MONITORING MICE TO IMPROVE REPRODUCIBILITY

Technology that remotely tracks mouse cages can reduce animal stress and reveal environmental factors that undermine the reliability of **EXPERIMENTAL DATA.**

Mice are creatures of habit and instinct. Even in the unnatural setting of a laboratory animal facility, they maintain many behaviour patterns exhibited in the wild, such as innate circadian rhythms. They also establish consistent captivity-specific behaviors, including aggressively enforced social hierarchies among cage-mates.

Interference with animal routines can have a profound and lasting consequence on their behaviour, and scientists who ignore this effect experience the consequences. Brun Ulfhake, a neuroscientist at the Karolinska Institutet in Stockholm, found that the act of moving lab mice from their home cage to a separate testing area was undermining his team's efforts to study the neurobiology of ageing. "Not only was moving them to the testing arena complicated, but the manual handling and observation were also causing a big problem," he says.

The key issue is stress, whether caused by a change of scenery, shifts in the temperature, noise of the laboratory, or the sensory overload associated with the sudden arrival of a laboratory technician. Stress sets off a chain reaction of physiological and behavioral changes that can make the instinct-driven mouse much less predictable, undermining efforts to draw robust conclusions from experiments involving that animal. Jeffrey Mogil, and colleagues at the University of Illinois at Urbana-Champaign, found that these types of non-experimental environmental factors accounted for 42% of the variability in how mice responded in a study of pain perception[1].

Mat Leblanc, senior director of animal resources at the Salk Institute for Biological Studies' Behavior Testing Core in La Jolla, California, points out that although this stress problem is recognized, it remains poorly characterized and addressed. "We've done an amazing job of controlling the variability that comes from the mouse," he says. "But we just don't control the environment."

Cages with automated monitoring systems could help mitigate this problem, by allowing researchers to surveil animals without entering the cage room. These systems monitor physical activities of residents and track key aspects of the cage, such as food and water levels or whether the bedding is due for a change. By reducing human interaction, these technologies can enhance animal welfare and reduce experimental variability caused by stress. But many of the available commercial systems require specialized facilities and are costly. A new generation of automated home cage monitoring systems promises to make these advantages more widely accessible to the animal research community.

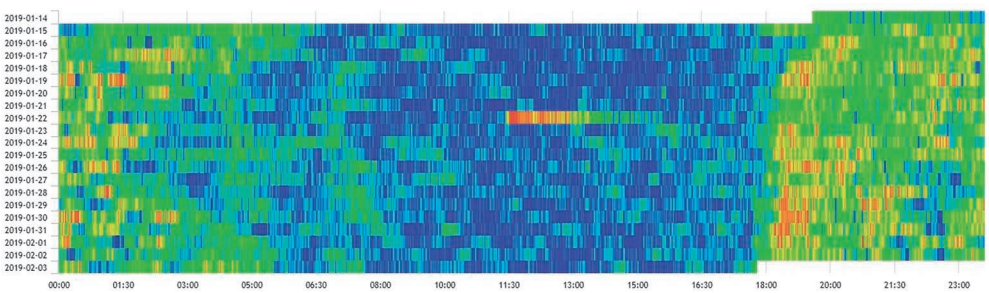
CAGES THROUGH THE AGES

Researchers have been exploring the use of automated caging systems for nearly 20 years. Perhaps the first such system was the IntelliCage, developed in 2002 by Hans-Peter Lipp[2] and commercialized by NewBehavior. This platform tracked animal weight and food and water consumption, and used infrared detectors to follow its movements. "This is a very clever system," says Ulfhake, "but it's kind of big, and not that affordable." In the ensuing years, several other systems have been developed by both individual labs and commercial manufacturers. Some of these offer sophisticated tracking capabilities such as video monitoring, but this also makes them expensive and difficult to scale[3].

In 2015, Italian company Tecniplast launched the Digital Ventilated Cage (DVC®) system[4] as a more accessible and scalable cage-monitoring solution. DVC®, based on electromagnetic field technology[5] (EMF), is compatible with existing cage racks and accessories so can be directly introduced into most animal facilities. DVC® racks are washable and autoclavable, and the EMF technology has been shown to have no long-term definite effects on mice[6]. "It's truly integrated into the holding unit, enabling automated high-throughput data collection from thousands of cages simultaneously," says Ulfhake, who began testing the DVC® system in 2017. This platform can track animal activity, food and water availability[7] and other parameters.

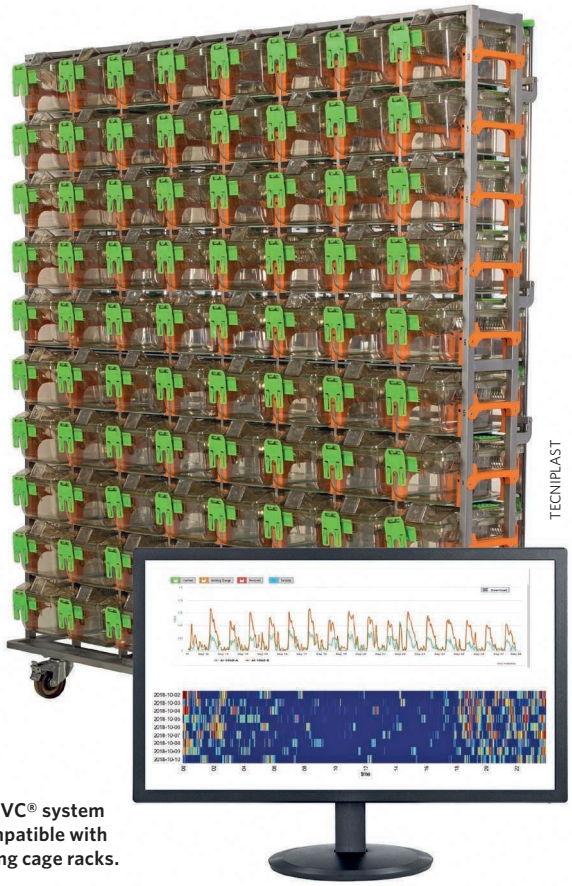
"BEDDING AND ENVIRONMENTAL ENRICHMENT HAVE A HUGE IMPACT ON ACTIVITY LEVELS."

DVC® employs an array of



TECNIPLAST

The DVC® system monitors animals' activity day and night



The DVC® system is compatible with existing cage racks.

electrodes that can monitor physical activity and reveal patterns of movement and behaviour based on changes in conductance as an animal passes over them. This enables researchers to quickly determine whether animals have experienced a disruptive stressor based on changes in activity. Giorgio Rosati, corporate marketing manager at Tecniplast, notes that upticks in daytime movement are often indicative of atypical behavior in the normally nocturnal mouse. This might happen after a cage change, for instance. "That could mean there is a stress in the mice because they have to rebuild their environment," says Rosati.

Every animal facility has its own ecosystem of potentially disruptive stimuli. Differences between research centres can manifest as irreproducible results. Ulfhake's team at the Karolinska recently employed the DVC® in a multi-centre test with The Jackson Laboratory, in the US, and Italy's National Research Council (CNR). Their goal was to determine the extent to which site-specific variability affects the behaviour of identical populations of mice receiving equivalent routine care. The results were somewhat alarming[8]. "We saw a huge variance between sites, despite having a well-controlled protocol," says Ulfhake. At CNR, for example, the animals' day–night activity patterns were completely different to those of their counterparts at the other two sites.

The multi-centre team is still trying to understand the sources of this variance, which will take a fair bit of detective work. However, the continuous tracking from automated cage systems makes the investigations easier. Leblanc's team have used their DVC® units to identify several confounding factors at Salk. "Bedding and environmental enrichment have a huge impact on activity levels," says Leblanc. This finding supports work from Craig Fletcher's team at the University of North Carolina at Chapel Hill, who used their DVC® system to show that adding an enriching 'mezzanine' platform in a cage can change aggressive behaviour among co-housed male mice[9].

Insights from these DVC®-enabled findings can help animal facilities to optimize their care protocols and reduce staff interactions with the animals to the minimum needed to keep the mice safe and healthy. This in turn can be beneficial for the animals, the researchers, and a facility's bottom line. "We have technicians checking hundreds and hundreds of cages every day," says Leblanc. "If we can have longer cage changing cycles, or monitor cages without having to look into them every day, that's going to decrease our costs."

SPECIALIZED TESTING

Equally exciting is the possibility of using systems like DVC® to conduct experiments without animals ever having to leave the security of their home cage. At Salk, researchers are using the system to study the behaviours specific to an anxiety-prone strain of genetically modified animals. "The activity patterns tracked by the DVC® correlated with some of the behavioural testing that we can do with specialized apparatus like the Y-maze or open-field test," says Leblanc. Rosati also highlights work from the Karolinska/CNR/Jackson group showing that DVC® can detect changes in locomotion patterns that track disease progression in mouse models of the neurodegenerative disorder amyotrophic lateral sclerosis.

This technology is still new, and early adopters are still figuring out how to make the most of the deluge of data that cage monitoring systems can produce. And this volume is only going to increase — the DVC® platform can accurately map animal-specific movement not only for single mice, but also for multiple mice within one cage.

Some early users already have an ambitious vision for cage-monitoring technologies as a means to reduce the use of laboratory animals in research. Ulfhake predicts that, as more researchers begin recording the activity profiles of their various rodent strains under both 'normal' and disruptive laboratory conditions, there is the potential to create a shared resource — a virtual control cohort for behavioral studies. "By leveraging high-throughput automated data collection technologies, such as DVC®, we can build huge libraries about behaviours of different strains, substrains, ages, sex, and have a knowledge base to draw on when we assess new behaviours," says Ulfhake. "That would be very valuable."

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