New approach helps rodents with spinal cord injury breathe on their own

October 17 2017

One of the most severe consequences of spinal cord injury in the neck is losing the ability to control the diaphragm and breathe on one's own. Now, investigators show for the first time in laboratory models that two different sets of neural signals control the movement of the diaphragm—one that originates in the brain and one that starts in the spinal cord. The researchers used a drug to turn on this alternative nerve pathway and restore breath-like movements in rodents. The study appears October 17 in *Cell Reports*.

"We realized that in order to further our research on how to direct nerve regeneration after spinal cord injury, we needed to first understand how this spinal activity arises," says first author Jared Cregg, a graduate student in neuroscience at Case Western Reserve University (CWRU) School of Medicine. "We took a basic approach to get at this question, which revealed the true nature of the mechanism."

Rhythms in the nervous system are controlled by neural networks within the brain called central pattern generators. For breathing, the central pattern generator is located in the medulla region at the base of the skull. When the connection between the brain and the nerves that control the diaphragm is interrupted due to injury, the ability to breathe is lost.

"It turns out, however, that not all activity signals come from the brain," says senior author Jerry Silver, a Professor of Neurosciences at CWRU. "We know that for movement of the limbs, the spinal cord sends signals to the motor neurons independent of the brain. This is why chickens are still able to run around after their heads are cut off."

He explains that with locomotion, sometimes the role of the central pattern generator is actually to send signals to inhibit movements, so they become more controlled. "We wanted to see if there are similar signals coming from the spinal cord that can control the movement of the diaphragm and whether we could bring them out by blocking the inhibitory signals," he adds.

There were three parts to the research. In the first set of experiments, the investigators used a laboratory model of ex vivo spinal cords that had been isolated from neonatal mice. When the spinal cords were bathed in drugs to block the inhibitory transmitters, the team detected spontaneous activity in the phrenic motor neurons—the nerves that control the movement of the diaphragm. Because there was no brain connected, this finding indicated that the spinal cord was able to control the diaphragm independent of signals that come from the medulla.

"But these signals were not like those that induce breathing," Cregg explains. "They were not as rhythmic, and they had longer durations between them. It was clear that this activity was not respiration but a different, independent system."

"We're not sure what this system does normally, but my speculation is that it has to do with the startle and gasp reflex," Silver adds. "This is a primitive response that has been kept in the spinal cord for emergencies, like gasping and screaming in response to danger."

In the next set of experiments, the investigators set out to determine whether these nerve impulses could be controlled to make them more like regular breathing. Using the same ex vivo model, they applied optogenetics technologies and showed that they could give the phrenic motor neurons a faster, breath-like rhythm with the use of light pulses to control the firing of the nerves.

Finally, they conducted experiments in adult rats and mice that had one side of their spinal cords severed at the C2 level (in the neck). The animals were then treated with drugs to block the inhibition of signals from the spinal cord. "When we did that, we saw these bursts of movement in the diaphragm on the side of the body where the spinal cord had been cut," Cregg says. "They were able to maintain this activity completely independent of the brain."

The next step in the research is to determine whether the optogenetics technology can be used to turn these spontaneous signals into real breathing in adult lab animals.

"Ultimately, the goal of this research would be to free people with these neck injuries from having to use mechanical ventilators," Silver concludes. "Infections and other complications from mechanical ventilators are a leading cause of death after spinal cord injuries."

More information: *Cell Reports*, Cregg et al. "A Latent Propriospinal Network Can Restore Diaphragm Function After High Cervical Spinal Cord Injury." www.cell.com/cell-reports/full … 2211-1247(17)31381-5 , DOI: 10.1016/j.celrep.2017.09.076

Provided by Cell Press