Home-Cage Monitoring and Its Effects on Research Capability and Outcomes

An Expert Panel Discussion

Moderator

Kevin Davies, PhD

Executive Editor

The CRISPR Journal

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Expert Panel

Dr. Kevin Davies (moderator) is the executive editor of *The CRISPR Journal* and editor-at-large for *Genetic Engineering & Biotechnology News* (GEN). He is the founding editor of *Nature Genetics* and author of four books including *Cracking the Genome* and *The $1,000 Genome*.

Dr. Amanda J. Kiliaan is associate professor in anatomy and neurosciences and principal investigator with Donders Institute for Brain, Cognition and Behaviour, at the Radboud Alzheimer Center and Preclinical Imaging Center (PRIME) of Radboud University Nijmegen Medical Center in The Netherlands. Dr. Kiliaan's research is focused on brain structure and function in human and mice models for neurovascular and neurodegenerative diseases (including hypertension, stroke, obesity, and Alzheimer's disease). With neuroimaging, cerebral hemodynamics as very early biomarkers for neurodegeneration are studied, but also gray/white matter integrity, in collaboration with clinical research groups (geriatrics, neurology, and radiology) and food industries. She is also interested in the influence of diets on brain function and structure.

Dr. Jan-Bas Prins is the director of the Biological Research Facility of the Francis Crick Institute, London, and professor of Laboratory Animal Science at Leiden University in The Netherlands and honorary professor of University College London. He is a member of the Netherlands National Committee for the protection of animals used for scientific purposes, chairman of Laboratory Animals Ltd, vice-chairman of the Scientific Committee of the Fondazione Guido Bernardini, vice-President of the Institute of Animal Technology, animal welfare officer and scientific advisor on animal welfare of Infrafrontier (IF2020), and an AAALAC ad hoc specialist.

Dr. Ernst Sigmund Brun Eric Ulfhake received his MD at Karolinska Instututet (KI) 1980. He was a practicing physician and served as designated diving physician in the naval forces until 2003. Dr. Ulfhake received a PhD at KI in 1982, became an associated professor in 1988, a lecturer of anatomy in 1994, and professor of anatomy from 2001 to present within the Neuroscience Department of KI. His main research interests are aging and the effect of dietary restrictions. During 1979–2019, Dr. Ulfhake published 119 articles in international journals including *Nature* and *Neuroscience* with >4000 citations. He has supervised 14 students to PhD and 15 visiting post docs. During 2013–2018 he served as director of Comparative Medicine, KI.

Home-Cage Monitoring and Its Effects on Research Capability and Outcomes

Moderator: Kevin Davies, PhD[1]

Participants: Jan-Bas Prins, PhD,[2,3] Brun Ulfhake, MD, PhD,[4] and Amanda Kiliaan, PhD[5,6]

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Experts discuss the current state of high-throughput home-cage monitoring, inherently enabled by digital ventilated cage technology and its effects on research capabilities now and in the future.

Dr. Davies: **First, what is home-cage monitoring and what impact can this have on mouse research?**

Dr. Prins: In general, home-cage monitoring is about monitoring the behaviors of animals—in my case mostly rodents—and also zebrafish in their home cage. It is the cage in which they are normally housed and it allows you to observe their behaviors in a 24/7 setting. It could include infrared monitoring and electromagnetic fields that you can apply. There are different modalities but that is what we mean when we say home-cage monitoring.

Dr. Ulfhake: We should stress that these recordings are not intrusive. With other methods, when we record behaviors, we handle the animals somehow. We bring them out of their normal context. That means that the recording includes a response to handling and taking them out of their standard context. So, we will have additional distortions of the data based on the fact that we are not recording them in their normal setting. I think that is a very important advantage for the home-cage monitoring and, as Jan-Bas said, there are a number of techniques with which to do this.

Dr. Davies: **With the ability to collect data directly from the rodent cages, what are the advantages versus traditional individually ventilated cage (IVC) systems? Is there an advantage performing night-time activity collection versus daytime collection, for example?**

Dr. Kiliaan: Yes, indeed, the advantage is that the animal's active period is during the night and, therefore, you can pick up subtle changes without stress for the animals, as Brun also described. You can monitor activity, walking patterns, because they move much more and turn much more during the night. For example, in stroke research, it is important to look at turnings and laterality—if they turn left or right as a preference. We could not pick that up during the day in our stroke experiments.

Dr. Prins: Amanda covered it well. It is very important for studying these animals, especially when you talk about rodents, mice and rats specifically, as they are nocturnal animals. The time that they are most active and you are more likely to find any behavioral differences is probably during the night. That is a great advantage; you actually pick up behaviors that you are normally not seeing when you are there when the animals are asleep, when you have daylight in your animal holding room.

Dr. Ulfhake: An IVC is a holding system that provides some protection for the animals living inside the cage, compared with open cages, but it is not a system by which you collect data. The home-cage monitoring is something you add-on to the IVC to get access to that information.

What is of clear importance, beyond what Amanda and Jan-Bas mentioned, is the capacity to integrate it into the holding quarters of the facility, so it is not an obstacle to obtain extended recordings. I have just been analyzing noninterrupted recordings for 16 months and it is amazing what you can get out of such a data set, which was completely unknown to me before I dug into it.

Also once these systems (digital ventilated cage [DVC®] and other systems) will enable us to integrate it into the standard housing units, we can have access to detailed data to a much larger extent. Perhaps 5–10 years from now, we can collect data from tens of thousands of cages across the world on different strains, and build up databases of behaviors, nocturnal activities, seasonal variations, etc. It is a very interesting era to be a behaviorist.

Dr. Davies: **Building off what Brun was describing, what kinds of data can you get from this new technology?**

Dr. Prins: In the DVC® system, the data that you can obtain are related to spontaneous behavior patterns. That is a bit different from when you have other systems that are actually more of an experimental setting most of the times, with limited number of cages. The advantage of the DVC® is that you can have multiple cages in a normal husbandry situation, which is quite different from those other systems.

Having said that, the information that you can get is limited to the spontaneous behavior that can be measured from a plate that is positioned below the cage. And that activity is determined on the number of animals in the cage. It might be possible to discriminate the activities from two animals in the cage; however, when you put more animals in the cage, it will give you more of an overall behavioral pattern of that entire group.

So, the major advantage of the DVC® is that it is in a real home-cage situation where they would sit in a normal husbandry environment in an animal holding room.

Dr. Kiliaan: Exactly, you can record normal behavior in the home-cage environment. In my research, we perform surgery to induce stroke, and the animals are, therefore, single housed in the DVC® so you can really monitor the walking patterns and the activity of one mouse. It is really an advantage to house the mice in DVC® cages because we study (dietary) treatments longitudinally in both male and female mice in stroke and obesity research, in other words large groups of mice followed for months 24/7, which is very labor intensive when performed with traditional behavior tests. Thereby with DVC® you can really detect subtle improvements and recovery after stroke, which we could not pick up with the normal open field experiments. Also, we can see the subtle differences between male and female in recovery after stroke.

In our obesity research, the animals are housed together. Then, you get the overall group activity information, 24/7 for months, just from the home cage.

Dr. Davies: **Are there other intangible benefits to the system? Perhaps benefits to your colleagues, your staff, the ability to spend more time on data analysis and data collection?**

Dr. Prins: The DVC® system is a technology that is now available for the home-cage monitoring in a normal husbandry situation, it is also very amenable, especially in situations wherein we are dealing with a lot of, for instance, genetically modified lines, some of them recently generated; it allows you, under these normal situations, to actually monitor these animals for any deviant behavioral phenotypes.

That is an additional benefit, not just to the staff but also it is of benefit to the researcher, because that gives you quickly and easily some additional information on these new strains. I find that is a benefit in the context of when you have a facility where you have a lot of genetically modified strains and also where you are generating quite a lot of these lines on almost a daily basis.

Dr. Ulfhake: We can learn much more from the signals that we generate across the DVC® sensors, and we are still in the very infancy of analyzing the data captured. Also, it is important to realize that what you record with the DVC® system is what is happening on the floor. So, that is a limitation that we must be aware of.

What we use the data for now is very simple analysis, wherein we use the activation or absence of an activation of the electrode. We do not have any pattern recognition, etc. Once we understand how to make the best use of the data, we will be able to apply much more clever algorithms than those we are using today.

That could certainly be of great value for phenotyping, as mentioned by Jan-Bas, to recognize deviation from the background strain and so on. Once we collect enough data we can build libraries, it will be quite easy to tell whether we have a deviant behavior of the mice living in the cage.

Also today we do not have any clever way to convert signals over time across the plate into activity patterns. But that is something I think we can use probability calculations for and at least come up with good approximation of what a certain record of activities corresponds to in real life.

Dr. Davies: **Which specific research applications, perhaps particularly in your group, could be improved or are being improved using this sort of technology?**

Dr. Kiliaan: As mentioned, we perform research models for stroke and Alzheimer's and obesity, as well

as movement disorders. What we could not detect before in a normal open field is recovery, turnings and walking patterns that are improving after a stroke and due to intervention. You cannot pick that up in the daytime open field. But you can during the night. You can really see the improvement and the recovery of animals after stroke because you can measure and monitor longitudinally over weeks or months. That could be very useful in studies on stroke, also Parkinson's and other movement disorders.

I also perform dietary studies—the diet as treatment in stroke, hypertension, obesity, and Alzheimer's. You can really pick up the impact of diet or diet components on different mechanisms and recovery of the animals.

Dr. Prins: We are using it for trying to identify phenotypes, we are looking at behavioral patterns, and also using it in preference situations, and to see whether aspects we change affects their behavioral patterns.

For instance, there is a big debate going on whether mice should be only handled by picking them up with a tube or by cup handling instead of picking them up by the tail. That is a debate in Europe. Actually, it is stimulated by Jane Hurst, who came out with the first publication[1] about this, that the anxiety levels go down, and it improves the welfare of the animals when they are cup handled or tube handled instead of tail handled.

So, those things can be looked at and analyzed also in this context. Of course, that is not the end of what you can do, but it is one of the instruments that can be used in context with other measurements.

Dr. Ulfhake: I think there are two aspects to it. The one brought up by Jan-Bas that actually can be very useful for refining husbandry of the animals. In the article we recently published,[2] we showed that cage change is a very dramatic event for the mice inside the cage and that there is a clear gender difference.

Male mice have less capacity to deal with it and show a crossover impact on their response to other events the following days. The response to, say, lights-on after a cage change is different than that the day before the cage change. It will have impact on the animal's behavior for several days when we talk about males, whereas the females appear to calm down within 24 h after the cage change. We can learn a lot about how they respond to events and procedures by interpreting their activity recorded by the sensors.

I have done behavior studies on mice and rats for ~20 years, and using the data, I can have access to now, 24/7, uninterrupted for a year or more shows me that they have a much more complex repertoire of behaviors than I thought they had. Then you have other seasonal variations, and all this was more or less not possible for me to take into consideration because I did not have any metrics for it. The DVC® can help us with that.

Dr. Kiliaan: You can also look at impact of different staff and animal caretakers on the animal behavior, or what happens during the weekends when no one is there. For example, the Alzheimer's mice models get seizures when they are stressed. Sometimes things happen during the weekends or because they are handled by one certain staff caretaker. Those things you can detect, find what is going wrong, and can adjust. Why are the animals dying or in stress? Is it due to renovation in the facilities or due to way of cleaning cages? Those are examples you can detect.

Dr. Davies: **How important is the possibility to measure anomalies, social behavior, environmental enrichment, cage density, and utilization or the influence of lack of food and water in the cages?**

Dr. Prins: I think for all of us, and with what has been said, it is definitely an additional and very helpful tool to get a better grip of what is going on in the cage and to measure all of that. So, it is important and very helpful, both in the context of experiments and in the context of just a regular colony management situation, where you want to not only be dependent on the daily or once-daily check during the daytime but also at least have a good feel of what is going on at other times of the day in those cages. From both views, it is very helpful and important to measure all of this.

Dr. Davies: **Do you think that automated 24/7 home-cage monitoring enables scientists to unveil information about animal behavior that would not be discoverable otherwise?**

Dr. Kiliaan: Yes, we found improvement of information on animal movement. Especially during the night, you can monitor, in my case, recovery over time, because you measure 24/7 and we could not pick this up with the conventional tests.

Dr. Prins: Yes, I think that is true for any 24/7 monitoring particularly for spontaneous behaviors. There are also times that your panel is not just based on the spontaneous behaviors; sometimes you need to do something like provoked behaviors or you need to do some additional things. Of course, then this system or any other home-cage monitoring system will not pick it up. So, yes, for spontaneous behaviors and that is true for any automated 24/7 home-cage monitoring system.

Dr. Ulfhake: The advantage here, I think, compared with other systems is that this is already integrated into standard home-cage units. You can use it in a large scale and that can provide us with much more rapid data collections to be used as baselines for recognizing deviations and so forth.

It has been impractical to do these recordings previously so we will learn new things about how these laboratory animals behave in the cage. It will be important and set the new standard for behavioral testing. And I think that the basis will be generated by data captured by home-cage monitoring systems, like the DVC® system. I foresee the next step in the development will be to add on devices to such a system, still being compatible with the cage system in the holding rooms. Then you can set up much more complicated challenges. If you would ask me 10 years from now, I would probably say yes, in the old days we brought out the animals. Now we bring in the challenges instead.

Dr. Davies: **Are there any points that you feel sets Tecniplast and the DVC® system apart from its competitors?**

Dr. Prins: As far as the 24/7 monitoring of spontaneous behavior in the true home cage, in a normal husbandry setting, that is not available through any other company I know of.

Dr. Kiliaan: Yes, I am thrilled by the possibilities that we have with these cages. As I said, I perform stroke research and if you want to study walking patterns and so on, there are very expensive setups that you can buy. Having the animals in the cage and letting them just walk normally is a great advantage. And as Brun said, you can also bring in other gadgets, such as running wheels, to measure other very important parameters in healthy aging/lifestyle research, etc.

Dr. Ulfhake: I agree with Amanda and Jan-Bas. This system is unique as of today, and there is much room for growth before we reach the limit on what kind of information we can get out from the stream of activities generated across the sensors.

It is important to note that the great advantage is that it is inside the standard holding unit. It does not take more room. It is easy to scale it up to thousands of cages and it is possible to collect all these data and analyze it. It can be done more or less in real time. That is very important, you can monitor the ongoing experiment. Still, it is limited to what is happening on the floor. To take this further, to really do more sophisticated behavioral analysis, we have to build on this system and add pieces to it. I think that will be the next step.

Dr. Davies: **Very good. Many thanks to our panel for your very interesting observations and comments.**

Disclosure Statement

All participants confirm they have no competing financial interests to disclose.

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Automated Home-Cage Monitoring Promotes Reproducibility and Data Capture

Christina Bennett, MS

Although mice are the cornerstone of animal experimental systems, they are fraught with challenges. When using mice, researchers must ensure good husbandry practices and document animal welfare on a daily basis, all while trying to generate reliable reproducible data for the scientific community. These tasks can prove particularly challenging because the animal handling required during routine cage changes for proper husbandry or experiments held outside of the cage can influence animal behavior and ultimately the results. In addition, the environment in animal rooms exposes the animals to a variety of disturbances, such as the presence of humans, noise, and lights. These environmental factors differ across facilities and impact animal behavior and activity patterns.

Automated home-cage monitoring systems can help address these issues because husbandry conditions are continuously monitored with nondisrupting technology, thus avoiding unnecessary cage changes and animal handling. In addition, such technology can help quantify potential impacts of environmental-related disturbances. In fact, a study recently published in *PLoS One*[1] demonstrated how automated home-cage monitoring systems not only reduced the amount of animal handling and improved reproducibility of experiments but also ultimately captured valuable data about animal behavior that typically is not possible with conventional cage setups.

"The study provides new insights into the undisrupted home-cage behaviors of group-held mice," says senior study author Brun Ulfhake, MD, PhD, Professor of Anatomy, Department of Neuroscience, Karolinska Insititutet, in Sweden. "We learned how mice respond to care-taking events, such as cage-change, and other events, such as transitions between day and night." Dr. Ulfhake explains that more detailed knowledge of the spontaneous behaviors in the home cage will assist in welfare surveillance and help improve husbandry, as well as improve experimental design and monitoring of ongoing experiments.

The study was designed to keep animals in their normal husbandry conditions and then perform routine tasks, such as cage change, all while capturing continuous data 24/7 with the digital individually ventilated cage system (DVC®) from Tecniplast. Female C57BL/6J mice were housed in DVC® systems at three facility locations: Consiglio Nazionale delle Ricerche in Rome, Italy; The Jackson Laboratory in Bar Harbor; and Karolinska Insititutet in Stockholm, Sweden. To observe gender differences, a fourth group of male C57BL/6J mice were housed in DVC® systems at Karolinska Insititutet.

All DVC® systems used a nondisrupting capacitive-based sensor board externally located under the standard individually ventilated home cage to collect data on animal activity within the cage every 250 ms, 24/7. The sensor board was made up of 12 capacitive-based planar sensing electrodes that measured changes in capacitance as animals moved across the cages and generated an activity metric for overall cage activity.

Mice were aged 6 to 8 weeks at the beginning of the study and activity was recorded with the DVC® system until 25 weeks of age. Fifteen home cages were set up at each site, and each cage housed five mice. Cages contained standard bedding, food, and cage enrichments, and the vivarium light cycles were set to 12 h on to simulate day and 12 h off to simulate night.

By continuously monitoring activity across several weeks, the DVC® system captured an abundance of data, such as the expected diurnal patterns of cage life. The data showed a gradual increase in mouse activity ~2 h after lights off followed by a high level of activity that varied over the next 4 to 5 h. This was followed by a decline in

activity and a subsequent burst of activity before lights on. Mice remained active for a short duration after lights on and then returned to their resting state. Overall, these findings suggest that the transition between day-to-night and night-to-day yields different responses in activity.

The DVC® system also elucidated considerable effects that normal procedures, such as handling, weighing, and cage change, can have on activity patterns. For instance, cage change led to an increase in activity that lasted for several hours for both female and male mice.

An evaluation of the activity across several weeks between two of the facility sites revealed statistically significant differences in the average activity and duration of activity. In addition, differences in peak activity, average activity, and duration of activity were seen among cages at the same site. Activity differences on the basis of gender were again observed, with female mice showing greater week-to-week variability in activity than male mice. Altogether, these variations underscore the complexity and sensitivity of animal behavior and indicate that activity is affected not only by site-specific factors, but also by group dynamics within each cage.

"The paper is a very clear example of the benefit that DVC® can have on the scientific community," says study coauthor Fabio Iannello, principal data scientist at Tecniplast SpA. He explained that under normal husbandry conditions, a cage-change operation is performed and no data are collected during this process. "If you had DVC® installed in every cage, you can actually perform a normal operation and collect the data and analyze the data, basically in real time," he says.

In addition, the DVC® system can extract continuous information when a researcher is running an experiment, and the data capture is quantitative metrics, not subjective observations. Such quantitative data capture is valuable for a wide variety of locomotion experimental tests. For example, a researcher conducing oncology-related experiments to understand the effect of tumors on mouse locomotion could collect these data during experimental tests as well as around the clock with the DVC® system without the need for animal handling. Furthermore, these data could be collected at night, when mice are naturally active. Dr. Ulfhake says, "You can really capture activity of the mice that otherwise you would not have."

Other monitoring systems can collect real-time information like DVC® does, but they lack scalability. For example, video camera-based systems can be installed on only a limited number of cages, whereas the DVC® system can be installed on a low number of cages as well as upward of thousands of cages. "There are no other tools that are as scalable as DVC®," says Mr. Iannello. "It's really a scalable system that can collect real-time information from thousands of cages."

In addition to being scalable, DVC® generates data with very low computational complexity, thus requiring minimal storage space. Video camera-based systems generate recordings that require significant storage space, limiting its utility. DVC® also has the advantage of being fully integrated into the facility management process and can be washed and autoclaved. Having these features, the authors explain, means DVC® does not change any of the conventional maintenance operations performed in a facility.

"Our results so far indicate that much remains to be done to harmonize the conditions of an experiment. If we make good use of this, the complementary data generated by the DVC® system will likely contribute to improved reproducibility of animal testing," says Dr. Ulfhake. "With a deepened understanding of how the DVC® data is best interpreted, I am convinced that more advanced behavioral testing will be done in the home-cage of the mice. This would be a highly significant refinement of the testing conditions."

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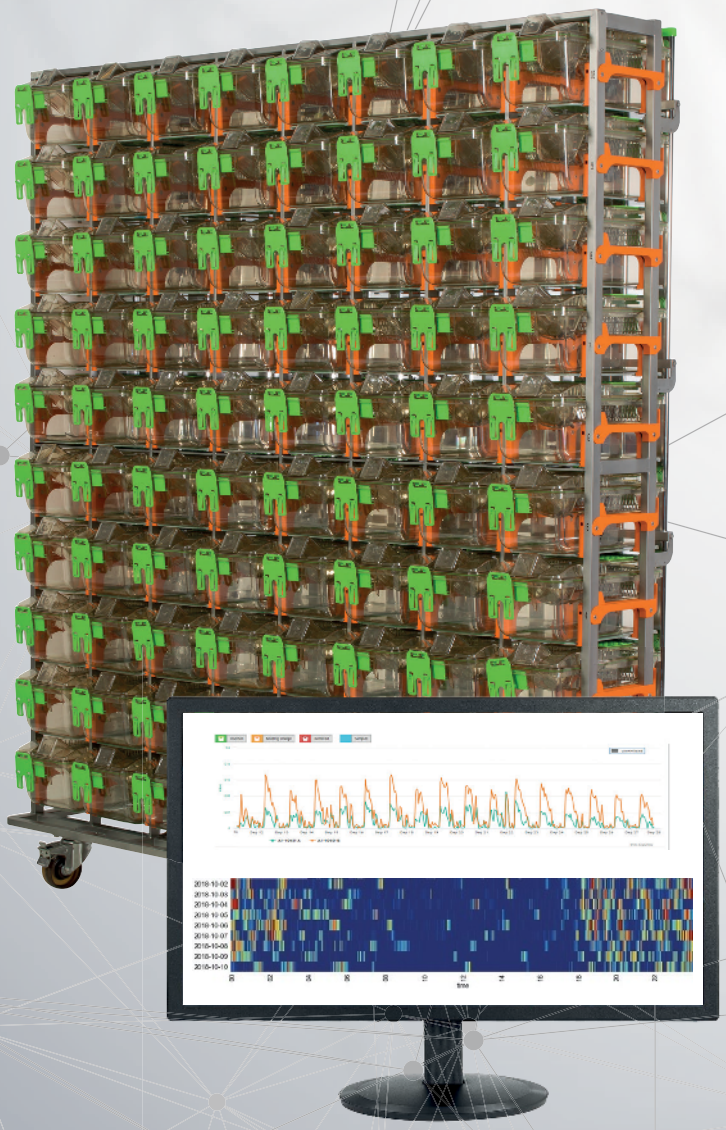

- Improve reproducibility of your experiments with automated and non-invasive data collection
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