CRACK IT

Workshop report:

NC3Rs rodent home cage monitoring workshop output

The NC3Rs is convening a working group to further support uptake of the Home Cage Analyser (HCA) home cage monitoring system developed through the Rodent Big Brother CRACK IT Challenge. The cross-company working group will be tasked with generating an evidence base which builds on current validation of the system for rodent CNS safety studies. This approach was identified at a workshop of industry scientists and regulators as the best opportunity to generate the scientific and 3Rs evidence to support adoption of the system.

This report describes the output from the workshop and how to get involved in the working group.

Workshop: 5 June 2017
Hallam Conference Centre, London

NC3Rs
National Centre for the Replacement Refinement & Reduction of Animals in Research

Introduction

Animals used in safety pharmacology assessments help inform the decision to progress new drug candidates in development towards the clinic. As part of these assessments, the behaviour, temperature and activity of the animals (predominantly rats) are recorded to evaluate the effects of the test compound and to monitor the welfare of the animals.

Activity monitoring generally requires rats to be singly housed, often in bespoke cages or testing arenas. Behavioural analysis for central nervous system endpoints of concern such as nausea, insolmnolence, convulsions, grip strength, etc. (e.g. FOB/Irwin test) requires manual observation by experienced observers, usually limited to 'snapshots' during the light phase when rats are naturally less active. Body temperature can be measured manually at intervals, or continuously using surgically implanted telemetry transducers. These conventional approaches to measure behaviour, activity and temperature are not generally compatible with 24 hour continuous monitoring or with being performed concurrently for example in a repeat-dose toxicology study. They also require large numbers of animals (when run as separate studies), removal of animals from the home cage for observational assessments on multiple occasions during the study (causing stress to the animals and increasing data variability), and are labour intensive. There are concerns regarding the translation of these studies to humans (Mead *et al.*, 2016).

The ability to monitor and assess CNS safety pharmacology endpoints in more detail and non-invasively, including subtle behaviours and changes in activity, could improve the predictivity of the tests to the clinic as well as reducing and refining the animal studies. Rodent home cage monitoring systems offer the potential to achieve this as well as having wider applications for example in toxicology studies and basic research. A number of systems for automated monitoring of individual animals are currently available, but usually require single housing of animals or surgical implantation of telemetry devices.

In 2011, Dr Will Redfern from AstraZeneca set the Rodent Big Brother CRACK IT Challenge to develop a home cage monitoring system that could be used in safety pharmacology and other studies involving the analysis of rat behaviour. The specific requirements of the system included:

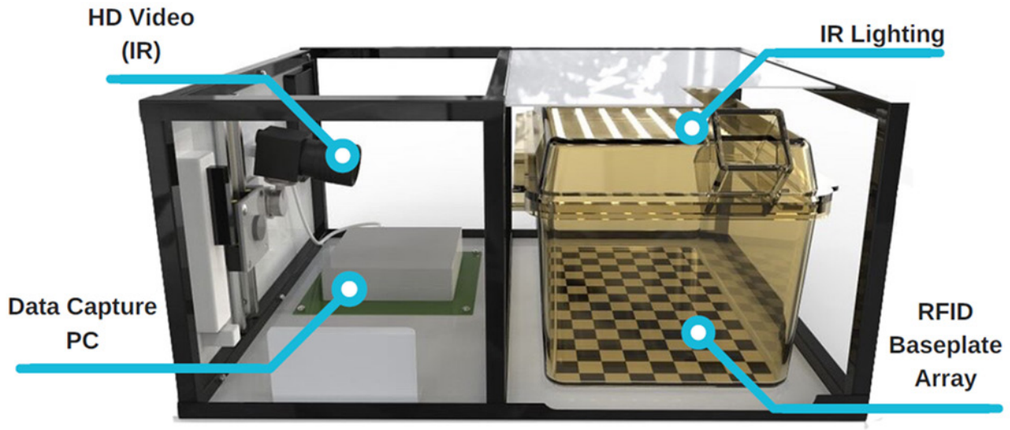
- Minimally invasive monitoring of group-housed rats in their home cage.
- Automated monitoring of temperature and ambulatory activity over 24 hours or longer.
- Ability to detect convulsions and other abnormal behaviours.
- Compatibility with standard Individually Ventilated Cage (IVC) racks, without having to modify the home cage.
- A portable, rack-based system

Addressing the Challenge

Professor Douglas Armstrong and colleagues from Actual Analytics Ltd, a spin-out company from the University of Edinburgh, were awarded a £0.5 million contract from the NC3Rs to deliver a product to address the Challenge. They developed the Home Cage Analyser system (HCA; Figure 1), which combines two technical approaches to the problems defined in the Challenge.

Each animal is tagged subcutaneously with an ISO radio-frequency identification (RFID) chip to record its temperature, location and identity using a 2D array of RFID readers that sit under each cage. The cage is also illuminated by infrared lighting and a side view HD camera captures 24 hour recordings of rodent behaviour. The system fits into existing IVC racks without having to modify the home cage, so animals can be monitored in their normal social groups and environments.

Figure 1. The Home Cage Analyser system (HCA).



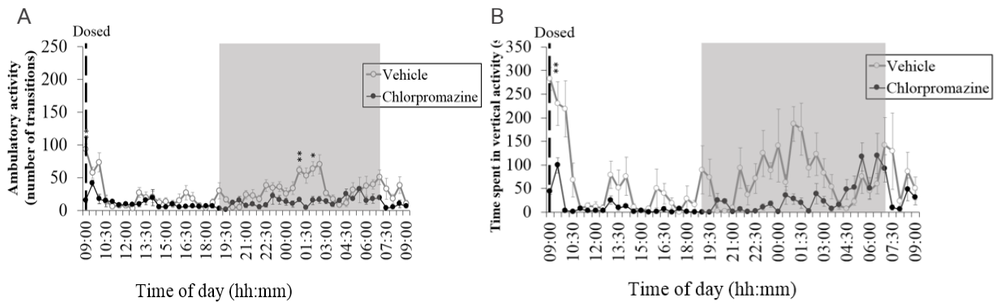
Optimisation and data validation

As part of their in-kind contribution to support the development of the HCA, AstraZeneca conducted a series of studies in group-housed rats. These began with optimisation work, progressing to data validation/verification and pharmacological validation (see below). The optimal implantation site for the subcutaneous RFID transponder was found to be a ventral abdominal midline location. Using this implantation site, good correlations were achieved between baseplate-derived ambulatory activity and manually verified ambulatory activity (using a vertically mounted video camera). Similarly, when compared over a longer time period (7-28 days) with whole-cage pixel movement (from the side-view HD video camera), there was a good correlation between baseplate-derived ambulatory activity and overall movement within the cage. The vertical activity measure was also manually verified and showed a good correlation. The system was considered fit-for-purpose for measurement of activity (ambulatory and vertical) for 24 hours and longer (up to 28 days) (Redfern *et al.*, 2017).

Pharmacological validation

Using three reference drugs (chlorpromazine and clonidine (sedatives) and amphetamine (stimulant)) in three different dosing protocols (dose during light phase, dose during dark phase, and dose during light phase then cage change at Tmax – to stimulate a temporary increase in activity), AstraZeneca was able to demonstrate that the HCA could detect changes in ambulatory activity, vertical activity and subcutaneous temperature in the light and dark phase in group housed rats (Figure 2), consistent with published data , but extending this into a 24 hour profile (Tse *et al*, 2017). Conventional snapshot recording performed during the light phase only would have missed these later observations (Tse *et al*, 2016, 2017).

Figure 2. Pharmacological validation of the HCA system. Ambulatory activity (A), vertical activity (B) and temperature (C) was recorded simultaneously in rats dosed with chlorpromazine during the light phase. Note that reduced activity was still evident in the dark phase many hours after treatment.



Maximising the impact

Despite optimisation and pharmacological validation provided by AstraZeneca, uptake of the HCA by the safety pharmacology community remains limited. To better understand the reasons for this, the NC3Rs hosted a workshop to showcase to the rodent CNS neurobehavioural assessment community the latest innovations in rodent home cage monitoring offered by the HCA system and to provide a forum for discussion about the opportunities for adoption of the system (or data derived from it) in their organisation.

Workshop participants were from a range of pharmaceutical companies, contract research organisations and regulatory agencies – see Box 1. A survey of CNS safety assessment experts from companies and CROs, including those not able to attend the workshop was also conducted prior to the meeting. The aim was to better understand what the barriers are to wider uptake of the system and what the opportunities might be to overcome these. Selected results from the survey are included in this report.

The workshop included the following presentations, full narrated slides sets for which can be found on the NC3Rs website.

- Setting the Challenge – Dr Will Redfern, AstraZeneca
- Developing the HCA system – Professor Douglas Armstrong, Actual Analytics
- Validating the system – Dr Will Redfern, AstraZeneca

Box 1: Organisations represented at the workshop, 5 June 2017, London, UK

- AstraZeneca
- Charles River
- Chugai Pharma
- Covance
- Envigo
- Finnish Medicines Agency
- GlaxoSmithKline
- Janssen
- MedImmune
- Medicines and Healthcare products Regulatory Agency
- Roche

Key highlights from the breakout session

The breakout session addressed three questions:

1. Where could the HCA system have utility in preclinical rodent CNS neurobehavioural safety assessment?
2. What are the barriers to wider uptake of the HCA system?
3. What strategy could be put in place to overcome these barriers and facilitate the uptake and application of the HCA system in preclinical safety studies?

The main findings from the breakout session and key survey data are described below.

1. Where could the HCA system have utility in preclinical rodent CNS neurobehavioural safety assessment?

Early, non-GLP rodent pre-candidate drug selection toxicology studies and dose range finding studies offer the greatest opportunities for the HCA system. This is perhaps not surprising considering the hurdles associated with carrying out later, GLP studies using novel systems. Integrating the HCA system into studies at an early stage would provide considerably more information on compound effects than is currently available due to the list of functionalities integrated into the system (see Box 2). This would facilitate earlier decisions about compound progression to be made, and support 'fail early, fail cheap' opportunities. When asked to rank how valuable the various functionalities of the system are for preclinical CNS safety hazard detection and risk assessment (1-5, where 1 is not valuable and 5 is very valuable), survey respondents* selected the following as their top choices as defined by the percentage of total responders selecting 4 or 5.

- Automated recording and analysis of multiple behaviours (92%)
- Concurrent temperature recording (92%)
- Capable of monitoring multiple group-housed animals concurrently (83%)
- Minimally invasive monitoring (83%)
- 24 hour continuous monitoring (75%)
- 24 hour continuous HD video recording (75%)
- Better quality scientific data (75%)

* 12 out of 15 total respondents

Box 2: Current fully automated behaviours

- Distance moved
- Speed
- Thigmotaxis
- Zone occupancy
- Circadian rhythm
- Social interactions
- Avoidance behaviour
- Following
- Clustering/grouping
- Climbing
- Rearing
- Feeding
- Drinking

These functionalities correlate well with the survey respondents' most commonly cited limiting factors of the conventional FOB/Irwin test battery (100% of survey respondents): the number of animals required and impact on animal welfare (67%), labour intensive approach (33%), low throughput (27%) and low confidence in translation to humans (27%).

Application of the HCA system in early non-GLP candidate selection studies as part of a standalone safety pharmacology package offers a good point of entry for early adoption of the technology and de-risking its future application in toxicology and GLP studies, but it also presents a number of potential issues. The 24 hour monitoring and HD video recording generates substantial volumes of data and information, raising questions around how this data is interpreted and stored. The ability to measure more data than is currently required for the FOB/Irwin package could add complexity and raise questions from decision makers (regulatory and within companies) about what this additional information means in terms of safety. This should not be seen as a barrier to the adoption of the HCA, or other novel systems which generate more data than is currently required as this information is likely to be valuable in understanding drug effects. Defining a path acceptable to all stakeholders to support its use and interpretation will be critical.

1.1 Opportunities beyond safety pharmacology

It was generally agreed by workshop participants that adoption of the HCA system for GLP and toxicology studies would be more difficult because of the constraints of the regulatory environment. However, the HCA system offers many opportunities beyond safety pharmacology and toxicology studies. The ability to unobtrusively monitor and measure subtle differences in behaviour and activity over time could have far reaching implications in areas such as pain research and inflammatory disease. An example is colitis where locomotor activity, together with the greater granularity in eating/drinking behaviour possible with 24 hour monitoring could identify trends and provide markers of an animals' welfare state previously not identified. More generally, the possibility of monitoring animals undergoing severe procedures (for example, spinal cord injury) or to identify behavioural markers that are predictive of animals whose health declines rapidly, could be invaluable at offering suitable interventions or removing the animals from study before this happens.

The application of the HCA system in these settings where it was not originally intended could offer a body of data and degree of validation of the utility and versatility of the technology, potentially providing greater confidence in the application of the technology in drug/chemical development where there is greater regulatory oversight.

2. What are the barriers to wider uptake of the HCA system?

Survey respondents* were asked to rank what they considered to be the biggest barriers to the adoption of the HCA system in preclinical safety studies (1-5, where 1 is not a barrier and 5 is a significant barrier). Highlighted below are the top choices as defined by the percentage of total responders selecting 4 or 5.

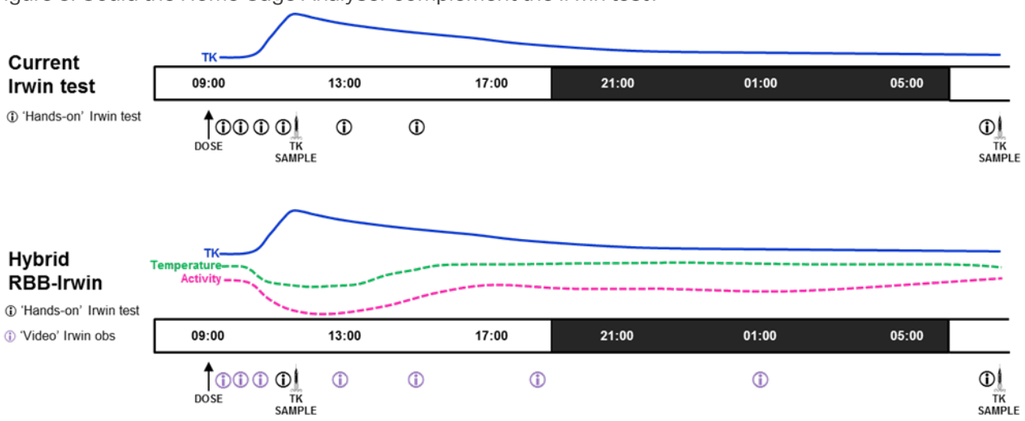
- Not all FOB/Irwin endpoints can be measured (100%)
- Cost of changing approach/infrastructure (91%)
- Limited pharmacological validation (82%)
- Regulators require data using current testing paradigm (73%)
- Lack of evidence of utility (64%)

* 11 out of 15 total respondents

Workshop delegates largely agreed that these represent the biggest barriers to the adoption of the HCA system in preclinical safety studies. The HCA system cannot currently measure all endpoints included in the FOB/Irwin test as many of these require manual interaction with the animal (e.g. grip strength, body tone, pinna reflex, etc.) or would need much greater video resolution to be detectable (e.g. pupil size, lacrimation, salivation, etc.). However validation studies conducted by AstraZeneca have demonstrated that the HD video quality enabled the majority of observational (i.e. non-interactive) elements of the Irwin test to be performed manually from the home cage video – see Table 1. Furthermore, effects on other parameters, such as gait and respiration that may be missed by the 'snapshot' nature of the Irwin test, could be detected on the video recording.

Approximately half of the observations in the Irwin test (17/32) are achievable by manual observations on the video recordings, at any time of day or night, without disturbing the animals. This presents an opportunity for combining the HCA and the conventional (manual) Irwin test (Figure 3). Those Irwin observations which require close observation and manual manipulation could be completed in ~one minute per rat (three minutes per cage) at two time points (Tmax and 24 hours), with HCA video observations in-between, to minimise disturbance to the animals. Integration of the HCA in this way will help de-risk its wider application and support regulators and other decision makers in accepting data generated in the HCA in a setting more familiar to them.

Figure 3. Could the Home Cage Analyser complement the Irwin test?



The cost of adopting a novel approach is always going to be a significant consideration. Just over half of survey respondents stated that if they were to adopt HCA studies they would outsource them to CROs. However, CROs would struggle to make a case to cover the initial outlay for new systems unless there is demand from their clients. It was also suggested that smaller pharmaceutical/biotech companies with limited resources would still favour the traditional FOB/Irwin studies as the minimum required by regulators. The proposed working group-driven validation study aims to generate the necessary data to provide end-users and CROs with the confidence to make the shift to adopting the HCA system.

The limited pharmacological validation and evidence of utility may be addressed to a degree by the work conducted by AstraZeneca which has recently been accepted for publication in PLoS One (Redfern et al., 2017). Regulators present at the workshop supported this, commenting that the data provides a good starting point for drugs developed to treat CNS disorders or diseases. However, far more data from a wider set of compounds, including those that failed in humans but which passed FOB/Irwin studies and compounds that do not have CNS effects, would be needed.

Regulatory agencies are keen to support the use of novel technologies/approaches which advance the 3Rs and which have been demonstrated to be fit for purpose. There are European wide regulatory initiatives that provide a platform for the development of such models, such as the European Medicines Agency (EMA) guideline on regulatory acceptance of 3Rs testing approaches. The UK Medicines and Healthcare products Regulatory Agency (MHRA) also offer scientific advice to researchers exploring the adoption of new approaches which will advance the 3Rs.

3. What strategy could be put in place to overcome these barriers and facilitate the uptake and application of the HCA system in preclinical safety studies?

Prior to the workshop, survey respondents were asked to comment on what strategy could be put in place to facilitate the uptake and application of the HCA system in preclinical safety studies within their company. Almost 100% of responses suggested an inter-company validation study with reference compounds. This would provide the necessary pharmacological validation and demonstrate the utility of the HCA system for rodent CNS neurobehavioural assessments, providing the evidence base to support wider adoption.

Based on this response, workshop delegates were presented with a proposed strategy for overcoming the barriers highlighted above and asked if they would support this. The proposal was:

- Do you support an international, inter-company validation study where:
    - Participating companies agree to provide compounds with CNS effects and preclinical/clinical data, where available
    - One or two CROs conduct the necessary studies
    - The data are published and presented widely
    - Regulatory agencies are engaged in the consortium

There was general support for this approach and agreement that it would provide the necessary evidence base to support adoption of the system, or to at least engage with senior decision makers on uptake. However, a number of considerations were raised around who would fund the work, what compounds would be used, the need to persuade senior management to allow their companies to participate in the current climate of limited resources, etc.

Next steps

The NC3Rs has a strong track record in an 'honest broker' role, bringing together companies to share data and experiences around specific problems. Based on this it was recommended at the workshop that the NC3Rs takes the lead on forming a working group to drive the consortium approach described above. The group would be tasked with defining a consensus view on scope for the project, resource requirements from participants, what studies would be conducted and what compounds should be used to give the greatest confidence in the HCA systems. The working group would analyse the study data and disseminate findings to engage other potential end-users of the HCA system.

We are now seeking participants from pharmaceutical companies, CROs and regulatory agencies to join, and contribute to this working group. Working group members will need to have approval from their companies to participate and to have discussed internally the potential extent of resources they would be able to contribute.

If you would like to discuss this further or join the working group, please contact Dr Kate Harris – kate.harris@nc3rs.org.uk.

References

Mead AN, Amouzadeh, HR, Chapman K, Ewart L, Giarola A, Jackson SJ, Jarvis P, Redfern W, Traebert M, Valentin JP, Vargas H. (2016) Assessing the predictive value of the rodent neurofunctional assessment for commonly reported adverse events in phase I clinical trials. *Reg Tox & Pharmacol.*; 80, 348-357

Redfern WS, Tse K, Grant C, Simpson D, Rimmer V, Leslie L, Keerie A, Klein SK, Sillito R, Chartsias A, Lukins T, Heward J, Vickers C, Chapman K, Armstrong JD. (2017) Automated recording of home cage activity and temperature of individual rats housed in social groups: The Rodent Big Brother project. *PLOS ONE* 12(9):e0181068.

Tse K, Keerie A, Sillito RR, Collier R, Grant C, Vickers C, Chapman K, Armstrong JD, Redfern WS. (2016) Rodent Big Brother: A Comparison to the Modified Irwin Test for Assessing Drug-Induced Changes in Activity and Temperature in Rats. *Safety Pharmacology Society 16th Annual Meeting, Vancouver, BC, Canada*.

Tse K, Sillito R, Keerie A, Collier R, Grant C, Karp NA, Vickers C, Chapman K, Armstrong JD, Redfern WS. (2017) Pharmacological validation of individual animal locomotion, temperature and behavioural analysis in group-housed rats using a novel automated home cage analysis system: a comparison with the modified Irwin test. (Submitted to *Journal of Neuroscience Methods*).

Table 1: How much of the Irwin test can be done via manual observation of the HD video?

	Detectable	Comment
Behavioural		
Arousal	YES	
Spontaneous activity	YES	
Aggressiveness	YES	
Sniffing	YES	
Grooming	YES	
Scratching	YES	
Rearing	YES	
Stereotypy	YES	
Bizarre behaviour	YES	
Vocalisation	NO	
Autonomic		
Rectal temperature	YES	24 h continuous (automated)
Salivation	NO	Insufficient video resolution
Lacrimation	NO	Insufficient video resolution
Piloerection	NO	Insufficient video resolution
Abnormal urination	NO	Not visible from side view
Pupil size	NO	Insufficient video resolution
Neuromuscular		
Abnormal posture	YES	
Abnormal gait	YES	
Ptosis	YES	
Straub tail	YES	
Tremor	YES	
Twitches	YES	
Convulsions	YES	
Body tone	NO	Requires manual interaction
Exophthalmos	NO	Insufficient video resolution
Grip strength	NO	Requires manual interaction
Traction response	NO	Requires manual interaction
Sensorimotor		
Touch response	NO	Requires manual interaction
Palpebral reflex	NO	Requires manual interaction
Startle reflex	NO	Requires manual interaction
Pinna reflex	NO	Requires manual interaction
Righting reflex	NO	Requires manual interaction