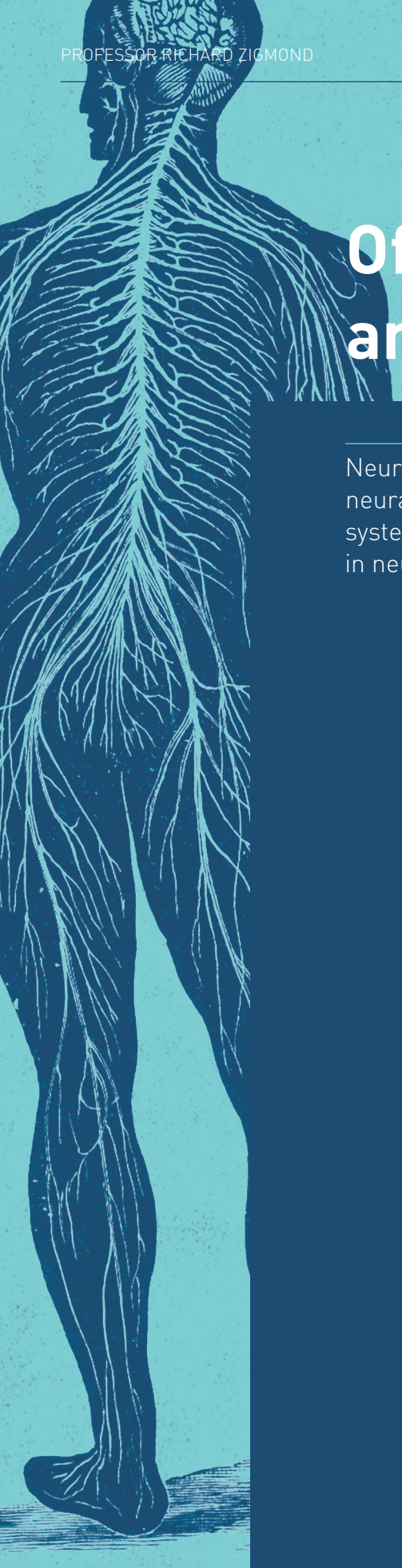

Of nerves and neurons

Neuroscientist **Professor Richard Zigmond** is exploring how neural damage and nerve activity impact the adult nervous system. Here, he talks about the trigger that sparked his interest in neurological research, and his studies on nerve injury

When and why did you first become interested in the adult nervous system?

I attended Harvard College, USA, with the intention of studying history; however, during my first year I took a fascinating course by Professor George Wald (winner of the 1967 Nobel Prize for Physiology and Medicine), which began by examining elementary particles and gradually built up to molecules, macromolecules, cells and finally organ physiology. I immediately switched my major to biology. From there, I decided to focus on the nervous system and behaviour, having spent a summer in Africa with Peter Marler and Jane Goodall studying chimpanzees in the wild.

Two particular alterations to the adult nervous systems – neural damage and changes in neural activity – are a key focus of your laboratory. How do these differentially impact the nervous system?

The way in which experience impacts most neurons is through changes in their electrical activity and hormonal inputs. Reproductive hormones alter behaviour and, in my thesis research, I found that areas of the brain where oestrogens act have specific receptors, located in neuronal cell nuclei where the hormone can alter gene expression. In my postdoctoral research, I examined the effects of changes in nerve activity on a neuron's biochemistry by electrically stimulating peripheral neurons. My laboratory has since shown that key proteins in neurons are regulated by changes in nerve activity and that these proteins are sensitive to the frequency, pattern and duration of this activity – in other words, to the 'language' by which neurons communicate.

Most recently, we have turned our focus to alterations produced after injury and found that neurons begin to express genes necessary for regeneration and depress expression of those required for intercellular communication.

Can you explain the purpose of your investigations into the limited recovery of pineal function after regeneration of preganglionic sympathetic axons?

The sympathetic nervous system is made up of two types of neurons; preganglionic nerves in the spinal cord project out and connect to collections of postganglionic nerves in structures called ganglia. This sympathetic system controls a variety of autonomic functions – those over which we have no voluntary control. In around 1900, British physiologist John Newport Langley published a classic paper that has been interpreted for over 100 years to indicate that, when damaged, the axons of preganglionic neurons are able to regenerate and restore normal autonomic function.

We decided to test this idea by focusing on the sympathetic innervation of the pineal – a gland that secretes the hormone melatonin. The answer we obtained was clear cut; while the axons did indeed regenerate, normal function was not restored. Our results were unexpected and strongly indicate that mistakes are made in the connections that the adult axons make in the ganglia.

In what ways do your goals for this study meet the overall objectives of your laboratory?

Knowing the basic anatomy, physiology and biochemistry of a neural circuit allows one

Advancing neural knowledge

A team of researchers based at **Case Western Reserve University**'s Department of Neurosciences is examining the molecular mechanisms underlying the regeneration of injured neurons, and how this knowledge has implications for understanding diabetic autonomic neuropathy

to ask important functional questions. We began by defining which sympathetic neurons project to the pineal gland and by which pathway they get there. We then examined in detail how the pineal function of secreting melatonin is regulated by these neurons. Melatonin is made at high levels at night (and during periods of nerve stimulation), and at low levels during the day (and during periods of no nerve stimulation). Our studies showed that, although after nerve injury this day-night rhythm in melatonin synthesis was not restored, a normal response could be obtained by directly stimulating the nerves. In other words, an adequate number of axons had regenerated but, when these animals were exposed to darkness and light, these axons did not convey the proper information to the pineal.

Could you reveal any personal highlights from your expansive research career?

One such highlight was the finding that when we stimulate preganglionic sympathetic neurons we can increase the postganglionic neurons' capacity to synthesise neurotransmitter; however, when we stimulated the postganglionic neurons directly, we have no effect. This suggests that both the release of neurotransmitter and the firing rate are important. Another example is the finding that the neurotransmitters involved in this biochemical regulation change depending on the pattern of nerve stimulation. Finally, I was excited to demonstrate that when sympathetic neurons are grown in cell culture they produce a different complement of neurotransmitters to normal. We discovered that this is a consequence of the axotomy produced by placing these neurons in culture, a finding that led us to two decades of investigations of what happens when neurons are injured.

THE HUMAN BRAIN and peripheral nervous system are inherently plastic. This reflects changes in both the biochemistry of individual neurons and in their ability to reorganise themselves by modifying their neural connections. Although much neuroplasticity occurs at the beginning of life during development, such rearrangements can also occur throughout adulthood whenever something new is learned and memorised, or in response to neuron injury where synaptic connections are altered in order to compensate for lost function.

Researchers in the laboratory of Professor Richard Zigmond at Case Western Reserve University, USA, are interested in the ways in which the chemistry of the adult nervous system – the biochemical and cellular mechanisms that underlie its modifiability – change in response to injury to the axons of neurons, and what the functional consequences of these changes are.

MAINTAINING MACROPHAGES

When an axon in the peripheral nervous system is damaged, there is a subsequent multicellular response that causes the damaged axon to degenerate and the cell body to express genes necessary for regeneration. The outcome is the development of a growth cone from which a cut axon then re-extends. These processes involve changes in many different cell types, including the Schwann and immune cells, and signalling molecules such as cytokines. It has been widely accepted for some time that macrophages, a type of immune cell, are involved in this repair process by digesting fragments of the degenerating nerve fibres, thus clearing the way for new growth. In the 1990s, research in Zigmond's group uncovered that macrophages also may have a second role to play in regeneration.

Many questions remained, however. Since this finding, the researchers have carried out further studies using two mutant mouse strains – a slow Wallerian degeneration mouse and a knockout of the chemokine CCL2's receptor CCR2 – to try and uncover the exact mechanisms underlying the activity of macrophages at the cell body. Their findings have revealed that the activity of macrophages is mediated by macrophage chemokine CCL2 signalling, and that the build-up of this immune cell at the nerve cell body is essential for promoting nerve regeneration. Contrastingly, however, in certain diseases explored by other groups, macrophages can be a significant contributor to nervous system damage. This collection of findings has left the team feeling inspired to continue down this path of discovery towards understanding the role of the immune system in mediating changes after injury. It is the reseachers' goal to determine the molecules by which macrophages act in both situations, ultimately aiming to enhance the regenerative processes and block the injurious effects.

IMPACT ON ACTIVITY

If a nerve is unable to repair itself following damage – or the damage is too extensive – the peripheral nervous system is able to

For Zigmond, performing basic research is a fundamental aspect of understanding neurology – one that is critical to the future of medicine

INTELLIGENCE

THE ZIGMOND LAB

OBJECTIVE

To understand the ways in which the chemistry of the adult nervous system can change and the functional consequences of such changes. Of particular interest are the alterations that occur in response to neural damage and changes in neural activity.

RESEARCH TEAM

Dr Angela Filous; **Dr Jaisri Lingappa**; **Alicia DeFrancesco-Lisowitz**; **Jon Niemi**; **Jane Lindborg**, Case Western Reserve University, USA

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PROFESSOR RICHARD ZIGMOND received his BA from Harvard University and his PhD from Rockefeller University, both USA, where he studied oestrogen receptors in the mammalian brain. He then undertook three years of postdoctoral research at the University of Cambridge, UK. In 1975, Zigmond joined the faculty in the Department of Pharmacology at Harvard Medical School, and took up his current position at Case Western Reserve University in 1989.

rearrange its connections, to a certain extent, in order to maintain function. In addition to investigating the underlying mechanisms of nerve regeneration, the group is also interested in how the alterations in connectivity that occur following injury affect recovery of normal function. One major priority is to identify molecules and cells involved in altering neuronal gene expression during response to axonal injury, particularly those changes related to nerve regeneration. "It is becoming increasingly obvious that the cells in the nervous system – nerve cells and their 'support' cells – are capable of considerable change in both health and disease," Zigmond elucidates. "Understanding the triggers for such changes and their functional consequences is key. Changes in nerve activity and responses to injury are two such triggers."

MAKING PROGRESS

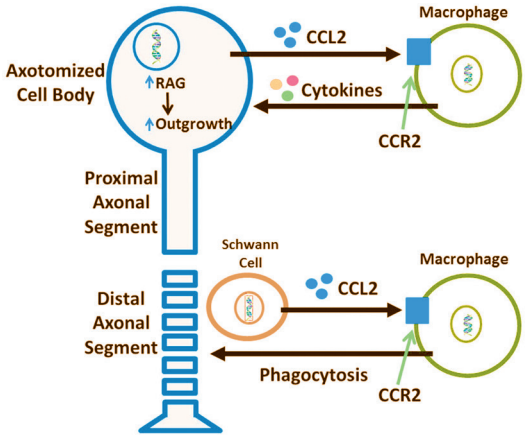
An example of how activity of the cells of the nervous system can change during disease is prominent in the case of diabetes, which has three primary complications, affecting the peripheral nerves (neuropathy), eye (retinopathy) and kidney (nephropathy). Researchers have been exploring the causes of these pathologies and how to reverse their effects for many years. A large part of Zigmond's work focuses on diabetic autonomic neuropathy – a serious yet common complication of diabetes that can lead to a range of conditions, including atrial fibrillation, stroke and sudden unexplained cardiac death, emphasising the urgency in developing new treatments and preventive strategies for regenerating ongoing axonal degeneration.

Twenty years ago, Zigmond's team made key advances in this field by showing that certain responses of sympathetic and sensory neurons to injury were mediated by a molecule called leukaemia inhibitory factor (LIF) – a member of the gp130 family of secreted molecules (cytokines). This prompted the researchers to question whether deficits in these cytokines play a role in diabetic neuropathy and neuronal injury.

The team went on to test diabetic mice over the course of two months: "We measured the levels of LIF and interleukin-6 (IL6) and found that the increases that normally occur after nerve injury were depressed," Zigmond explains. "Since these cytokines produce their biological effects primarily by causing the chemical modification of a factor that controls gene expression, STAT3, we examined whether the modification of this factor was reduced, and found that it was." Moving forwards with this knowledge, the team then monitored various regeneration-associated genes and discovered that their increased expression after injury was reduced. Consequently, Zigmond is now keen to explore the potential role of decreased gp130 signalling in producing diabetic neuropathy.

ANYTHING BUT BASIC

Disorders of the nervous system are particularly complicated for researchers to study. For Zigmond, performing basic research is a fundamental aspect of understanding neurology – one that is critical to the future of medicine. "In my field, it is hard to conceive of a clear division between translational and basic research; it is more a question of the time course over which findings are likely to be translated," he notes. "If we dismantle the apparatus for studying basic biological processes, humankind will suffer in the future and talented students and young scientists will choose other careers." Zigmond and his team recognise that in order to explore such clinical issues in an effective way, scientists must begin with basic research and build from there. It is their hope that through conducting basic neurological research, their work will lead to the development of improved diagnoses and better treatments for conditions such as diabetes.



The role of macrophages and intercellular signalling in the repair of an axotomised neuron.

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