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New Strides In Understanding How Walking Is Coordinated

Researchers have devised a genetic technique to distinguish the neurons in the spinal cord that control the sequential stepping of the left and right limbs. Their findings have literally taken them a step closer to understanding the neural circuitry that coordinates walking movements - which has been one of the main obstacles in developing new treatments for paralysis.

According to the researchers, using genetic techniques to identify specific spinal neural networks could greatly enhance our knowledge of the intricate neural circuitry involved in spinal motor control. Moreover, a better understanding of this area is absolutely crucial in developing strategies to restore motor function caused by paralysis due to spinal injury or disease.

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— **Thomas M. Jessell**

The approach that the researchers used to identify these neurons will likely find broader application and be useful in defining the "local" circuitry that governs other rhythmic processes such as breathing, as well as reflex behaviors that do not involve the brain.

The research team—led by Martyn D. Goulding at The Salk Institute for Biological Studies and Howard Hughes Medical Institute (HHMI) investigator Thomas M. Jessell at Columbia University—published its findings in the May 13, 2004, issue of the journal *Neuron*. Joint lead authors on the paper were Guillermo M. Lanuza and Simon Gosgnach in Goulding's laboratory.

The objective of the research, said Goulding, was to identify unequivocally the neurons involved in the circuit called the "central pattern generator" that

generates the rhythmic left-right signal that makes walking possible.

“Of course, people had known that the motor neurons were involved in the circuit operating the muscles,” he said. “But no one knew the identity of the ‘interneurons’ upstream of those motor neurons that coordinated the rhythmic drive governing these motor neurons.” Earlier anatomical studies had not provided any molecular or functional signatures that could be used to distinguish those interneurons, said Goulding.

In earlier studies, co-author Alessandra Pierani in Jessell’s laboratory had established that a particular set of interneurons, called V0 interneurons, require a genetic switch called *Dbx1* to develop their identity. Such genetic switches, called transcription factors, control the activity of groups of genes during embryonic development.

Pierani and colleagues also traced the anatomic wiring of these *Dbx1* neurons in the mouse spinal cord—showing that they arose on one side of the spinal cord, but crossed to the other side to connect to motor neurons. Pierani knew that such a “wiring diagram” would be characteristic of circuitry that controls left-right locomotor activity.

To explore whether such V0 interneurons actually contribute to the central pattern generator controlling left-right alternation, Goulding and his colleagues performed electrophysiological studies on isolated mouse spinal cords. They found that, while normal spinal cords showed standard alternating left-right phasing of electrical activity of motor neurons, genetic knockout mice lacking *Dbx1* showed abnormal simultaneous activity. This abnormal pattern of activity would be characteristic of an ataxic walking gait in the *Dbx1* mutant mice.

According to Goulding, the dissection of the central pattern generator offers a valuable model system for understanding autonomous local spinal circuitry. “There are few such systems in which you can hope to understand how each component within the circuit generates some aspect of a behavior, and arrive at an overall understanding of the system,” he said.

Jessell views this as the beginning of what could be a rich harvest of new knowledge using this approach. He said the genetic technique used to distinguish V0 neurons represents only the first of what are likely to be many derivative techniques that will be used to identify specific subsets of neurons in both the brain and spinal cord.

“For example, there are probably a dozen different sets of interneurons, and the expectation is that each of these sets will have a genetic identity,” said Jessell. “And if that’s the case, then one can make molecular inroads into selectively manipulating those neurons.” Such manipulation, said Jessell, could involve selectively inactivating specific neuron subsets by using their distinctive transcription factors to change their identity or to introduce substances to silence or kill them.

“With silencing techniques, for example, you could analyze locomotor behaviors when those neurons are functioning, then silence them and look at the changes, then reactivate them once again,” said Jessell.

Such identification and manipulation techniques may help scientists develop clinical strategies to restore spinal cord function, he said. “Part of the problem of restoring motor function in patients with spinal cord injury is re-establishing connections between the brain and the spinal cord,” he said. “But another major part is understanding enough about the spinal cord motor system to figure out how to restore the wiring correctly to achieve function.

“This technique represents a small step toward understanding the logic of spinal locomotor control. Once we understand that logic, we should in principle have a better insight into how to manipulate the damaged spinal cord in a way that restores function,” said Jessell.

Genetic identification techniques could enable researchers to distinguish which types of neurons are most affected in animal models of spinal cord injury, and which types are most critical for restoring function, said Goulding.

“That knowledge may also enable strategies of selectively focusing on regrowing essential core components of the damaged spinal cord and paying less attention to less critical cells,” he said.