Rodent Big Brother: Development and validation of a home cage automated behavioural monitoring system for use in repeat-dose toxicity studies in rats

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In 2011 AstraZeneca set a challenge under the NC3Rs inaugural CRACK IT scheme, calling for novel technology to record locomotor activity, behaviour and temperature of individual rats continuously for up to 30 days (ie, '24/30' monitoring), noninvasively, when group-housed in standard home cages.

Although methodology has been available for several decades to measure locomotor activity of rats, this requires them to be placed singly in bespoke arenas, for brief recording periods. Analysis of behaviour generally requires manual observation by experienced observers, usually limited to 'snapshots' during the light phase. Body temperature can be measured manually at intervals, or continuously using surgically implanted telemetry transducers. Such approaches are not always compatible with repeat-dose toxicity studies[1].

We required the technology to be unobtrusive, and incorporated into standard IVC cage racks rather than as a benchtop system.

Here we describe a preliminary evaluation of a prototype system.

Rodent Big Brother

- Positional information and temperature via a subcutaneous RFID chip, detected by a baseplate reader under the cage;
- Behaviour captured via a high-res camera using IR lighting above each cage;
- Software trained by expert behavioural annotation of 24 h video of rats in their home cage
- The hardware sits in an IVC cage rack
- Home cages are positioned and removed in the normal way

Schematic of the Rodent Big Brother module

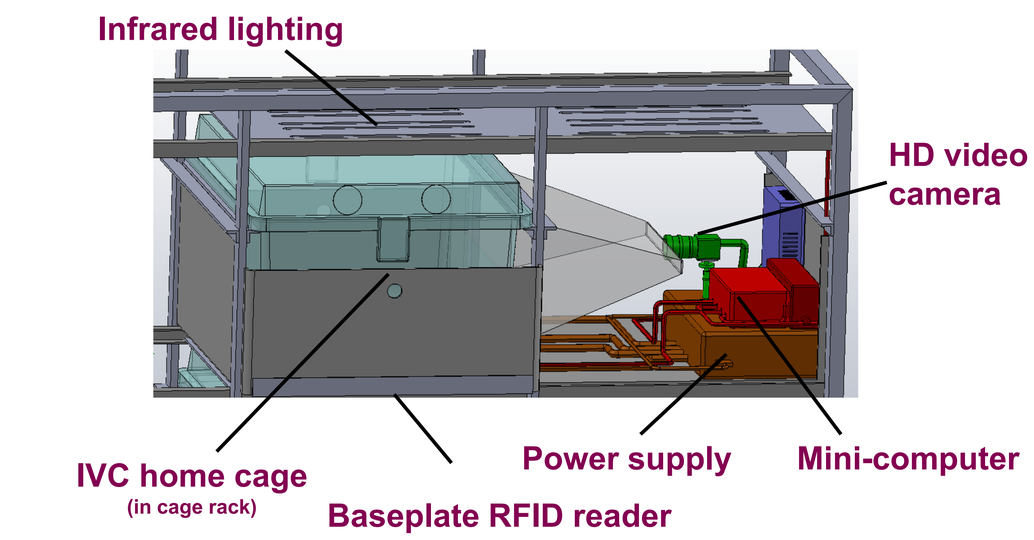


Image from side-on view of 3 rats in an IVC cage

The high quality video enabled identification and annotation of behaviours (28 defined behaviours in total). The table shows the 10 commonest behaviours in a single-housed rat over a 22 h period (entire 12 h of dark phase; 10 h of light phase). Annotation of 1 h of video takes ~2h per rat.

'Top Ten' commonest behaviours

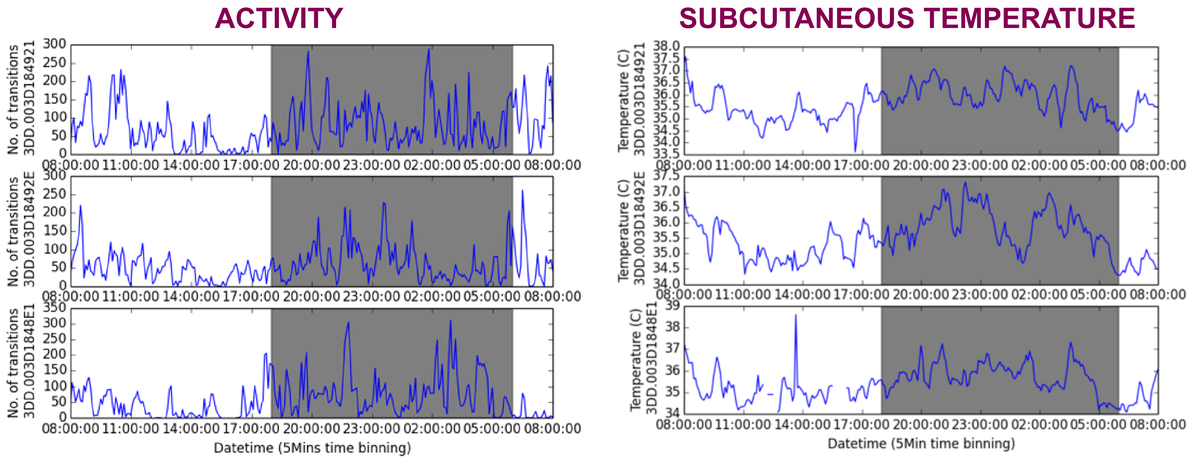
Behaviour	Number of events
Scratching	593
Rearing	579
Walking	475
Chewing hind paw	334
Licking/chewing coat	298
Immobile	266
Face washing	205
Eating from forepaws	203
Feeding from hopper	190
Drinking	177

Protocol for pharmacological validation with clonidine

- Male Han Wistar rats, 3 rats per cage
- Software records temperature and ambulatory activity for each rat individually
- Behaviour captured via a high-res camera using infrared lighting above each cage
- Rats dosed with vehicle (10 mL/kg po) and clonidine (0.3 mg/kg po) on separate occasions using a crossover design
- Monitored for 24 h post-dose
- Irwin observations run manually from the video at 30 and 60 min post-dose

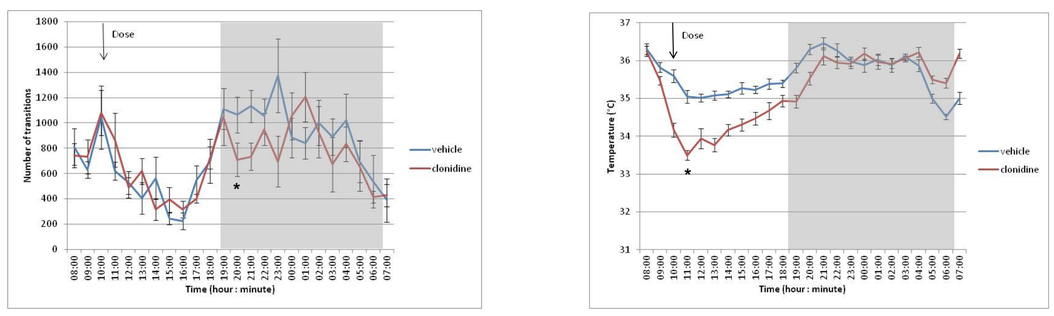
Results:

Individual 24 h activity and temperature plots from 3 rats housed together



Data collected from the individual RFID microchips by the baseplate reader.

Effects of clonidine (0.3 mg/kg po) on activity and subcutaneous temperature (n = 6)



Group mean data plotted. Home cage activity during the light phase is already low so no further suppression by clonidine was detectable. There appears to be a reduction in activity by clonidine during the first part of the dark phase (* P < 0.05; paired t-test). A hypothermic response to clonidine was detected (* P < 0.05; paired t-test on change from pre-dose).

Manual Irwin observations from home cage video cf. published data with clonidine

	Abnormal gait	Abnormal respiration	Flat body posture	Piloerection	Decreased spontaneous activity
Published Irwin data[2]					
60 min (AZGBR)[2]	0/6	5/6	3/6	3/6	6/6
60 min (AZUSA)[2]	0/6	0/6	6/6	0/6	6/6
60 min (CR)[2]	6/6	6/6	2/6	2/6	6/6
Home cage (video)					
15 min	2/6	0/6	0/6	0/6	6/6
30 min	0/6	6/6	6/6	0/6	6/6
60 min	0/6	1/6	0/6	0/6	6/6
120 min	0/6	0/6	0/6	0/6	6/6

The high quality video enabled the majority of observational (ie, 'non-interactive') elements of the Irwin test to be performed manually from the home cage video. The Irwin observations listed were those affected by clonidine at 60 min in the paper by Ewart et al. (2013)[2]. n = 6 for both studies. Given the variability in the Irwin data from the 3 laboratories ('AZGBR', 'AZUSA' and 'CR'), the outcome from the home cage video analysis is reasonable (within ± 60 min). Note that abnormal gait can only be detected when the animals walk around.

Summary and Conclusions

We have developed new technology to record locomotor activity, temperature and behaviour of individual rats when group-housed in their standard home cage.

The technology fits neatly into IVC cage racks and has applications in repeat-dose toxicity studies.

Ongoing work to improve on the prototype includes:

- Tuning the baseplate activity readout to optimise accuracy during low activity levels
- Evaluate and validate software for automated behavioural analysis (including convulsions)
- Further pharmacological validation.

3Rs benefits include being able to greatly increase the information content of existing study types to assess effects of compounds on activity, behaviour and temperature, without requiring surgery, and the potential to reduce the number of animals needed overall[1].

References:

1. Redfern WS et al. (2013) Functional Assessments in Repeat-dose Toxicity Studies: The Art of the Possible. *Toxicology Research* **2**: 209-234.
2. Ewart L et al. (2013) A multi-site comparison of in vivo safety pharmacology studies conducted to support ICH S7A & B regulatory submissions. *J Pharmacol. Toxicol. Methods* **68**: 30-43.

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