Teaser: **Window on the brain**

Headline: **Researchers look into the living brain**

Standfirst: **Dr Vincenzo De Paola's cutting-edge experiments reveal the brain's latent healing ability.**

The brain is the new frontier in life science. Neuroscientists don't fully understand its overall structure and what its individual cells do. What they also need to know is how those cells are organised and how they change over time in response to experience and disease. Especially important is how they recover from injury and why sometimes they don't.

The brain's secrets are in its connections. Neurons are sprawling, tentacular cells (see image) adapted to conduct electrical signals. They bear many small projections, called dendrites, and one long one, called axon. With these, a neuron can exchange signals with thousands of others. The connections, called synapses, encode all the information held in your brain – the thoughts, feelings, memories, skills and everything else.

In the rest of your body, broken neural connections can be repaired. But this just doesn't happen in the brain or spinal cord. Brain cells refuse to regrow and replace synapses destroyed by conditions like stroke and Alzheimer's or by wounds. This makes brain damage permanent, and it's one of the biggest problems in neuroscience.

What stands between us and the answers is that you have only one brain. It's very difficult to decode it without taking it apart, or do experiments to discover what stops it healing.

Dr Vincenzo De Paola is finding ways to look into the brain, and watch it regenerate in real time. He leads the Neuroplasticity and Disease Group at the Medical Research Council Clinical Sciences Centre at Imperial College London. His group uses a combination of techniques to track the development of individual neurons in a living, working mouse brain for up to a year. And by making miniscule injuries, they have been able to reveal its healing capacity. All this without touching its surface.

"It really excites everyone here in the lab," Dr De Paola told me. "The possibility of looking into the living brain while it's functioning… The fact that it's alive and you're watching the neurons and their connections while they're working. It's completely different to looking at a fixed sample."

A stubborn refusal to heal

If you hurt your arm and paralyse your hand, the axons sending signals to your hand muscles have been severed. If you're lucky, the bodies of those neurons are still alive. In that case they can grow new axons along their original routes and restore some of the broken connections.

This doesn't happen in the brain. "We don't completely understand why this is," says Dr De Paola, "but we know some of the reasons. Some neurons seem to lose their ability to grow after they become mature. But their main problem is their environment."

Neurons are supported by glial cells, which surround them and regulate their environment. To grow, neurons need to be exposed to encouraging substances and not inhibitory ones. In the healthy adult brain, most of the encouraging substances are lacking and glial cells release inhibitory ones instead.

The situation is even worse after injuries because scar tissue forms. Dead and dying neurons are cleared away and replaced by glial cells. They seal off the area with a physical barrier and release doses of growth inhibitors. "The brain seems to hold back its own growth capacity when it most needs it," says Dr De Paola.

Neuroscientists want to get around this. '***Strokes, Alzheimer's, Parkinson's, multiple sclerosis and blows to the head take brain function away from millions of people each year. A major goal for neuroscience is finding a way to give it back. This means remaking broken connections.'***

A window on the brain

To investigate the potential of neurons to regenerate, Dr De Paola had to find a way to see inside the living brain and look at individual axons.

The first step is to implant a glass window on the brain. Though it sounds strange, this is actually the most straightforward part. “Removing sections of skull is quite routine, even in human patients, for treatments such as deep brain stimulation for Parkinson’s disease,” explains Dr De Paola. He and other members of the lab, are experienced in the procedure, which is performed under general and local anaesthetic, and routine use of analgesia.

“The wound heals within a few days”. This is the only invasive procedure that they have to undergo, and the brain itself is not disturbed.

The next technique is called two-photon fluorescence microscopy. The lab’s mice are genetically modified so that a small fraction of their brain cells glow green when triggered by laser light. The microscope filters out all colours but green and produces very clear, detailed images of the fluorescent cells.

It can focus on cells up to about 0.5 mm deep under the surface, which doesn’t sound like much but is enough to see several different layers.

The third technique, which Dr De Paola and his collaborators pioneered in the mammalian brain, is ultra-precise laser surgery. If the lasers that make the cells fluoresce are made as narrow as possible and powered up by about ten times, they concentrate so much energy on the point where they cross that the tissue there is destroyed. Only a tiny area is affected (thousand times smaller than one mm), and no damage is done at all except to the target. This allows the researchers to slice through a single axon, among millions of others, without putting a blade into the brain or causing any collateral damage.

Hidden potential

Using these techniques, Dr De Paola and his team watched what happens when a single axon is severed inside the brain, away from the restrictive effects of scar tissue. This had never been done before.

Eliminating the scar tissue did not allow all neurons to repair their original connections. “Without the molecular signals that guided them during their development, they were lost,” explains Dr De Paola. “They never regained their original function.”

Not every neuron regrew a new axon. The team looked at two groups of neurons, from different layers of the cortex. Some were more active than others, meaning that they rearranged their synapses more often. Active neurons are always making and breaking synapses, while less active neurons generally keep the same ones for a long time.

In the adult, active neurons regrew a new axon about half the time. Only about a fifth of less active neurons regrew. During early stages of development, both types of neuron regrew about twice as often. This reveals that even in the brain some neurons have more intrinsic growth potential than others. It also confirms that young mammals, whose brains aren’t fully developed, have much more regenerative capacity.

What was extraordinary was that the axons that could spontaneously regrow consistently formed new connections. Axons could extend for distances larger than the amount cut (i.e. distances unseen before in the adult cortex) at speeds comparable to peripheral nerves. “The regrown axons formed, on average, just as many connections as the originals had. This suggests that the capacity to make new connections isn’t restricted by the environment or by injury, which is good news if we want to stimulate regrowth in people with brain damage”.

“It’s also very exciting because it shows that the neurons took on new functions. We might be able to use this to repair injuries, because the new connections could take over the jobs of the old ones.”

Salvaging function

Dr De Paola’s group went on to look at what happened to the part of the neuron that survived the surgery. When an axon is cut, the part separated from the cell body decays quickly. The rest can still function but reacts differently in different neurons.

Firstly, some portion of it dies back, retracting away from the injury site. The exact amount of retraction varies for unknown reasons, but an interesting trend was found: the less the neuron died

back, the more new axon grew out. “This is promising too. It takes a few months for dieback to happen. As the axon dies off, it loses more synapses and more functions. But if we can find a way to reduce dieback, we might be able to reduce brain damage at any time over those months.”

Secondly, the team found that the surviving synapses tend to react in one of two ways, depending on whether they are on more or less active neurons. More active neurons lose a lot of connections in the few days after the surgery. In contrast, the less active neurons reacted as if nothing had happened. They kept almost all their connections, even the ones right next to the stump. It may be that the active connections, which tend to be newer, are lost to save resources and help deal with the injury.

“Altogether, we think these findings show there is a window for effective treatment of brain injury soon after it occurs, but also that the window is larger than we previously thought,” summarises Dr De Paola.

Unlocking new insights

These studies are just the start of what Dr De Paola hopes he and others can achieve. “We’ve characterised the baseline capacity for regeneration in the adult brain. We’ve found differences between different kinds of neuron. The logical next step is to understand what controls these capacities. Ideally, we want to be able to change them at will.”

The techniques give researchers the opportunity to test chemical or genetic treatments and see whether these help or hinder neuron regrowth and remodelling. It may also be possible to find out what some neurons do by cutting their axons and looking for changes in the animal’s behaviour. But Dr De Paola plans to go further into the basic biology and discover what controls regrowth and the synaptic reorganization.

“Our basic idea is that it’s electrical activity that regulates the regenerative capacity – that signalling to other neurons stimulates injured neurons to regrow or to rearrange their synaptic connections. So we plan to trigger different activity patterns in the brain by using optogenetics.”

Optogenetics is another cutting-edge technique that allows scientists to control the electrical activity of neurons by shining a light on them. It will combine well with the existing experimental set-up. “It’s a powerful, precise and non-invasive way to control the activity of neurons.”

Incorporating optogenetics will make the lab’s methods even more sophisticated, but keep them non-invasive. This is a scientific advantage because it minimises direct interference in the brain, examining it in a more natural state.

“We’re looking at a very dynamic process in several steps. The best way to do this is to visualise them all as they happen. This allows you to interpret the data better, with fewer assumptions.”

Besides providing unique and valuable insights into the repair and regeneration potential of the mammalian brain these studies are already stimulating research into novel biomaterials (e.g., ceramic-based transparent nanocrystalline) in order to gain optical access to the human brain and allow potentially beneficial laser-based therapies in neurology.

It may be even possible in the near future to do similar live imaging experiments using neurons derived from human skin biopsies (e.g. from induced pluripotent stem cells), to learn more about what is special about human synapses compared to other species and about their regenerative and plastic potential.

“I think this is the way forward,” says Dr De Paola.

Sidebar

In a study published in March, Dr De Paola’s group looked at how synapses, and the ability to create new ones, change with age. It had been thought that as we get older, it becomes harder to form new connections between neurons. But this turned out not to be the case. In fact, older mice appear to lose new connections faster, which might be what makes them more forgetful. Dr. De Paola now plans to extend these findings by studying the involvement of genes known to alter mammalian lifespan.

References

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(*See also highlight in Nature*).

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(*Highly accessed*, see also *highlight in Nature* & *F1000 recommendation*).