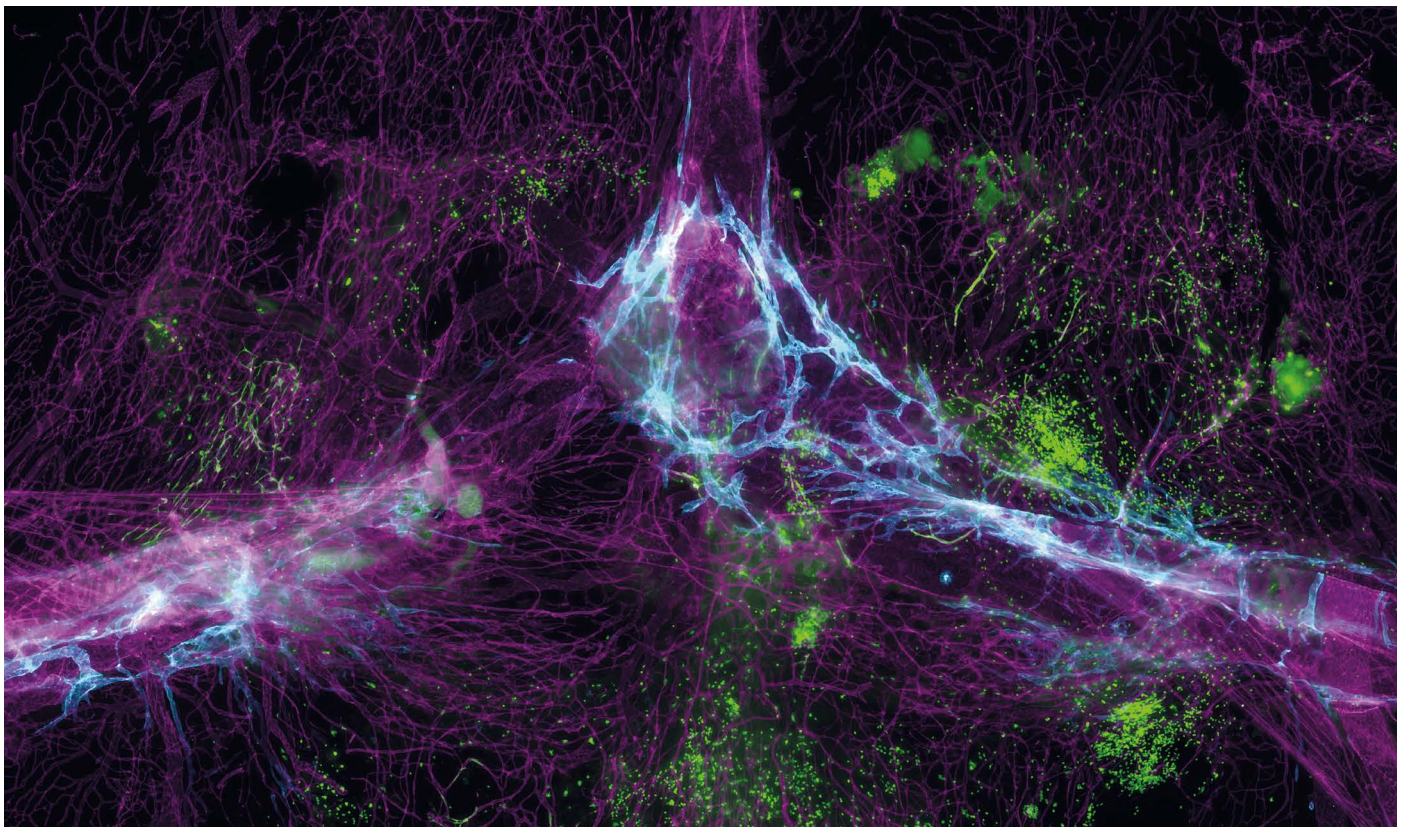


SILING DU, KIPNIS LAB, WASHINGTON UNIV. IN ST. LOUIS

The brain's immune system includes a network of transport vessels (blue) and its own immune cells made in the bone marrow (green).

# GUARDIANS OF THE BRAIN

## The brain's borders teem with an army of immune cells that monitor and protect it.

**By Diana Kwon**

The brain is the body's sovereign, and receives protection in keeping with its high status. Its cells are long-lived and shelter inside a fearsome fortification called the blood–brain barrier. For a long time, scientists thought that the brain was completely cut off from the chaos of the rest of the body — especially its eager defence system, a mass of immune cells that battle infections and whose actions could threaten a ruler caught in the crossfire.

In the past decade, however, scientists have discovered that the job of protecting the brain isn't as straightforward as they thought. They've learnt that its fortifications have gateways and gaps, and that its borders are bustling with active immune cells.

A large body of evidence now shows that the brain and the immune system are tightly intertwined. Scientists already knew that the brain had its own resident immune cells, called microglia; recent discoveries are painting more-detailed pictures of their functions and revealing the characteristics of the other immune warriors housed in the regions around the brain. Some of these cells come from elsewhere in the body; others are produced locally, in the bone marrow of the skull. By studying these immune cells and mapping out how they interact with the brain, researchers are discovering that they play an important part in both healthy and diseased or damaged brains. Interest in the field has exploded: there were fewer than 2,000 papers per year on the subject in 2010, swelling to more than 10,000 per year in 2021, and researchers have made several major findings in the past few years.

No longer do scientists consider the brain to be a special, sealed-off zone. "This whole idea of immune privilege is quite outdated now," says Kiavash Movahedi, a neuroimmunologist at the Free University of Brussels (VUB). Although the brain is still seen as immunologically unique — its barriers prevent immune cells from coming and going at will — it's clear that the brain and immune system constantly interact, he adds (see 'The brain's immune defences').

This shift in attitude is widespread in the community, says Leonardo Tonelli, chief of the neuroendocrinology and neuroimmunology programme at the US National Institute of Mental Health in Bethesda, Maryland. In his experience, almost every neuroscientist who reviews grant proposals for the agency accepts

the connection, he says, although many still need to catch up with the latest discoveries in neuroimmunology, which have started to reveal the underlying mechanisms.

The rush to understand how the brain and immune system knit together has prompted a wealth of questions, says Tony Wyss-Coray, a neuroimmunologist at Stanford University in California. "How important is this in normal brain function or disease? That is a very hard question to answer."

## Privileged space

More than two decades ago, when neuroimmunologist Michal Schwartz had just set up her laboratory at the Weizmann Institute of Science in Rehovot, Israel, she couldn't stop asking herself an unpopular question: could it really be true that the brain is completely cut off from immune protection? "It was completely axiomatic that the brain cannot tolerate any immune activity — everyone thought that if you have any immune activation, this was a sign of pathology," she says. "But it didn't make sense that tissue that is so indispensable, like the brain, cannot enjoy the benefit of being assisted by the immune system."

The idea that the brain was off limits to the immune system took root decades earlier. In the 1920s, the Japanese scientist Y. Shirai reported[1] that when tumour cells were implanted in a rat's body, the immune response destroyed them, but when placed in the brain, they survived — indicating a feeble or absent immune response. Similar findings followed in the 1940s.

Most scientists also thought that the brain lacked a system for ferrying immune molecules in and out — the lymphatic drainage system that exists elsewhere in the body — even though such a system was first described in the brain more than two centuries ago[2]. The prevailing view, then, was that the brain and the immune system lived largely separate lives. The two were thought to collide only under hostile circumstances: when immune cells went rogue, attacking the body's own cells in diseases such as multiple sclerosis.

So when, in the late 1990s, Schwartz and her team reported[3] that after an acute injury to the central nervous system, two types of immune cells, macrophages and T cells, protected neurons from damage and supported their recovery, many scientists were sceptical. "Everyone told me, you're absolutely wrong," Schwartz recalls.

Since those early experiments, Schwartz's team and others have amassed a large body of evidence showing that immune cells do, indeed, have a significant role in the brain, even in the absence of autoimmune disease. Researchers have shown, for example, that in mice engineered to lack an immune system, neurodegenerative diseases such as motor neuron disease (amyotrophic lateral sclerosis) and Alzheimer's disease seemed to progress more rapidly[4], whereas restoring the immune system slowed their progression. Scientists have also revealed a potential role for microglia in Alzheimer's disease.

More recently, scientists have shown that immune cells at the brain's edges are active in neurodegenerative diseases. After examining the cerebrospinal fluid of people with Alzheimer's, Wyss-Coray and his colleagues found evidence of a rise in numbers of T cells in the brain's fluid-filled borders[5]. The expansion of these immune-cell populations suggests that they might have a role in the disease, Wyss-Coray says.

But whether immune cells hurt or help the brain is an open question. In their studies of Alzheimer's and other neurodegenerative disorders, Wyss-Coray and his colleagues suggest that the immune system could be damaging neurons by releasing molecules that boost inflammation and trigger cell death. Others have suggested that T cells and other immune cells could instead be protective. For example, Schwartz's group has reported[6] that in mouse models of Alzheimer's, boosting the immune response leads to a clearance of amyloid plaques — a pathological hallmark of the disease — and improves cognitive performance.

## Busy borders

It's now becoming clear that the brain's margins are immunologically diverse: almost any type of immune cell in the body can also be found in the area surrounding the brain. The meninges — the fluid-filled membranes that wrap the brain — are an "immunological wonderland", says Movahedi, whose work focuses on macrophages in the brain's borders. "There's so much happening out there."

Some residents are exclusive to the frontiers. In 2021, Jonathan Kipnis, a neuroimmunologist at Washington University in St. Louis, Missouri, and his colleagues reported[7] that there is a local source of immune cells: the bone marrow of the skull.

**"We are very far away from understanding what's happening in healthy brains."**

When they explored how the bone marrow mobilizes these cells, Kipnis and his colleagues demonstrated[8] that, in response to an injury to the central nervous system or in the presence of a pathogen, signals carried in the cerebrospinal fluid were delivered to the skull bone marrow, prompting it to produce and release these cells.

What role these locally produced immune cells have remains to be seen, but Kipnis's group thinks that they might have a gentler role than immune cells from elsewhere in the body, regulating the immune response rather than being primed to fight. Kipnis says that this distinction, if true, has implications for treatment. In diseases such as multiple sclerosis, he says, symptoms could perhaps be improved by preventing immune cells from other parts of the body from coming in. By contrast, with a brain tumour, he adds, "you want the fighters".

His team has also detected a network of channels that snake and branch over the surface of the brain, and which swarm with immune cells, forming the brain's own lymphatic system[9]. These vessels, which sit in the outermost part of the meninges, give immune cells a vantage point near the brain from where they can monitor any signs of infection or injury.

## In sickness and in health

As evidence builds for the involvement of immune cells during brain injury and disease, researchers have been exploring their function in healthy brains. "I think the most exciting part of neuroimmunology is that it's relevant to so many different disorders and conditions and to normal physiology," says Beth Stevens, a neuroscientist at Boston Children's Hospital in Massachusetts.

Many groups, including Stevens's, have found microglia to be important to the brain's development. These cells are involved in pruning neuronal connections, and studies suggest that problems in the pruning process might contribute to neurodevelopmental conditions.

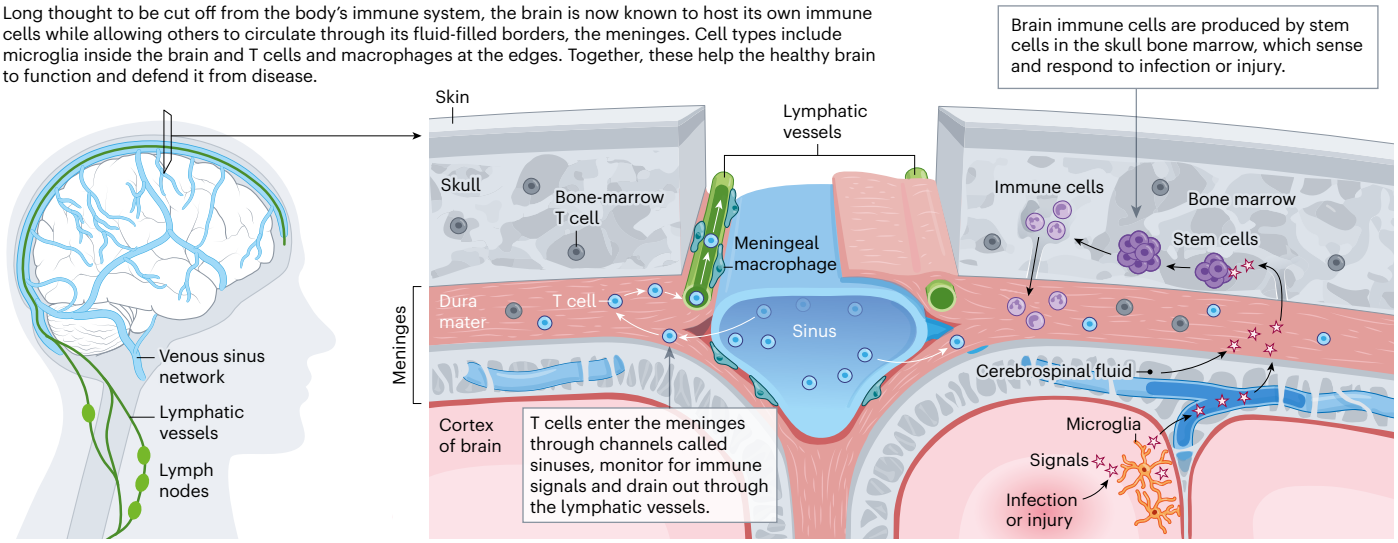
Border immune cells, too, have been shown to be essential in healthy brains. Kipnis, Schwartz and their colleagues, for example, have shown that mice that lack some of these cells display problems in learning and social behaviour[10]. Others reported[11] in 2020 that mice that develop without a specific population of T cells in both the brain and the rest of the body have defective microglia. Their microglia struggle to prune neuronal connections during development, leading to excessive numbers of synapses and abnormal behaviour. The authors propose that during this crucial period, T cells migrate into the brain and help microglia to mature.

One big mystery is how exactly immune cells — particularly those around the borders — talk to the brain. Although there is some evidence that they might occasionally cross into the organ, most studies so far suggest that these cells communicate by sending in molecular messengers known as cytokines. These, in turn, influence behaviour.

Researchers have been studying how cytokines affect behaviour for decades, finding, for example, that cytokines sent out by immune cells during infection can initiate 'sickness behaviours' such as increased sleep[12]. They have also shown in animal models that alterations in cytokines — induced by depleting them throughout the body or knocking out specific cytokine receptors on neurons — can lead to alterations in memory, learning and

# THE BRAIN'S IMMUNE DEFENCES

Long thought to be cut off from the body's immune system, the brain is now known to host its own immune cells while allowing others to circulate through its fluid-filled borders, the meninges. Cell types include microglia inside the brain and T cells and macrophages at the edges. Together, these help the healthy brain to function and defend it from disease.

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social behaviours[13]. How cytokines travel into the brain and exert their effects remains an area of active study.

Cytokines might also be a link between the immune system and neurodevelopmental conditions such as autism. When Gloria Choi, a neuroimmunologist at the Massachusetts Institute of Technology in Cambridge, and her colleagues boosted cytokine levels in pregnant mice, they saw brain changes and autism-like behaviours in the offspring[14].

Although these insights are tantalizing, much of the work on how immune cells, especially those in the borders, operate in the brain is still in its infancy. "We are very far away from understanding what's happening in healthy brains," Kipnis says.

## A two-way street

Communication between the immune system and the brain also seems to go in the other direction: the brain can direct the immune system.

Some of these insights are decades old. In the 1970s, scientists conditioned rats to become immunosuppressed when they tasted saccharin, an artificial sweetener, by pairing it with an immunosuppressive drug for several days[15].

In more recent work, Asya Rolls, a neuroimmunologist at Technion – Israel Institute of Technology in Haifa, and her team explored the link between emotion, immunity and cancer in mice. They reported[16] in 2018 that activating neurons in the ventral tegmental area, a brain region involved in positive emotions and motivation, boosted the immune response and, in turn, slowed tumour growth.

Then, in 2021, her group pinpointed neurons in the insular cortex – a part of the brain involved in processing emotion and bodily sensations, among other things – that were active during inflammation in the colon, a condition also known as colitis.

By activating these neurons artificially, the researchers were able to reawaken the intestinal immune response[17]. Just as Pavlov's dogs learnt to associate the sound of a bell with food, causing the animals to salivate any time they heard the noise, these rodents' neurons had captured a 'memory' of the immunological response that could be rebooted. "This showed that there is very intense crosstalk between neurons and immune cells," says Movahedi, who wasn't involved with this work.

Rolls suspects that organisms evolved such immunological 'memories' because they are advantageous, gearing up the immune system in situations when the body might meet pathogens. She adds that in certain cases, they can instead be maladaptive – when the body anticipates an infection and mounts an unnecessary immune response, causing collateral damage. This pathway might help to explain how psychological states can influence the immune response, providing a potential mechanism for many psychosomatic disorders, according to Rolls.

It could also inspire therapies. Rolls and her team found that blocking the activity of those inflammation-associated neurons lessened inflammation in mice with colitis. Her group hopes to translate these findings to humans, and is examining whether inhibiting activity using non-invasive brain stimulation can help to alleviate symptoms in people with Crohn's disease and psoriasis – disorders that are mediated by the immune system. This work is in the early phases, Rolls says, "but it'll be really cool if it works".

Other groups are exploring how the brain controls the immune system. Choi's team is tracing out the specific neurons and circuits that modulate the immune response. One day, she hopes to be able to generate a comprehensive map of the interactions between the brain and immune system, outlining the cells, circuits and molecular messengers responsible for the communication in both directions – and connecting those to behavioural or physiological readouts.

One of the biggest challenges now is to tease apart which populations of cells are involved in these myriad functions. To tackle it, some researchers have been probing how these cells differ at the molecular level, by sequencing genes in single cells. This has revealed a subset of microglia associated with neurodegenerative disease, for example. Understanding how these microglia function differently from their healthy counterparts will be useful in developing treatments, Stevens says. They could also be used as markers to track the progression of a disease or the efficacy of therapies, she adds.

Researchers have already begun using these insights into the immune ecosystem in and around the brain. Schwartz's team, for example, is rejuvenating the immune system in the hope of fighting Alzheimer's disease. This work has opened up new avenues for therapeutics, particularly for neurodegenerative conditions, Schwartz says. "It's an exciting time in the history of brain research."

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