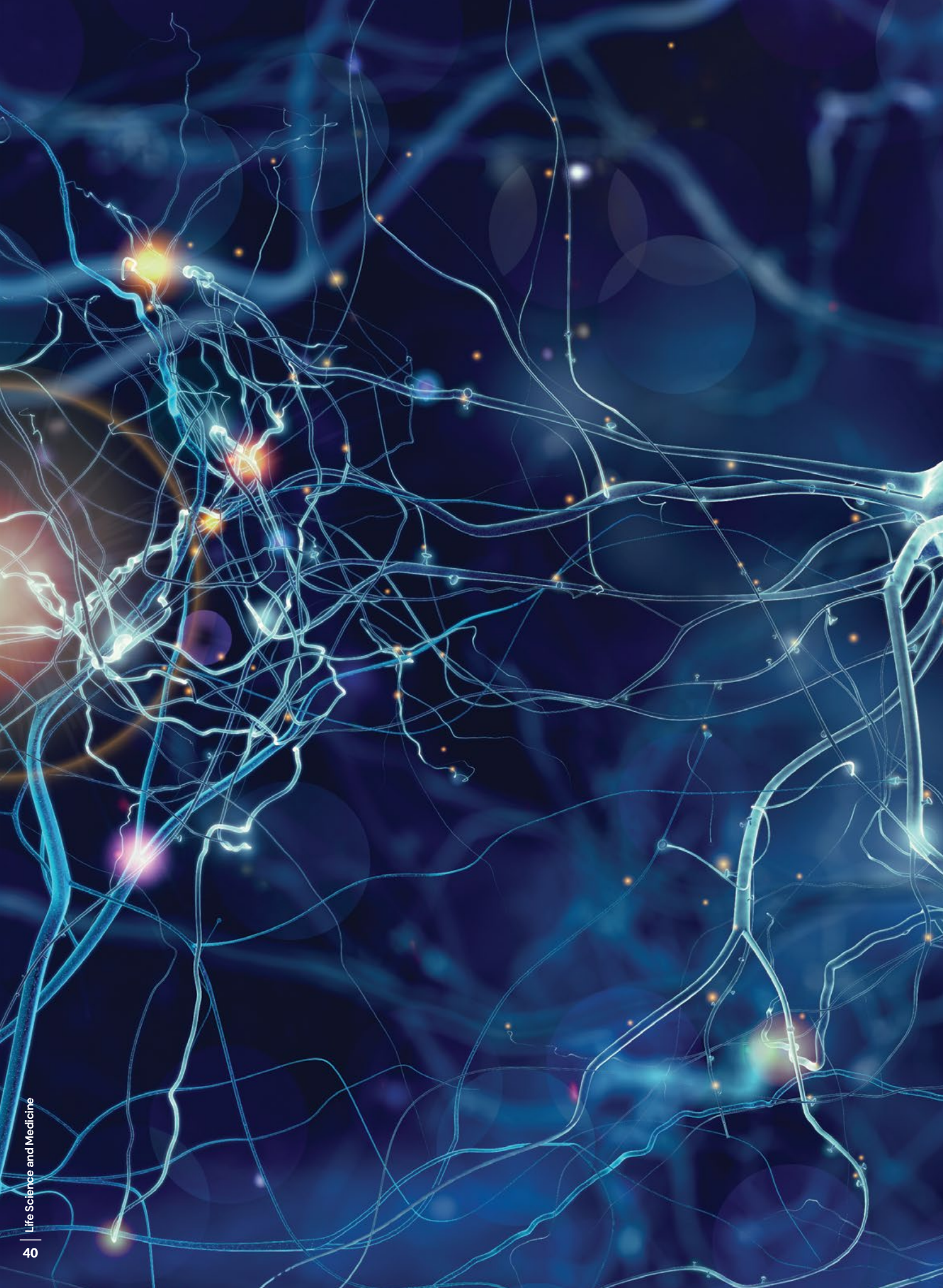


Protecting White Matter:

The Key to Preserving Brain Function after Injury

Author: **Antónia Ribeiro**

The destruction of white matter in the brain, which can occur during a stroke, is an irreversible process that can cause serious cognitive decline. While unravelling the effect of drugs that may protect against white matter injury, new research at the University of Malta is giving hope to people at higher risk of strokes.

The brain is a marvellously complicated organ. It oversees all our bodily functions and controls our perception of the world. Yet, it is also highly sensitive. Heavy impacts, infections, or intoxication can lead to alterations in the brain that deal great damage.

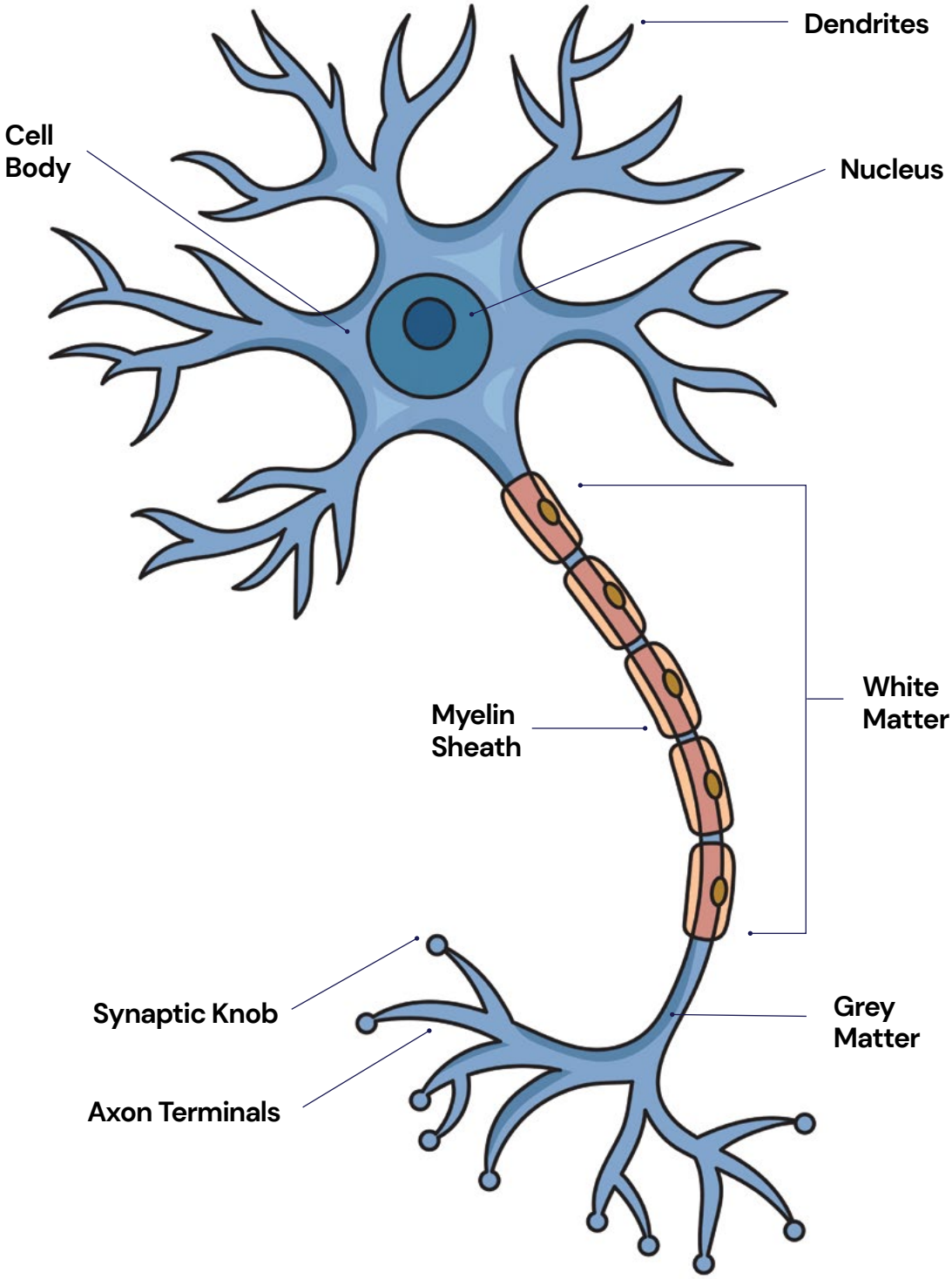
Look at strokes, for example. They are either caused by an obstruction of the brain's blood supply, which deprives it of oxygen and nutrients (a phenomenon called ischemia), or by the rupture of a blood vessel supplying the brain (referred to as a haemorrhagic stroke). In both cases, this can lead to the destruction of neurons and affect a person's ability to speak, move, and think. Or consider diabetic patients, prone to periods of extremely high (hyperglycemia) or extremely low (hypoglycemia) blood sugar; both conditions can also trigger neuronal death.

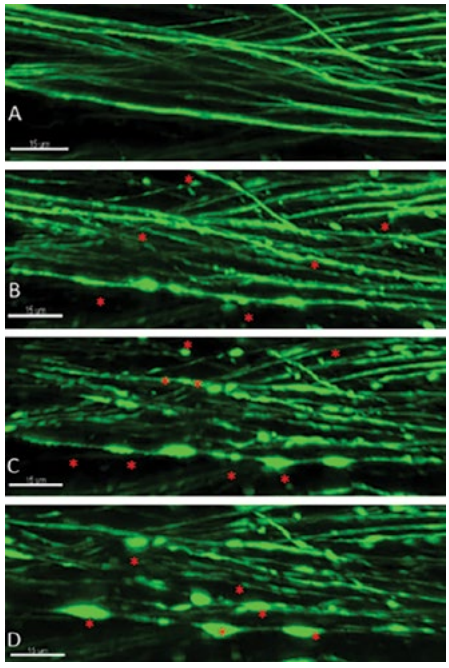
Neurons are specialised cells which are responsible for transmitting information throughout the body, allowing us to think, feel, move, and respond to our environment. When neurons die, it's like losing a part of our communication network. Neurons cannot easily be replaced, so finding a way to protect them is crucial.

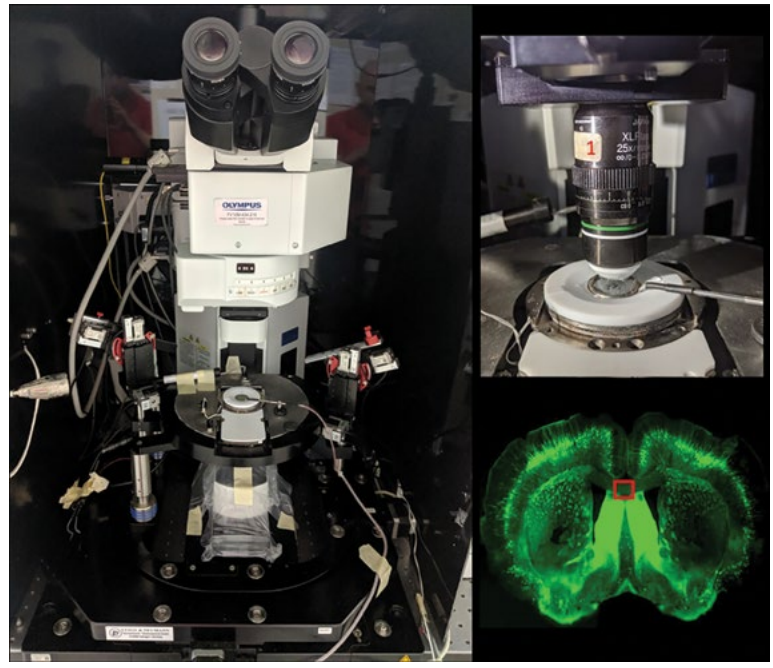
WHITE AND GREY MATTER

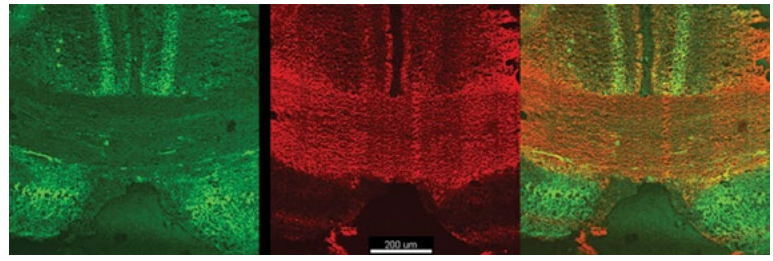
Neurons are an important part of the brain, which can be divided into white matter and grey matter. 'Grey matter includes the cell bodies of neurons, which work similarly to the CPU in a computer,' explains Dr Christian Zammit, a researcher at the Laboratory for the Study of Neurological Disorders, Faculty of Medicine & Surgery, UM, and a lecturer at the Department of Anatomy of the same faculty. Grey matter is the area where information is processed, while white

Neuron Structure









Top left: Visual showing progressive axonal injury

Top right: Two-photon microscopy

Bottom: Neuronal profiles in green and myelin stained in red

Images by Dr Christian Zammit

matter is made up of axons: extensions from the cell bodies of neurons that work as connection wires.

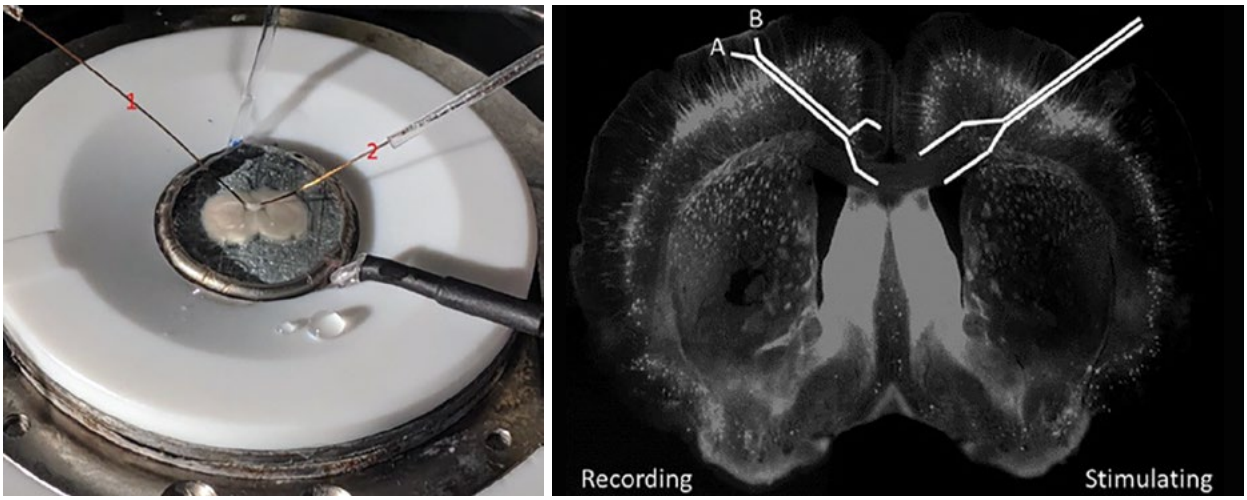
In addition to neurons, there is another group of cells that make up the brain, known as glial cells, which act as 'helper cells' to adjacent neurons. One particular cell type is called the oligodendrocyte, and its function is to cover the axons with an insulating layer that helps the electrical message travel quicker, known as the myelin sheath.

Led by Prof. Mario Valentino, the group at the Laboratory for the Study of Neurological Disorders where Zammit works is trying to protect white matter components during periods of hypoglycemia and ischemia to hopefully limit brain damage. Zammit guided **THINK** through their research.

The group is studying two drugs that target different components in white matter. The first drug, QNZ-46, blocks specific receptors present on the myelin sheaths of axons that are usually activated by the neurotransmitter glutamate. During ischemia and hypoglycemia, an excess of glutamate floods the spaces outside the cells in the brain. This glutamate binds to specific receptors in the surrounding cells and initiates a cascade of events that ultimately may lead to cell death. This process is called excitotoxicity.

Imagine that receptors are like doors and glutamate is the key. When glutamate is inserted in the keyhole, the door opens, and people are let in. QNZ is a master key that fits the keyhole usually used by glutamate, but instead of opening the door, it locks it. This prevents glutamate from binding to the myelin sheath and limits neuronal death.

The second drug (CP-465,022) acts on oligodendrocytes, which when damaged via excitotoxicity, are weakened in their ability to produce myelin sheaths. Valentino's group is trying to stop glutamate from binding to oligodendrocytes during ischemia and hypoglycemia. While QNZ-46 locks the 'doors' on the myelin sheaths, CP-456,022 guards the entry into the oligodendrocytes. The group hopes that by protecting oligodendrocytes – and consequently the myelin sheath – the drug will help limit neuronal death.



The above images show how electrodes are placed to measure axonal electrical conductivity
Images by Dr Christian Zammit

Zammit, in collaboration with a research group based in Plymouth University (led by Prof. Robert Fern, Associate Dean-Research, Faculty of Health, Plymouth University) has already shown that QNZ-46 protects neurons during ischemia. The group in Malta is now assessing how the two drugs act together when the brain is deprived of glucose.

EVALUATING NEURONS' STRUCTURE AND FUNCTION

Studying the mechanisms that lead to neuronal injury when the brain is deprived of oxygen and glucose is a challenge in its own right. To do so, the group uses genetically modified mice. For this particular research project, they extract their brains, slice them, and keep each section in a special solution that mimics the fluid that surrounds our brains. With this method, the brain slices are kept alive for up to 7 hours and react to stimuli just as if they were still a whole brain inside the head of a mouse.

As Zammit explains, he can remove glucose (the energy source of neurons) from the solution, creating an environment that mimics the human brain during periods of hypoglycaemia. In addition, he can also replace the oxygen in the solution by displacing it with nitrogen to create an ischemic-like environment. Then, he can assess how the injured brain slices react to the drugs.

To determine whether the drugs are effective in protecting the neurons, Zammit and his colleagues need to assess both the structure and function of these cells. For this particular research project, two different types of genetically modified mice are used: one type produces fluorescent proteins in neurons, the other in oligodendrocytes. By utilising a special type of microscopy known as multi-photon microscopy, the researchers can visualise these fluorescent proteins in real time, thus enabling them to establish whether the cells are healthy or not. In addition, they can also add colourful tags to specific proteins they are interested in (a technique known as immunocytochemistry) to obtain more information about the health status of these cells.

Axons possess a remarkable property: the ability to transmit information from one part of the brain to the other via electrical impulses. Researchers determine whether a neuron is still functioning or not by checking if the axons are still conducting electricity. To do so, they insert two pairs of electrodes in two places along a bundle of axons and measure the

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electrical current between them. Healthy neurons are able to transmit a constant electrical signal.

In his research, Zammit has found that neurons lose their structural integrity and their ability to transmit electrical signals when there is a low level of glucose. But if brain slices are treated with the two drugs, either alone or in combination, there is a good level of protection to neurons during prolonged periods of low glucose supply.

To consolidate their findings, the group now has to prove specifically how these two drugs protect neurons, without technically acting on them. Their hypothesis is that protecting the nerve insulation (myelin sheath: QNZ-46) and the neighbouring helper cell (oligodendrocyte: CP-465,022) offers secondary protection to the neurons.

So far, it seems like the drugs Zammit is studying can protect against brain damage. Even more exciting, when used together, they increase each other's effects.

A LONG ROAD BEFORE GETTING TO MARKET

As demonstrated by previous research, these two drugs are full of advantages. They target specific types of receptors found in the myelin sheath and on oligodendrocyte, decreasing the possibility of side effects. Additionally, they cross the blood-brain barrier (BBB), a crucial aspect for the potential future drug development process, since the effectiveness of a drug doesn't matter if it cannot enter the brain through the BBB.

QNZ-46 and CP-465,022 are proving to be effective at dampening stroke-induced damage, yet, as Zammit puts it, 'translating something that is effective in rodents into human use is a long way.' There is a long process to bring drugs to market. The chemical formulations used by the research group will have to be adapted by pharmaceutical companies into actual medicines that can be given to patients and tested for safety and effectiveness in humans. If developed, these drugs could potentially be given to patients who are at a significant risk of developing a stroke in order to limit neuronal damage.

Further Reading:

Doyle, S., Hansen, D. B., Vella, J., Bond, P., Harper, G., Zammit, C., Valentino, M., &Fern, R. (2018). Vesicular glutamate release from central axons contributes to myelin damage. *Nature Communications*, 9(1). https://doi.org/10.1038/s41467-018-03427-1