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Progress report

Focus on novel approaches: Home-cage monitoring of laboratory mice

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Summary

Assessment of behavioural phenotype is crucial for the evaluation of various disease models, particularly in laboratory rodents. Traditionally, this includes performing a variety of conventional tests where animals are removed from their home-cages and placed in behavioural test apparatuses. This approach can be affected by micro-environmental stress (removal from cage, handling, moving to an unfamiliar setting, and the test itself) and other biases by capturing animals' responses in a short time-window and potentially missing subtle or circadian effects. Overall, serious concerns have been expressed regarding the validity and reliability of such measurements. To address some of these concerns, researchers are increasingly resorting to automated home-cage monitoring (HCM) technologies, which allow continuous recording of behavioural and physiological parameters of undisturbed animals. In 2021, a pan-European network of researchers started the 4-year COST Action "Improving biomedical research by automated behaviour monitoring in the animal home-cage" (CA20135 TEATIME, https://www.cost-teatime.org/). For this project, experts from different fields joined forces to critically assess the potential of available technologies, to develop guidelines and identify where further technological development is needed, including analysis of big data. The opportunities opened by HCM for daily health and welfare monitoring of laboratory mice in a contactless, stress-free, and continuous fashion are also being explored. We provide a short overview of the progress made by the Action during the first year and a half (presentation available at https://osf.io/5dgz7).

Introduction

For over a decade, reproducibility of scientific results has been a hot topic, particularly in preclinical research with animal models (van der Worp et al. 2010). One main target of criticism is behavioural testing of laboratory rodents (Bespalov and Steckler 2018). This is mostly done in novel arenas, for a short time ("snapshot") and sometimes at an inappropriate time, i.e. by studying nocturnal animals during their inactive period. Behavioural testing is also often focused on a limited set of variables, neglecting the richness of behavioural expressions, as well as being susceptible to handling stress, human interference and environmental factors. All these factors have been shown to contribute to difficulties in interpreting the behaviour of rodents and in replicating the results across laboratories, thereby impacting on the translational value of animal experiments (Kafkafi et al. 2018; Blenkuš et al. 2022). In order to improve the *status quo*, a transition to automated and continuous monitoring of animals in their home-cage has been

suggested. HCM allows undisturbed animals in their familiar and social environment to be studied without human interference, while allowing monitoring of disease development and treatment effects with reduced observer bias. Such an approach can benefit preclinical research, while also improving animal welfare monitoring. It should be noted however that HCM, as a concept and approach, is not new. For example, adding running wheels to the home-cage, when studying circadian activity, has been a standard approach for decades. However, with recent developments in available technology, the field is evolving very rapidly (Voikar and Gaburro 2020; Baran et al. 2022).

COST Action Teatime

Through discussions at several international meetings throughout 2019-2020, it was clear that rodent behavioural phenotyping researchers should share experiences and collaborate to improve scientific outcomes (Restivo et al. 2021). This led to the submission of a successful funding application to set up the COST Action TEATIME – "Improving biomedical research by automated behaviour monitoring in the animal home-cage" (Hölter et al. 2022). For four years TEATIME will fund an interdisciplinary research network on HCM (Figure 1), while promoting excellence, openness, inclusiveness, gender and geographical diversity, and empowering young researchers (<40 years of age). As working with laboratory animals requires broad expertise beyond basic neuroscience, TEATIME also includes experts in animal welfare, veterinary and laboratory animal science. Moreover, as recording animal behaviour has advanced through artificial intelligence and machine learning, making routine tasks previously done by the experimenter more automated and sophisticated, it became evident that additional expertise from data science and machine learning was also warranted (Kenkel 2023). TEATIME has therefore grown significantly since its original proposal. It was signed by 57 researchers from 23 countries in October 2020, growing in 2023 to a network of ~160 researchers from 34 countries.

One of the first activities of the Action was to set up a comprehensive catalogue of existing HCM systems that have a user base in the network (https://www.cost-teatime.org/about/technologies/). Each system is accompanied by a reference to one or more experienced users who can provide relevant and professional information. Further work will be done to add more detailed descriptions and operating procedures from reference laboratories. We have also realised that the definitions of HCM may vary, thus we produced a graphical description and a more precise definition of HCM (Figure 2; https://www.cost-teatime.org/about/hcm-definition/). It is important to stress at this point that there is no all-in-one system available. The parameters that can be recorded depend on the technology applied, and the housing conditions. Therefore, researchers and core facilities

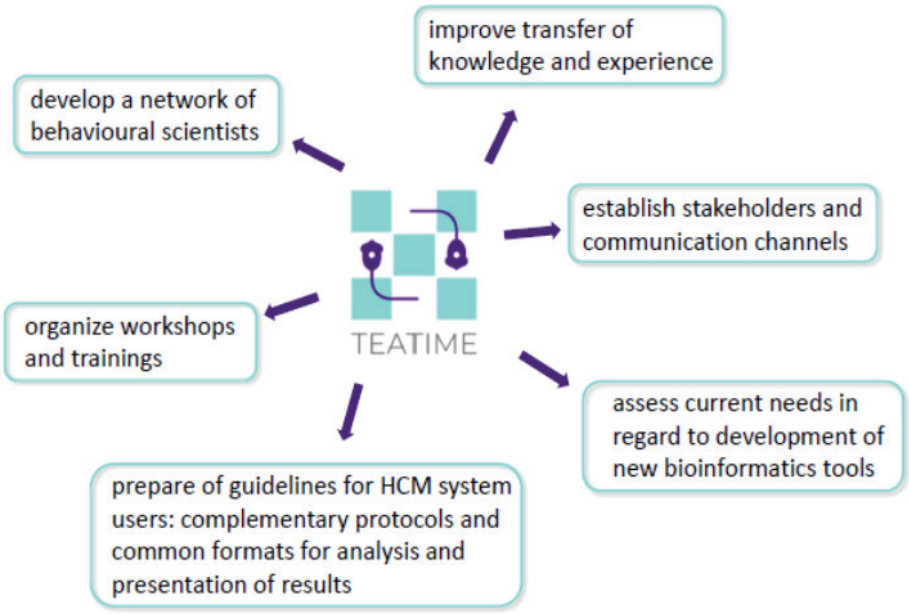

Figure 1: The main aims and objectives of the TEATIME network

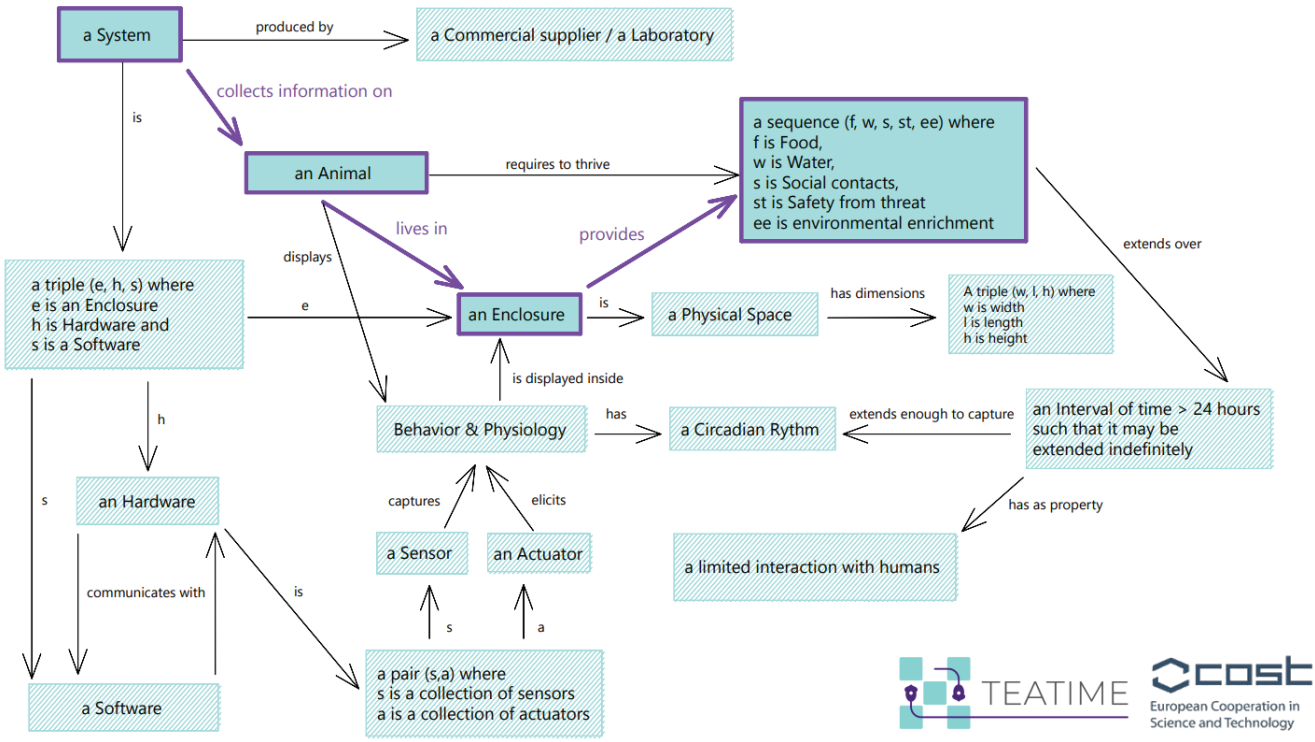

Figure 2: Home Cage Monitoring definition by TEATIME. The definition of a HCM using natural language can be given by following the purple arrows and connecting the boxes with words like "which" or "that". In this case the definition is: **A system that collects information on an animal which lives inside an enclosure that provides food, water, social contacts and environmental enrichment.** While this definition is correct, it fails to capture the details that make up a full-fledged definition of HCM. This enhanced version is an attempt at capturing, using a more formal structure, the multifaceted nature of HCM systems. In principle, by following boxes and arrows of the Olog, it should be possible to 1) Decide whether an unknown system is an HCM system; 2) Identify key parameters needed to be reported in an article to define the experimental settings used for an HCM experiment.

managers need to carefully weigh all pros and cons of any HCM equipment and its scalability along with their research questions and models.

We also carried out a systematic review that has revealed a significant increase in publications focusing on HCM, for 24 hours or more continuously, in the past decade (Kahnau et al. 2023). One of the concerns has been that HCM requires single housing of the animals. However, our review revealed that the number of studies in which laboratory rodents were individually housed has slightly decreased in the 2000s and 2010s when compared to the previous two decades. Moreover, female mice, in contrast to male mice, were more likely to be kept in groups. In the TEATIME catalogue, 27 out 49 HCM systems currently listed there, allow group housing. However, development of social structure and hierarchy must be considered when interpreting the data. For mapping the current landscape of HCM, we conducted a survey, where 279 respondents (mostly from Europe, but also from US, Japan, Canada) shared their experiences. Based on expertise and background, the survey participants represented four major stakeholder groups. The largest group were biomedical researchers (>70% of responders), followed by animal caretakers and welfare specialists (~15%), facility managers (~10%) and equipment developers (<5%). More than half of the responders (161/279) do not currently use HCM systems, yet the large majority (215/279) agreed that there is a need and demand for this approach. The major obstacles preventing the widespread use of HCM identified were the cost of ownership, difficulty in obtaining technical and IT-support, IT-infrastructure limitations, reluctance in accepting these new behavioural testing approaches (namely from trusting their added value as compared to current "gold standard" i.e. "out-of-cage" testing), limited awareness of capabilities, lack of space and/or qualified personnel. Interestingly, the question of how well the current, conventional methods suit the needs of research in various types of studies (understanding basic behaviour in disease models; regulatory testing / toxicology; translational validity; disease progression; complex traits; treatment effects), revealed only moderate satisfaction (on a 5-point rating scale). The outcome of the survey justifies

several of the objectives of the Action: to encourage use of the HCM systems by using the breadth of knowledge and expertise available in the network, to exchange knowledge through training, improve communication and dissemination, and to develop new lasting forums to bridge behavioural and data science in order to achieve breakthroughs in the integration and analysis of complex datasets.

Training and knowledge-sharing are crucial for early career researchers and innovation acceptance, therefore COST provides financial support for young researchers to attend conferences and visit other laboratories (Short-Term Scientific Missions). Moreover, TEATIME Action has already held two successful summer schools, an introductory one in Varese (Italy) and an advanced one in Porto (Portugal). The schools promote active discussion and interaction between the participants, thus establishing new networks and disseminating best practice. Based on the number of high-quality applications we have received, it is clear that there is an unmet need and demand for in-person interactive training and workshops. Our working group dedicated to training and knowledge transfer is identifying the existing gaps in this area and is developing a long-term program.

One of the outcomes of the Action so far is the establishment of an online discussion forum (https://www.thebehaviourforum.org/). This forum is for discussion of animal behaviour experiments with a special focus on HCM techniques. It is designed for scientists, laboratory technicians, veterinarians and animal welfare staff and is intended to be a resource where people can ask questions and receive guidance on best practices for behavioural experiments. Our aim is to improve scientific excellence and animal welfare in equal measure. Training and knowledge sharing are further supported by our webinar series on current topics in behavioural monitoring, freely accessible on our YouTube channel (www.youtube.com/@cost_teatime).

We are increasingly exploring the potential of HCM systems to monitor animal health and welfare parameters (e.g. body temperature, general activity, eating and drinking), and to facilitate intervention if an animal shows early signs of distress or goes beyond a predefined threshold (e.g. hypothermia). The ability of HCM to detect subtle changes in animal behaviour or physiology that traditional observational methods cannot, aside from clear Refinement benefits, also has Reduction implications. Firstly, more data can be obtained without the need for additional animals. Secondly, by reducing handling stress-related variability - a source of experimental 'noise' - smaller sample sizes are needed for detecting a given effect size. Our intention to contribute to better science while adhering to animal welfare and 3Rs principles is emphasised by the fact that TEATIME Action has been actively participating in the national and international laboratory animal science (LAS) conferences (in 2022 – FELASA; in 2023 – Scandinavian (Scand-LAS) conference, Central-East European LAS Congress (CELASC), Slovenian and Turkish LAS meetings, 12th World Congress on Animal Use and Alternatives in the Life Sciences).

Conclusion

As not all "out-of-the-cage" conventional testing can currently be replaced, HCM approaches should be viewed as complementary at the moment. However, the benefits and potential are undeniable and therefore their application should be promoted as widely as possible. With this in mind, at TEATIME we are seeking further collaboration and interaction with like-minded groups, and are open to professionals willing to contribute to our Action.

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Conflict of interest

The authors declare that they have no conflict of interests.

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