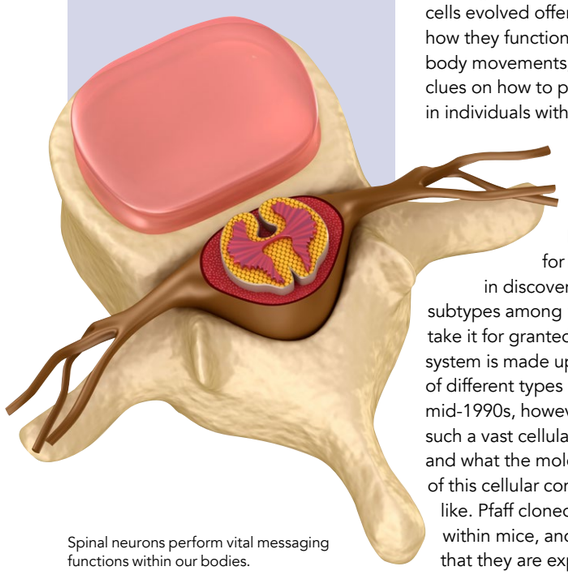
# Using genetic signatures to better classify spinal neurons

*Professor Samuel Pfaff at the Salk Institute for Biological Studies builds on previous work revealing important principles related to neural development, gene regulation, axon guidance and connectivity, and spinal motor circuit function. Spinal neurons were traditionally grouped into around 12 cardinal classes – but this doesn't describe their full diversity. Now, the Salk Institute for Biological Studies team has developed genetic tags that can be used to further classify spinal neurons, paving the way for easier future study of these vitally important cells.*



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Spinal cord nerve cells play vital roles within our bodies. Otherwise known as spinal neurons, they're responsible for the transmission of messages between the spinal cord and the rest of the body. When visualised, spinal cord nerve cells resemble trees, with branches stemming from a central trunk and then fanning off throughout the body in every direction.

This is where the work of Samuel L Pfaff, Benjamin H Lewis Chair in Neuroscience and Professor at the Gene Expression Laboratory, Salk Institute for Biological Studies comes in. Pfaff and his colleagues have developed a new way of classifying spinal neurons by analysing their genetic signatures. Gaining understanding into how these cells evolved offers fresh insight into how they function and regulate our body movements, as well as providing clues on how to potentially repair them in individuals with neuron damage.

## BUILDING ON LANDMARK STUDIES

Pfaff is best known for his contributions in discovering the distinct subtypes among neurons. We now take it for granted that the nervous system is made up of literally thousands of different types of neurons. In the mid-1990s, however, it was unclear how such a vast cellular diversity could arise, and what the molecular underpinnings of this cellular complexity might look like. Pfaff cloned a family of genes within mice, and was the first to show that they are expressed in unique combinatorial arrays. These early landmark studies helped to define the different subtypes of motor neurons and interneurons.

His most recent work, recently published in the prestigious journal *Science*, expands on neuron classification even further.

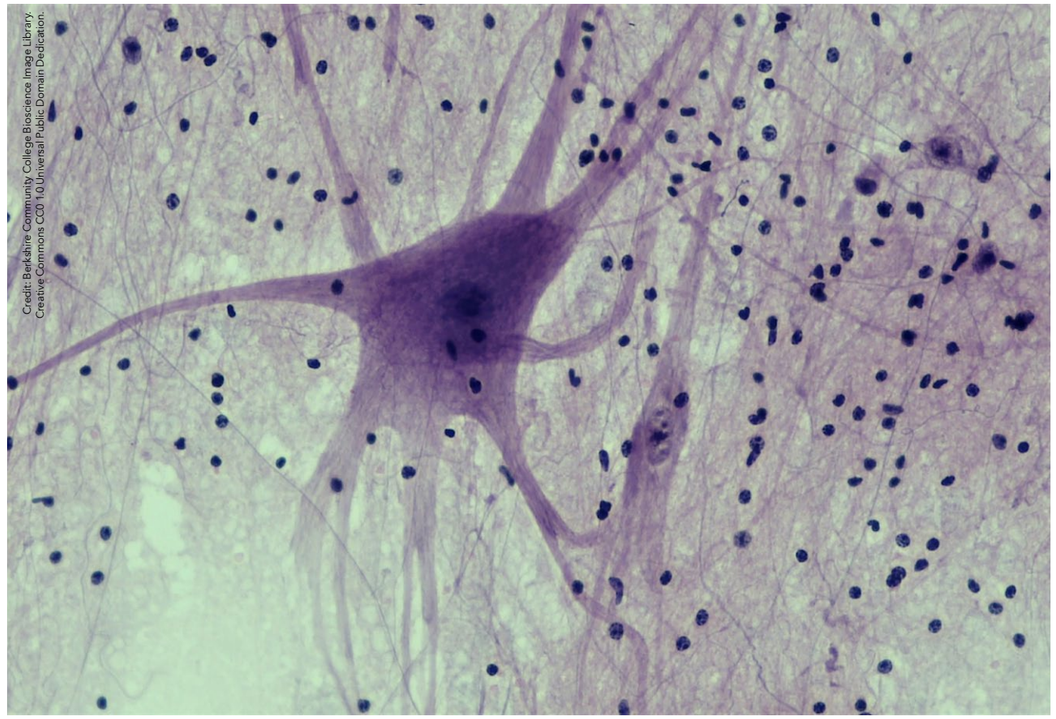
## WHY ARE SPINAL NEURONS SO IMPORTANT?

Spinal neurons perform a vital messaging function within our bodies. When they're not functioning properly, there's no way for our brains to tell our muscles how and when to move. Put simply, if the messenger in the middle is out of action, communication becomes impossible.

The spinal cord is an integral part of the body's central nervous system, and contains millions of these neurons. Something as simple as picking up a glass, or typing on a keyboard, relies on huge amounts of sensory and motor information being conveyed to and from the brain via spinal neurons.

People with damaged spinal neurons can become paralysed. Their brains function normally but they are unable to make their bodies move in the way they want to, or even move at all. There are also a group of debilitating illnesses, known as neurodegenerative diseases, where the health and function of these cells are gradually worn down over time. Any research that can help people who have become paralysed through accidents or who experience neurodegenerative disease is highly valuable, and of extreme importance to the medical community.







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## HOW ARE SPINAL NEURONS CLASSIFIED?

For some time, scientists have sorted spinal neurons into so-called 'cardinal classes'. This classification system is mainly based on where in the developing foetus the nerve cells first appear. However, there is significant diversity among the neurons within these cardinal classes.

It's an important goal to classify spinal neurons further than this, as it could help with understanding how the spinal cord neurons control movements and what goes wrong in neurodegenerative diseases or spinal cord injuries. Cardinal classes are useful, but ultimately incomplete when it comes to describing spinal neuron diversity.

Previous attempts to diversify these cells further have been challenging, until Pfaff and his colleagues harnessed the power of single-cell genetic analysis to show that spinal neurons are more diverse than previously realised.

## NEW DISCOVERIES

The key to being able to further classify spinal neurons was gaining a better understanding of their evolutionary history. In a recent study, Pfaff and his colleagues discovered distinct genetic signatures that can be used to classify cells beyond the cardinal classes.

Using single-cell RNA sequencing technologies, almost 7,000 different spinal neurons from mice were analysed. The aim was to see the differences in which genes were being activated, then use this data to try and group the neurons into closely related clusters. Essentially, it was similar to building an evolutionary tree of closely related organisms.

***A study like this provides the first molecular handles for scientists to go in and study the function of spinal cord neurons more precisely than ever before.***

The researchers hoped to discover this evolutionary history by looking for conserved and then distinct, specialised gene-expression signatures in the different neural subtypes.

## CHARACTERISING SPINAL NEURAL CIRCUITS

Pfaff's lab has also made additional significant strides in our understanding of motor neuron gene regulation and axon guidance. His lab has conducted a series of studies that unravelled the spatial and temporal genetic-control mechanisms that establish motor neuron connectivity. These studies have helped the scientific community to understand how a relatively small number of signalling proteins contribute to the enormous wiring complexity of the nervous system.

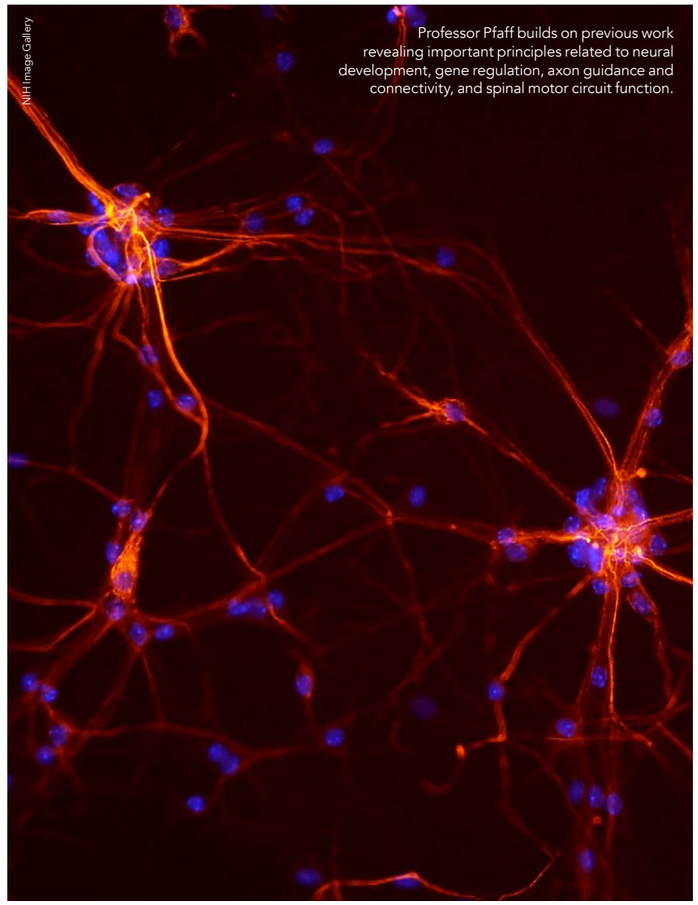
Most recently his group has uncovered a small RNA (microRNA) that is selectively expressed within motor neurons. This microRNA is critical for maintaining neuromuscular synapses (messages) and the survival of these neurons, and once levels drop below a critical threshold motor degeneration occurs. These new findings might help to understand why motor neuron degeneration occurs in amyotrophic lateral sclerosis (ALS).

**NEW GENETIC SIGNATURES**

By identifying genetic relationships shared across neuronal populations in the spinal cord, the researchers uncovered an orderly series of neuronal systems. Firstly, the neuronal components comprising sensory and motor circuits emerged as having profoundly different genetic underpinnings. Sensory-related neurons relay information about our limbs back to the spinal cord and prompt us to pull away reflexively when we touch painful stimuli – for example, when your hand automatically flinches away from touching a hot frying pan. Motor circuitry enables us to do things like swing our arms while running to help maintain balance. Despite the heterogeneity of the neurons comprising sensory and motor circuits, the researchers identified simple gene patterns that distinguish these two fundamental divisions.

The more surprising division came when the researchers further analysed the motor circuitry. These cells were clearly split into two distinct groups based on a new genetic marker. When the team used a biological dye to visualise cells belonging to each group in the spinal cord, it became clear that the markers differentiated neurons based on whether they had long-range or short-range connections in the body.

Further experiments then revealed that the genetic patterns specific to long-range and short-range properties were common across all the cardinal classes tested. Notably, this study could open up exciting possibilities for the future treatment of spinal cord injuries. Pfaff explains: 'A study like this provides the first molecular handles for scientists to go in and study the function of spinal cord neurons in a much more precise way than they ever have before. This also has implications for treating spinal cord injuries.'



NIH Image Gallery

Professor Pfaff builds on previous work revealing important principles related to neural development, gene regulation, axon guidance and connectivity, and spinal motor circuit function.

***Thanks to the new findings, just two genetic tags are now needed to flag very specific populations of neurons.***

**FUTURE OUTLOOK**

In the past, it's been difficult for researchers to use genetic tags to narrow down the one particular neuron type they wished to study, as using many markers is technically challenging. Thanks to these new findings, just two genetic tags are now needed to flag very specific populations of neurons. Firstly, the previously known tag for cardinal class and secondly, the newly discovered genetic tag for long-range versus short-range connections.

While the study was carried out in mice, the researchers have reason to predict that the same genetic patterns would be seen across most living animals with spinal cords. This could help when researchers are studying which groups of neurons are affected by spinal cord injury or neurodegenerative disease in humans.

It could also one day help to pave the way for research into how to regrow those specific cells in affected individuals. Having an easier way to classify and research neurons could lead to important future breakthroughs in the medical field.



# Behind the Research

## Professor Samuel L Pfaff

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## Research Objectives

Professor Samuel Pfaff's lab used a novel bioinformatic approach to reveal a new organisational pattern of neuronal subtypes in the spinal cord.

## Detail

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**Bio**

Dr Samuel L Pfaff is the Benjamin H Lewis Chair in Neuroscience and Professor in the Gene Expression Laboratory at the Salk Institute for Biological Studies. He also holds appointments with the Biology, Bioengineering, and Neuroscience programmes at the University of California, San Diego. He received his BA in biology from Carleton College (Northfield MN) and his PhD in molecular biology from the University of California, Berkeley. He completed postdoctoral training at Vanderbilt University with William Taylor on gene regulation followed by Columbia University with Thomas Jessell on neural development. He is the recipient of the Javits Neuroscience Investigator Award, McKnight Scholar Award, PEW Scholar Award, and an Alfred P Sloan Research Fellow Award.

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## Personal Response

***What do you believe will be the most interesting practical application of this research in the future?***

"With a relatively simple toolset of molecular markers that can be used in pairwise combinations, researchers can now label and characterise very discrete populations of spinal neurons. A major research question that remains is to understand how spinal circuits integrate volitional and subconscious sensory commands and compute precise limb movements that involve many highly coordinated muscle groups. Scientists are now in a position to selectively study the function of very discrete neuron populations to understand how complex motor circuits function. The principles that underlie spinal motor circuit computations will help to better understand mechanistically how behaviour is controlled."

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