Challenges of a small world analysis for the continuous monitoring of behavior in mice

Edgar Bermudez Contreras, Robert J. Sutherland, Majid H. Mohajerani, Ian Q. Whishaw.

Canadian Centre for Behavioural Neuroscience, University of Lethbridge, Canada.

Corresponding authors: edgar.bermudez@uleth.ca & mohajerani@uleth.ca

Highlights

- Discuss challenges of continuous and extensive small world mouse behavior analysis and describe how to uncover insights into phenotypical expression, development and aging, and neurological disease.
- Discuss data collection and emphasize the role of machine learning in uncovering the organization of behavioral over extended periods of time.
- Highlight the advantages and limitations of extending acute behavioral testing methodology to the small world of laboratory small world monitoring.
- Describe a behavioral methodology for assessing organized behavior across the lifespan.

Keywords: mouse behavior, mouse home-cage, homecage behaviour, behavioral phenotype, computational ethology, deep learning.

Abstract

Documenting a mouse's "real world" behavior in the "small world" of a laboratory cage with continuous video recordings offers insights into phenotypical expression of mouse genotypes, development and aging, and neurological disease. Nevertheless, there are challenges in the design of a small world, the behavior selected for analysis, and the form of the analysis used. Here we offer insights into small world analyses by describing how acute behavioral procedures can guide continuous behavioral methodology. We show how algorithms can identify behavioral acts including walking and rearing, circadian patterns of action including sleep duration and waking activity, and the organization of patterns of movement into home base activity and excursions, and how they are altered with aging. We additionally describe how specific tests can be incorporated within a mouse's living arrangement. We emphasize how machine learning can condense and organize continuous activity that extends over extended periods of time.

1. Introduction

The French scientist Jean-Jacques d’Ortous de Mairann’s 1729 innovation for studying daily rhythms was to devise a recording drum to document the 24-hour movement of the plant *Mimosa pudica* (Klarsfeld, 2013). His study has been followed by improvements in behavioral monitoring and their application to many animals, including humans. Continuous monitoring of rodents in their home environment has led to an understanding of the molecular and genetic basis of circadian rhythms (Kohsaka and Bass, 2007), the structure and neural basis of sleep waking cycles and the rhythms of many other behaviors (Hoffman et al., 2007; Kohsaka et al., 2007; Tapia-Osorio et al., 2013). Advances in automated monitoring methods combined with analysis using computer vision and machine learning are opening up the possibility that most mouse behavior can be concurrently and continuously monitored and analyzed over long periods. It is the application of this idea to the study of laboratory mouse behavior in the “small world” that comprises its laboratory cage that is discussed in this review.

In the following sections, we discuss how and whether a mouse’s cage can be designed to serve as a small world in which ethologically relevant behavior can be extracted as if it were obtained in the mouse’s real world. We then review methodologies for continuous automated behavioral data acquisition and machine learning analysis of mouse behavior in the small world over long time periods. We emphasize that engineering approaches and advances in the automation of continuous data acquisition can uncover the organization of mouse behavior in a small world over a mouse lifespan. We describe the organization of behavior in terms of home base and excursion behavior and describe how their organization changes with age. We conclude by discussing the contributions and limitations of this approach as a supplement to acute behavioral testing.

1.1 Can mouse behaviour analyses in natural environments guide phenotypic studies?

The house mouse, *Mus musculus*, has become the most widely used species of laboratory animal for the study of brain and behavior (Bonhomme and Orth, 2013). An ideal setting for the study of its behavior and brain considers the mouse in its natural habitat (Berry, 1970; Berry and Bronson, 1992). The mouse is *commensal*, meaning that it thrives near the houses, barns and fields of humans (**Fig. 1, left**). Males are polygamous and mouse families usually comprise a male, one or more females, and their offspring. Males defend their territory and females have many litters of up to 15 pups each throughout the year. Mice are crepuscular and nocturnal, meaning that they are active at twilight and at night. They can have a home range as large as one or more hectares (2.47 acres) but within this territory, they center their activity at a home nest that they construct of soft material (Chambers et al., 2000). They are omnivores, but their main diet is plant material, and they are scatter hoarders in that they cache food in a variety of locations in their territory. They are subject to conflict with other mice and they are subject to predation by many other animals. A mouse can live up to two to three years, but seldom lives longer than one year. Admittedly many of these features of the natural habitat of mice are difficult to subject to analysis in the laboratory. To date, however, research on the mouse in its natural habitat does not provide insights into the daily behaviour of a mouse or the changes in that activity extending over a lifespan, questions that are usually the focus of laboratory research. Hopefully future study of the mouse in its natural habitats will fill in this area of research.

1.2 The small world; problems in miniaturizing a natural world

We suggest that the term “small world” should replace the usual designation of a laboratory mouse’s living quarters as “homecage” or “cage”. The latter terms are experimenter-centric and do not address the problem of studying the mouse in a context that is as natural as possible. Clearly,

1 life for a mouse in a laboratory is different to its counterparts in nature (Gonder and Laber, 2007).
2 Because its cage is small, its world is small and comparatively impoverished (**Fig 1, right**). A cage
3 usually has a sawdust substrate and is provisioned with some nesting material. A mouse is often
4 housed with some littermates or alone (Martin and Brown, 2010), and it is provided continuous
5 food and water at proximate locations. Lighting is typically on-off on a fixed schedule and room
6 temperature and humidity are constant. Its movements are limited by its small cage and it usually
7 does not come into conflict with other mice and it is not subject to predation (Gonder and Laber,
8 2007). These housing facilities are usually determined by economic and animal welfare
9 considerations (Makowska and Weary, 2020) and are not perfectly defined (Whittaker et al., 2012).
10 For example, the periodic cage cleaning in which bedding and nesting material are removed is a
11 laboratory stress far removed from the stress of a natural world (Burns, 1786, aside). Any
12 consideration of the proper continuous analysis of laboratory mouse behavior must also consider
13 the proper design and maintenance of the small world in which ongoing behavior is shaped (Fonio
14 et al., 2012b; Weissbrod et al., 2013; Thompson et al., 2018).

15
16 *Figure 1. Contrast of a mouse real world (left) and its laboratory small world (right). Allentown™*
17 *Caging Equipment's (Allentown, NJ).*

1
2 We recognize that at present commercial cages are likely to be a mainstay until more dedicated
3 housing directed toward miniaturizing a natural world is designed. We suggest that studies in
4 which the home cage is systematically modified combining information about how the
5 environment shapes the behavior of the mouse could be used to optimize a laboratory small world.
6 For example, Berry and Bronson, (1992) present a review of the bioeconomy of the house mouse
7 through its lifetime that suggest life history has seven stages (up to birth, nest life, sex life, social
8 structure, population statics and stability, senescence, and death). As a beginning, these stages may
9 serve as brackets for small world analysis in which the small world is specifically designed to
10 reveal aspects of one or another stage. Some aspects of mouse ecology may be difficult to monitor,
11 including a large home range containing both a home base and foraging territory (Hayne, 1949),
12 variations in home ranges as function of sex (Chambers et al., 2000) and changing home ranges
13 during the breeding season (Li et al., 2020). Of course, the most difficult feature of natural mouse
14 behavior relates to its large natural range varying from 0.0002 to 8.024 ha (MacKay et al., 2011;
15 Tomich, 1970). Strategies such as changing the configuration and contents of a small world may
16 model some of the features of a natural world. However, current design approaches of commercial
17 home cages are not directed for this purpose but should be a subject of further research. In the
18 following subsection, we review the two main design approaches to collect behavioral data in
19 conventional housing units.

20 *1.3 Automated data collection in a small world*

21
22 Obviously, the continuous monitoring of a mouse in its natural habitat is difficult but monitoring
23 behavior in a laboratory also has its challenges. In most cases, the small world cannot be lighted
24 evenly for filming due to lighting changes over the day/night cycle. There are objects under which
25 a mouse can hide; e.g., beneath the material that it has used to construct its nest. Nevertheless, the

1 automation of continuous data collection is becoming feasible with the development of new
2 technology. For example, electronic devices that are either automated or triggered by the animal
3 itself can collect behavior continuously (Neff, 2018). The general approach is directed to
4 maximizing the range and duration of behavioral sampling while minimizing intervention. This is
5 important because the continuous behaviour monitoring over long periods, or even the entire life
6 span, opens the possibility to study behaviours that might be overseen by traditional behavioral
7 assay, which last from minutes to hours at most. An additional advantage could be an increase in
8 the reliability and validity of experiments. Importantly, this approach is consistent with the 3Rs
9 (replacement, reduction and refinement) principle of animal welfare that it increases information
10 obtained during an experiment (Richardson, 2012; Recordati et al., 2019).

11 In addition to free-behavior data collection in a small world, an automated approach can
12 be used to voluntarily train animals on diverse tasks without human intervention (Balzani et al.,
13 2018). Salameh et al., (2020) used this approach to study fine motor skills and assess recovery of
14 motor function after brain injury. Similarly, Woodard et al., (2017) designed a lever-pulling
15 automated system to study motor and cognitive function in a mouse model of Huntington's disease.
16 In addition, rodents have been conditioned to voluntarily position themselves in an apparatus for
17 brain imaging in an automated manner (Scott et al., 2013; Murphy et al., 2016). In the following
18 section we review advancements in automated data collection from a small world.

19 There are two approaches for automated data collection for a small world analysis. One in
20 which data is collected by a dedicated acquisition system that is separated from the housing rack
21 (e.g. (Robinson and Riedel, 2014). This approach has the limitation that it cannot be scaled up to
22 a large number of cages (**Fig. 2A-B**). A different and more recent approach consists of a data
23 collection system integrated into the cage rack and even into the cage itself; i.e., by integrating

cameras and other sensors into the structure of each cage (**Fig. 2C-D**). The second approach allows monitoring to be scaled up to include multiple cages and multiple housing racks (Lim et al., 2017; Peng et al., 2019; Singh et al., 2019; Abe et al., 2020; Voikar and Gaburro, 2020). In principle, an entire animal colony having every cage serving as a small world could be the target of continuous data collection.

Figure 2. Approaches for the automated mouse behavior classification in the homecage. (A-B) Example of a highly-specialized monitoring systems for dedicated modified homecage systems (Wolfer et al., 2012). (C-D) Examples of fully-integrated monitoring system in a normal homecage (Lim et al., 2017; Singh et al., 2019).

Despite the methodological differences used to acquire behavioral information, these two approaches have propelled an explosion of systems and techniques (Vogt, 2018). There are three key factors that have contributed to this explosion. The first factor is hardware, the advent of low-cost microcomputers and off-the-shelf components have made behavioral data collection

affordable. For example, the introduction in 2012 of the Raspberry Pi, a single-board low-cost micro-computer capable of integrating multiple sensors, including a video-camera and full networking capabilities, reduces the expense of monitoring many animals concurrently. The second factor is software, the development of machine learning for data analysis has made it possible to manage and analyze high-dimensional (results with many variables, many of which may be interrelated) data. Deep learning algorithms (see (Schmidhuber, 2015) for a review) enable the processing of multiple variables from video recordings and the organization of the results into meaningful generalizations (Mathis et al., 2018; Mathis and Mathis, 2020; van Dam et al., 2020). Third, contemporary open-source approaches to software and hardware development make automated small world monitoring quickly accessible to all investigators (White et al., 2019).

The advantages of the automation of data collection and training in a small world are an increase in the efficacy and throughput of unbiased behavioural and physiological data. This approach provides a new and unprecedented opportunity to increase reproducibility of scientific experiments and to accelerate preclinical research; for example, by providing a high-throughput data collection and analysis system for the purposes of evaluating drug administration in mouse models of disease.

2. Machine learning approaches for the analysis of rodent behavior

As noted above, mouse behavior can be analyzed at different scales depending on the coarseness of the behavior of interest (see (Pereira et al., 2020) for a more extensive review). Algorithms can classify behaviour as a circle or ellipse (blob) that moves or not (**Fig. 3A**). More complex behavior classifications can be implemented using the same representation when multiple animals are present to study social interactions. Moreover, it is possible to increase the complexity (reduce the coarseness) of the features used to analyze behavior in more detail. Instead of only keeping track

of the position of the animal, there are systems that decompose mouse behavior of a sequence of ‘behavioral syllables’ that consists of characteristic body poses or movements (**Fig. 3B**). Analogously, the analyses of mouse behavior can be classified into three different approaches: rule-based, supervised or unsupervised behavior classification depending on the type of algorithm that is used. It is important to note that these approaches are not mutually exclusive. Recent automated behavioral phenotyping systems use a combination of these approaches (Egnor and Branson, 2016; Gris et al., 2017). In the following subsections we review approaches that have been applied to study mouse behaviour and that are promising for data acquired in a small world.

Figure 3. Behaviours in the small world. (A) Organization of features used to classify mouse behavior with increasing levels of complexity. Inspired from (Pereira et al., 2020). (B) Examples

1 *of natural mouse behavior observed in the small world include sleeping, grooming, eating,*
2 *walking, drinking, stopping, nesting and rearing amongst others.*

3 4 *2.1 Rule-based approaches*

5 Rule-based approaches usually use a manually tuned threshold-based criteria to classify behavior.
6 These approaches do not require the classifier (model) to be ‘trained’. Usually, this approach is
7 relatively easy to implement because the criteria are determined by the experimenter with few
8 parameters. The features over which the thresholding can be applied varies from simple motion-
9 based sleep/wake classification in mice (Singh et al., 2019) to more complex social interaction
10 based on the distance between mice (De Chaumont et al., 2012).

11 Given that these approaches are based on simple thresholding algorithms, they are not
12 adaptive and therefore not very robust to variations in environmental conditions such as
13 illumination, camera field of view, video resolution, etc. Therefore, the rule-based systems are
14 required to adjust the features and other parameters to every variation of the experimental
15 conditions. This makes these approaches not easy to scale or to apply easily to the homecage in
16 the home rack.

17 *2.2 Supervised approaches*

18 In supervised approaches the classification model is ‘trained’ using human-labeled examples,
19 defined as ‘ground-truth’ that determine how to tune the model parameters to improve its
20 performance by minimizing the distance between the model estimation and the provided examples.
21 Once the model is trained, it can be used to classify behavior in new data sets (**Fig. 4A**). Typically,
22 to classify mouse behavior using this approach, the classification is made using predetermined
23 features that depend on body and spatial information (e.g. speed, posture, etc.). During training,
24 the model is supposed to be exposed to diverse experimental conditions to increase its robustness

1 to these variations (**Fig. 4A**). These approaches rely on the learning algorithm to extract relevant
 2 features that are general enough to be applied to different situations but specific enough to still
 3 recognize the behavior of interest (see bias vs variance trade-off) (Datta, 2019). Examples of
 4 supervised approaches include support vector machines, linear and logistic regression and artificial
 5 neural networks amongst others (Russell and Norvig, 2010).

Figure 4. Machine learning approaches to analyse rodent behavior. (A) Cartoon representation of supervised and unsupervised classification algorithms Inspired from (Ma et al., 2018). (B) Schematic representation of a shallow and a deep artificial neural networks.

Deep learning applied to mouse behavior classification in the homecage.

Recent developments of machine learning and computer vision algorithms have propelled an advancement of the tools to analyze mouse behavior. One of the most important supervised approaches that have been applied to the automated analysis of mouse behavior is deep learning (Graving et al., 2019; Lee et al., 2019; Mathis and Mathis, 2020; Pereira et al., 2020; Bohoslav et al., 2021). This is an area of machine learning that employs artificial neural networks (ANNs), a computational formalism originally inspired in biological brains. This formalism uses deep neural networks (DNNs) consisting of layers of simple units that are stacked in series to form ‘deep networks’ (**Fig. 4B**). The units are connected with weights that represent the strength of the connection. These weights are optimized during ‘training’ periods based on examples to extract information from raw data to solve the task at hand, in this case, behavioral classification. DNNs have allowed faster, more accurate and more reliable automated behavioral analyses with finer details. For example, DeepLabCut uses deep learning to track body postures of mice in diverse conditions (Mathis et al., 2018). Being able to track body parts reliably provides a great starting point to study body kinematics but also opens the possibility to study complex behaviors in an automated and efficient manner. This tool is revolutionizing how neuroscientists study behavior (Wei and Kording, 2018; Mathis and Mathis, 2020; Mathis et al., 2020).

Another example of deep learning being applied to rodent behavior classification is described by van Dam et al. (2020). In this work, the authors show that using a multi-fiber network architecture can improve the performance for automated rat behavior recognition compared to a more conventional hand-crafted feature based unsupervised classification algorithm. Similarly, Bohoslav et al., (2021) used a deep convolutional network to build a video-based ethogram in an

1 automated manner. In this work, the authors use user-defined features to classify each video frame
2 into sequences of previously defined behaviors. The deep learning revolution is changing the way
3 rodent behavior is analyzed, including domains in which previous computer vision approaches
4 were not very successful in the development of automated systems.

5 *Social behavior and tracking multiple animals in the small world using deep learning*

6 One of the behaviors that is important to understand brain function is the way individuals interact
7 with conspecifics (Hari and Kujala, 2009). One of the limitations of traditional video-based
8 approaches to study social interactions in mice is the complexity of tracking the position of
9 multiple animals simultaneously. In general, this is a complex problem in computer vision because
10 the variability of illumination conditions, image background, mouse visual similarity and,
11 occlusions and superposition. Artificial deep neural networks have been applied to tackle this
12 problem. For example, (Geuther et al., 2019) trained different architectures of artificial neural
13 networks to track a single mouse of different strains and under different environmental conditions.

14 For the small world approach, tracking multiple mice becomes even more complex because
15 the highly dynamic and non-uniform conditions of the small world and limitations of the recording
16 apparatus, compared to the more controlled and uniform conditions in separate dedicated
17 experimental apparatuses. There are multiple approaches for this problem, including providing
18 multiple cameras to provide additional perspectives to disambiguate occlusion and superposition.
19 For example, (Ebbesen and Froemke, 2020) used multiple 3D video cameras and deep learning to
20 monitor postures of multiple mice engaged in social interactions. In principle, a single camera and
21 a system of mirrors can also be used to monitor behavior from a variety of perspectives (Bova et
22 al., 2019). Although these approaches solve the problems of tracking multiple mice to study social

1 behavior using depth information from different perspectives, they employ settings that are
2 challenging for the study of small world behavior at scale.

3 One approach that has been used to improve the accuracy of tracking of multiple animals
4 is to incorporate the dynamics of body parts to infer motion trajectories. Deep learning has been
5 successfully applied to this form of pose estimation in humans and other animals (Mathis and
6 Mathis, 2020). This approach uses several pipelines to perform multiple-mice tracking for the
7 study of social interactions (Jiang et al., 2019b). The authors decompose the problem of tracking
8 multiple mice into three components: body-part detection, body-part position predictions and
9 associations of such body-parts across frames. Their use of a deep convolutional network and a
10 Bayesian inference approach shows promising tracking results. The conditions in which this work
11 has been developed might be not very similar to the ones encountered in a small world. Other
12 approaches that use deep learning for tracking multiple mice include siamese networks for
13 segmentation of multiple mice. These networks consists of two (or more) copies of the same
14 network (same architecture and weights) to estimate the similarity of two (or more) different
15 inputs. For example, Chen et al., (2020) use a siamese architecture and deep convolutional
16 networks to track multiple mice in the homecage in near real-time.

17 One limitation of automated supervised approaches for behavioral classification and the
18 study of social interactions is that all approaches require labeled examples to train the models.
19 Usually, the initial labelling is made by humans but because supervised algorithms need a large
20 number of examples (e.g. deep learning), this can be problematic either because it can be difficult
21 to acquire labeled data or because it can be time-consuming and biased to label them (Leng et al.,
22 2020). Moreover, the initial labelling of behaviors for the training datasets requires a certain level
23 of expertise (in animal behavior and machine learning) to adapt the methods to different laboratory

and experimental conditions. Therefore, the application of these approaches sometimes can be limited (Datta, 2019).

Synthetic approaches to training a program

A novel approach to alleviate the problem of supervised algorithms needing a large number of training labeled samples for behavioral classification is to generate synthetic labeled data with controlled variations using 3D models (**Fig. 5A**) (van Vugt and Kamilaris, 2019; Seib et al., 2020). This approach has been applied to generate synthetic data to train cattle behavior classifiers (Maraghehmoghaddam, 2020), and mice (**Fig. 5B**) (Bolaños et al., 2021). Similar approaches haven been applied to study behaviour in a synthetic simulated rat (Merel et al., 2019). As 3D animal models, scene and motion pattern generation continue to improve, this approach has a great potential to be applied to automated rodent behaviour analysis in a small world where the variability in the environmental conditions are not as uniform and controlled as in more traditional behavioral experiments. That is, being able to produce synthetic labeled training data that are difficult to acquire due to the number of occurrences or the lack of controlled conditions in the real world, will improve the classification models to analyse rodent behaviour in an automated manner.

Figure 5. Examples of synthetic 3D models of rodents to generate training data sets for behaviour analysis models. (A) Example of a 3D model of a mouse with labeled tracking points

1 *for pose estimation. (B) Pipeline for generation of training datasets for a skilled reaching task*
2 *using a 3D model of a mouse (top) and example of pose estimation using synthetic data. Both*
3 *images courtesy of Tim Murphy. Used with permission.*

4
5 In attempts to lower the entry barrier for non-programmer users of these systems, there are
6 open source approaches that are user-friendly and that lower the entry barrier considerably. For
7 example, SimBA is a simple toolkit for classification of social behavior in experimental animals
8 that uses pose-estimation features (e.g. DeepLabCut) using deep learning (Nilsson et al., 2020).
9 Similarly, (van den Boom et al., 2017) use an user-friendly annotation package (JAABA) to
10 identify grooming behavior in mice videos with a graphical interface. An attractive feature of deep
11 networks is the high-level of abstraction of the data-driven features that are exploited for
12 classification. There are attempts to use this approach to study behavior in less restricted or
13 controlled settings (Nourizonoz et al., 2020).

14 In summary, with the progress in the field of deep learning, deep artificial neural networks
15 is becoming popular in computational ethology. Although, to date there have been very few studies
16 using this approach in the home cage. This is because supervised algorithms need to be trained
17 labeling the behavioural components of interest that the model will classify. In many cases the
18 experimenter will not know what the behavioral components are. If this is the case, unsupervised
19 algorithms can be used to find the meaningful units of an animal's behavior.

20 *2.3 Unsupervised approaches*

21 In unsupervised approaches, statistical regularities parse data into distinct groups (or classes) of
22 behavior (**Fig. 4B**), instead of using human-labeled examples (Berman, 2018). There are a number
23 of approaches that classify mice behavior in an unsupervised manner. For example, Wiltchko et
24 al., (2015) used an autoregressive (AR) process to capture stereotyped trajectories through pose
25 space using a 3D camera and modeling the transitions between different behavioral modules using

1 a hidden Markov model. The authors found that mouse open-field behavior is composed of
2 modules that last on average 350 ms. They applied this approach to study how behavioral
3 phenotypes change in response to modifications of the environment, and to genetic and neuronal
4 manipulations in mice. More recently, Wiltchko et al., (2020) use a similar approach to analyze
5 the behavior of hundreds of mice in an unsupervised manner. Their method called motion
6 sequencing (MoSeq) decomposes animal behavior captured with 3D cameras into a sequence of
7 ‘syllables’. They use MoSeq to predict the probability that a mouse has been treated with a certain
8 drug and dose.

9 An advantage for unsupervised approaches in automated homecage behavioral
10 classification is that the repertoire of behaviors in the homecage is larger than in more restricted
11 experimental paradigms. Unsupervised approaches are particularly important for the phenotyping
12 of animal models of disease in which the affected behaviors might not be known a priori. For
13 example, Huang et al., (2020) used a 3D camera and unsupervised clustering to separate mouse
14 behavior into components of motion features to produce a mouse feature space. The authors
15 distinguished the motor activity of a mouse model for autism from that of control mice. Similarly,
16 Luxem et al., (2020) used a variational auto-encoder algorithm to decompose a low dimensional
17 mouse behavior space using spatiotemporal pose estimation features of a freely exploring mouse.
18 They demonstrate this unsupervised behavior decomposition distinguished a mouse model of
19 Alzheimer’s disease from a control group.

20 It is important to note that supervised and unsupervised approaches are not necessarily
21 exclusive. There are mixed approaches in which supervised deep learning is used for pose
22 estimation and the unsupervised classification of the kinematics of the identified body parts is
23 performed. For example, Hsu and Yttri (2020) used an unsupervised approach for behavioral

segmentation of bottom-up view of mice in an open-field. The authors first reduced the dimensionality of the feature space extracted from the identified body parts by clustering them using t-distributed stochastic neighbor embedding and then classified the clusters into behavioral groupings using support vector machines.

3. Getting at the organization of behavior in a small world

There are a lot of targets to choose from if one wants to monitor behavior in a small world (Levitis et al., 2009). The mouse like other vertebrates is a movement machine that has about 250 degrees of motion within its body enabled by about 650 muscles. Together, this motor and physiological activity can be directed toward about 15 different goals such as walking (Gire et al., 2016), eating (Czyzyk et al., 2010), nesting (Deacon, 2006; Hess et al., 2008; Okayama et al., 2015), grooming, etc. These behaviors have a temporal dimension in that they can occur in relation to each other and to time and they can have organizational features in that they optimize territory, mates and safety. Over the life span of an animal all of these aspects of behavior change in their frequency, duration and relations to each other (Berry and Bronson, 1992; Logan and McClung, 2019).

Figure 6. Hierarchical analysis of mouse behaviour. The lowest level of analysis is to count behavioural acts (A). At a midlevel of this hierarchy, the temporal occurrence and durations of acts can be measured (B). At the top of the hierarchy, behavioural components (stopping, eating, rearing, sleeping, drinking, etc.) are grouped into spatiotemporally organized patterns in the environment (C). This schematic representation of the possible analyses using this approach shows the location in the small world of each behavioural act (stopping, eating, rearing, sleeping and drinking) as coloured circles. The duration of each act is represented as the size of the circle. The home base (most occupied space) is represented as the light blue shaded area and animal trajectories are represented as red dashed lines.

One approach to making the potential complexity of behavior more manageable is to use a hierarchical approach. At the first level of the hierarchy, a mouse can be treated as a solid object that spends its time engaged in different acts. Acts include stopping, walking, rearing, eating, drinking etc. (**Fig. 6A**). At this level of analysis, a simple count of the frequency of acts can provide a numerical account of mouse activity (Singh et al., 2019). At the second level of the hierarchy, behavior can be classified in terms of its temporal and spatial features, such as location, time and duration (**Fig. 6B**). This description of behaviour across time is termed an ethogram (Eibl-Eibesfeldt, 1970). For example, circadian measures and sleep waking behavior document the

1 temporal occurrence of such behaviors, their changes in relation to modifications in the
2 environment and during development and aging (Kolker et al., 2004; Nakamura et al., 2011;
3 Thompson et al., 2018). At the third level of the hierarchy behavior can be parsed by its
4 organization in space and time and with respect to the environment or its features, (**Fig. 6C**). In
5 the following sections we will describe results directed toward analyzing the organization of
6 behavior.

7 Activity in a small world over periods of weeks to months can be related to theories of
8 mouse ecology in which an animal has a home that serves as a refuge from which its behavior is
9 organized. For example, optimal foraging theory proposes that patterns of behavioral organization
10 are directed at maximizing benefits at the least cost (Houston and McNamara, 2014). That is, the
11 behavior of a mouse has evolved to minimize its exposure to potential predation while foraging,
12 defending its territory, seeking mates by organizing its excursions with respect to time and
13 duration. All of this activity is centered on the home, which potentially provides maximum security
14 (Whishaw et al., 2006). The value of having a continuous and extended record of behavior from a
15 small world is that a good representation of a mouse's natural behavior can be analyzed in the
16 small world of the laboratory not only with respect to a phenotypic description but also to test
17 explanations of behavior that otherwise would remain untested (Fonio et al., 2012b, 2012a;
18 Reynolds, 2018).

19 *3.1 The home base*

20

21 In acute studies using open fields, the home base is the location at which mouse spends most of its
22 time and from which the animal performs excursions or trips into the environment (Eilam and
23 Golani, 1989; Clark et al., 2005, 2006; Golani and Benjamini, 2018; Thompson et al., 2018). In an
24 examination of behavior in small world behavior, we have found with this definition, the home

1 base is the nest in a small world (Bermudez Contreras et al, unpublished manuscript). As occurs
2 in acute studies, using the home base as the centroid of behavior, we find that this is the location
3 in which the mouse sleeps and the location from which the mouse makes various excursions. Each
4 excursion can be then defined with respect to its purpose and as a route of varying distance and
5 complexity.

6 **Fig 7** illustrates the home base in the small world. We first calculated the occupancy maps
7 during the light and dark cycles using the position of the mouse to find the home base location and
8 size by calculating the region in the small world where the mouse spends most of the time (95
9 percentile threshold) using deep learning (Mathis et al., 2018). This approach was applied to
10 quantifying the changes in the home base across a mouse's life span during both the light and dark
11 cycle of each day (**Fig. 7A**). Using a similar approach, were also able to track the motion of the
12 nose and ears as an index of a mouse's mobility within the home base (**Fig. 7Bi**). This measure
13 also indicated sleep/wake patterns including REM sleep (**Fig. 7Bii**).

Figure 7. Example of the home base in the small world. (A) Occupancy maps of the mouse location in the small world during the light cycle (top) and dark cycle (bottom). The light-blue line denotes the home base region. (B) Example of sleep analysis of the same animal at the nest area in the small world. (i) Sleep analysis during quiet wakefulness periods in the nest. The line denotes the position of the nose of the animal during 4 seconds after waking up. (ii) Spectrogram of pixel intensity of a region of interest (ear) (top) showing when the animal wakes up and falls asleep (bottom).

3.2 Excursion as the unit of locomotor activity

Previous studies have demonstrated that excursions or trips made by animals are structured (Golani and Benjamini, 2018; Kafkafi et al., 2005; Clark et al., 2006; Thompson et al., 2018; Wexler et al., 2018). Excursions begin and end at the home base and can be interrupted by a number of stops. The nature and purpose of excursions is influenced by the environment and previous experiences (Whishaw et al., 2006; Thompson et al., 2018). These environmental features are not exclusive to horizontal spaces but extend to volumetric space (Wexler et al., 2018; Jedidi-Ayoub et al., 2021).

Figure 8: Excursion analysis in the homepage. (A) Mean number of excursions per hour during the day over 3 weeks at young age (left) and old age (right). The error bars represent the standard error of the mean. The shaded areas represent the daily dark cycle. (B) Example of excursions during 20 mins at the first hour of the dark cycle at a young age (left) and old age (right). The dark lines represent excursions during 1 min within the 20 min (shaded lines). The blue and red dots represent the initial and the end of the trajectories respectively.

We find that in the small world, excursions are similarly recognizable but in addition can have a number of purposes; to eat, to drink, to explore or to build and organized the nest across time (**Fig. 8A**). Each excursion type can be identified by measures of counts and durations (see **Fig. 6C** for some examples of different types of excursions). Excursions can also take the form of patterns that are repeated, they can be interrupted to stops, and they can also be interrupted by rearing or climbing (**Fig. 8B**). This organization of excursions is analogous to the way we might classify our own behavior. Some excursions are for shopping, some are for work and others for play whereas at home activities are related to feeding, house cleaning and sleeping. Excursion may

be the unit of locomotor activity and so they offer a richer insight into day/night activity and simpler levels of time or distance travelled.

3.3 The organization of behavior altered by aging

The hierarchical approach that we have outlined above can be applied to practical problems of phenotyping mice at different ages. Here we demonstrate some of the possible analyses for a mouse at young adulthood and old ages (around 4 and 11 months old approx., respectively) (Bermudez Contreras et al., 2022. Unpublished manuscript). These time points are illustrated within a mouse life span in **Fig. 9A**. Using the richness of video recordings to extract behavioural features over extended periods of time provided more behavioral elements to compare than would be obtained from a simple motion quantification. For example, a power spectrum analysis shows the strong circadian cycles revealed by the motion signal, both at young and old age of the animal, did not reveal behavioural differences (**Fig. 9B**). This is not to discard that a more refined analysis of the motion, such as a comparison of the motion onset and offset with respect to the light/dark cycles, might reveal behavioural differences produced by aging (Fuochi et al., 2021; Pernold et al., 2021). Other measures did reveal differences, however.

Using an automated analysis of the spatial distribution of the location and size of the home base gave differences in its behavioural organization in young adulthood vs old age (**Fig. 9C**). The small world was more dispersed at old age than at young age, particularly during the dark cycle (**Fig. 9D**, top row). Continuous automated mouse position tracking for long periods of time allows the comparison of the size of the small world at young and old ages. At old age, this mouse has a significantly smaller small world than at the younger age (**Fig. 9C**, left column) during both dark and light cycles. Finally, a detailed analysis of the excursion behaviour from the home base revealed reduced numbers of excursions mainly during the dark cycle as a mouse ages. Finally,

1 the number of stops across these excursions increased with age (**Fig. 9C**, middle and right
2 columns).

3 We also automated the classification of behavior of a mouse in the small world using
4 *DeepEthogram* (Bohnslav et al., 2021). We trained this tool to classify moving, rearing, eating,
5 drinking, sleeping and nesting behaviours, at young adulthood and old age. All other behaviours
6 (such as jumping, freezing, etc.) we defined as “background”. We found that not all behaviours
7 were modified in the same manner by aging (**Fig. 9E**). Although some of these measures were
8 unchanged as a function of age, others were different. For example, although total time spent in
9 the nest was not different, there was more time spend nesting at a young age during the light cycle
10 but not during the dark cycle. In contrast, there was more time spent sleeping in old age during the
11 dark cycle but not during the light cycle. Nesting quality, based on a ranking score of 1 to 5, where
12 1 corresponds to scattered nesting material and 5 corresponds to a fully rounded cocoon nest
13 (Deacon, 2006; Hess et al., 2008; Gaskill et al., 2013; Okayama et al., 2015) also changed with
14 age (**Fig. 9F**).

Figure 9. Automated analysis of mouse behaviour in a small world. (A) Diagram of the lifetime of a mouse. Data is analyzed from two time points at young and old age during adulthood (around 4 and 11 months approx.). Diagram adapted from (Brust et al., 2015). (B) Motion in the small world across 15 days during young age (blue) and old age (orange) in the same animal (top). a.u. denote arbitrary units. Power spectrum of the small world motion signal of frequencies corresponding to hours per cycle (bottom). (C) Excursion analyses during 15 days at young and old ages. Comparison of the mean small world size (left) during the dark (top) and light cycles

(bottom). Comparison of the mean number of excursions (middle) during the dark (top) and light cycles (bottom). Comparison of the mean number of stops (right) during the dark (top) and light cycles (bottom). (D) Spatial organization of the small world for the same mouse at young age and old age during the dark (top) and light (bottom) cycle. (E) Comparison of the total mean occurrences of eight behaviour classes for the same mouse at young and old ages. (F) Comparison of the distribution of nesting scores for the same mouse at young and old ages.

4. Future applications

The longitudinal and automated data acquisition and analysis of mouse behavior in the small world is a promising approach for advancing clinical and scientific research. In the following subsections we summarize the main advantages and limitations of this approach and highlight possible future directions.

4.1 Advantages and limitations

The automated small world monitoring approach described here has advantages over manual assessments of natural unrestricted rodent behaviour. First, automation allows the continuous and extended recording of signals during periods where human interventions are difficult, including dark cycles, night shifts, limited access to animal facilities, lockdowns, etc. (Poe and Cai, 2020). Second, continuous behavior monitoring can be extended to the entire lifespan of a mouse at scale, thus increasing the chances of recording sporadic or intermittent events that can be important for behavioral phenotyping (Fonio et al., 2012a, 2012b; Bains et al., 2018; Mingrone et al., 2020). Compared to typical behavioural assays that last from a few minutes to some hours, or even days, being able to study the behaviour organization over the lifespan of mice opens novel research avenues that otherwise would remain elusive without heroic efforts by laboratories personnel. Third, the reduction of stress to mice due to the elimination of human interventions increases the sensitivity and reproducibility of experiments (Neff, 2018; Frommlet, 2020). Finally, this approach presents the opportunity to continuously allow animals to voluntarily perform training thus decreasing the time required to achieve a desired performance (Spruijt and DeVisser, 2006; Jhuang

et al., 2010; Murphy et al., 2016; Woodard et al., 2017; Balzani et al., 2018; Neff, 2018; Singh et al., 2019). Although the focus of this review is mouse behaviour, similar approaches can be applied to other nocturnal species such as rats, and diurnal species such as degus, nonhuman anthropoids and humans.

Continuous automated behaviour monitoring also presents challenges. First, the high-throughput approach of the automation of continuous video data acquisition in the small world requires managing, storing and analyzing a large volumes of data (Spruijt and DeVisser, 2006; Gomez-Marin et al., 2014; Sejnowski et al., 2014; von Ziegler et al., 2020). Second, despite the great advances in machine learning for the automated analyses of behaviour in the recent years, more testing of such approaches is needed, particularly on more naturalistic conditions (e.g. multiple housed animals, social interactions, object interactions, etc.). Third, the approach requires data access platforms that share data and compare models. Initial attempts to make progress in this direction include the OpenBehavior project (Jiang et al., 2019a; White et al., 2019) but more efforts in this direction are needed (von Ziegler et al., 2020). Finally, a limitation is the advanced computer science knowledge required to implement and adapt the latest algorithms to neuroscientific and clinical settings (Mathis and Mathis, 2020). Clearly, training in behavioral sciences will require training in AI methods.

4.2 The future of automated mouse behaviour analysis in a small world

Just as improved research methodology is being directed to the analysis and monitoring of mice in a small world, improved research methodology can be applied to the structure and furnishing of that small world. Improvements in the methodology for monitoring small world behavior described in this paper in conjunction with alterations to the small world can lead to modifications of the mouse's small world so that it is an analogue of the natural world of the mouse. Some changes to

laboratory housing in aid of obtaining ideal small world conditions are relatively easy to make. For example, lighting changes can be scheduled to take place gradually, food and water dispensers can be automated so that a mouse must work (forage), and the shape of cages and the location of objects in a cage can be modified in the interests of creating a more realistic small world as well as facilitating behavioral monitoring. Other changes are more difficult to make, including opportunity for territorial competition (Makowska et al., 2019) and continuous breeding (Finn, 1963; Chatkupt et al., 2018).

Beside methodological and structural improvements for the study of mouse behavior in the small world, comparative studies need to be made with both acute behavioral tasks and studies of the organization of mouse behaviour in natural environments. First, these acute tasks can include novel object recognition, highly-skilled motor tasks, and navigation tasks, amongst others. Other kinds of studies may be more difficult to perform. There is a large body of research that uses aversive or stressful conditions to motivate mice to perform a task. The application of such methods in the small world might be challenging (Würbel, 2001; Hess et al., 2008; Hendershott et al., 2016; Zocher et al., 2020). However, there are important advances in brain activity monitoring for behaving mice at multiple scales that could be incorporated to a small world setting (Gagnon-Turcotte et al., 2015; Bermudez-Contreras et al., 2018; Rynes et al., 2020). Second, as more quantitative studies of the organization of behaviour in natural environments are done, a more complete comparison with its counterpart in the small world needs to be carried out (Weissbrod et al., 2013). Based on the studies reviewed here and the examples we provided, we have outlined a good starting point such as the structure of excursions, small world and home-ranges in the small world, as well as the spatiotemporal organization of more complex behaviours such a sleeping, drinking, eating, rearing, nesting and walking. Future work that combines ethologically relevant

1 automated behavioral monitoring in the small world with motivational manipulations,
2 electrophysiological and optophysiological recordings will also be instrumental to complement
3 and expand use of the small world approach (Fan et al., 2011; Idogawa et al., 2020; Sanguinetti-
4 Scheck and Brecht, 2020).

5 **5. Conclusion**

6
7 The automated small world monitoring of behavior over extended periods with minimal human
8 interference along with technological developments and progress of machine learning will
9 continue to advance the field of computational ethology, translational and basic neuroscience
10 research. Here, we provide examples of how the understanding of organized behavior in a small
11 world with respect to a home base and dedicated excursion and their age-related changes can
12 provide insights into the mouse phenotype. In recommending automated monitoring, the
13 recommendation is not to replace well-established and successful specialized experimental
14 approaches. Instead, automated monitoring of behavior in the small world should be seen as a
15 complementary approach that provides new vistas for phenotypic analyses of the mouse.

16 **6. Author contributions**

17 All authors discussed the concepts and contributed to and approved the submission of the final
18 manuscript.

19 **7. Acknowledgments**

20 This work was supported by Natural Sciences and Engineering Research Council of Canada
21 (NSERC) Discovery Grant #40352 and #RGPIN-2017-03857 (MHM), Campus Alberta for
22 Innovation Program Chair, Alberta Alzheimer Research Program, Alzheimer Society of Canada
23 (MHM), NSERC Discovery Grant and Alberta Neuroscience Program Grant (RJS)

8. Disclaimer

The authors have a financial interest in the company “NeuroCage Systems Inc.” that is developing a product “HomeCage Monitoring System” and have a patent application that includes some of the functionality presented in this work. Pending patent for SYSTEMS AND METHODS OF HOMECAGE MONITORING; patent application # 62/857,879 (US) and 3,045,478 (Canada).

9. References

- Abe Y et al. (2020) Behavioral and electrophysiological evidence for a neuroprotective role of aquaporin-4 in the 5xFAD transgenic mice model. *Acta Neuropathol Commun* 8:1–15.
- Bains RS, Wells S, Sillito RR, Armstrong JD, Cater HL, Banks G, Nolan PM (2018) Assessing mouse behaviour throughout the light/dark cycle using automated in-cage analysis tools. *J Neurosci Methods* 300:37–47 Available at: <http://dx.doi.org/10.1016/j.jneumeth.2017.04.014>.
- Balzani E, Falappa M, Balci F, Tucci V (2018) An approach to monitoring home-cage behavior in mice that facilitates data sharing. *Nat Protoc* 13:1331–1347.
- Berman GJ (2018) Measuring behavior across scales. *BMC Biol* 16:1–11.
- Bermudez-Contreras E, Chekhov S, Sun J, Tarnowsky J, McNaughton BL, Mohajerani MH (2018) High-performance, inexpensive setup for simultaneous multisite recording of electrophysiological signals and mesoscale voltage imaging in the mouse cortex. *Neurophotronics* 5:1.
- Bermudez Contreras E, Sutherland RJ, Mohajerani MH, Whishaw IQ (2022) Challenges of a small world analysis for the continuous monitoring of behavior in mice - Methods. OSF Available at: <https://osf.io/2dhxf/>.
- Berry RJ (1970) The Natural History of the House Mouse. *F Stud* 3:219–262.
- Berry RJ, Bronson FH (1992) Life history and bioeconomy of the house mouse. *Biol Rev Camb Philos Soc* 67:519–550.
- Bohnslav JP, Wimalasena NK, Clausing KJ, Dai YY, Yarmolinsky DA, Cruz T, Kashlan AD, Chiappe ME, Orefice LL, Woolf CJ, Harvey CD (2021) DeepEthogram, a machine learning pipeline for supervised behavior classification from raw pixels. *Elife* 10.
- Bolaños LA, Xiao D, Ford NL, Ledue JM, Gupta PK, Doebeli C, Hu H, Rhodin H, Murphy TH (2021) A three-dimensional virtual mouse generates synthetic training data for behavioral analysis. *Nat Methods*.
- Bonhomme F, Orth A (2013) *Mus musculus*. *Brenner’s Encycl Genet Second Ed* 3:515–517.
- Bova A, Kernodle K, Mulligan K, Leventhal D (2019) Automated Rat Single-Pellet Reaching with 3-Dimensional Reconstruction of Paw and Digit Trajectories. *J Vis Exp* 149.
- Brust V, Schindler PM, Lewejohann L (2015) Lifetime development of behavioural phenotype in the house mouse (*Mus musculus*). *Front Zool* 12:1–14.
- Burns R (1786) Poems, chiefly in the Scottish dialect. :138–140 Available at: https://archive.org/details/cihm_39559.
- Chambers LK, Singleton GR, Krebs CJ (2000) Movements and social organization of wild house mice (*Mus domesticus*) in the wheatlands of northwestern Victoria, Australia. *J Mammal* 81:59–69.

- Chatkupt TT, Libal NL, Mader SL, Murphy SJ, Saunders KE (2018) Effect of continuous trio breeding compared with continuous pair breeding in ‘shoebox’ caging on measures of reproductive performance in estrogen receptor knockout mice. *J Am Assoc Lab Anim Sci* 57:328–334.
- Chen X, Zhai H, Liu D, Li W, Ding C, Xie Q, Han H (2020) SiamBOMB: A Real-time AI-based System for Home-cage Animal Tracking, Segmentation and Behavioral Analysis. In: *Proceedings of the 29th IJCAI*, pp 5300–5302.
- Clark BJ, Hamilton DA, Whishaw IQ (2006) Motor activity (exploration) and formation of home bases in mice (C57BL/6) influenced by visual and tactile cues: Modification of movement distribution, distance, location, and speed. *Physiol Behav* 87:805–816.
- Clark BJ, Hines DJ, Hamilton DA, Whishaw IQ (2005) Movements of exploration intact in rats with hippocampal lesions. *Behav Brain Res* 163:91–99.
- Czyzyk TA, Sahr AE, Statnick MA (2010) A model of binge-like eating behavior in mice that does not require food deprivation or stress. *Obesity* 18:1710–1717 Available at: <http://dx.doi.org/10.1038/oby.2010.46>.
- Datta SR (2019) Q&A: Understanding the composition of behavior. *BMC Biol* 17:1–7.
- De Chaumont F, Coura RDS, Serreau P, Cressant A, Chabout J, Granon S, Olivo-Marin JC (2012) Computerized video analysis of social interactions in mice. *Nat Methods* 9:410–417 Available at: <http://dx.doi.org/10.1038/nmeth.1924>.
- Deacon RMJ (2006) Assessing nest building in mice. *Nat Protoc* 1:1117–1119.
- Ebbesen CL, Froemke RC (2020) Automatic tracking of mouse social posture dynamics by 3D videography, deep learning and GPU-accelerated robust optimization. *bioRxiv*.
- Egnor SER, Branson K (2016) Computational Analysis of Behavior. *Annu Rev Neurosci* 39:217–236.
- Eibl-Eibesfeldt I (1970) *Ethology: the biology of behaviour*. New York: Holt, Rinehart and Winston.
- Eilam D, Golani I (1989) Home base behavior of rats (*Rattus norvegicus*) exploring a novel environment. *Behav Brain Res* 34:199–211.
- Fan D, Rich D, Holtzman T, Ruther P, Dalley JW, Lopez A, Rossi MA, Barter JW, Salas-Meza D, Herwik S, Holzhammer T, Morizio J, Yin HH (2011) A wireless multi-channel recording system for freely behaving mice and rats. *PLoS One* 6.
- Finn CA (1963) Reproductive Capacity and Litter Size in Mice: Effect of Age and. *J Reprod Fertil* 6:205–214.
- Fonio E, Benjamini Y, Golani I (2012a) Short and Long Term Measures of Anxiety Exhibit Opposite Results. *PLoS One* 7.
- Fonio E, Golani I, Benjamini Y (2012b) Measuring behavior of animal models: Faults and remedies. *Nat Methods* 9:1167–1170.
- Frommlet F (2020) Improving reproducibility in animal research. *Sci Rep* 10:1–2 Available at: <https://doi.org/10.1038/s41598-020-76398-3>.
- Fuochi S, Rigamonti M, Iannello F, Raspa M, Scavizzi F, de Girolamo P, D’Angelo L (2021) Phenotyping spontaneous locomotor activity in inbred and outbred mouse strains by using Digital Ventilated Cages. *Lab Anim (NY)* 50:215–223 Available at: <http://dx.doi.org/10.1038/s41684-021-00793-0>.
- Gagnon-Turcotte G, Kisomi AA, Ameli R, Camaro COD, LeChasseur Y, Néron JL, Bareil PB, Fortier P, Bories C, de Koninck Y, Gosselin B (2015) A wireless optogenetic headstage with multichannel electrophysiological recording capability. *Sensors (Switzerland)*

15:22776–22797.

Gaskill BN, Karas AZ, Garner JP, Pritchett-Corning KR (2013) Nest building as an indicator of health and welfare in laboratory mice. *J Vis Exp*:51012.

Geuther BQ, Deats SP, Fox KJ, Murray SA, Braun RE, White JK, Chesler EJ, Lutz CM, Kumar V (2019) Robust mouse tracking in complex environments using neural networks. *Commun Biol* 2:1–11 Available at: <http://dx.doi.org/10.1038/s42003-019-0362-1>.

Gire DH, Kapoor V, Arrighi-Allisan A, Seminara A, Murthy VN (2016) Mice develop efficient strategies for foraging and navigation using complex natural stimuli. *Curr Biol* 26:1261–1273.

Golani I, Benjamini Y (2018) Home base. *Encicl Anim Cogn Behav* Available at: <https://www.baseperformance.net/>.

Gomez-Marin A, Paton JJ, Kampff AR, Costa RM, Mainen ZF (2014) Big behavioral data: Psychology, ethology and the foundations of neuroscience. *Nat Neurosci* 17:1455–1462.

Gonder JC, Laber K (2007) A renewed look at laboratory rodent housing and management. *ILAR J* 48:29–36.

Graving JM, Chae D, Naik H, Li L, Koger B, Costelloe BR, Couzin ID (2019) Deepposekit, a software toolkit for fast and robust animal pose estimation using deep learning. *Elife* 8:1–42.

Gris K V., Coutu J-P, Gris D (2017) Supervised and Unsupervised Learning Technology in the Study of Rodent Behavior. *Front Behav Neurosci* 11:1–6.

Hari R, Kujala M V. (2009) Brain basis of human social interaction: From concepts to brain imaging. *Physiol Rev* 89:453–479.

Hayne DW (1949) Calculation of Size of Home Range. *J Mammal* 30:1–18.

Hendershott TR, Cronin ME, Langella S, McGuinness PS, Basu AC (2016) Effects of environmental enrichment on anxiety-like behavior, sociability, sensory gating, and spatial learning in male and female C57BL/6J mice. *Behav Brain Res* 314:215–225 Available at: <http://dx.doi.org/10.1016/j.bbr.2016.08.004>.

Hess SE, Rohr S, Dufour BD, Gaskill BN, Pajor EA, Garner JP (2008) Home improvement: C57BL/6J mice given more naturalistic nesting materials build better nests. *J Am Assoc Lab Anim Sci* 47:25–31.

Hoffman KL, Battaglia FP, Harris K, MacLean JN, Marshall L, Mehta MR (2007) The upshot of up states in the neocortex: from slow oscillations to memory formation. *J Neurosci* 27:11838–11841 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/17978020> [Accessed December 29, 2014].

Houston AI, Mcnamara JM (2014) Foraging currencies, metabolism and behavioural routines. *J Anim Ecol* 83:30–40.

Hsu A, Yttri E (2020) B-SOiD: An Open Source Unsupervised Algorithm for Discovery of Spontaneous Behaviors. *BioRxiv*:1–40.

Huang K, Han Y, Chen K, Pan H, Yi W, Li X, Liu S, Wei P, Wang L (2020) Mapping Mouse Behavior via an Unsupervised Spatio- temporal Sequence Decomposition Framework.

Idogawa S, Yamashita K, Sanda R, Numano R, Koida K, Kawano T (2020) A Lightweight, Wireless Bluetooth Low-Energy Neuronal Recording System for Mice. *Sensors Actuators B Chem*:129423 Available at: <https://doi.org/10.1016/j.snb.2020.129423>.

Jedidi-Ayoub S, Mishchanchuk K, Liu A, Renaudineau S, Duvelle É, Grieves RM (2021) Volumetric spatial behaviour in rats reveals the anisotropic organisation of navigation. Springer Berlin Heidelberg. Available at: <https://doi.org/10.1007/s10071-020-01432-w>.

- 1 Jhuang H, Garrote E, Yu X, Khilnani V, Poggio T, Steele AD, Serre T (2010) Automated home-
2 cage behavioural phenotyping of mice. *Nat Commun* 1:1–9 Available at:
3 <http://dx.doi.org/10.1038/ncomms1064>.
- 4 Jiang Z, Crookes D, Green BD, Zhao Y, Ma H, Li L, Zhang S, Tao D, Zhou H (2019a) Context-
5 Aware Mouse Behavior Recognition Using Hidden Markov Models. *IEEE Trans Image*
6 *Process* 28:1133–1148.
- 7 Jiang Z, Liu Z, Chen L, Tong L, Zhang X, Lan X, Crookes D, Yang M-H, Zhou H (2019b)
8 Detecting and Tracking of Multiple Mice Using Part Proposal Networks. *arXiv* Available
9 at: <http://arxiv.org/abs/1906.02831>.
- 10 Klarsfeld A (2013) At the dawn of chronobiology. *BibNum*:1–12 Available at:
11 [https://docs.google.com/viewer?a=v&pid=sites&srcid=ZGVmYXVsdGRvbWFpbnxrbGFy](https://docs.google.com/viewer?a=v&pid=sites&srcid=ZGVmYXVsdGRvbWFpbnxrbGFyc2ZlbGRhbmRyZXxneDo0OTIyYzUwY2IyYWVjMjg2)
12 [c2ZlbGRhbmRyZXxneDo0OTIyYzUwY2IyYWVjMjg2](https://docs.google.com/viewer?a=v&pid=sites&srcid=ZGVmYXVsdGRvbWFpbnxrbGFyc2ZlbGRhbmRyZXxneDo0OTIyYzUwY2IyYWVjMjg2).
- 13 Kohsaka A, Bass J (2007) A sense of time: how molecular clocks organize metabolism. *Trends*
14 *Endocrinol Metab* 18:4–11.
- 15 Kohsaka A, Laposky AD, Ramsey KM, Estrada C, Joshi C, Kobayashi Y, Turek FW, Bass J
16 (2007) High-Fat Diet Disrupts Behavioral and Molecular Circadian Rhythms in Mice. *Cell*
17 *Metab* 6:414–421.
- 18 Kolker DE, Hotz Vitaterna M, Fruechte EM, Takahashi JS, Turek FW (2004) Effects of age on
19 circadian rhythms are similar in wild-type and heterozygous Clock mutant mice. *Neurobiol*
20 *Aging* 25:517–523.
- 21 Lee S, Hayakawa T, Nishimura C, Yawata S, Yagi H, Watanabe D, Ishikawa M (2019)
22 Comparison of Deep Learning and Image Processing for Tracking the Cognitive Motion of
23 a Laboratory Mouse. *2019 IEEE Biomed Circuits Syst Conf*:1–4.
- 24 Leng X, Wohl M, Ishii K, Nayak P, Id KA (2020) Quantifying influence of human choice on the
25 automated detection of Drosophila behavior by a supervised machine learning algorithm.
26 *PLoS One* 15:1–27.
- 27 Levitis DA, Lidicker WZ, Freund G (2009) Behavioural biologists don't agree on what
28 constitutes behaviour. *Anim Behav* 78:103–110 Available at:
29 <https://www.sciencedirect.com/science/article/pii/S0003347209001730>.
- 30 Li D, Hao J, Yao X, Liu Y, Peng T, Jin Z, Meng F (2020) Observations of the foraging behavior
31 and activity patterns of the Korean wood mouse, *apodemus peninsulae*, in China, using
32 infra-red cameras. *Zookeys* 2020:139–155.
- 33 Lim MA, Louie B, Ford D, Heath K, Cha P, Betts-Lacroix J, Lum PY, Robertson TL, Schaevitz
34 L (2017) Development of the Digital Arthritis Index, a novel metric to measure disease
35 parameters in a rat model of rheumatoid arthritis. *Front Pharmacol* 8:1–18.
- 36 Logan RW, McClung CA (2019) Rhythms of life: circadian disruption and brain disorders across
37 the lifespan. *Nat Rev Neurosci* 20:49–65 Available at: [http://dx.doi.org/10.1038/s41583-](http://dx.doi.org/10.1038/s41583-018-0088-y)
38 [018-0088-y](http://dx.doi.org/10.1038/s41583-018-0088-y).
- 39 Luxem K, Fuhrmann F, Kürsch J, Remy S, Bauer P (2020) Identifying Behavioral Structure from
40 Deep Variational Embeddings of Animal Motion. *bioRxiv*:1–19.
- 41 Ma Y, Liu K, Guan Z, Xu X, Qian X, Bao H (2018) Background augmentation generative
42 adversarial networks (BAGANs): Effective data generation based on GAN-augmented 3D
43 synthesizing. *Symmetry (Basel)* 10.
- 44 MacKay JWB, Murphy EC, Anderson SH, Russell JC, Hauber ME, Wilson DJ, Clout MN
45 (2011) A successful mouse eradication explained by site-specific population data. ...
46 *Invasives Erad* ... 1989:198–203 Available at:

http://www.issg.org/pdf/publications/Island_Invasives/pdfwebview/2MacKay.pdf.
 Makowska IJ, Franks B, El-Hinn C, Jorgensen T, Weary DM (2019) Standard laboratory housing for mice restricts their ability to segregate space into clean and dirty areas. *Sci Rep* 9:1–10.
 Makowska IJ, Weary DM (2020) A Good Life for Laboratory Rodents? *ILAR J* 00:1–16.
 Maraghehmoghaddam A (2020) Synthetic data generation for deep learning model training to understand livestock behavior.
 Martin AL, Brown RE (2010) The lonely mouse: Verification of a separation-induced model of depression in female mice. *Behav Brain Res* 207:196–207.
 Mathis A, Mamidanna P, Cury KM, Abe T, Murthy VN, Mathis MW, Bethge M (2018) DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. *Nat Neurosci* 21:1281–1289 Available at: <http://dx.doi.org/10.1038/s41593-018-0209-y>.
 Mathis A, Schneider S, Lauer J, Mathis MW (2020) A Primer on Motion Capture with Deep Learning: Principles, Pitfalls and Perspectives. *Neuron* 108:44–65 Available at: <http://arxiv.org/abs/2009.00564>.
 Mathis MW, Mathis A (2020) Deep learning tools for the measurement of animal behavior in neuroscience. *Curr Opin Neurobiol* 60:1–11 Available at: <http://arxiv.org/abs/1909.13868>.
 Merel J, Aldarondo D, Marshall J, Tassa Y, Wayne G, Ölveczky B (2019) Deep neuroethology of a virtual rodent. *arXiv*:1–20.
 Mingrone A, Kaffman A, Kaffman A, Botterill JJ (2020) The Promise of Automated Home-Cage Monitoring in Improving Translational Utility of Psychiatric Research in Rodents. *Front Neurosci* 14:1–8.
 Murphy TH, Boyd JD, Bolaños F, Vanni MP, Silasi G, Haupt D, LeDue JM (2016) High-throughput automated home-cage mesoscopic functional imaging of mouse cortex. *Nat Commun* 7:11611 Available at: <http://www.nature.com/doi/10.1038/ncomms11611>.
 Nakamura TJ, Nakamura W, Yamazaki S, Kudo T, Cutler T, Colwell CS, Block GD (2011) Age-related decline in circadian output. *J Neurosci* 31:10201–10205.
 Neff EP (2018) Home sweet home cage. *Lab Anim (NY)* 47:261–264 Available at: <http://www.nature.com/articles/s41684-018-0151-3>.
 Nilsson SR, Goodwin N, Choong JJ, Hwang S, Wright H, Norville Z, Tong X, Lin D, Bentzley B, Eshel N, McLaughlin R, Golden S (2020) Simple Behavioral Analysis (SimBA) – an open source toolkit for computer classification of complex social behaviors in experimental animals. 02:1–29.
 Nourizonoz A, Zimmermann R, Ho CLA, Pellat S, Ormen Y, Prévost-Solié C, Reymond G, Pifferi F, Aujard F, Herrel A, Huber D (2020) EthoLoop: automated closed-loop neuroethology in naturalistic environments. *Nat Methods* 17 Available at: <http://dx.doi.org/10.1038/s41592-020-0961-2>.
 Okayama T, Goto T, Toyoda A (2015) Assessing nest-building behavior of mice using a 3D depth camera. *J Neurosci Methods* 251:151–157 Available at: <http://dx.doi.org/10.1016/j.jneumeth.2015.05.019>.
 Peng Q, Mechanic J, Shoieb A, Pardo ID, Schaevitz L, Fenyk-Melody J, Vitsky A, Boucher M, Somps C, Cook JC, Liu CN (2019) Circulating microRNA and automated motion analysis as novel methods of assessing chemotherapy-induced peripheral neuropathy in mice. *PLoS One* 14:1–17.
 Pereira TD, Shaevitz JW, Murthy M (2020) Quantifying behavior to understand the brain. *Nat Neurosci* Available at: <http://dx.doi.org/10.1038/s41593-020-00734-z>.

- 1 Pernold K, Rullman E, Ulfhake B (2021) Major oscillations in spontaneous home-cage activity
2 in C57BL/6 mice housed under constant conditions. *Sci Rep* 11:1–13 Available at:
3 <https://doi.org/10.1038/s41598-021-84141-9>.
- 4 Poe GR, Cai DJ (2020) The lab on lockdown: thinking back and looking ahead. *Nat Rev*
5 *Neurosci* 21:447–448 Available at: <http://dx.doi.org/10.1038/s41583-020-0353-8>.
- 6 Recordati C, De Maglie M, Marsella G, Milite G, Rigamonti A, Paltrinieri S, Scanziani E (2019)
7 Long-Term Study on the Effects of Housing C57BL/6NCrl Mice in Cages Equipped With
8 Wireless Technology Generating Extremely Low-Intensity Electromagnetic Fields. *Toxicol*
9 *Pathol* 47:598–611.
- 10 Reynolds AM (2018) Current status and future directions of Levy walk research. *Biol Open* 7:1–
11 6.
- 12 Richardson CA (2012) Automated homecage behavioural analysis and the implementation of the
13 three Rs in research involving mice. *Altern Lab Anim* 40:7–9.
- 14 Robinson L, Riedel G (2014) Comparison of automated home-cage monitoring systems:
15 Emphasis on feeding behaviour, activity and spatial learning following pharmacological
16 interventions. *J Neurosci Methods* 234:13–25 Available at:
17 <http://dx.doi.org/10.1016/j.jneumeth.2014.06.013>.
- 18 Russell SJ, Norvig P (2010) Artificial Intelligence: A Modern Approach.
- 19 Rynes ML, Daniel S, Linn S, Laroque M, Rajendran V, Dominguez J, Haddjstamolou O, Navabi
20 ZS, Ghanbari L, Johnson GW, Nazari M, Mohajerani M, Kodandaramaiah SB (2020)
21 Miniaturized head-mounted device for whole cortex mesoscale imaging in freely behaving
22 mice. *bioRxiv*.
- 23 Salameh G, Jeffers MS, Wu J, Pitney J, Silasi G (2020) The home-cage automated skilled
24 reaching apparatus (HASRA): individualized training of group-housed mice in a single
25 pellet reaching task. *Eneuro* 7:ENEURO.0242-20.2020.
- 26 Sanguinetti-Scheck JI, Brecht M (2020) Home, head direction stability, and grid cell distortion. *J*
27 *Neurophysiol* 123:1392–1406.
- 28 Schmidhuber J (2015) Deep Learning in neural networks: An overview. *Neural Networks* 61:85–
29 117 Available at: <http://dx.doi.org/10.1016/j.neunet.2014.09.003>.
- 30 Scott BB, Brody CD, Tank DW (2013) Cellular Resolution Functional Imaging in Behaving Rats
31 Using Voluntary Head Restraint. *Neuron* 80:371–384 Available at:
32 <http://dx.doi.org/10.1016/j.neuron.2013.08.002>.
- 33 Seib V, Lange B, Wirtz S (2020) Mixing Real and Synthetic Data to Enhance Neural Network
34 Training - A Review of Current Approaches. *arXiv*.
- 35 Sejnowski TJ, Churchland PS, Movshon JA (2014) Putting big data to good use in neuroscience.
36 *Nat Neurosci* 17:1440–1441.
- 37 Singh S, Bermudez-Contreras E, Nazari M, Sutherland RJ, Mohajerani MH (2019) Low-cost
38 solution for rodent home-cage behaviour monitoring. *PLoS One* 14:e0220751.
- 39 Spruijt BM, DeVisser L (2006) Advanced behavioural screening: automated home cage
40 ethology. *Drug Discov Today Technol* 3:231–237.
- 41 Tapia-Orsorio A, Salgado-Delgado R, Angeles-Castellanos M, Escobar C (2013) Disruption of
42 circadian rhythms due to chronic constant light leads to depressive and anxiety-like
43 behaviors in the rat. *Behav Brain Res* 252:1–9 Available at:
44 <http://dx.doi.org/10.1016/j.bbr.2013.05.028>.
- 45 Thompson SM, Berkowitz LE, Clark BJ (2018) Behavioral and Neural Subsystems of Rodent
46 Exploration. *Learn Motiv* 61:3–15.

- Tomich PQ (1970) Movement Patterns of Field Rodents in Hawaii. *Pacific Sci* 24:195–234.
- van Dam EA, Noldus LPJJ, van Gerven MAJ (2020) Deep learning improves automated rodent behavior recognition within a specific experimental setup. *J Neurosci Methods* 332:108536 Available at: <https://doi.org/10.1016/j.jneumeth.2019.108536>.
- van den Boom BJG, Pavlidi P, Wolf CJH, Mooij AH, Willuhn I (2017) Automated classification of self-grooming in mice using open-source software. *J Neurosci Methods* 289:48–56 Available at: <http://dx.doi.org/10.1016/j.jneumeth.2017.05.026>.
- van Vugt M, Kamilaris A (2019) The potential of synthetic training data for training deep learning models.
- Vogt N (2018) Machine learning in neuroscience. *Nat Methods* 15:33.
- Voikar V, Gaburro S (2020) Three Pillars of Automated Home-Cage Phenotyping of Mice: Novel Findings, Refinement, and Reproducibility Based on Literature and Experience. *Front Behav Neurosci* 14:1–16.
- von Ziegler L, Sturman O, Bohacek J (2020) Big behavior: challenges and opportunities in a new era of deep behavior profiling. *Neuropsychopharmacology*:1–14 Available at: <http://www.nature.com/articles/s41386-020-0751-7>.
- Wei K, Kording KP (2018) Behavioral tracking gets real. *Nat Neurosci* 21.
- Weissbrod A, Shapiro A, Vasserman G, Edry L, Dayan M, Yitzhaky A, Hertzberg L, Feinerman O, Kimchi T (2013) Automated long-term tracking and social behavioural phenotyping of animal colonies within a semi-natural environment. *Nat Commun* 4.
- Wexler Y, Benjamini Y, Golani I (2018) Vertical exploration and dimensional modularity in mice. *R Soc Open Sci* 5.
- Whishaw IQ, Gharbawie OA, Clark BJ, Lehmann H (2006) The exploratory behavior of rats in an open environment optimizes security. *Behav Brain Res* 171:230–239.
- White SR, Amarante LM, Kravitz A V, Laubach M (2019) Novel Tools and Methods The Future Is Open: Open-Source Tools for Behavioral Neuroscience Research Why Open Source? *Significance Statement. Commentary* 6:223–242.
- Whittaker AL, Howarth GS, Hickman DL (2012) Effects of space allocation and housing density on measures of wellbeing in laboratory mice: A review. *Lab Anim* 46:3–13.
- Wiltschko AB, Johnson MJ, Iurilli G, Peterson RE, Katon JM, Pashkovski SL, Abaira VE, Adams RP, Datta SR (2015) Mapping Sub-Second Structure in Mouse Behavior. *Neuron* 88:1121–1135 Available at: <http://dx.doi.org/10.1016/j.neuron.2015.11.031>.
- Wiltschko AB, Tsukahara T, Zeine A, Anyoha R, Gillis WF, Markowitz JE, Peterson RE, Katon J, Johnson MJ, Datta SR (2020) Revealing the structure of pharmacobehavioral space through motion sequencing. *Nat Neurosci* 23:1433–1443 Available at: <http://dx.doi.org/10.1038/s41593-020-00706-3>.
- Wolfer DP, Voikar V, Vannoni E, Colacicco G, Lipp HP (2012) Mouse Phenotyping in the IntelliCage : From Spontaneous Behavior to Cognitive Function Spontaneous behavior. 2012:66–69.
- Woodard CL, Bolaños F, Boyd JD, Silasi G, Murphy TH, Raymond LA (2017) An automated home-cage system to assess learning and performance of a skilled motor task in a mouse model of huntington’s disease. *eNeuro* 4:1–13.
- Würbel H (2001) Ideal homes? Housing effects on rodent brain and behaviour. *Trends Neurosci* 24:207–211.
- Zocher S, Schilling S, Grzyb AN, Adusumilli VS, Lopes JB, Günther S, Overall RW, Winter Y, Kempermann G (2020) Early-life environmental enrichment generates persistent

individualized behavior in mice. Sci Adv 6.

Supplemental Material

Methods

Animals.

One female C57BL/6J mouse was continuously monitored in its homecage for more than 10 months. Several behavioural features of this mouse were compared at two time points in its lifetime, one during its young adulthood (around four months old) and one during its old age (around 11 months old). Animal housing and all experimental protocols were approved by the University of Lethbridge Animal Care Committee in line with the guidelines from the Canadian Council for Animal Care.

Video data.

All analyses presented were performed on continuous videos of a single-housed mouse in its homecage (Singh et al., 2019). The video-based analyses were performed using videos corresponding to 15 days at both time points (young vs old). The videos were recorded using an automated homecage monitoring system (Neurocage Systems Ltd). The videos were recorded at 30 frames per second and 640x480 pixel resolution with the infrared (IR) Picamera module (RaspberryPi) and divided into segments of 20 min each. IR light emission diodes (LEDs) were used during the dark cycle and white LEDs during the light cycle to maintain constant uniform illumination.

Automated animal tracking.

DeepLabCut (DLC) was used to track 7 body parts in the homecage videos (Mathis et al., 2018). We used 200 frames from 6 different 20 min videos to train DLC for 1,000,000 iterations. DLC

was trained in an Nvidia GeForce 1080 GTX Ti graphics card on a Dell Workstation T7500. The positions of the body parts for all the videos were preprocessed and analysed using custom Python code for all the excursion, homebase and stops measurements.

Homebase calculation.

The homebase of a mouse in the homecage was defined as the region of homecage where mice spend most of the time and where excursions originate and terminate (Clark et al., 2005; Eilam and Golani, 1989; Golani and Benjamini, 2018). To determine this location and its areas, we calculated an occupancy map thresholded at the 95 percentile and calculated the centroid and area of the region using the OpenCV library. The area units corresponded to pixels and were denoted as arbitrary (a.u.) given that due to the lens optics (Fish-eye lens), the visual field is distorted and does not have a direct mapping to spatial units (e.g. cm^2). This can be corrected using algorithms to transform the distorted visual field appropriately (Lee et al., 2019).

Excursions and stops

Excursions were defined as “trips” that a mouse executes starting from the homebase and terminate back in the homebase. During an excursion, a mouse stops for different periods of time displaying different behaviours such as grooming, rearing, eating, and drinking. A stop was defined as an event that occurred when a mouse stopped moving for at least 1 sec staying in the same location (Eilam and Golani, 1989).

Animal motion in the homecage.

Animal motion estimation in each recorded video in the homecage was calculated using the automated vector motion data using the Picamera library 1.13 (<https://picamera.readthedocs.io/en/release-1.13/>).

Ethograms

The automated behavioural classification in time (ethogram) was calculated using *DeepEthogram* (Bohnslav et al., 2021). We trained *DeepEthogram* to classify mouse behavior into nesting, sleeping, drinking, eating, moving, rearing, grooming and background classes using manually classified labels in a small dataset of eight 20 min videos (four during the light cycle and four during the dark cycle). Using this trained network, we classified the behaviour on a single mouse for 2 hours every day during 15 days, one during its young adult age and another during its old age.

Nesting analysis

The quality of the nest was scored in an automated manner using deep learning. We trained a deep convolutional neural network (CNN) to score the nest quality of the same animal using frames from three-hour videos in every day during 15 days at two time points in its lifetime, one during its young adult age and another during its old age (defined above).

The trained CNN consisted of 2 convolutional layers and 2 pooling layers. To train this deep network, we used manually nest scoring using 1-5 score labels (1 poorest to 5 good nest) described in (Deacon, 2006). The human scoring was done by two experienced experimenters.

Code.

All the analyses were implemented in Python 3.5 and is available upon reasonable request.

References

- Bohnslav, J.P., Wimalasena, N.K., Clausing, K.J., Dai, Y.Y., Yarmolinsky, D.A., Cruz, T., Kashlan, A.D., Chiappe, M.E., Orefice, L.L., Woolf, C.J., Harvey, C.D., 2021. DeepEthogram, a machine learning pipeline for supervised behavior classification from raw pixels. *Elife* 10. <https://doi.org/10.7554/elife.63377>
- Clark, B.J., Hines, D.J., Hamilton, D.A., Whishaw, I.Q., 2005. Movements of exploration intact in rats with hippocampal lesions. *Behav. Brain Res.* 163, 91–99. <https://doi.org/10.1016/j.bbr.2005.04.007>
- Deacon, R.M.J., 2006. Assessing nest building in mice. *Nat. Protoc.* 1, 1117–1119. <https://doi.org/10.1038/nprot.2006.170>
- Eilam, D., Golani, I., 1989. Home base behavior of rats (*Rattus norvegicus*) exploring a novel environment. *Behav. Brain Res.* 34, 199–211. [https://doi.org/10.1016/S0166-4328\(89\)80102-0](https://doi.org/10.1016/S0166-4328(89)80102-0)
- Golani, I., Benjamini, Y., 2018. Home base. *Encicl. Anim. Cogn. Behav.*
- Lee, M., Kim, H., Paik, J., 2019. Correction of Barrel Distortion in Fisheye Lens Images Using Image-Based Estimation of Distortion Parameters. *IEEE Access* 7, 45723–45733. <https://doi.org/10.1109/ACCESS.2019.2908451>
- Mathis, A., Mamidanna, P., Cury, K.M., Abe, T., Murthy, V.N., Mathis, M.W., Bethge, M., 2018. DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. *Nat. Neurosci.* 21, 1281–1289. <https://doi.org/10.1038/s41593-018-0209-y>
- Singh, S., Bermudez-Contreras, E., Nazari, M., Sutherland, R.J., Mohajerani, M.H., 2019. Low-cost solution for rodent home-cage behaviour monitoring. *PLoS One* 14, e0220751. <https://doi.org/10.1371/journal.pone.0220751>