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Tracking 'tails': refining motor activity monitoring in rats and mice

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Abstract

Motor activity monitoring is used in specialist regulatory toxicology studies to investigate test item related neurobehavioral effects.

Introduction of a new video tracking software system which detects the centre point, tail base and nose tip of rodents to individually track the movement of each animal in a non-invasive way.

The system was successfully validated and demonstrated it could be used within the constraints regulatory toxicology studies in both rats and mice.

The data generated demonstrated that the system accurately identified the targeted parameters on the animal which aligned with the needs of the study.

Introduction

A variety of regulatory rat and mouse toxicology studies for pharmaceuticals, agrochemicals and chemicals require the inclusion of neurobehavioral testing. These include water maze tests for learning, memory and motor activity monitoring which must be performed at specific times within the toxicology studies.

Motor activity assessments measure parameters such as distance moved, time at rest and count of total activity to determine whether a test item has a neurobehavioral effect.

The modern technology uses video capture and analysis can not only measure the required motor activity parameters but can assess additional measurements later from recordings of the same dosing and recording sessions as refinement.

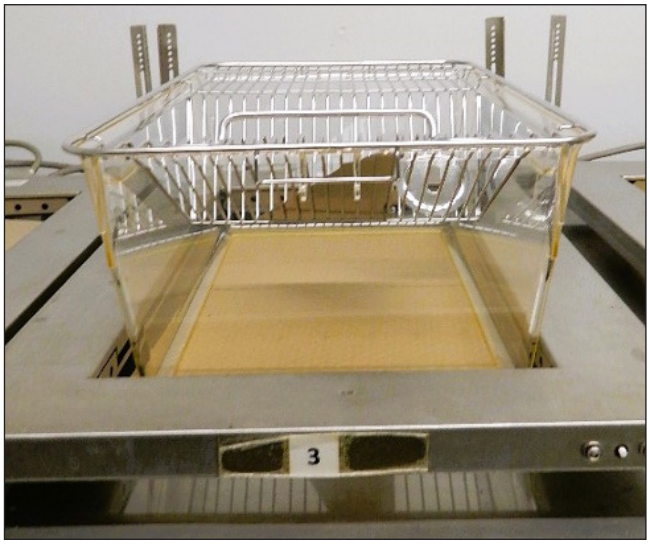


Figure 1. Legacy motor activity recording system which used beam break technology.

Figure 2. Cage set up with metal grid top was unsuitable for use with data capture.

Aim

To develop a caging, orientation and lighting system that enabled simultaneous video capture of sufficient animals to allow efficient assessment during toxicology studies.

To extend the video tracking software we already used for Morris Maze to provide robust measurements of distance travelled, time at rest and count of total activity.

Method

The Motor activity module detects contrast between the animal subject and the background.

The contrast between the subject and their background was maximised by using:

– 1700 x 1300 mm infrared light box (two used per recording session).
– Clear plastic rodent cage bases (W 255 mm x L 475 mm x H 210 mm).
– Perforated Perspex base lids with clasps (custom manufactured).
– Ethernet cameras mounted above each light box.

This format allowed measurement from 20 cages (1 animal per cage) simultaneously in one session.

The software tracks the centre point, base of tail and nose in rodents. The software algorithm was set to measure:

- total distance moved (cm)
- mean velocity
- cumulative duration of movement using the centre point (center-point)
- cumulative duration of movement using the nose reference point (nose-point)

Validation study

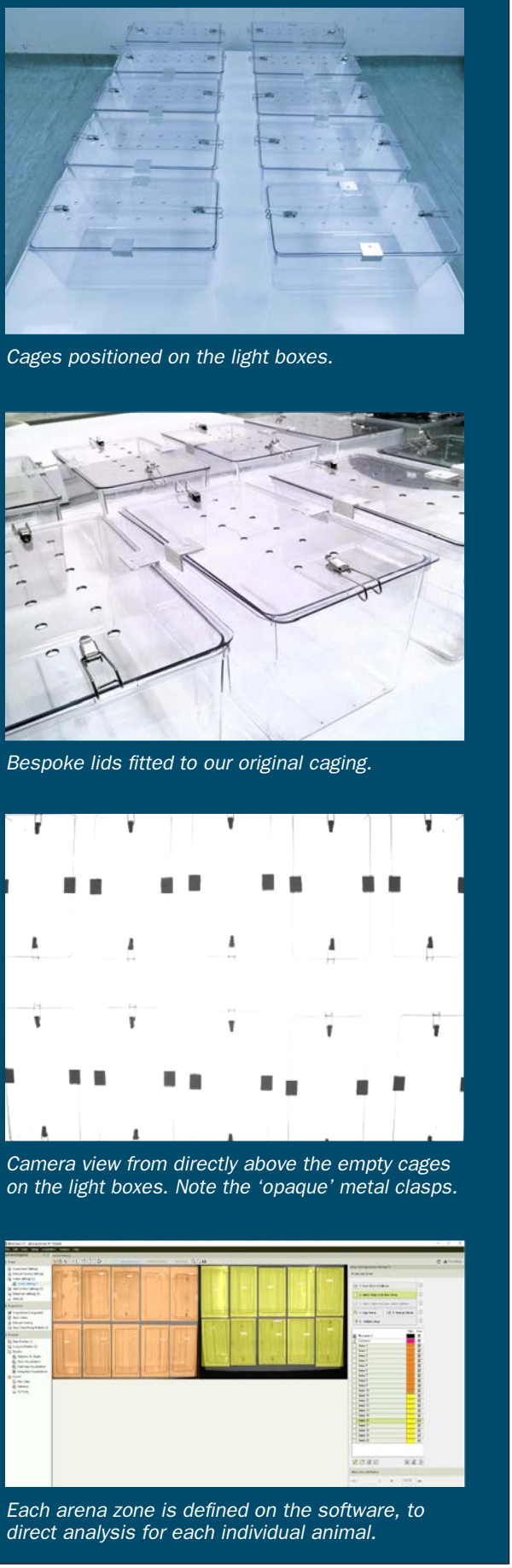
This was conducted to ensure results were robust and comparable to previous methodology.

3 male and 3 female Wistar Han rats and 2 male and 2 female CD-1 mice were used, which are common strains.

These animals were used to define the settings, algorithms and validation.

Running the experiment

- one animal placed into each cage in a random order
- main room lights are turned off
- white noise generator (set to 75 dB +/-5) minimises impact of any sudden background noise
- technologists exit the room and monitor Animal Welfare remotely from a computer in an adjoining room
- data acquisition was for 1 hour



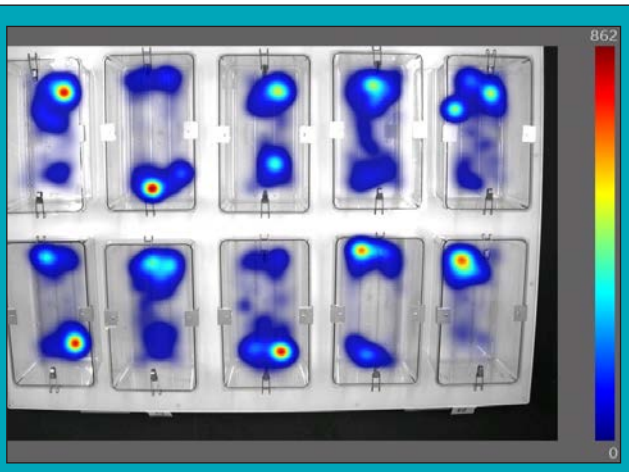
Cages positioned on the light boxes.

Bespoke lids fitted to our original caging.

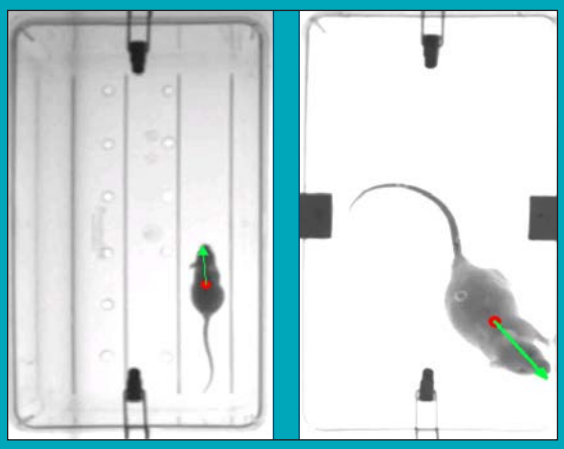
Camera view from directly above the empty cages on the light boxes. Note the 'opaque' metal clasps.

Each arena zone is defined on the software, to direct analysis for each individual animal.

Figure 3.



Heat map showing areas animals spent the most time in during the experiment (Dark blue lower time; Red is highest time).



Mouse (left) and rat (right) within the cages. Tracking nose and centre of gravity can differentiate sitting, rearing and sleeping behaviours.

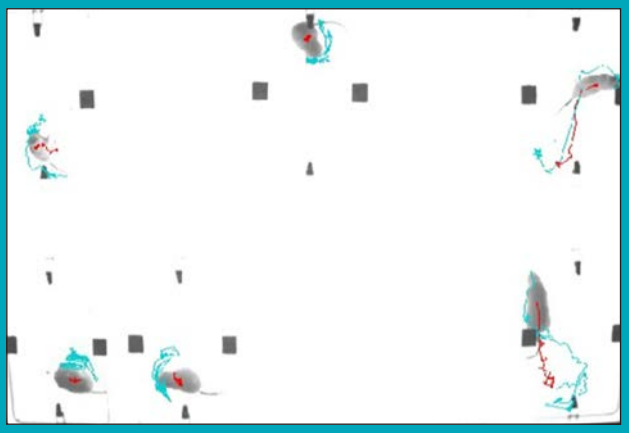
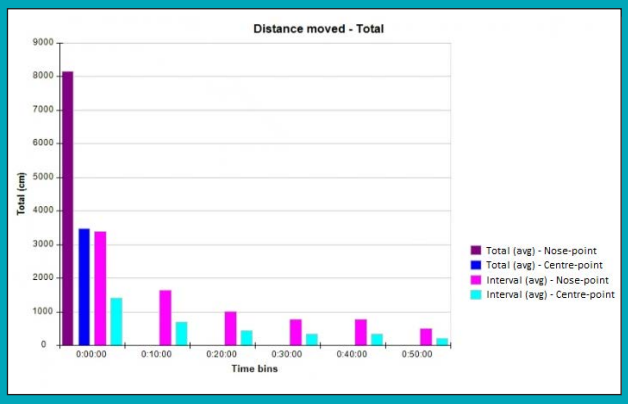


Image of nose tracking patterns (blue) and central point tracking (red).



Expected decrease in movement over time after placed in cage. Expected increase in movement when nose is used vs. centre point (rat n=6).

Figure 4.

Obtaining and analysing data

- Live video tracking or by running previously recorded footage through the software.
- Data exported to Excel, visualised in heat maps or integrated visualisation in video.

Individual animal visualisation chart shows:

- The distance moved (TOP PANEL for the center-point and nose-point).
- Time moving/resting using Centre-point (middle panel).
- Time moving/resting using Nose-point (lower panel).
- Nose-point tracking picks up finer head movements.
- Centre-point tracking only measures whole body movement.

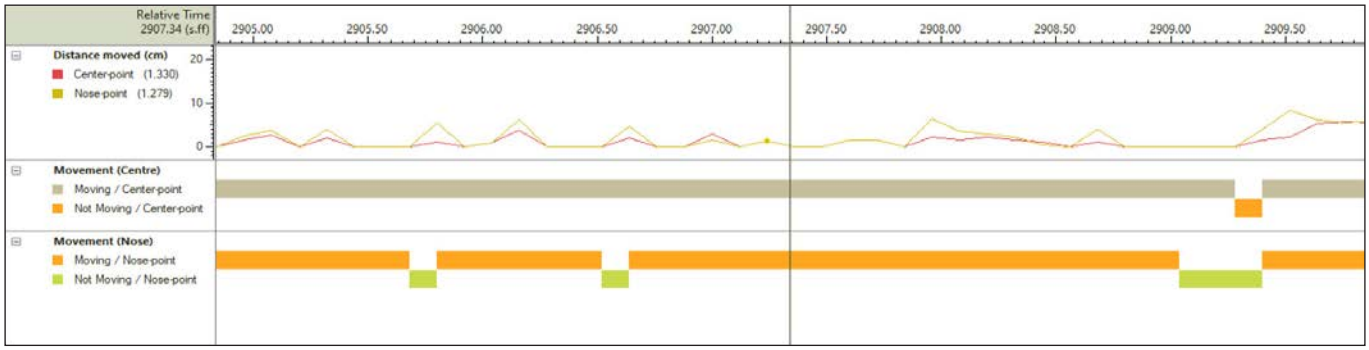


Figure 5. Individual animal visualization chart.

Results

The data was reported in 10-minute segments (intervals) and as a 60-minute overall count (totals). In rats, fine tuning of the base algorithm produced robust data. In mice, some blind spots, where the mice seemed to disappear, were evident initially; altering the camera settings within the software resolved this without making changes to the physical camera set up.

As a result of the increased activity generally in mice versus rats, the file size of the captured video is notably larger (1 hour session with 20 animals is approximately 300 mb in rat and 1.1 gb in mice).

Templates are easily created for each species to ensure efficient and reproducible set-up.

Acceptance criteria were met on the following requirements

Software was able to detect the correct anatomical features of each animal.

- Tracking was visibly accurate when replayed.
- A difference in activity levels between the start (higher activity due to novel environment) and end of the trial (lower activity due to habituation) was clear.

Conclusion and next steps

We have confirmed video tracking can be used to measure motor activity at the scale necessary to be included in rodent regulatory toxicology studies.

Retrospective analysis of these video files is also possible so removing the potential need to perform additional animal studies should additional parameters be needed following completion of the study, providing a significant refinement in Animal Welfare.

With thanks

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Suppliers

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