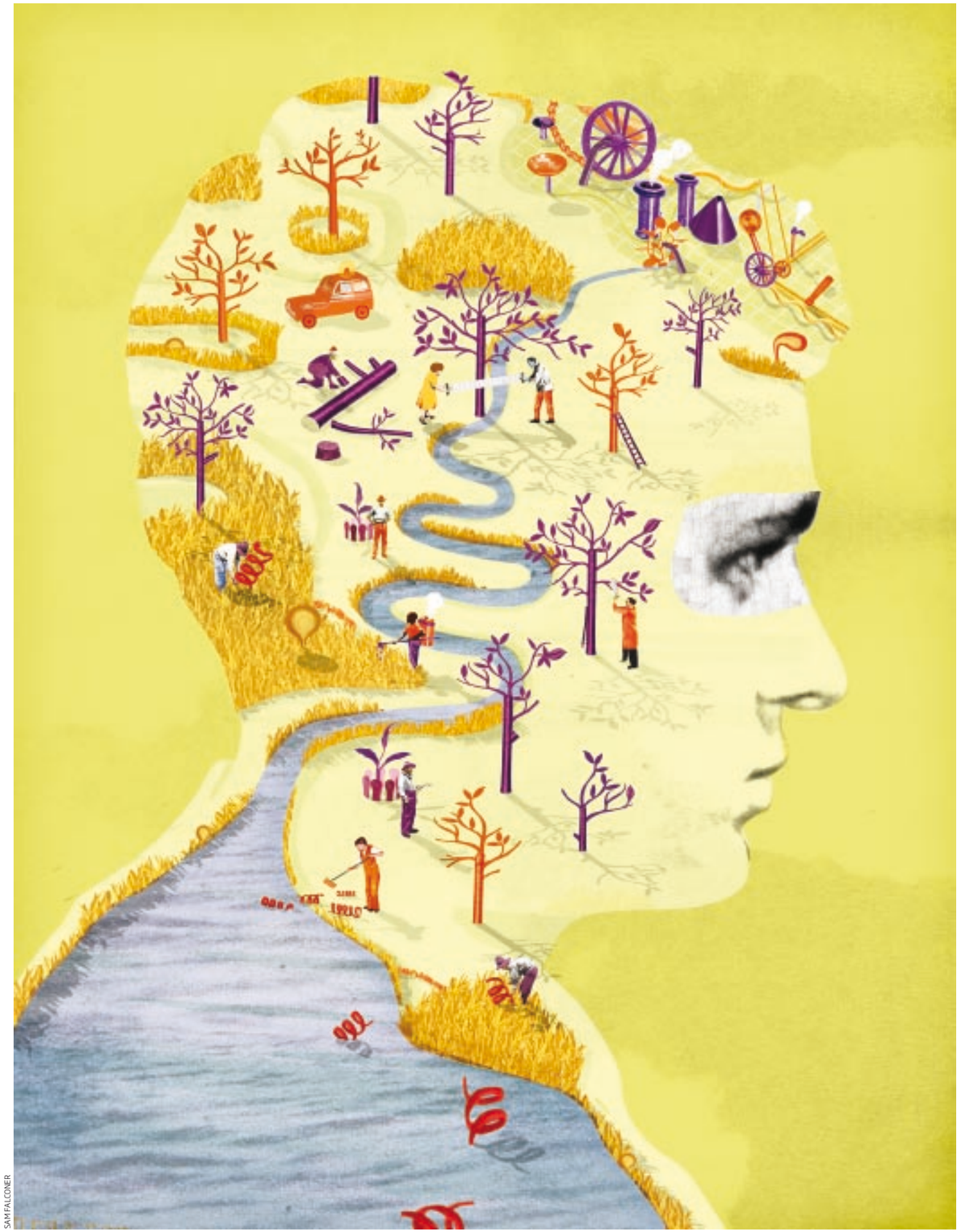


SAM FALCONER

THE MIND MINDERS

A fertile, flexible brain couldn't live without its roving band of caretakers. Moheb Costandi meets the microglia

YOU may not realise it, but your brain is home to an army of invaders. Riding in on blood vessels and nerve fibres during your first months in the womb, these amorphous creatures colonised every part of your brain, where they transformed into a strange, tentacled form, before lying still, waiting like spiders at the centres of their webs.

It sounds sinister, but these shape-shifters, known as microglia, are no cause for alarm. Rather, they are an underappreciated ally. While neurons, sparking with electrical activity, steal the limelight as the makers of thought, we now know that microglia are just as critical for a fertile, flexible mind.

As master multitaskers, microglia play many different roles. On the one hand, they are the brain's emergency workers, swarming to injuries and clearing away the debris to allow healing to begin. On the other hand, during times of rest, they are its gardeners and caretakers, overseeing the growth of new neurons, cultivating new connections and pruning back regions that threaten to overgrow. They may also facilitate learning, by preparing the ground for memories to form.

In this way, microglia empower us with the ability to adapt to life's experiences. Yet their work can backfire, and neuroscientists now believe that microglia will provide insights to neurological conditions such as Alzheimer's disease and autism.

Our first view of these mercurial cells came in the 1840s, when pathologist Rudolph Virchow first noted the presence of microglia near sites of brain injury. As our understanding of the brain's building blocks grew, they were later considered to be part of the mysterious "third element" of the brain. The first element was the neuron; the second was the astrocyte. Astrocytes were thought to pack the spaces around neurons and give them nutrients, but now also seem to be involved in other duties, such as controlling blood flow through the brain (see diagram, page 46).

The third element remained a puzzle, however, until the pioneering work of Pío del Río Hortega in the 1920s and 30s. His stroke of genius was to find dyes that allowed him to stain the cells so that he could view each more clearly under the microscope. He proved that neuroscientists had previously confused the microglia with another kind of cell, muddying the picture of their activity. He then used his microscope to track the microglia's shape-shifting life cycle – including their early migration, and their spidery, dormant state.

According to Río Hortega's observations, it was only in the face of disease that they started to spring into action again, assuming the form of an amoeba to crawl towards a site of injury. Once there, they cleared away damage, devouring microbes and dead neurons to allow healing to set in.

Río Hortega's work would establish microglia as the brain's emergency workers, yet it also contributed to the idea that they are mostly inactive under other circumstances, leading their other duties to be overlooked for the rest of the century. "If there's a disturbance, they will react," says Axel Nimmerjahn, a biophysicist at the Salk Institute for Biological Sciences in La Jolla, California. "The question is: what do they do in the healthy brain? Do they just hang out?"

In some ways, the delay was a matter of technology. "We've been aware of microglia for over 100 years, but it's been very hard to study them because we didn't have the right tools," says neurobiologist Ben Barres of Stanford University in California. But in the 21st century better microscopic techniques have set the stage for a new understanding.

The breakthrough came 10 years ago, while Nimmerjahn was doing his PhD at the Max Planck Institute for Medical Research in Heidelberg, Germany. At the time, his team was more interested in those other mysterious brain cells, astrocytes, which they hoped to mark and identify using a chemical dye. But they were concerned that the dye might also stick to microglia, confounding their results. So they ran a follow-up experiment, injecting the dye into mice that had been genetically engineered so that their microglia glowed green with a fluorescent protein. They then used cutting-edge time-lapse imaging to watch the mice's brains in action.

The films confirmed that their dye only bound to the astrocytes, as intended. But although they were untouched by the dye, ›

it was the glowing microglia that really caught Nimmerjahn's attention. Rather than lying dormant, they were teeming with activity, stretching out their spider-like tentacles, called processes, to stroke the nearby neurons, synapses and blood vessels. "They don't just sit there and wait," he says. "They actively patrol their environment."

He showed the videos to his colleague, Frank Kirchhoff of the University of Saarland in Germany. "I realised immediately that we were looking at something spectacular," says Kirchhoff. "They were constantly moving." The two wrote a paper that is now considered a landmark in the field (*Science*, vol 308, p 1314).

What were the microglia doing? Kirchhoff and Nimmerjahn's findings seemed to show microglia in the midst of some kind of surveillance. Each cell sat in a territory just 80 micrometres across, with its processes patrolling the surroundings during the course of 2 to 4 hours. One possibility was that they were performing regular health checks on the surrounding neurons to detect disease. But clues soon began to emerge that they were looking for something else entirely, starting with the work of Beth Stevens, then a postdoc in Barres's lab at Stanford.

Once again, it was a surprise finding. Stevens was examining the genetic activity of the synapse at the time, and she was puzzled by a gene that coded for complement proteins, a set of molecules normally associated with the immune system. These proteins were known to circulate in the blood and help antibodies and other immune cells to clear away pathogens – but what were they doing at a synapse in the healthy brain? As she delved deeper, she found that these complement proteins were acting as the mark of death for the synapse, attracting nearby microglia that would then rip out and engulf the neural connection.

Brain topiary

This finding put microglia at the heart of the brain's synaptic pruning process. As our brains grow, they form synaptic connections somewhat haphazardly. Later on, particularly during adolescence, the brain prunes back and refines the neural circuits so that they become more efficient at processing information. Far from being dormant bystanders, microglia were shaping and guiding the brain through one of its biggest upheavals.

Such topiary can also bring about the gentler transformations that help us adapt to new situations. One of the best pictures of this process in action comes from Marie-Eve Tremblay, now at Laval University in Quebec, Canada. She reared a group of mice in the dark for a few days and then exposed them to light. All the while, she examined activity in their visual cortices using an electron microscope and a technique called two-photon in vivo imaging.

As expected, she found that many of the neurons were rewiring their connections in the changing conditions, particularly at the dendritic spine structures that form one side of the synapse. When the mice were reared in the dark, the spines seemed to shrivel up, but when the lights came on, the spines recovered and grew larger.

Importantly, Tremblay found that microglia dramatically alter their behaviour in response to these changes. Light deprivation made them associate more closely with shrinking spines. "Microglial processes not only touched these synapses, but actually engulfed them," says Tremblay. In contrast, exposure to light had the opposite effect – the microglia shrank away from the synapse as it grew and strengthened (*PLoS Biology*, vol 8, e1000527). All in all, it appeared that the microglia were closely monitoring their territory to hunt out and prune weaker connections that were not being used so that the brain could refine its circuits and reserve resources.

Exactly how the microglia know which connections are ripe for the chop is a matter of debate. Stevens's work on complement proteins suggests one possibility: the neurons themselves may be using proteins to mark the synapses that are not pulling their weight, ready for the microglia to come in and do the dirty work. But there may be other mechanisms, too. For example, active neurons seem to signal their health by producing a substance called fraktaline, which might warn the microglia to leave its synapses alone.

Whatever the mechanism, it is now clear that microglia are busy tending forests of synapses throughout the brain. "I look at development, adolescence, young adults and aged animals, and I've seen the same phenomenon everywhere," says Tremblay.

And the more they look, the more neuroscientists are finding that the microglia's remit goes far beyond pruning – with many other activities that might each contribute to the brain's adaptability. For instance, besides pruning synapses, microglia cultivate their development, by secreting nutrients called growth factors that promote the sprouting of new neural connections. And once the synapse is formed, they may monitor and tweak the receptors that help pass messages between two neurons. Such changes, dubbed synaptic plasticity, fine-tune the communication across neural networks, and are thought to be a key mechanism for learning. Indeed, Tremblay has found signs of high microglial activity in the hippocampus – a brain region that is central to memory.

Microglia may also watch over the early stages of brain development to make sure it doesn't become overgrown with neurons. Earlier this year, researchers at the University of California, Davis, showed that microglia control the size of the cerebral cortex by regulating the numbers of neural stem cells, which give rise to immature neurons in the developing brain. Examining the brains of rats and macaque monkeys, the researchers found that microglia are concentrated in areas at which neural progenitor cells divide to give rise to young neurons. Activating the microglia significantly reduced the number of cells, whereas deactivating or eliminating them led to a boom (*Journal of Neuroscience*, vol 33, p 4216).

This growing résumé of duties is leading some neuroscientists to wonder what happens when these important cells malfunction, and whether that might help us to understand certain brain disorders. The role of microglia in synaptic pruning, for instance, implicates them in neurodegenerative diseases such as Alzheimer's. In the earliest stages of Alzheimer's, cells begin to die off in the hippocampus. This degeneration spreads throughout the brain, and is accompanied by the widespread loss of synapses.

Alzheimer's involves the deposition of several mutated proteins within and around brain cells, and deposits of one of these proteins, called beta-amyloid, are widely believed to be toxic to neurons. One popular

"During Alzheimer's disease, microglia may become over-excited, stripping away too many synapses"

idea is that microglia might gradually lose their ability to mop up the beta-amyloid deposits.

But their newly discovered role in synaptic pruning suggests they may be involved in another way: perhaps the microglia become over-excited and strip too many synapses, says Barres. For instance, the brain may produce too many of those complement proteins, inadvertently tagging large swathes of synapses for destruction. Along these lines, Barres has found that complement proteins do seem to amass at synapses as we age, while genetic studies have found that a mutation in a gene involved in the tagging process can put people at increased risk of the disease.

A clear verdict on an Alzheimer's connection is a long way off, however. Alexei Verkhratsky at the University of Manchester, UK, is one neurophysiologist who would like to see direct evidence of hyperactive microglia before he will be convinced. But if they are found to be involved, it raises the possibility of new therapies. Barres, for instance, has developed a drug that targets the complement proteins and so could potentially prevent microglia from harmfully engulfing synapses.

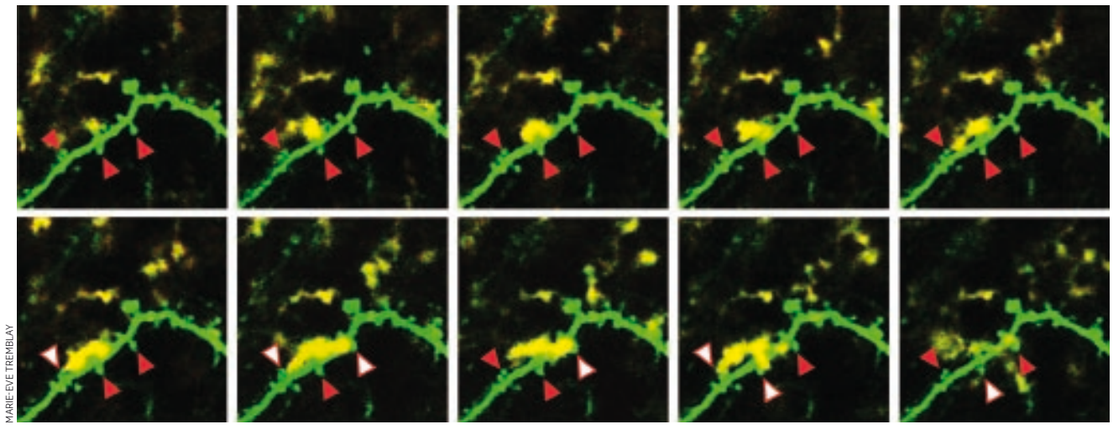
The role of microglia in synaptic pruning also implicates them in autism, which is associated with improper wiring of neuronal circuits during brain development. Intriguingly, the microglia of people with autism do seem to behave differently. They lie closer to their neighbouring neurons and their "tentacles" are more likely to be hugged around the cells, though much more work will be needed to understand whether this interaction contributes to the symptoms of autism. Similarly, some promising leads suggest that microglia are more active in people with depression, potentially changing the chemical signalling in areas of the brain that are thought to regulate our mood.

It is now clear that learning more about the biology of microglia could help us gain a better understanding of both healthy and diseased brains. Among the most pressing questions is how microglia interact with neurons and astrocytes. Barres, Stevens and Tremblay are all working to identify the signals microglia use to communicate.

The revelation that "resting" microglia are anything but dormant also raises the possibility that these shape-shifters may exist in many more forms than we realise. "Activation of microglia is not an all or none process, but rather a continuum which produces many different phenotypes that we still don't know anything about," says Verkhratsky. And that could have implications for the whole of neuroscience. "It's obvious that we have to redefine our approach to the brain. We need to stop dividing it into neurons and glia, because we are dealing with a tissue containing many cell types with distinct functions, which are working together."

Barres agrees that microglia are harbouring many more mysteries. "Who knows what microglia are doing to neurons in terms of synaptic activity?" he says. Indeed, microglia may even play a direct role in the electrical communication that makes thought itself. "We're still at the tip of the iceberg." ■

Moheb Costandi is a science writer in London, who blogs at guardian.co.uk/science/neurophilosophy



MARIE-EVE TREMBLAY

Microglia (yellow) probe neural connections (green) ready for pruning

Three elements of the brain

As neuroscientists in the late 19th and early 20th centuries examined the brain in increasing detail, they identified three distinct building blocks – neurons, astrocytes and a more mysterious "third element"