Tracking of Individual Mice in a Social Setting Using Video Tracking Combined with RFID tags

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Background

Rodents used in laboratory research are housed in small groups in cages where they eat, sleep, drink, groom and interact socially. Procedures and behavioural tests to analyse an animal's capabilities and fitness are often laborious, slow, subjective and unnatural. Experimenter influence is a particularly difficult issue; even if the data capture itself can be automated, or controlled, the presence of the scientist during the experiment may have an influence [e.g. 1]. These challenges are not new but with increasing interest in longitudinal studies, for example the analysis of effects of aging or the impact of neurodegenerative diseases the ability to accurately and consistently measure behaviour over prolonged times becomes extremely important [2].

We designed, implemented and validated a system for collecting longitudinal data on individual animals who are, importantly, still housed within a normal social group. A range of homecage analysis systems already exists but none quite meet the constraints we had: Most of the existing systems are focussed on single animals or use essentially bespoke environments. Instead we sought to develop a system that was completely compatible with modern high-density IVC caging systems and that could slot seamlessly into high-throughput facilities [3].

System Design

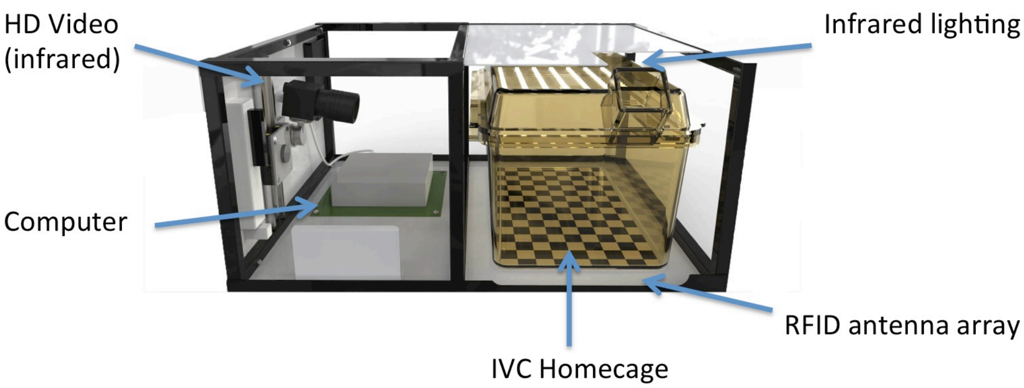

Figure 1: Schematic of the Home Cage Analysis System.

The system is entirely built around a normal IVC homecage designed for a small social group of mice (Fig 2). All the studies described here were performed using Techniplast Sealsafe IVC Blue line cages. The general design is compatible with other cages and racks of similar dimensions and we have also developed a similar system for rats [4]. Identity tags are already widely used in the field and involve the non-surgical implantation of minute, low-cost RFID asset tags into each animal. To achieve spatial monitoring of individual location and detect animal activity, the home-cage is then placed on a low profile base-plate that contains a 2D array of RFID antennae. Each of the antennae in the baseplate is designed to energise a small spatial area within the cage and read the identity of a tagged animal within that space. We also included an infrared light source and infrared camera to record video footage for validation and to allow automated behaviour recognition. A small computer is included to record the data; and a frame to match the rack it is installed into; and the appropriate power supply units that complete the system. The complete physical system occupies two spaces in a standard IVC mouse rack and holds one standard, unmodified cage (i.e. 50% occupancy in a full rack).

Identification of locomotor behaviours of individuals

Locomotion of individuals can be extracted from the social group using the unique ID from the RFID tags. Each antenna under the homecage returns an approximate position and time. To validate the system we recorded a series of top-down videos and manually annotated the centre of mass of each animal once every 25 frames (once per second). We then looked at the correlation of distance travelled from the manual annotations compared to the same distance inferred from the RFID array of the baseplate (Fig. 2). Since the antennae position the animals at the spatial centre of the electromagnetic fields then the distance moved should, on average, be a slight underestimate of actual distance moved but nonetheless shows a strong rank correlation (Spearman's rank coefficient $\rho = 0.911$, $p = 7.55 \times 10^{-16}$, $N = 39$).

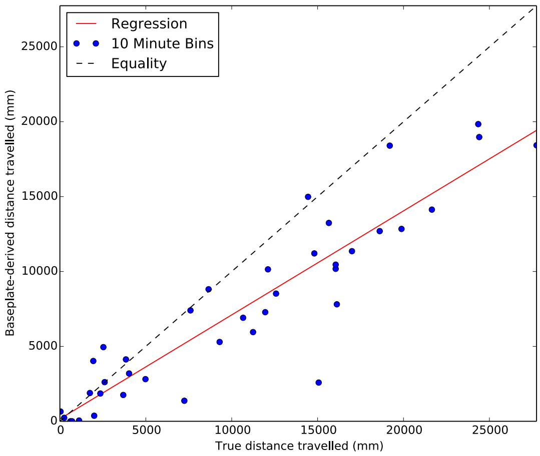

Figure 2. Correlation plot of distance (mm) moved by each animal in 10 minutes measured by annotators using top-down video footage or estimated from the RFID antenna array under the homecage.

When applied to individuals, cages and entire cohorts over multiple days, patterns of activity can be measured accurately. For more details, see the accompanying presentation by Nolan.

Identification of individual behaviours from video

Infrared video provides a means to extract more complex behaviours of the animals at any point in the light-dark cycle. We obtained a series of training videos with a range of common, spontaneous behaviours annotated by three experts. One example is 'drinking'. We trained an SVM classifier to identify drinking based utilising histograms of spatiotemporal features similar to optic flow, extracted at the drinking spout. Bouts of drinking can then be assigned to the most likely animal based on RFID-based position estimates. At the time of writing, drinking events are detected with an average frame-by-frame accuracy of 91.2% and a mean accuracy of ~84.2% when normalised to take into account the low probability of observing drinking events. When applied to a set of more than 40 half hour videos, automated estimates of the proportion of time spent drinking correlate well with human estimates although tending to overestimate with current parameters (Fig 3).

Figure 3 Left: Shows the correlation of drinking bouts as detected by the automatic classifier compared to a human expert. Right: Example frame from video.

Behaviour of animals as a social group in the homecage

The tracking of individuals within the group also unlocks a potential to measure spontaneous social interactions within the cage. While our work on this aspect is at a very early stage we can show clear patterns that naturally emerge. We recover a range of parameters including spatial positions and average pairwise distances between individuals onto a spatial map we can easily visualise where in the cage the animals congregate (huddle). Dividing these data into time bins allows us to see the animals huddled together during the light phase and then splitting up to explore their environment more actively (Fig 4) and independently just in advance of the dark phase (anticipation).

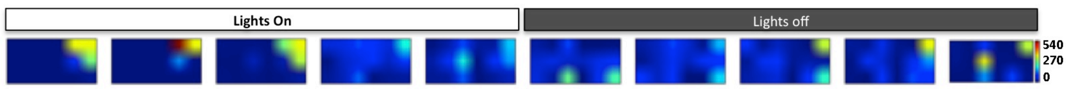

Figure 4. Each frame shows the average position of the animals over a 6minute period coloured by the seconds spent in that position. . The lights are on during the first half (left) and off for the second half (right). The animals were reared under a constant 12:12 light:dark cycle. The social group disperses just before the expected lights off time.

Discussion

The system we present is highly compatible with modern, high-density animal facilities. It has minimal disruption to locally established animal husbandry procedures. The ability to work in normal social groups has profound welfare implications and helps simplify colony management. The combination of HD video and RFID tracking provides the data needed to observer behaviours yet recover the identity of individual animals. Most importantly it allows the automated extraction of a range of spontaneous behaviours (some examples described above) and can be used in longitudinal studies spanning days/weeks. Retaining the data also means that all automated analysis performed by the system can easily be validated. The automatic extraction of behaviours from video is a complex challenge and algorithms are under constant development and refinement. However the platform is stable and the data can be retained so as new algorithms are developed, existing datasets can easily be re-examined. The first studies using these systems are nearing completion and we see very clear patterns of behaviour that vary with the genetic strain under investigation and which will be presented at the workshop.

Mouse Husbandry and Ethical Approval

Animal studies described here were subject to the guidance issued by the Medical Research Council (UK) in Responsibility in the Use of Animals for Medical Research (July 1993), were dependent on an institutional Animal Welfare and Ethical Review Body evaluation and were carried out under UK Home Office Project Licenses #30/2995 and #30/3206.

Availability

Data used in this study including video and annotations will be made publically available under an open license at the time of full publication. Early access may be requested by contacting the authors. The system (hardware and software) is marketed by Actual Analytics Ltd (Edinburgh, UK) www.actualanalytics.com

Acknowledgements

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