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Some soldiers are destined to relive the horrors of the battlefield for the rest of their lives.

SHADOWS ON THE MIND

Scientists went searching for the causes of PTSD and came back with an unlikely answer: the immune system

BRETT SZMAJDA reports.

DANIEL* DEPLOYED TO IRAQ AGED 24. He thought he was invincible. His first firefight was intoxicating and Daniel quickly became the sort of squad leader everyone looked up to – unshakeable, the first into the line of fire. The problems began after one of Daniel's squad took a bullet to the face.

A COUPLE OF WEEKS LATER Daniel and his squad were en route to an engagement when panic overwhelmed him: his heart pounded, he couldn't breathe and he was so dizzy he had to brace against the door of the Humvee. Fortunately no one in his squad seemed to notice. Daniel wiped clammy hands down the sides of his pants, forced himself to breathe and focus on the mission ahead.

Three months into his tour of duty, Daniel was acting erratically. On one occasion he opened fire before the order was given; on another he froze. He told combat stress medics that he felt numb, that he was losing his grip on reality. Counselling didn't help. They sent Daniel home.

Daniel's family found that a different person had returned to them. Someone who flew into an explosive rage when a Jehovah's Witness knocked at the door; someone who shrank from crowded streets or malls. Daniel's war had not ended. Cut off in traffic, he was back in the Humvee as it lurched after an explosion. Sleeping next to his wife, he tasted the blood of his fallen comrade. More than anything else, Daniel wanted to forget – but that was the one thing that he seemingly could not do.

TWO MILLION AMERICAN troops have fought in Iraq and Afghanistan since 2001. Various studies estimate that between 8-13% will be diagnosed with post-traumatic stress disorder (PTSD). For most people, a traumatic event will leave them in shock and reliving their terror. In time, however, the memory of the event becomes dissociated from the "life and death" feeling that went with it. But not for people with PTSD. They may be out of danger but any slight trigger pushes the replay button. Instead of getting better with time, they get worse.

After both World Wars, the common term for soldiers' trauma was "shell shock". In the aftermath of the Vietnam War, with huge numbers of veterans unable to readjust to normal life, the term PTSD entered the lexicon. PTSD is not only a huge problem for the military. In the 21st century, the theatre of war has relocated to our streets and coffee shops. And then there are the victims of natural disasters, violent attacks and rape. Women appear to be twice as susceptible to the disorder as men.

So far, predicting who will be smitten by PTSD has proved impossible. Why, for instance, can 10 soldiers in the same combat unit be exposed to the same traumatic events, yet only one develops PTSD?

Studies of those who live with the disorder are starting to give us answers. They point to a crucial dialogue between two parts of the body that were thought not to communicate: the brain and the immune system. That dialogue has now been established – and it provides a new way of understanding PTSD. As Indian neurobiologist Sumantra Chattarji put it, "it's a synergy people always suspected was there. This is an entire paradigm shift."

THE HIPPOCAMPUS in the brain plays an important part in PTSD. Named for its resemblance to a seahorse, it is the brain's librarian – the hippocampus files away short-term experiences into long-term memory. It's also highly vulnerable: it disintegrates in Alzheimer's disease and shrinks in people exposed to chronic stress. Hints that differences in the hippocampus could predispose people to PTSD emerged when Mark Gilbertson and colleagues at Harvard Medical School decided to scan the brains of Vietnam vets suffering from the disorder. Sure enough, they revealed the hippocampus was noticeably smaller in those with PTSD. But the Harvard researchers thought to ask another question. Did those vets who developed PTSD have a smaller hippocampus to begin with? They found 40 veterans with twin brothers who didn't fight in the war. As they published in *Nature Neuroscience* in 2002, it turned out the untraumatised twins had a smaller

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Predicting which soldiers will develop PTSD has so far proved impossible. The disorder affects one in 10 combatants.

hippocampus – like their traumatised brothers. So along with the colour of their eyes, the twins had also inherited an undersized hippocampus – and it seems a predisposition to PTSD.

IT'S A LONG WAY FROM the jungles of Vietnam to the white stone minarets, churches and synagogues of the Jerusalem hills. Nestled in one of the cobble-stoned streets in a leafy garden lies a colonial villa. Tranquil and romantic with its rose-gold limestone walls and green-shuttered windows, it appears to be a world apart from the conflicts of the day – until you read the plaque proclaiming your arrival at the Jerusalem Crisis Intervention Centre.

Jerusalem is far from peaceful. The City of Gold is also Israel's poorest city, a reflection of a demographic mix of ultra-religious, immigrant and Arab communities – and that 40% of the population is under the age of 14. The city also boasts the country's highest rate of stress disorders – often linked to terrorist attacks like those that targeted civilians in bus stops, city squares, pizza parlours and cafes in the early 2000s. That's the reason the children and adolescents of Jerusalem need the intervention centre.

Softly spoken child psychiatrist Esti Galili is the founding director. Daily she engages youngsters from her consulting room where a giant teddy bear and shelves of dolls and toys preside over the sessions. Soft skills are important but Galili is also at the forefront of bringing hard science to bear on the problems of PTSD.

Why are some children at more risk of developing the disorder than others? If there is a physiological basis, perhaps they might better be able to help the children; they might also be able to predict who will go on to develop the disorder.

That's how Galili came to collaborate with Ronen Segman, director of the National Institute of Psychobiology, based close to the intervention centre at Hadassah University Hospital. When questioned about the genetic markers of PTSD, Segman is cautious. "Human reactions are multifaceted and complex," he says, warning against distilling a complex disorder down to the action of a few genes. Nevertheless his studies over the past 10 years

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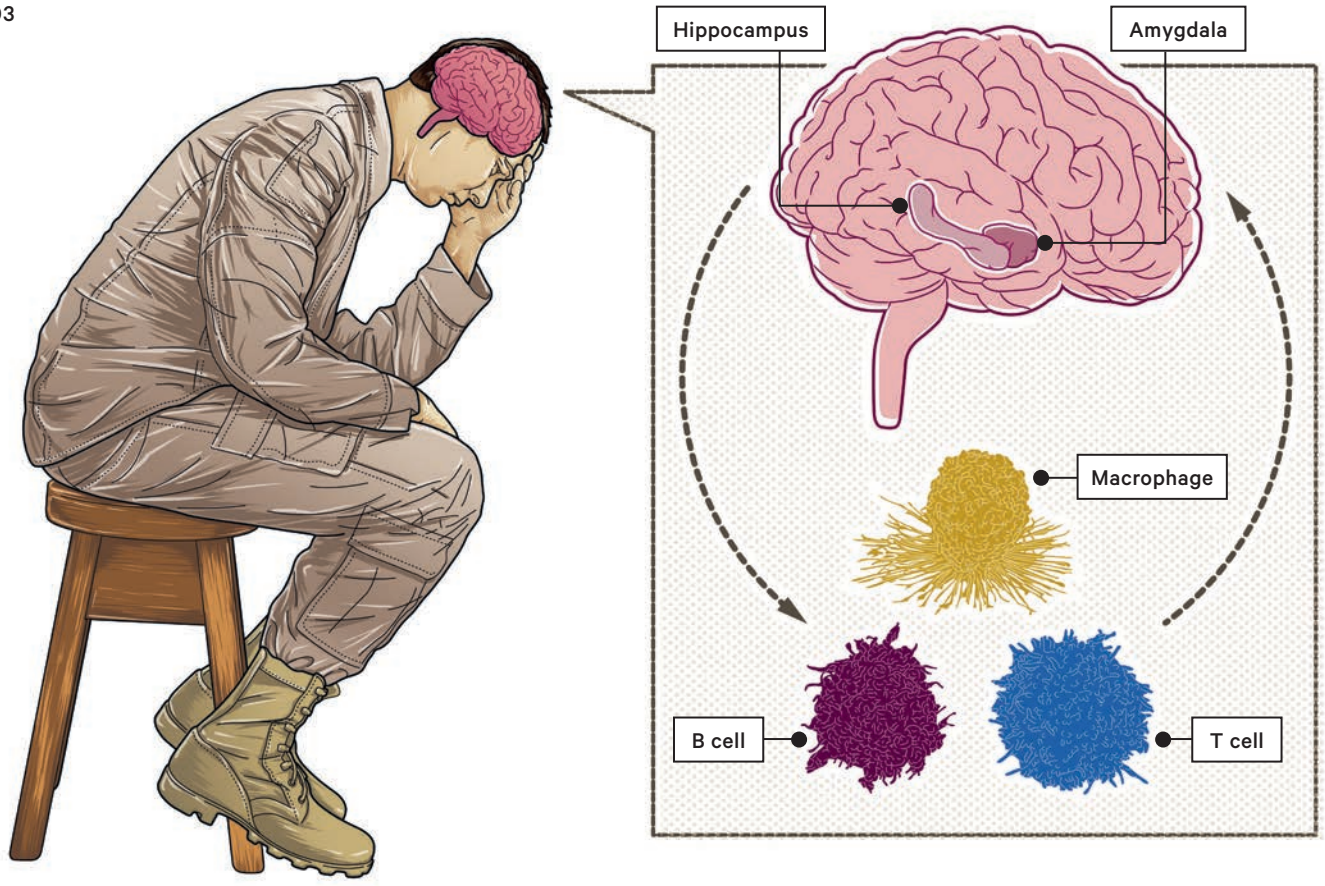

The brain of a person with PTSD changes. In the hippocampus, which plays a role in sorting memories, circuits weaken. In the amygdala, which stores emotional memories, they grow stronger. Contrary to what we once believed, the cells of the immune system play a role in restoring the balance of the brain.

suggest that certain patterns of gene activity might be uniquely tied to PTSD – like a "fingerprint" for the condition.

An obvious place to start looking was the fight-or-flight response. Once aroused by danger, signals travel from the brain to the adrenal glands to release the stress hormones adrenalin and cortisone into the bloodstream. They prepare the body for action: the heart starts thumping and sugar pours into the bloodstream to fuel the muscles. Changes also occur in the brain. The stress starts chipping away at the connections in the hippocampus, while the connections in the amygdala – the part of the brain that stores emotional memories – grow stronger.

To find out if genes in the fight-or-flight pathway were different in PTSD sufferers, Segman and his colleagues recruited 24 people admitted to the emergency ward of Hadassah University Hospital after a traumatic event. One and four months later, the subjects were psychologically evaluated and blood samples taken. The signal strength of thousands of individual genes was gauged by measuring their output of so-called "messenger RNA".

Eight subjects showed symptoms of PTSD. Memories of explosions, dismembered bodies, screams and sirens, refused to fade. But were the emergency sirens inside their cells also blaring, unable to turn off? The results, published in the journal *Molecular Psychiatry* in 2005, answered with a resounding yes.

More than 650 genes had different signal strengths in those who developed PTSD compared with those who remained resilient. Like an intelligence agent tracking text messages, Segman could pick out familiar networks in the cellular data. As he'd guessed, many of these pathways related to the fight-or-flight response. One signal in particular came from a gene called FKBP5. (Later work by Rachel Yehudah and colleagues at Mt. Sinai School of Medicine in New York also showed the same changes to this gene in people who developed PTSD after the September 11 attacks.)

But another activated pathway stood out. It was what you'd see when the cells of the immune system were being called to battle. So what does a system designed to fight microbes have to do with the development of PTSD? The question had Segman and his colleagues scratching their heads. The simplest explanation was that chronic stress was changing their immune systems – cortisol is well known to tamp down immunity. But Segman couldn't help wondering if the data was trying to tell him something else: could the unusual activity of the immune system explain why these people developed PTSD in the first place?

ONE PERSON UNSURPRISED by Segman's results was Michal Schwartz, now chairwoman of neuroimmunology at Israel's Weizmann Institute of Science. Schwartz is small in stature, but big on passion – she speaks with an intensity underscored by nearly two decades of assaults on her scientific ideas.

"I was the first in the world to suggest that the immune system can help the brain," says Schwartz.

It was an assertion that tilted at one of medicine's most established beliefs: the immune system and the central nervous system (comprising the brain and spinal cord) supposedly led separate lives. The blood-brain barrier provided clear evidence for that belief. Immune cells execute their sorties via the bloodstream. Like five star generals, they merely flash their credentials (specific proteins on their surface) and squeeze through blood vessel walls into the target tissue. But if that target tissue happens to be the brain, access is denied. If they did gain access, disaster might ensue. Multiple sclerosis, for instance, is a disorder in which the immune system breaches the barrier and attacks the insulating sheath around brain cells.

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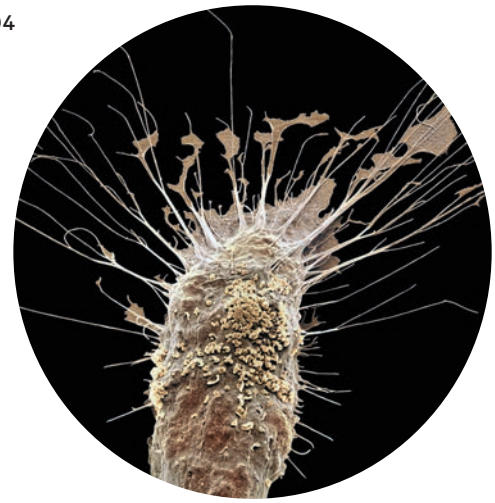

Microglia are deployed by the brain to carry out surveillance against microbial invaders. They are also implicated in the development of PTSD.

The brain meanwhile carries out its own surveillance against microbial invaders via a domestic militia of cells called microglia. But microglia never cross out of the brain and the cells of the blood-borne immune system never cross in.

Schwartz questioned the dogma. The blood-borne immune system was crucial for repairs in every other part of the body: the scab on your sore would not form without it. Might it not also help repair the central nervous system? In 1998, her lab tested the idea by injecting macrophages, one of the many

"HUMAN REACTIONS ARE MULTIFACETED AND COMPLEX," SEGMAN SAYS, WARNING AGAINST DISTILLING A COMPLEX DISORDER DOWN TO THE ACTION OF A FEW GENES.

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Tragically, the Middle East has provided fertile ground for studying PTSD in children.

types of immune cells found in the bloodstream, into the crushed spinal cords of mice. The result was stunning: the macrophages helped repair the damaged spinal cords.

Then they tested to see what effect the immune system might have on the brain's day-to-day functions – for instance the ability of mice to learn a maze. The classic test involves throwing them into a murky pool with a submerged platform. With the first dunk, the mice frantically swam around until their feet found the hidden platform. But with a bit of practice, they headed straight for safety – and they remembered their lesson. Schwartz did the test on mice with an impaired immune system that were missing T cells. It took them much longer to learn the position of the platform and the next day they appeared to have forgotten their lesson. The mice were also exceptionally jittery. When Schwartz fixed their immune system with a bone marrow graft, the slow learners came up to par. They also became much calmer.

How could the T cells of the immune system be affecting learning, memory and even stress responses?

Schwartz found a clue when she took a closer look at the brains of the mice with a defective immune system.

Mostly, the brain is rather poor at regenerating itself. But in 1998, Fred Gage and colleagues at California's Salk Institute discovered one part of the human brain can generate new cells in a process termed *neurogenesis*. That part turned out to be the librarian: the hippocampus.

So why, of all parts of the brain, is it the hippocampus that generates new cells? Is wiring new cells into the memory circuit the physical correlate of converting short-term to long-term memories? It turns out something more subtle is going on. The role of these new neurons is to help distinguish between memories that are closely associated – so called "pattern separation."

In everyday life, this could influence such things as recalling where you parked the car this morning versus where you parked it yesterday morning. Or it could influence how a person recalls a traumatic event.

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Hippocampal neurons regenerate – a process which is needed to distinguish between closely associated memories. In PTSD this mechanism is disrupted.

Many studies have shown that chronic stress dampens neurogenesis. And so, explains Gage, a soldier seeing his friends blown up will experience a slow-down in his production of new neurons. Even once returned to safety, fewer hippocampal neurons will make it harder for him to discriminate his memories: "He'll remember but he won't be able to separate the emotions then from the emotions now."

Gage says that psychologists are taking advantage of the new research to try and boost neurogenesis in their patients while they try to layer a more positive association with the traumatic event. And one of the best ways of boosting neurogenesis, it turns out, is exercise. Mouse studies show that a month on a

running wheel can boost the hippocampal brain cells of a mouse by up to 40%, says Gage. A stimulating environment, sex and anti-depressant drugs also boost neurogenesis – at least in mice.

And so does the immune system.

As Schwartz reported in 2006 in a paper in *Nature Neuroscience*, mice lacking T cells were not only slow learners, they were also worse at making new cells in the hippocampus.

The findings suggested some sort of crosstalk was going on between the immune system and the brain. Neurogenesis, learning and a normal stress response all seemed to rely on it. "That led us to think that the immune system is a partner in the maintenance of the brain," summarised Schwartz. T cells normally home in on cells infected with viruses. But the T cells of the mice were travelling to the brain – which is what happens, to devastating effect, in autoimmune diseases such as multiple sclerosis. Schwartz coined a new term for the paradoxical behaviour of the T cells: "protective autoimmunity".

To make sense of all this requires taking a broader view. An immune system is an army but it can be commanded to do battle or carry out disaster relief. Schwarz believes that a stressed brain needs the immune army to provide the disaster relief. Seared by the chronic effects of cortisol, the stressed brain ends up activating the resident microglia. As if responding to an infection, they release toxins that inflame the surrounding tissue and hamper neurogenesis. Toning down the microglia and mopping up the damage relies on the support services of cells from the blood-borne immune system.

How do they breach the blood-brain barrier? The Schwartz lab found the entry point in 2013: the *choroid plexus*. It's a leaky set of structures near the base of the brain that during injury provides a back door for cells of the immune system. T cells can also relay their messages via the back door without actually passing through.

SEGMAN'S STUDIES in people have continued to implicate the immune system in traumatic stress disorders. More recently his studies have focused on a related disorder – post-partum depression. With one in seven new mothers at Hadassah Medical Centre suffering clinical depression, it is a serious problem. But the sheer number also provides Segman with plenty of subjects to study. He found a clear difference in the blood of women who will go on to develop depression – they show activation of genes known to rouse the immune system just 48 hours after giving birth.

Segman has also been tracking the survivors of the Jerusalem suicide bomb attacks. First seen by Esti Galili and her team more than a decade ago, they are now in their early 20s. Some have proved resilient; others developed chronic PTSD. How their immune systems differ is yet to be fully deciphered.

"We are still lacking biomarkers that would allow us to predict who will go on to develop chronic PTSD and we do not know how to prevent its development during the immediate time window after trauma," Segman says. He hopes that the soon to be published long-term data set from the residents of Jerusalem will help clarify what distinguishes the immune systems of the resilient from the vulnerable.

MANY SOLDIERS STILL SUFFER from the lingering spectre of PTSD – not only Iraq and Afghanistan veterans such as Daniel; even Vietnam War veterans still report symptoms. More than a third of PTSD sufferers will never fully recover. "There generally seems to be low efficacy for medications or psychotherapy," says Segman. Yet billions have been spent on various treatments according to a 2014 report from the US Institute of Medicine.

Could manipulating the immune system provide a remedy?

Schwartz has found that a vaccine that recruits immune cells to the brain for a limited time makes mice perform better in stressful situations – cat odours don't petrify them and loud noises are less startling. When it comes to people, Schwartz speaks of a new immune-modulatory treatment her lab is developing that would also recruit immune cells to the back door of the brain. She suggests that if used in the hours after a trauma, it might be the beginning of a treatment to prevent the development of PTSD; a treatment that might be used to slow the procession of victims making their way to Galili's crisis centre.

Or it could be used prophylactically for those about to be exposed to traumatic situations. One in 10 soldiers risk becoming debilitated by PTSD. Before deploying, they might be given a preventative "booster" to make them more stress resilient. "This is our vision," says Schwartz.

*Daniel is not his real name

"THE BEST ANSWER COULD BE TO SHORE UP THE IMMUNE DEFENCES, BEFORE EXPOSING THE MIND TO STRESS."

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