Thought control

The brain isn't supposed to need an immune system. So what are some of its key players doing there, asks **Bijal Trivedi**

THROUGHOUT most of the body our immune system reigns supreme. Molecular whistle-blowers seek out bacteria, viruses and sickly cells and tag them for destruction. Trigger-happy demolition crews patrol the area killing invaders and infected cells, then efficiently clean up the mess. They are a crack team that takes no prisoners. Even so, there is one place where the immune system is not invited: the brain, or so we've been led to believe.

With 100 billion neurons linked via a quadrillion connections, the brain has always been considered too delicate – and too important – to be subjected to the destructive power of a molecular search-and-destroy team. Even a small mistake could damage or destroy the essential wiring that keeps us alive. Instead, the brain is protected by the blood-brain barrier, a highly selective filtration system which keeps out invaders and the army of patrolling white blood cells.

Now, though, this view is changing. In the past decade key immune molecules have been discovered in the brain. And researchers working out exactly what they are doing there are making some surprising discoveries. The brain has taken potentially destructive molecules and put them to work as architects, fine-tuning networks of neurons and removing any unwanted connections. Now they believe these same molecules, when the system fails, might be involved in conditions as diverse as Alzheimer's, glaucoma, schizophrenia and autism.

The brain's connections, the synapses, can potentially link each neuron to thousands of others, forming complex electrical pathways that control the body and behaviour. By far the most important changes to our brain's wiring happens in key stages of development – in the uterus as the brain wires up, in childhood as we grow and learn, and again during puberty as the brain prepares for adulthood. Each of these growth spurts is followed by a period of pruning, where unnecessary connections are cut back. In adulthood this process continues, with the brain constantly updating its connections, adding branches as we learn a skill or make a new memory, and cutting back those that it doesn't need. Without this constant trimming the brain risks becoming confused by competing information and crossed wires.

The immune brain

The first hint that the immune system was involved in this process came in 1998 when Carla Shatz, then at the University of California, Berkeley, stumbled across one of the body's key immune markers – the major histocompatibility complex (MHC) – in the brains of fetal cats. MHC Class 1 (MHC1) is a group of genes that encode proteins found on the surface of almost every cell in the body and flags each cell as "self". Any cell displaying the wrong MHC1 proteins – called human leukocyte antigen (HLA) in humans – is deemed foreign, and is tagged for destruction by specialised groups of white blood cells.

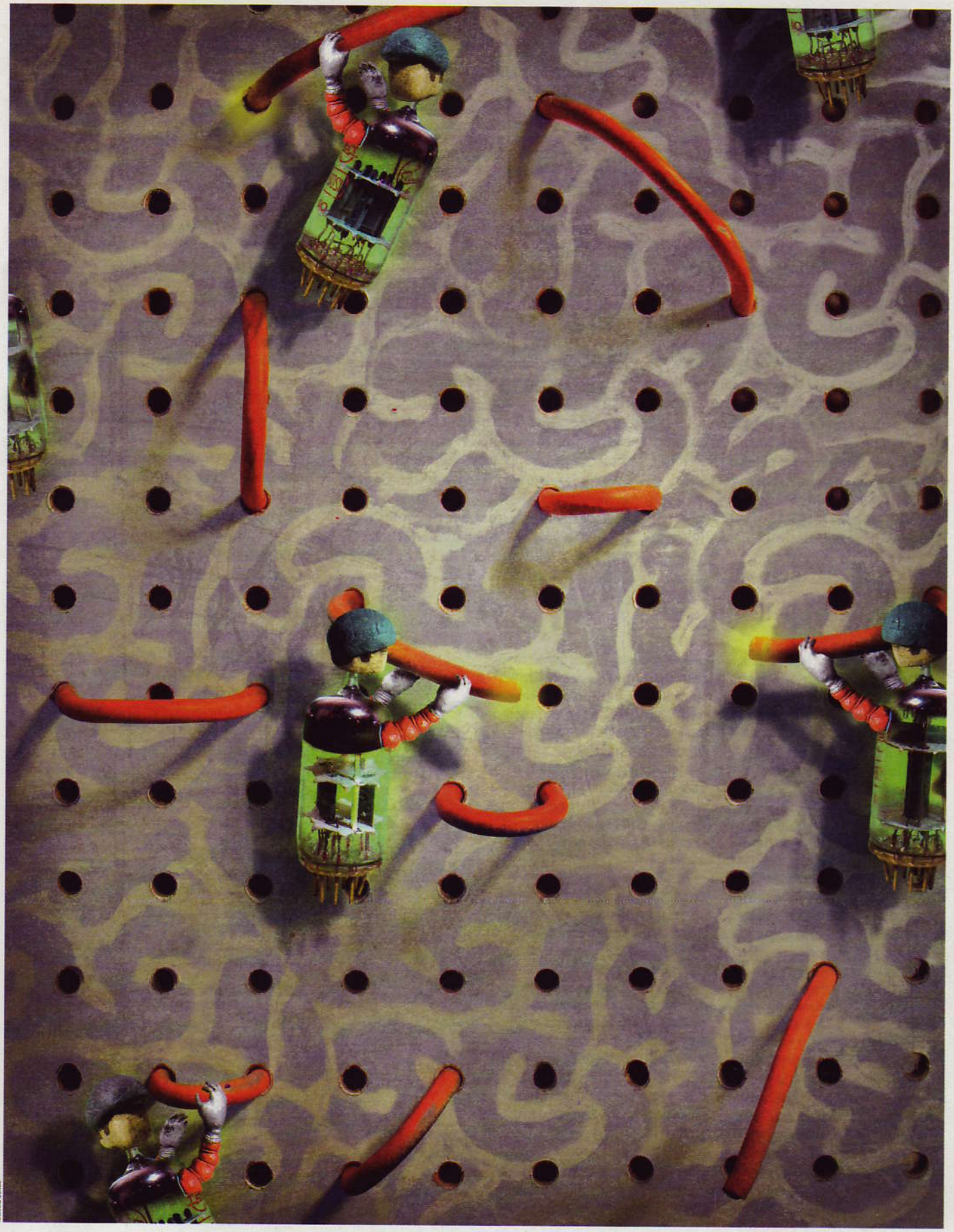
Organ transplants, for example, must have similar MHC markers to the recipient's to prevent rejection. Chunks of brain tissue transplanted into genetically different individuals can survive, however, and since no one had found MHC1 gene expression in the brain, this made perfect sense. After all, without the immune system's circulating sentinels, brain cells would have no need to prove they belonged. Now, though, it seemed that MHC1 had been there all along.

Shatz's discovery came about while she was investigating how the visual system wires up during fetal development and early life. She wanted to know which genes control the organisation of neurons as they grow from the eye to the visual centres of the brain and become organised into distinct zones – one from the right eye and one from the left.

The brain was known to send electrical "test signals" along these new pathways in the uterus, signals that somehow reinforce existing synapses and eliminate those that aren't required. What wasn't clear was which genes control this process. Shatz set about answering this question by stopping electrical signals being sent along the neural pathways in fetal cats, first with drugs to halt electrical activity in the neurons, and later by keeping mice in the dark or closing one eye at birth. She then analysed messenger RNA from brain tissue to discover which genes were turned on or off compared to the controls. To her surprise, when the visual pathway was blocked, MHC1 was one of the gene families most affected, with expression dropping significantly. "[I thought], this is really nuts," says Shatz. "What is this doing in the brain? It's not even supposed to be there."

In follow-up studies Shatz and her team compared normal mouse brains with those of mice lacking MHC1. They found that without MHC1 the wiring in their visual cortex became unruly, compared to the neatly arranged connections of controls. In recent experiments they have knocked out MHC1's partner molecule in the immune system, a protein called PirB, to similar effect.

Shatz now believes that MHC and PirB work together as a molecular brake to stop the neurons making too many connections. "When you lack these molecules the circuits change way too much. You get excessive synaptic plasticity – too many connections and a lack of balance of inputs," she says. ▶



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MHC1 is not the only immune molecule moonlighting in the brain. Ben Barres, a neuroscientist at Stanford University in California, discovered that another, called C1q, was also involved in tweaking connections.

Like Shatz, Barres was intrigued by the formation of the visual system, and keen to understand how some synapses are stabilised to form a permanent part of the adult brain while others are lost. He focused his attention on brain cells called glia – in particular a type of glial cell called an astrocyte. Textbooks dismiss astrocytes as "boring support cells", says Barres, "the glue that holds the neurons together", and credit them with menial tasks like mopping up cell corpses, ions, and spilt neurotransmitters. Barres believed the cells were doing much more. "When you look under the electron microscope, anywhere you see synapses you see glia," he says.

By culturing neurons with and without glial cells, Barres discovered that neurons need astrocytes to build synapses. Without them there was almost no synaptic activity, but when he mixed neurons with astrocytes, or bathed them in liquid where the astrocytes had been, synapse activity exploded 100-fold.

In 2005 Barres reported that astrocytes were secreting a molecule called thrombospondin, which stimulated synapse formation. He later found that the job of pruning fell to C1q, which was secreted by the neurons themselves. The two molecules were working together to make and break connections.

In the rest of the body C1q forms part of the "complement cascade" – a gang of protein molecules that stick to bacteria and other invaders and target them for destruction. In the visual system, though, C1q clustered specifically on synapses. Mice lacking C1q formed too many connections and their visual system failed to develop properly. "We have shown it is required... it is getting rid of the bad synapses," says Barres.

He thinks that this process is analogous to the immune system. In the body C1q tags bacteria to alert white blood cells called macrophages to come in and eat the invaders, like a cellular Pac-Man. In the brain C1q tags synapses for destruction, and they are then engulfed by microglia – the macrophages of the nervous system, Barres believes.

Marc Freeman, a neurobiologist at the University of Massachusetts Medical School in Worchester, says that whether redundant synapses are actually engulfed still needs to be proved, although "it's a good bet". It makes sense that the immune and nervous systems would share some of the same molecules, says Freeman. "C1q is a way to very specifically tag a synapse for removal."

Barres believes that aberrant C1q activity could be involved in neurodegenerative diseases. Working with Simon John, a geneticist at The Jackson Laboratory in Bar Harbor, Maine, Barres is exploring the role of C1q in a mouse model of glaucoma.

In glaucoma, neurons in the retina and the optic nerve – the bundle of nerves carrying images from the retina to the brain – begin to die. In mice C1q levels rise dramatically from almost zero and the molecules cluster on retina early in the disease. As the mouse ages, more and more C1q is produced and more and more synapses are lost. Only late in the disease does this lead to the death of neurons.

Make or break

In people with glaucoma, says Barres, neurons only die when they have lost most of their synapses. Others such as Eliezer Masliah and Robert Terry, molecular pathologists at the University of California, San Diego, have shown that Alzheimer's also results in massive synapse loss. It is estimated that when a person first visits their neurologist with the earliest detectable form of dementia, they have already lost 60 per cent of their synapses in the parts of their brain involved in memory. "That's interesting because in Alzheimer's C1q goes through the roof," says Barres.

This raises the possibility of blocking the C1q cascade to prevent the death of neurons. It might not cure Alzheimer's or glaucoma, but such a treatment could potentially

Spine mending

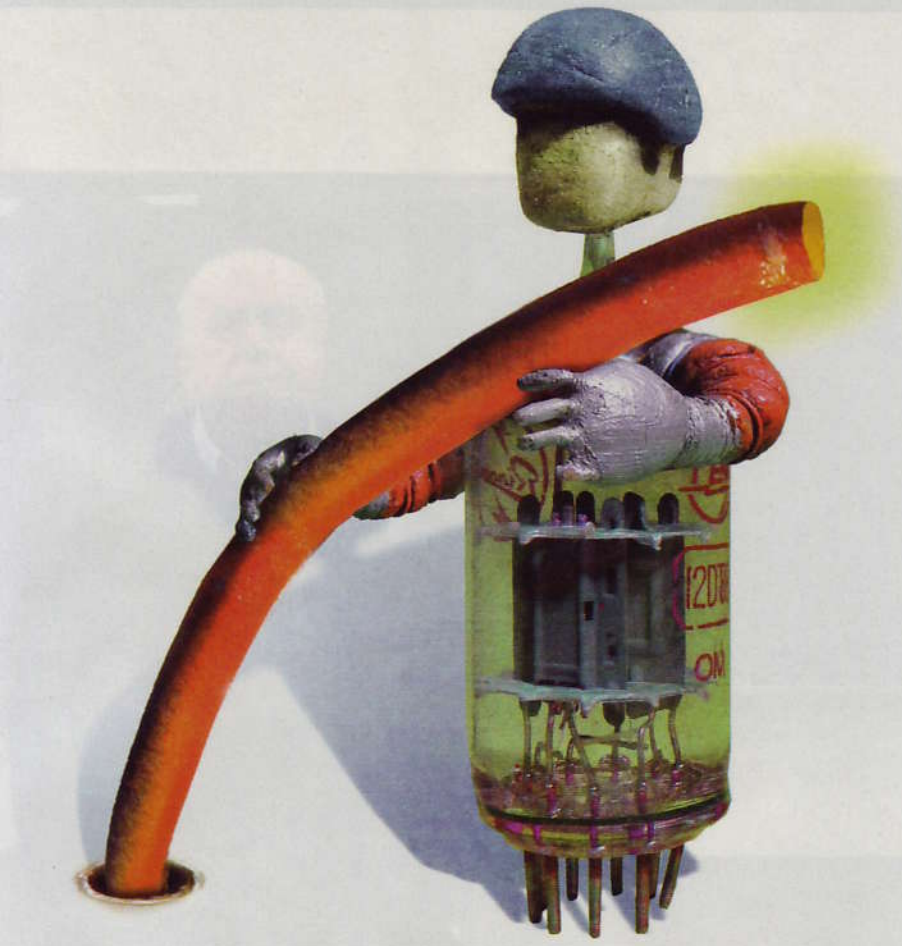
Molecules found in the immune system are turning up unexpectedly, not just in the brain, but in other parts of the nervous system where they don't belong.

Like the brain, motor neurons, which link the spinal cord to the muscles, are exempt from the patrolling white blood cells of the immune system so have no need to tag themselves with immune proteins as "self". But researchers have found that one of these proteins, the major histocompatibility complex 1 (MHC1) is not only expressed in motor neurons, it plays a role in mending damaged nerves. The finding could help efforts to heal spinal cord injuries.

When Staffan Cullheim, a neuroscientist at the Karolinska Institute in Stockholm, Sweden, discovered MHC1 in motor neurons back in 1998, it came as a complete surprise. Since then he and his team have been working out exactly how MHC1 helps repair damaged nerves. After several years of experiments in mice, Cullheim believes that MHC1 helps the neuron to repair by breaking synaptic connections between the neuron and muscle fibres. This strategy, known as synaptic stripping, allows the cell to divert energy from communication to repair. But breaking all connections would make it difficult for the neuron to resume communication with the muscles once it was repaired, so MHC1 breaks only synapses that excite the neuron, while retaining inhibitory ones, which prevent the neuron firing. This ensures the neuron remains connected, but is kept quiet enough to allow recovery.

In recent experiments they cut motor neurons in the hind limbs of mice with variable levels of MHC1. Sure enough, the damaged axon showed greater regrowth in animals with higher levels of MHC1.

While Cullheim's experiments concentrated on peripheral motor neurons, he points out that experiments by other researchers have shown that synaptic stripping also occurs as spinal injuries attempt to heal. Stimulating MHC1 alone may not be enough to repair a broken spinal cord, says Cullheim, but it could be combined with other approaches to provide an environment which makes it as easy as possible for spinal neurons to regenerate.



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"This is really nuts... what's this doing in the brain? It's not even supposed to be there"

delay the progression of the disease by reducing the death rate of neurons.

So could errors in the MHC1 system also be responsible for disease? Perhaps. Lisa Boulanger, a neuroscientist at the University of California, San Diego, is investigating a possible link between the chances of developing schizophrenia or autism and stimulation of the immune system during fetal development. In genetically susceptible individuals, the risk of a child developing schizophrenia later in life is significantly increased if the mother develops a viral infection during the second trimester of pregnancy. Boulanger wonders whether the explanation could lie in the impact of the mother's immune response on the developing brain of her baby.

"Our hypothesis is that mom's immune system gets stimulated, she releases factors known as cytokines that change MHC expression – and you don't want to change MHC expression during fetal brain development because it is busy building the fetal brain," she says.

A common symptom of both schizophrenia and autism is sensory overload, where the person affected reports feeling bombarded with an overwhelming mixture of sensory information. Could a lack of pruning, due to underactive MHC1 be behind a confused and over-connected brain? In an effort to find out, Boulanger is now looking for behaviours analogous to autism or schizophrenia in mice whose mothers had challenges to their immune system during pregnancy, and in mice with genetically altered levels of MHC1. Initial tests show that MHC1-deficient mice do indeed exhibit symptoms of sensory overload in a diagnostic test called "prepulse inhibition".

This test is also used in humans as a measure of the brain's ability to filter out overwhelming sensory information. In the test, a loud tone causes people to startle and flinch, but playing a soft tone 50 milliseconds before the loud one reduces the startle response. This is a sign that the brain is filtering out information coming in too fast to process – a normal brain can't handle it so the second tone is filtered out and not heard. But some people with schizophrenia and autism lack the ability to filter, so startle just as much on hearing two tones as one. In Boulanger's tests, mice without MHC1 startle in much the same way as people with schizophrenia and autism. They also have a similar imbalance in the levels of excitatory neurotransmitters.

She admits that so far "all of these things are just correlated in a very interesting way" and more experiments are required to discover whether there really are changes in the MHC in the brains of people who develop schizophrenia. To do this she is using tissue samples from brain banks and is collaborating with local physicians to test whether levels of secreted MHC might be altered in the blood or urine. That could potentially pave the way for a diagnostic test for autism or schizophrenia. "It's a long shot," she admits. Schizophrenia symptoms may not manifest themselves until 20 years after an initial insult, perhaps during gestation. "It is like if someone tripped and then 20 years later you tried to figure out why their nose was broken... the clues just might not be there any more."

MHC1 may also shed light on the causes of cognitive deficits and diseases of normal ageing. MHC1 is expressed in the brain throughout life, says Shatz. If the relative amount of MHC1 increases, then this imbalance could lead to overpruning. In the elderly, a relative increase in MHC1 in the hippocampus might be responsible for loss of memory and spatial awareness. "If you could get rid of MHC1 you might be able to facilitate recovery of learning and memory," adds Shatz.

Potential treatments are a long way off, but the idea that the brain is an immune-free zone is beginning to change. "These are very unexpected results and they go against the dogma in the field," says Shatz. "The fields of immunology and neuroscience are so separated that if you talk to immunologists about these ideas, they are likely to say that the neuroscientists are crazy, which is great... This is a wonderful moment in science." ●

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