinstating MECP2 expression in only the astrocytes of mice lacking MECP2 eased some of the animals’ Rett-like traits and extended their lifespans.

Astrocytes lacking MECP2 grow shorter and less elaborate processes than healthy astrocytes, according to Ballas’ **subsequent work** — providing further evidence that the knock-on effects could be large: Because neurons and astrocytes make physical contact, abnormally-shaped astrocytes can’t support synapses as well, she says.

At the time, the finding was met with skepticism, “as always happens, when you come out with a new finding that was maybe not going along with an old view,” Ballas says. “But I think now there’s no question about it anymore.”

Related papers:

- Non-cell autonomous influence of **MECP2-deficient glia** on neuronal dendritic morphology (2009)
- A role for glia in the progression of **Rett’s syndrome** (2011)

A role in excitation:

Since then, researchers have found evidence that astrocytes contribute to other autism-linked conditions, including **fragile X syndrome**. Mice missing **FMR1**, the responsible gene, in only their astrocytes show **traits reminiscent of fragile X syndrome**, including social difficulties and learning problems, Yang and his colleagues found. The traits seem to stem from decreased astrocyte expression of GLT1, a protein transporter for the chemical messenger glutamate. With GLT1 suppressed, astrocytes don’t clear glutamate from synapses quickly enough, making neurons hyperexcitable.

The mechanism also involves disrupted expression in astrocytes, but not in neurons, of metabotropic glutamate receptor 5 (MGLAR5), which is most active during development, Yang and his colleagues discovered. “It’s really an astrocyte-specific pathway,” he says.

Crucial constructors: Eliminating a type of neuroligin protein in only the astrocytes of mice stunts the formation of synapses.

Courtesy of Maria Pia Rodriguez Salazar / Eroglu Laboratory, Duke University

According to a leading theory, autism involves hyperexcitability in the brain, caused by an imbalance between **excitatory and inhibitory signaling** of neurons. But Yang's findings in astrocytes suggest another culprit: "It's also dysregulation from extracellular glutamate levels that promote excitation as well," he says.

Related papers:

- Astroglial FMRP-dependent translational down-regulation of mGluR5 underlies **glutamate transporter GLT1 dysregulation** in the fragile X mouse. (2013)
- Selective deletion of astroglial FMRP dysregulates glutamate transporter GLT1 and **contributes to fragile X Syndrome** phenotypes in vivo (2016)
- **Astroglial FMRP modulates** synaptic signaling and behavior and phenotypes in FXS mouse model (2020)
- **Astroglial FMRP deficiency** cell-autonomously up-regulates miR-128 and disrupts developmental astroglial mGluR5 signaling (2020)

Stem cell evidence:

The discovery of MECP2's expression in astrocytes "present[ed] a new research direction," says **Qiang Chang**, professor of medical genetics and neurology at the University of Wisconsin at Madison.

When Chang and his team grew wild-type neurons derived from human stem cells together with astrocytes derived from the stem cells of people with Rett syndrome, they saw that the "neurons don't grow as well; they don't make as many connections," Chang says.

Rett astrocytes have unusually high calcium levels in the endoplasmic reticulum and cell body. And when those levels briefly fluctuate, known as transients, it occurs at a higher frequency and amplitude than in wild-type astrocytes.

> "All this time we were looking at neurons, and then suddenly we discovered that maybe the guys that we should look for were the astrocytes." — Patricia Braga

The same phenomenon occurs in astrocytes from MECP2 mutant mice, Chang's team found, and, as a consequence, neural networks in the animals are hyperexcitable — not a surprise, given the frequent seizures seen in people with Rett syndrome, Chang says. "We know there is a neuronal contribution to that phenotype. But what our work revealed was that for the same network excitability problem, there is an astrocyte component."

Astrocytes lacking MECP2 function differently from wild-type astrocytes, too, Chang's team found. They have more transporters for the inhibitory messenger gamma aminobutyric acid (GABA) and thus remove more GABA from synapses. This free-floating GABA contributes to what is called "tonic inhibition": If MECP2 astrocytes mop up too much GABA and inhibit neurons, that could also contribute to the overall hyperexcitability of the brain in people with Rett syndrome.

It isn't just in syndromes such as fragile X and Rett that astrocytes seem to stunt neuronal growth. Neurons derived from non-autistic people's stem cells grown with astrocytes derived from autistic people's **baby teeth** have fewer branches and synapses than usual, a 2017 **study** shows. But when autism-derived neurons grow alongside astrocytes from non-autistic people, the neurons recover their normal shape and ability to form synapses, "which is amazing," says co-lead investigator **Patricia Braga**, professor of genetics and embryology at the University of São Paulo in Brazil.

"All this time we were looking at neurons," **Braga** adds, "and then suddenly we discovered that maybe the guys that we should look for were the astrocytes."

Related papers:

- Mutant astrocytes differentiated from Rett syndrome patients-specific iPSCs have **adverse effects** on wild-type neurons (2014)
- Modeling the **interplay between neurons and astrocytes** in autism using human induced pluripotent stem cells (2018)
- Mechanism and consequence of **abnormal calcium homeostasis** in Rett syndrome astrocytes (2018)
- An astrocytic influence on **impaired tonic inhibition** in hippocampal CA1 pyramidal neurons in a mouse model of Rett syndrome (2020)

Activity regulators: Astrocytes (shown here derived from human stem cells) may contribute to the overall hyperexcitability of the brain in people with Rett syndrome.

Courtesy of Yirui Sun / Wellcome Images

Genetic leads:

Autism-linked genes may hold yet another role in astrocytes. For instance, astrocytes in mice express neuroligins, autism-linked proteins that guide the arrangement of synapses. Eliminating any of three types of neuroligins in only an animal's astrocytes **stunted the cell's growth**; eliminating only one type impaired the formation of synapses, a 2017 **study** showed.

"You can eliminate those proteins only in astrocytes and still impact pretty profoundly neuronal connectivity, again proving a point that glial function, astrocyte function, is very important for constructing a proper brain," says Eroglu, who led the work.

Individual astrocytes and microglia cells in postmortem brain samples from autistic people express 513 genes differently, 26 of them strongly linked to autism, **according** to a 2019 **study**. What's more, **many autism-linked genes are active in glial cells**, and two — **KANK1** and **PLXNB1** — were most highly expressed in astrocytes rather than neurons, another team **reported** in 2021.

"I would love to see that people are getting the message about glial cells," says **Mohammed Uddin**, associate professor of human genetics at Mohammed Bin Rashid University in Dubai, United Arab Emirates, who led the 2021 study. "Otherwise they'll be spending years and years on this one cell [neurons] that might leave out a major portion of the risk factor for autism."

Related papers:

- Astrocytic neuroligins control astrocyte **morphogenesis and synaptogenesis** (2017)
- Single-cell genomics identifies cell type-specific **molecular changes in autism** (2019)
- Single-cell transcriptome identifies molecular subtype of autism spectrum disorder impacted by de novo loss-of-function variants **regulating glial cells** (2021)

Counting stars:

Astrocytes from the frontal cortex of autistic people are also **smaller and have fewer and shorter processes** than the astrocytes of non-autistic people, research shows. And postmortem brain samples from autistic people have heightened levels of glial fibrillary acidic protein (GFAP), a marker of astrocytes, according to a 2005 **study**.

> “There are [fewer] astrocytes, but they are more activated. It points to a chronic inflammation in the brain in autism.” — Verónica Martínez Cerdeño

The findings suggest that **people with autism have more astrocytes** in general, but a 2022 **study** counted fewer of the glial cells in autistic than non-autistic people, although the amount of GFAP each astrocyte produced was higher. “There are [fewer] astrocytes, but they are more activated,” says lead investigator **Verónica Martínez Cerdeño**, professor of pathology and laboratory medicine at the University of California, Davis. “It points to a chronic inflammation in the brain in autism.”

Why people with autism should have fewer astrocytes is unclear, Martínez Cerdeño says. It could be that they’re not generated during the flurry of astrocyte development and neurogenesis in the weeks before birth, or they could be generated and later die off. “We are in the very beginnings of understanding,” she says.

Related papers:

- **Glial fibrillary acidic protein** is elevated in superior frontal, parietal and cerebellar cortices of autistic subjects (2005)
- **Decreased number and increased activation** state of astrocytes in gray and white matter of the prefrontal cortex in autism (2022)

Targets for treatments:

Each new result opens up possible new targets for treatment. For instance, a chemical that blocks GABA transporters mildly improves traits in mice that model Rett syndrome, Chang’s **research shows**. His team is also searching for a compound that might normalize calcium levels in astrocytes and alleviate traits: “We are working to translate these findings into ways that could help the patient, that’s the next step of our work,” he says.

Meanwhile, Braga and her colleagues have noticed another potentially treatable mechanism: Astrocytes from autistic people produce more immune molecules called **cytokines**, and particularly interleukin-6, than do those from controls. Blocking IL-6 improved the growth of synapses in co-cultures of neurons and astrocytes derived from autistic people. “So maybe this is a possible treatment in the future,” she says. Even if neurons are ultimately affected, when it comes to developing treatments, “maybe the astrocytes are the key.”

In April this year, Colak and her colleagues found that wild-type mice who had astrocytes cultured from autistic people implanted into their brains demonstrated repetitive behaviors and memory

problems. And culturing the wild-type neurons with these astrocytes led them to grow fewer protrusions and show less electrical activity.

Calcium chatter: Astrocytes communicate with one another via changing calcium ion levels in response to stimuli.

Courtesy of Yirui Sun / Wellcome Images

Certain characteristics of autism may have a primary cause in astrocytes, not neurons, Colak says. “We transplanted these astrocytes into an intact, healthy environment,” she says. “That shows that [autism] astrocytes initiate or introduce this.”

The culprit seems to be that autism astrocytes release higher amounts of calcium. When the team modulated calcium, the behavioral differences disappeared. “Of course, it’s in the long run, but this study at least shows that astrocytes could be targeted as well,” she says.

Related paper:

- Astrocytes derived from ASD individuals **alter behavior** and destabilize neuronal activity through aberrant Ca^{2+} signaling (2022)

More to discover:

Research on astrocytes and autism is still in its infancy, and astrocytes themselves remain little understood. Scientists don’t fully grasp the chemical signals astrocytes release to regulate neuronal development, Allen says. And several subtypes of astrocytes have different, and as yet poorly studied, characteristics, Colak says.

Another big question for future work is how environment affects astrocytes during development, either in the womb or soon after birth, and thus brain development. “There is definitely an immune component,” Eroglu says. Environmental exposures to a developing fetus, whether infections or even pollution, could have long-term implications for both astrocytes and neurons. “Any immune challenge will shape their trajectory for life,” she says — and potentially lead to overactive astrocytes.

It’s exactly what Braga and her colleagues are trying to discover: Why do astrocytes overproduce cytokines? And is there a cause during development and pregnancy, such as infections? These

days, Braga says, she and her team are more interested in astrocytes than neurons.

“I think I am an astrocyte researcher,” she says with a laugh.

There’s even evidence from evolution that astrocytes could be more crucial to brain function than scientists ever suspected, Eroglu adds. As brain size increases, say, from mice to humans, astrocytes not only increase in number, which would be the case if they were simply support cells for the increased number of neurons — they also become bigger and more complex. That hints at a more important role in making sophisticated forms of cognition possible, she says. “Not paying attention to them and just thinking, ‘Oh, they are support cells,’ is going to be shortsighted,” Eroglu says. “They must be doing something really fundamentally important for the brain to function.”

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