AUTOMATED MOUSE CAGES HELP REVEAL SUBTLE DISEASE SIGNS

Automated home cages deliver big data that let scientists monitor animals' activity, food and water availability, and other parameters around the clock, opening NEW DOORS TO DISCOVERY.

For people with amyotrophic lateral sclerosis (ALS), early diagnosis matters. They survive just 3 years on average after diagnosis of the motor neuron disease, which affects 50,000 people worldwide, and is the most common adult-onset neurodegenerative disease. Those who receive an early diagnosis benefit most from drug therapy, are most likely to be eligible for clinical trials, and can access early medical care to prolong survival and improve quality of life. Yet ALS symptoms mimic those of other neurological diseases, and on average it takes doctors more than a year to offer a definitive diagnosis after a patient first presents with symptoms.

That's why neuroscientist Silvia Mandillo, a neuroscience researcher from of the Institute of Biochemistry and Cell Biology at the National Research Council in Rome, was intrigued to learn that a common mouse model of ALS known as $SOD1^{G93A}$ was behaving oddly. Mice are supposed to be nocturnal, but scientists at Tecniplast, a leading manufacturer of automated mouse cages based in Milan, Italy, told her that they were seeing the transgenic mice move more during the day when they should have been sleeping, and more than their wild-type littermates did.

Mandillo knew from previous studies that sleep disturbance was common in ALS patients, and that as the disease progressed, it contributed to declines in their quality of life. "Seeing irregularities in day activity only in the transgenics made us think that [it] could be related to sleep," Mandillo recalls.

A few years earlier, a team led by Andrew Grierson of the Sheffield Institute for Translational Neuroscience monitored $SOD1^{G93A}$ mice on odometer-equipped running wheels to detect early motor dysfunction. $SOD1^{G93A}$, today's most popular mouse model for ALS, overexpresses a human superoxide dismutase 1 mini-gene containing a single mutation at position 93, leading to motor neuron disease, limb paralysis, and eventual death at 5 to 6 months of age. Grierson's team monitored $SOD1^{G93A}$ mice activity daily for more than 4 months, and they reported in *PLoS One* in 2014 that the mice showed signs of motor function decline as early as day 40, with rapid decline thereafter.[1]

Grierson's study was based on a single exercise measurement, but Mandillo knew that she needed a more detailed picture of the animals' movement patterns to understand the connection between sleep disturbances and ALS. To understand these patterns in detail, sophisticated monitoring was required. That's where automated home cages came in.

"AUTOMATED MONITORING IN HOME CAGES MAY BE JUST STARTING TO REACH ITS POTENTIAL."

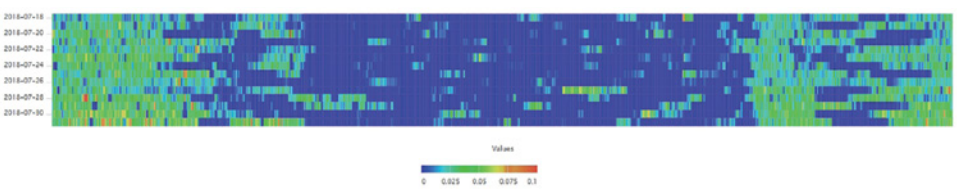
Troubling Signs

Mandillo and her colleagues used Tecniplast's DVC® automated cages to monitor each SOD1G93A mice in its home cage — the cage where the animal typically resides and where it experiences minimal stress. Unlike earlier mouse cages, DVC® home cages let researchers make sophisticated observations of an animal's activity, its food and water availability, and other parameters, providing volumes of physiological and behavioral data.[2] Mandillo's team monitored these parameters 24 hours a day for more than four months. "This is very important in longitudinal studies, where you want to capture events along the whole progression of the disease from the asymptomatic to the end stage," Mandillo says.

To identify sleep pattern changes, the researchers developed a quantitative measure called the Rest Disturbance Index (RDI) that captures the odd animal activity and movement during the day when the mice should be asleep. In unpublished work posted in December to BioRxiv, Mandillo and her colleagues suggested that RDI could detect sleep-related disturbances in the ALS mice, and it proved highly correlated with a test for mouse motor function and with body weight declines.[3]

"The RDI rise started earlier than the commonly used measures of symptoms onset, making it even more clinically relevant, since sleep disturbances are not only common, but also premorbid

UNDER OBSERVATION

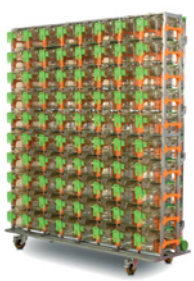


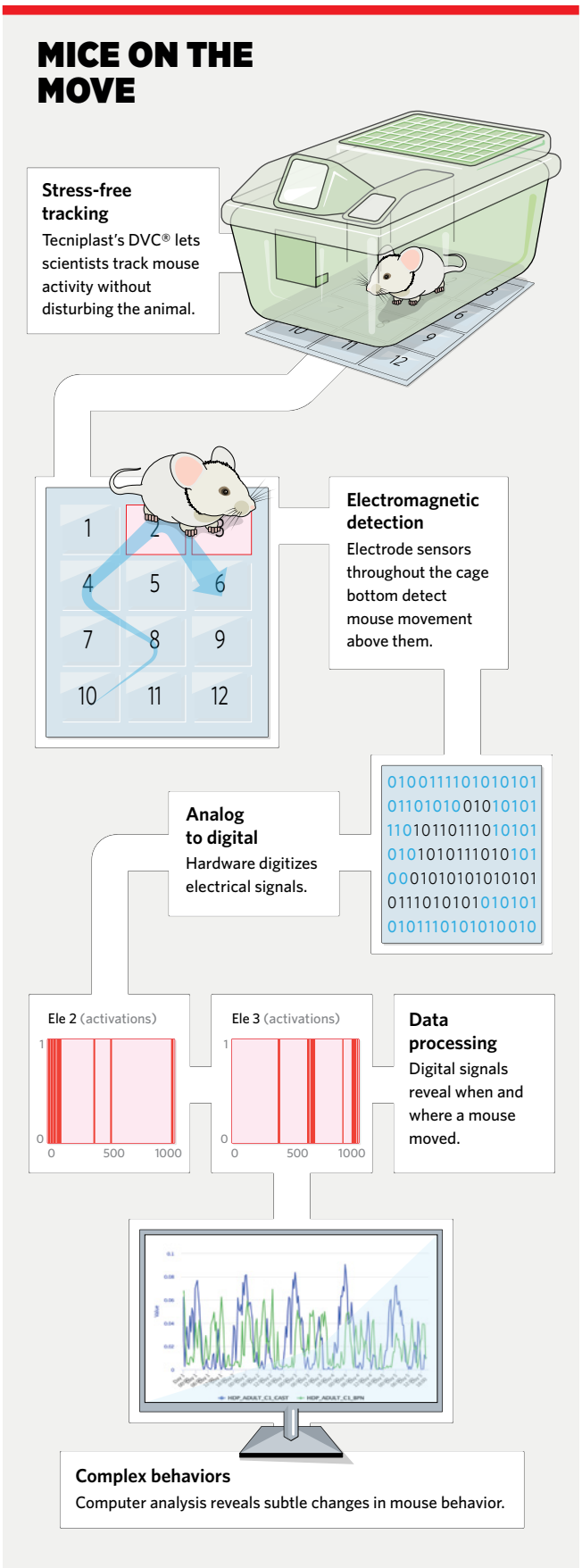
PATTERN DETECTION

Digitally ventilated cages (DVC®) generate a rich array of ongoing data on mouse activity, answering questions such as how far and how fast they walk, when they sleep and wake, and how they interact socially.

HOME RACK

Monitoring mice without disturbing them enhances animal welfare.





in many neurodegenerative diseases," Mandillo says. If the results hold up in peer review, then measurements of rest disturbances may one day be useful in measuring early ALS progression.

Cages of the Future

Tecniplast's DVC® home cages use electromagnetic field technology (EMF), employing a series of electrode sensors located underneath the cage bottom to monitor mouse movement. The DVC® system allows for fewer staff interactions with mice than in a typical animal facility, and the EMF exposure causes no short- or long-term clinical or biological effects. It also does not raise mouse anxiety, or alter their cognition or overall welfare, according to recent studies, including one reported last year in Toxicologic Pathology.[4] By monitoring individual mice 24 hours a day, 7 days a week for months on end, the DVC® home cages enable researchers to detect activity patterns that can't be detected by removing animals from their cages to the lab and observing them.

This long-term monitoring also reduces an animal's stress from being handled and taken out of its safe environment of the home cage, notes Amanda Kiliaan from Radboud University. Animal stress can lead to experimental problems and irreproducible results that make it difficult to spot subtle physiological or behavioral effects.

That's one reason Kiliaan used the DVC® cage to help investigate whether Mediterranean diets with extra-virgin olive oils protect mice from cardiovascular and cerebrovascular events. In 2019, she asked if hydroxytyrosol (HT), the most common polyphenol in olive oil, could impact recovery from stroke.

Spotting Complex Behaviors

Following a stroke, mice can be paralyzed on one side, leading to a directional preference when they turn. Kiliaan believed she could use this preference and how it changes over time to investigate stroke damage and recovery. To detect such a subtle motor change, she used DVC® cages, which employ sensitive capacitive sensors that enable detailed and precise measurements. "You can monitor turning preferences and how often and how fast [the mouse] turns," she explains.

Kiliaan and her colleagues fed mice that had undergone an induced ischemic stroke high-HT diets, then used daily physiological tests and motor-function assays to monitor their movements and behaviors. They also continuously monitored mouse movement, especially at night — which uncovered something interesting. One week after the induced stroke, nighttime activity decreased, and 2 weeks afterwards, the left-turning preference appeared, suggesting that HT can improve stroke recovery.[5]

By monitoring complex behaviors, the DVC® system could also help test experimental drugs. For example, Mandillo hopes to use the DVC® and Rest Disturbance Index to test potential treatments for sleep-related symptoms in ALS.

Automated monitoring in home cages may be just starting to reach its potential, both Mandillo and Kiliaan say. Researchers may soon be able to monitor a variety of complex behaviours, yielding new insights on neurological diseases. ■

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