Report of the 2011 RSPCA/UFAW Rodent Welfare Group meeting – harms and benefits of new technologies

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Introduction

The RSPCA/UFAW Rodent Welfare Group holds a one-day meeting every autumn so that its members can discuss current welfare research, exchange views on rodent welfare issues and share experiences of the implementation of the 3Rs of replacement, reduction and refinement with respect to rodent use. A key aim of the Group is to encourage people to think about the whole lifetime experience of laboratory rodents, ensuring that every potential negative impact on their wellbeing is reviewed and minimised.

The 2011 meeting focused on the application of technologies such as imaging, biotelemetry and automated blood sampling in studies involving rodents. These techniques can help to implement reduction and refinement, as well as providing scientific benefits, but they can also cause harms that need to be identified and minimised. The Rodent Meeting explored these issues and enabled members to discuss how these harms and benefits can be considered against one another, so as to help improve decision-making about techniques and protocols. Other presentations addressed refinements in behavioural experiments and epilepsy research, and provided an overview of welfare research at the Royal Veterinary College.

Overview: Harms and benefits of new technologies

Ngaire Dennison, Home Office Inspectorate, Dundee

Technology is developing rapidly in many fields, which has led to an increased application of technological developments in animal research and testing. This can help to implement reduction, by facilitating the collection of more, better quality, data from each experimental animal. For example, repeated imaging enables animals to be used as their own controls, reducing variability and therefore numbers as well as enabling endpoints to be refined. Some technologies may also allow animals to have fewer restrictions on their behaviour during the time they are on experiment, e.g. telemetry can be used to collect blood pressure, electrocardiograms (ECG) or body temperature measurements from animals in their home cages.

However, there can also be harms associated with the application of these technologies to rodents. Some, such as automated blood sampling and telemetry, can result in single housing of social animals, which is a major stressor, and telemetry also often requires

invasive surgery. Scanning can require animals to be immobile for relatively long periods, involving either repeated anaesthesia or training and restraint. Hence, while the number of animals used may be reduced by applying technology, each animal used may experience greater pain or distress and this increased negative impact on individuals can mean that decisions about using these techniques may not be straightforward.

For example, careful thought needs to be given to questions such as "how does the harm of surgery weigh against the potential refinement of reduced handling or human interaction during a study?" and "how many episodes of anaesthesia are acceptable, and at what intervals, for scanning procedures, both from the point of view of the welfare of the animals but also from the point of view of possible effects on scientific data?".

The use of these technologies therefore requires a case by case harm-benefit analysis. The benefits, harms and technological challenges associated with each application will depend upon a number of factors including the species, size and age of the animal, their physiological state and the goals of the study. Consideration should also be given to the *cumulative* severity, or overall harm to the animal throughout the lifetime experience. It is essential to refine both how the methods are applied to the animals (e.g. effective perioperative pain management for telemetry device implantation surgery), and the technologies themselves (e.g. developing smaller, lighter telemetry devices). In the case of telemetry, the involvement of personnel from a variety of disciplines, such as bioengineers and mathematicians, can help to develop smaller devices, improve the accuracy of readings and ensure that maximum benefit is derived from the data obtained. With respect to blood sampling, improved analytical technology can mean that smaller samples are required to obtain useful data, e.g. by using blood spots instead of collecting larger volumes. The concept for all technological applications should be to obtain 'more from less' while minimising any additional impact on the individual – preferably to zero.

Preclinical imaging technologies: animal welfare considerations

Jordi L. Tremoleda, MRC Clinical Sciences Centre, Imperial College London

A variety of dedicated small animal imaging devices is now available, including computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT) and optical based technologies (Figure 1). These have provided the ability to obtain detailed, *in vivo* anatomical and functional data, which can help to both refine procedures (e.g. by better implementation of humane endpoints) and reduce the number of animals used. These technologies have also dramatically increased the efficiency of preclinical studies, providing a powerful and non-invasive way to monitor disease progression, test new therapies and help to phenotype animals.

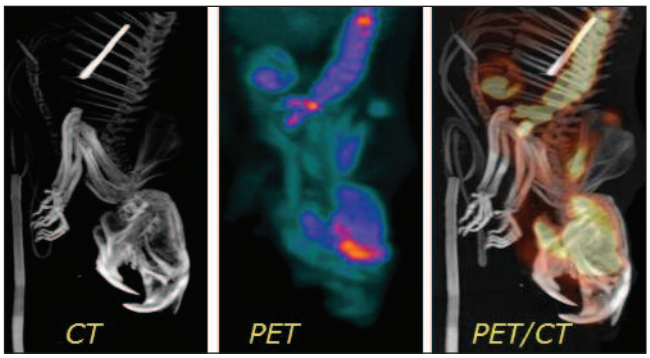

Figure 1. *In vivo* PET/CT image of a rat used in a study of cardiovascular disorder

The CT image of the skeleton has been combined with PET images to detect the specific uptake of a glucose analogue biotracer. This enables identification of anatomical areas with strong metabolic activity and/or diseased, inflamed organs or tissues.

Photo: Biological Imaging Centre. MRC Clinical Sciences Centre, Imperial College London

While non-invasive imaging is clearly a refinement over exploratory surgery, there are still adverse effects associated with the procedure that need to be identified so that refinement can be implemented. Each technique has its particular applications and technical limitations, and there are some challenges in rodent bio-imaging relating to prolonged or repeated anaesthesia, such as the use of appropriate anaesthetic regimens and supporting the animal's physiologic balance. It is essential to ensure the animal's well-being and to minimise any stress-related responses that would compromise both welfare and the imaging outcomes[1,2].

The best approach to achieve this is to consider the animal's entire experience, from the time when they arrive in the facility to the end of the project. Each scan will involve capture; handling, transport to the imaging unit; restraint for pre-medication and anaesthesia and recovery from anaesthesia. Fasting will often be necessary for some techniques, such as PET, to ensure better uniformity of the tracer uptake. For all of these potential welfare compromises, it is important to review the literature on refinement and good practice, as well as consulting senior animal technologists and the attending veterinarian.

Technique	Application	Welfare implications
Micro-CT (Computed Tomography) technology	X-ray based 3D imaging modality; excellent for imaging bones in high resolution (10 µm)	Radiation exposure in repeated studied; exogenous agents required to improve soft tissue contrast
PET (Positron Emission Tomography)	Functional imaging; high resolution localisation of a radiotracer (biomarker), e.g. for tracking biological or disease process. Short half-life radiotracers	Radiation exposure; injection of radiotracers (tail vein, which is challenging); may need to be repeated due to short half-life
SPECT (SIngle-Photon Emission Computed Tomography)	Tomographic functional imaging, as above but used to produce virtual 'slices'	Radiation exposure; injection of radiotracers; long half-life so used in long duration studies which require careful monitoring
MRI technology (Magnetic Resonance Imaging)	High contrast tissue imaging; anatomical and metabolic data	No radiation exposure but long term anaesthesia may be required
Bioluminescence	Functional imaging; light is generated within the animal, emitted and detected	Requires genetic alteration of animal/cells and injection of luminescent substrate; fast acquisition so no prolonged anaesthesia
Fluorescence	Functional imaging; light is shone onto the animal to excite a fluorescent marker then emitted for detection	Important to minimise background autofluorescence; fast acquisition so no prolonged anaesthesia
Multimodal imaging, e.g. PET/SPECT/CT scanning	Enables 3D visualisation of the PET/SPECT tracer within skeleton and/or soft tissues (see Figure 1).	Radiation exposure; more than one tracer may be imaged; refinement and reduction can be achieved BUT essential to ensure wellbeing and health status

Table 1. Imaging techniques, their applications and welfare implications

During anaesthesia, there is an inevitable autonomic nervous system depression which induces cardiovascular depression, respiratory depression and hypothermia. Therefore it is important critically to assess the anaesthetic regimen, including the appropriate depth of anaesthesia, what side effects there might be, and the length of time that the animals will be under the effects of anaesthesia. Injectables and inhalation anaesthetics both have advantages and disadvantages, for example with respect to levels of respiratory depression and recovery times, and the optimal agent with respect to animal welfare and the science should be decided in consultation with the veterinarian. Monitoring respiration using techniques such as clinical observations (e.g. a respiratory sensor), a capnograph or blood gas analysis can help to prevent hypoxia/hypercapnia, which can lead to welfare problems and produce confounds such as impaired drug metabolism.

Maintaining body temperature and heart rate is also extremely important, due to the high body surface/weight ratio and high metabolic rate of rodents. These aspects of rodent physiology mean that animals undergoing lengthy imaging may become dehydrated. This can be addressed by humidifying inspired gases, parenterally administering warmed fluids and applying eye ointment. The above considerations apply to all imaging techniques, but each individual technology has its own specific welfare issues, as listed in Table 1.

These challenges can be successfully addressed through an appropriate understanding of the imaging technologies and the impact on the animal, together with the implementation of adequate physiological monitoring systems. Special attention should be paid to health screening; adequate animal acclimatisation and preparation; effective monitoring of homeostasis during anaesthesia with support if necessary; good animal monitoring before, during and after imaging and care to avoid excessive or unnecessary radiation exposure, from equipment or radiotracers. The result will be not only better animal welfare but also better data with reduced confounds due to physiological or psychological stress, along with the refinement and reduction benefits that be achieved using imaging techniques[3].

The Curvet™ rat training simulator

Martin Heath, The Learning Curve

Developments in materials technology have permitted the design of new, realistic simulators that can be used in education and training. One example is the Curvet™ rat training model, a purpose-designed training aid that can help to develop the skills required for humane, confident animal handling and competence in conducting procedures without using a live animal

(Figure 2). The aim is to avoid stress to both animals and their handlers, by enabling some practice before training in handling and dosing live animals. Features include:

- Realistic skin, suitable for injections by most conventional routes; micro-chipping identification; handling and restraint (suitable for scruffing if required).
- The absence of fur enables this model to be cleaned and sanitised with an appropriate chemical sterilant.
- Realistic spinal and lateral and ventral head movement, suitable for practising oral administration, rear limb movement.
- Removable tail with two lateral tail veins, ideal for blood sampling; intravenous administration; insertion of a flexible catheter (angiocath). The tail is made from a self sealing material so it can be repeatedly used and additional tails can also be purchased separately. An internal reservoir can hold artificial blood (also available) or remain empty to practise intravenous administration of saline.
- Realistic features including: eyes, whiskers, hard palate with incisors, pink extremities, fore and rear limbs, anus and rectum with limited aperture to practise inserting a thermometer, adult rat size and weight (approximately 350g).

For further information see: http://www.vet-tech.co.uk/

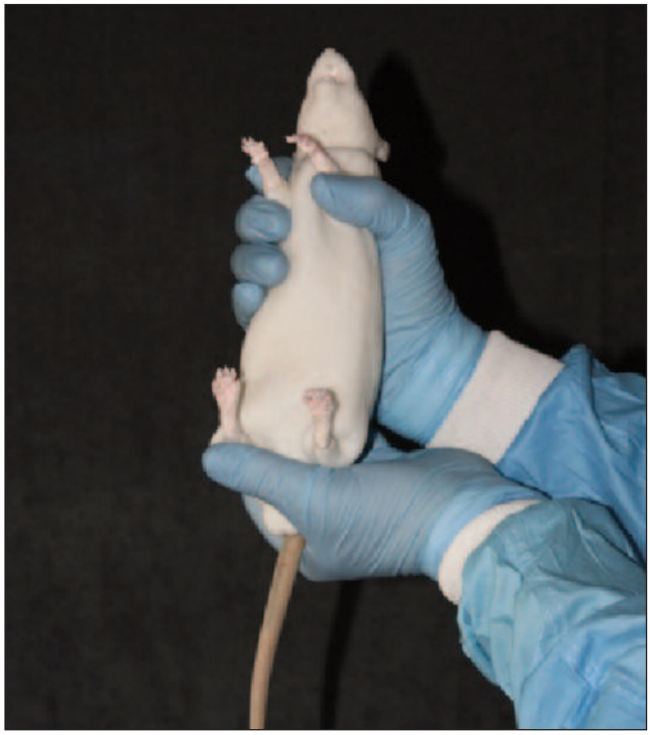

Figure 2. The Curvet™ Rat Training Simulator

Practising handling with the Curvet™ Rat Training Simulator

Photo: VetTech

The mouse hospital: cage to bedside, ultrasound scanning in a mouse 'model' of pancreatic ductal adenocarcinoma (PDA)

Paul Mackin, Cancer Research UK, Cambridge Research Institute

Imaging technology can be used in cancer studies to reduce animal numbers and to monitor tumour development more accurately, so that humane endpoints can be better implemented. For example, our laboratory employs imaging as an integral part of our research into pancreatic ductal adenocarcinoma (PDA), which is the fifth most common cause of cancer mortality in the UK. The prognosis is extremely poor and survival of patients with advanced disease is about 6 months. Despite major efforts in basic and clinical research, most therapies still fail to improve survival. Research into this fatal disease is thus ongoing, including animal studies (see www.cancerresearchuk.org.uk).

As part of this, our laboratory has produced a genetically altered mouse line (KPC) that recapitulates the human disease, tumour microenvironment, occurrence and site of metastasis as well as the clinical symptoms. We believe that this mouse 'model' is likely to be more predictive than previously used strains for identifying novel treatments that can be translated to patients, but we are also mindful of the need to recognise and minimise any adverse effects on the animals. For example, PDA can be painful in humans when tumours become large, so we use scanning technology to help us keep tumour size to a minimum in our mice and to closely monitor tumour growth.

Our 'Mouse Hospital' team coordinates the breeding of the KPC mice, with the aim of minimising wastage and closely monitoring the health and welfare of the animals. Tumour growth is closely tracked by palpating the mice for tumours weekly; it is generally possible to feel tumours of 1 to 3 mm from two months old. Once their presence is confirmed by palpation, tumours are measured weekly by non-invasive ultrasound imaging to follow the growth of the PDA. Studies begin once tumours are 6 to 9 mm in diameter, as measured by scanning. However, we wanted to reduce the need for repeated anaesthesia, as this is a stressor, so we worked to increase the precision of our palpations in order for these to provide a sufficiently accurate estimate of tumour size before scanning begins (Figure 3).

A high resolution ultrasound scanner is used to image the mice and because good quality images are obtained more rapidly, there is a welfare benefit in that

anaesthesia and recovery time are reduced. Fur from the abdomen is removed under general anaesthesia, using a commercially available depilatory cream, on the day before scanning so that the animals do not have to experience the two stressors on the same day. Saline is administered intraperitoneally to enable the internal organs and tumour to 'float' freely, which separates the organs slightly for better quality imaging (Figure 4).

Figure 3. Palpation of KPC mice

Mice are enrolled onto study when tumours are between 6 and 9 mm, identified by accurate manual palpation

Photo: Cancer Research UK

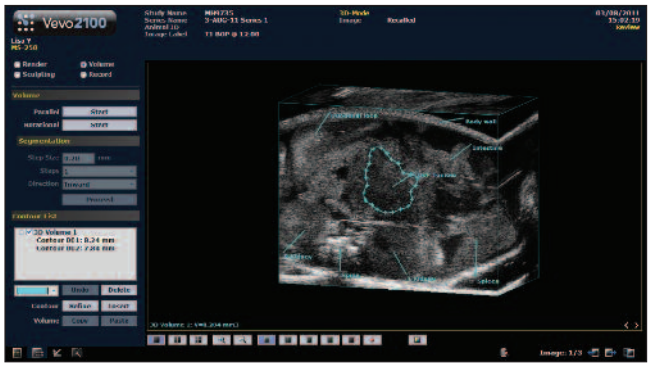

Figure 4. Organ suspension and identification

Injection of a small volume of saline enables more rapid and easy identification of tumours (outlined)

Photo: Cancer Research UK

With our previous scanning system we would have to administer around 3 to 4 ml of saline but since upgrading we have found that we can acquire very clear images using approximately half the amount of saline and without distending the abdomen.

Each animal is scanned from three to four different angles because the pancreas in mice is relatively diffuse in shape (unlike the human organ) and it is important to ensure that tumours are not missed (Figure 5). Post scanning, each mouse is cleaned of imaging gel before being placed in a heated recovery chamber. They are then monitored for recovery of righting reflex and mobility before being replaced into the home cage. Each scanned mouse has a 'Scanned' card placed over the cage card stating the mouse ID and scan date. The following morning all scanned mice are physically checked to ensure diuresis has taken place and that they are fully recovered from the scanning process. The acquired images are uploaded to allow each mouse's set of images to be evaluated and measured independently. If the tumour is between 6 and 9 mm, the mouse will be entered onto the therapeutic study.

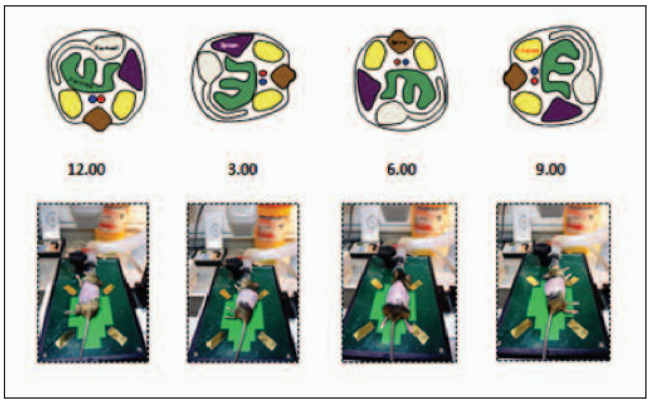

Figure 5. Mouse orientation

Each mouse is scanned from four different angles; '12.00', '3.00', '6.00' and '9.00'

Photo: Cancer Research UK

The scanning technology enables us to build up a detailed, three dimensional picture of each tumour and to quantify its volume over time. Candidate therapies can be evaluated more accurately than before using this technique, and humane endpoints can be implemented with greater precision. Previously we would routinely run four scan sessions per week, with each session lasting around 3.5 hours. With the new scanner we are able to halve the number of scanning sessions for each individual which should significantly reduce the cumulative stress experienced by the animals. Due to the success of our training the animal facility staff to palpate more accurately, we are better able to identify and estimate the size of tumours manually, which also reduces the number of scans for each mouse.

Rodent telemetry: When, how and why?

Anthony Webb, Chiron Bioscience Limited

Telemetry, in the context of biomedical research, usually refers to the use of devices that transmit signals such as temperature, ECG or blood pressure to a remote receiver for capture, storage and analysis. A wide range of biological parameters can be converted into signals and transmitted, although there are still some technical limitations (Table 2). Telemetry devices may be remote from the animal, surgically implanted or externally attached, but the important point is that the animals are not restrained and there is no need for sedation or anaesthesia when recording data. The use of telemetry thus has a welfare benefit because it can minimise or even eliminate stress associated with monitoring parameters, and the science is often improved because the resulting data are free from artefacts associated with stress or physical/chemical restraint.

✓	Identity (probe serial number or animal ID)
✓	Position, movement, activity
✓	Body temperature
✓	Pressures – e.g. blood vessels, ventricles, bladder, intraocular, pleural cavity, uterus, intracranial
✓	Electrical potentials (biopotentials) – e.g ECG, EMG (skeletal or smooth muscle), EEG
✓	Impedance, therefore volumes – e.g. respiration
✖	Blood flow – high power demands
✖	Chemical parameters – problems with biofilms forming on sensors, preventing parameters of interest being detected

Table 2. Parameters that can – and cannot – be measured using telemetry

Implantable telemetry devices allow animals to be studied in their established social groupings, which benefits both welfare and science because stressful disruptions of social groups and artefacts associated with this, are eliminated. However, there are harms associated with implantation, including the anaesthesia and surgery required, possible post-operative complications and the impact of the transducer mass and volume on the animal's physiology. These can be minimised by ensuring that surgical technique has been fully refined, including aseptic technique, and by exploring the potential to reduce device bulk[4-6]. For example, batteries can add significantly to the size of a device, so using passive transponders instead of battery-powered devices can help to reduce both mass and volume but the feasibility of this depends upon the parameter required. Current passive transponder technology does not permit complex wave forms such as ECG to be transmitted, although it can be used for animal identity, body temperature, position or activity.

As well as improving data quality, telemetry can generate longitudinal data during experiments conducted over significant periods of time, such as disease pathogenesis or responses of pathophysiology to experimental therapies. This application of the technology can significantly reduce animal numbers. Animal welfare can also be studied using telemetry, allowing objective physiological data to be obtained during common procedures such as humane killing, animal transport or husbandry procedures such as identification. These data can be used, in conjunction with other information such as behavioural observations, to indicate whether animals are in pain or distressed – or whether welfare is good.

Having looked at why and when telemetry may be used in biomedical research, the "how" also needs to be addressed – this is vital from a refinement point of view. Many aspects of refinement in telemetry were reported by the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement[4]. Developments in both the application of telemetry and in telemetry device development, may have big impacts on welfare – both positive and negative. For example, progress in microelectronics and precision engineering is allowing more parameters to be transmitted or recorded per telemetry device. In reality, however, it can be detrimental to animal welfare to implant too many sensor transducers and numbers should be not be reduced if it results in causing significant additional suffering to individuals.

It is also important to ensure that refinement is applied to the housing and care of animals on telemetry studies wherever necessary. Managing social groupings of animals when using devices where limited numbers of frequencies are available remains a challenge at present. If devices all transmit at the same frequency, there is a risk that social animals may be individually housed. This is not necessary, however, and many establishments address this by using the 'buddy' system, where a non-telemetered individual is housed with the study animal, or by using devices that can be switched on and off one at a time[7]. In addition, telemetry devices that transmit at different frequencies and are small enough for rodents are beginning to become available. Ongoing reviews of new knowledge and best practice are thus essential for all aspects of the application of telemetry to animals.

Turning point discussion session on new technologies

Ngaire Dennison, Home Office Inspectorate, Dundee

We rounded off the session with an interactive discussion using Turning Point handsets and software, which allowed delegates to vote on the severity classifications that they would assign to various procedures involving applications of new technologies. There were some interesting variations in responses and some delegates' views changed when they were given additional information about the procedures to consider.

For example, people were asked to consider a protocol in which a rat underwent repeated MRI scanning under general anaesthesia, involving eight scans at four week intervals. One delegate felt that this would be below the threshold for regulation; 24 classified it as mild; 35 as moderate; and 11 as severe. However, there was a shift in views when data were shown from work on aversiveness to isoflurane[8]. Nobody classified the protocol as below threshold; there were 12 votes for mild; 44 for moderate; and 7 for severe, while one delegate felt that it would be above the 'upper threshold' – there were also more abstentions than for the previous part of the question.

The responses and accompanying discussions showed that, whilst there is appreciation of the benefits of new and emerging technologies, these are accompanied by concerns about the potential for additional harm to the individual animals involved in some circumstances. There is also some uncertainty about how such harms can be identified, quantified and minimised.

Conclusions on new technologies

Considering the harms and benefits of the application of new technologies proved to be a very useful and interesting exercise and there are some common factors that apply to any proposed use of a new technological application in an animal study:

- the use of a new technology requires a detailed harm-benefit analysis, taking full account of the animals' lifetime experiences and cumulative suffering with and without the technique;
- the local Ethical Review Process (ERP) can play a useful role in the above, by bringing a range of perspectives and priorities to the discussion;
- some techniques may enable reduction in numbers but this is not the most ethical approach if it means that the burden on individuals is increased;
- whether or not the application of a technology will genuinely 'add value' to the project should be critically considered;
- some technologies may be refinements, but these should still be reviewed to see whether they need refining themselves – telemetry is one example;
- when assessing the severity of a protocol involving a new technology, the animal's lifetime experience should be broken down into components and refinement applied to each one;
- input from animal technologists and veterinarians is essential when considering the application of new technologies and how these should be refined.

Other presentations on refinement and animal welfare

Other speakers gave presentations on applying practical refinements to procedures relating to behavioural and neurophysiology research, including a refined rodent 'model' of epilepsy. A presenter from the Royal Veterinary College, who hosted the meeting, also gave an overview of the animal welfare research conducted there.

Head implants and paddling mice

Robert Deacon, University of Oxford

Head implants

Head implants are widely used in a range of species, including rodents but little has been done to evaluate their impact on animals and their welfare. For example, head implants are often prepared by mixing methyl methacrylate (MMA) with acrylic powder. This has a strong odour and causes electroencephalogram (EEG) changes in rodents similar to those that occur when they are exposed to the odour of predators such as weasels and foxes. Since this typically elicits stress and arousal reactions, it is possible that exposure to methyl methacrylate vapour in the laboratory might not only affect welfare but also alter the induction time or depth of anaesthesia in rodents. The latter could have further animal welfare implications if it means that the action or efficacy of anaesthetic agents is affected.

To evaluate effects on welfare and anaesthesia efficacy, mice were first tested in a T-maze to determine whether methyl methacrylate was aversive to them. They were also exposed to a high concentration of methyl methacrylate vapour and the time to the loss and subsequent recovery, of the righting reflex was measured following administration of sodium pentobarbitone anaesthesia. Results showed that mice tended to avoid the odour of methyl methacrylate but it did not affect the parameters of anaesthesia. Therefore, while exposure to methyl methacrylate odour does not affect the induction or depth of anaesthesia, it should be avoided for animal welfare reasons.

Paddling mice

The Morris water maze has been widely used in studies of spatial cognition in rodents and has become an internationally recognised standard procedure, but it presents some significant welfare problems for mice because, unlike rats, they evolved in the deserts of central Asia and are not natural swimmers. While rats generally swim well and persistently until they find the hidden platform, mice often have considerably more difficulty with swimming. Their small size and high surface area to volume ratio also mean that they can lose heat rapidly, either while in the water or afterwards while still wet, so careful drying and a warm recovery chamber are required.

Recognising this, natural mouse behaviour was used to design a more 'mouse-friendly' version of the Morris maze. Mice readily attempt to escape from shallow water. They also have a strong tendency to run into dark tunnels, particularly when escaping a stressful environment or potential predator. These aspects of mouse behaviour were used to develop a 'paddling pool' that combined elements of the Morris water maze and the Barnes holeboard maze. A white circular arena was filled with water to a depth of 2 cm (Figure 6). Twelve potential exits were located around the perimeter of the paddling pool, only one of which was connected to an escape tunnel leading back to the animal's home cage (Figure 7). The other 'exits' looked the same as the real exit from the mouse's point of view, but were blocked by black wooden plugs. The real exit was always in the same spatial location, and the mice readily attempted to escape into it using the various extramaze cues located around the laboratory.

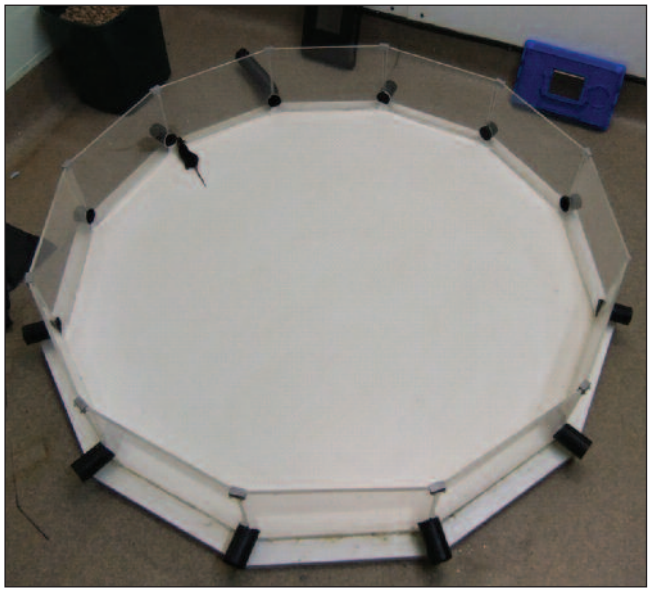

Figure 6. The 'paddling pool' used for behavioural tests as an alternative to the Morris water maze

The pool is 1.2 m in diameter and has transparent Perspex sides. Each 'exit' is a 50 mm diameter plastic pipe.

Photo: Robert Deacon

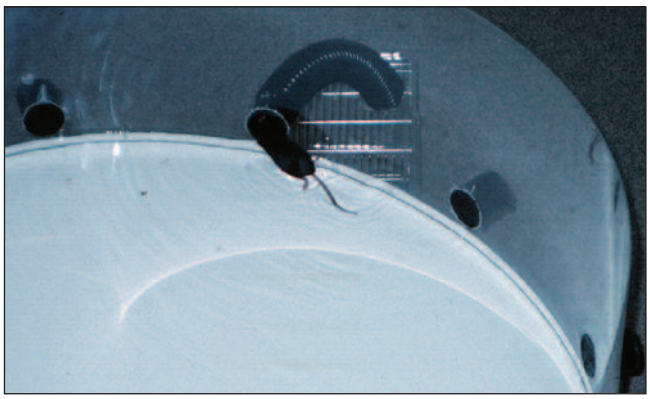

Figure 7. A mouse returning to the home cage

The mouse has correctly located the exit tunnel, which leads to the home cage.

Photo: Robert Deacon

The exits were placed at the side of the pool, to take account of the strong thigmotaxic behaviour displayed by rats and mice, in which they tend to stay in contact with the walls of an arena and spend much less time in the middle area. In the Morris water maze, rodents swim constantly around the periphery in an apparent attempt to cling to, and escape via, the solid opaque wall. Until this tendency is overcome, learning about the existence or location of the escape platform cannot occur. Besides this aspect of rodent behaviour, the natural instinct of most terrestrial animals (including humans) wishing to escape from a body of water is to swim towards the side, not the centre, so it makes more sense to place the animal in the centre of the water and the exits at the side.

This ethologically based approach to evaluating cognition is likely to reduce stress and to improve data quality, because behavioural procedures may yield more scientifically useful results if they are grounded

Figure 8. The paddling Y-maze

The transparent arms of the 'Y' are filled with water to a depth of 2 cm and the mouse has to learn which arm leads to the exit tube.

Photo: Robert Deacon

on an understanding of the natural history of the study species[9]. A simplified version of the pool, the paddling Y-maze, has now been developed and validated (Figure 8). Both the pool and Y-maze have yielded useful scientific data while minimising animal stress. There are also practical advantages, as a full Morris maze is very heavy, whereas the paddling pool is much lighter and no structural reinforcements to the laboratory are needed.

Using the Three Rs to develop a refined rodent 'model' of chronic epilepsy

Gavin Woodhall, Aston University

We aimed to develop a refined 'model' of epilepsy that would reduce suffering and result in minimal mortality. Epilepsy is a term that includes many neurological syndromes characterised by recurrent seizures. Epilepsy affects as many as 50 million people worldwide and, of those who are treated, only 60% respond well to drug therapies, with a further third of drug responders going on to become either difficult to treat or unresponsive to drugs. This difficulty in treating epilepsy relates to its complexity and multiplicity of causes, and childhood epilepsies, in which neurodevelopment is added to the already complex neurological problems, are often amongst the most difficult to treat.

Understanding epilepsy is often attempted through the creation of animal 'models', which may be reflective of the type of epilepsy to be studied. The most common epilepsy, temporal lobe epilepsy, is often produced in experimental animals by chemical induction of seizures. This process, which may use a variety of pro-convulsant agents, is often high mortality (10 to 50%) and therefore of substantial severity. This is obviously highly undesirable from both ethical and animal welfare aspects and there was clearly a very strong incentive to refine the protocol.

A project funded by the NC3Rs enabled us to develop a new, refined approach to producing temporal lobe epilepsy in experimental animals by:

- using a muscle relaxant, xylazine, to reduce the intensity of the seizures;
- reducing the dose of the epilepsy-causing agent, pilocarpine, so as to avoid causing *status epilepticus* (a long-term seizure with significant welfare implications);
- using a multi-drug approach to terminate seizure activity once the required data have been obtained;
- improving the efficiency of the *in vitro* aspects of the epilepsy research programme;
- ensuring that multiple researchers were able to obtain data from each epileptic rat (via CARMEN, see below).

This has resulted in the development of a lower-intensity 'model' of epilepsy, which induces a lower level of seizure activity and reduces mortality to below 2%. Human tissue was used to validate the new approach and, in an unexpected benefit, the new 'model' was found to show features that are strikingly similar to childhood epilepsy.

Our philosophy is to obtain the best 'ethical value' from the epilepsy studies. Besides using the refined approach above, we also pool data with other epilepsy researchers online, via CARMEN (*Code Analysis, Repository and Modelling for e-Neuroscience*, see http://www.carmen.org.uk/).

These studies demonstrate the value of applying Three Rs principles to biomedical research, both in terms of animal welfare and scientific utility – this is a clear example of the Three Rs driving improvements in the science.

Animal welfare research at the RVC

Charlotte Burn, Royal Veterinary College

The Centre for Animal Welfare (CAW) at the Royal Veterinary College includes animal welfare scientists with backgrounds in biology, zoology, animal science and veterinary science, alongside ethicists, clinical veterinarians, epidemiologists, physiologists and environmental engineers. Research covers laboratory, farm, companion, zoo, 'pest' and working animal welfare. Typical projects explore questions such as: 'Can we define healthy limits to extreme body conformation in domestic dogs?', 'How do noise, light and ammonia affect farm animal welfare and productivity?', 'Do feline amputees experience phantom limb sensations?', 'What welfare indicators best identify the 'tip-of-the-iceberg' in terms of underlying problems?', 'How can we increase the humaneness of slaughter and pest control?' and 'How can we refine the olfactory environment and identification marking schemes for laboratory mice?'.

An important area of work relates to public understanding of welfare issues and how best to educate people about animal behaviour and needs. For example, brachycephalic (or 'short-snouted') dogs such as pugs often have breathing difficulties, but 58 % of people who own these dogs say that they do not have breathing problems, even when their animals have frequent and severe respiratory noise[10]. After formal veterinary diagnosis of breathing difficulties, 41 % of owners still say that their dogs do not have problems

breathing! As another example, of 400 videos uploaded to the internet that showed dogs chasing their tails, 33% showed clinical signs. Dogs in these videos were over six times more likely to be described as 'funny' or 'stupid' than dogs in other videos showing tail-chasing[11]. It is clearly very important to educate the public about issues such as these, as animal suffering cannot be alleviated if health and welfare problems go unrecognised, and the RVC receives funding from a number of bodies including UFAW, the BBSRC and the Wellcome Trust for these projects. Another project in collaboration with the RSPCA is exploring the effects of education about farm animal welfare on adolescents, and what barriers there might be to students implementing what they have learned (for example, not buying higher-welfare products because they do not perceive that this can make a difference).

Other ongoing work at the RVC CAW relies on detailed non-invasive behavioural analysis, targeted physiological measurements, DNA microarray technology, statistical analyses and ethical decision-making frameworks. The animal welfare work at the RVC is strengthened through collaborations with other groups, such as those working on animal anatomy and biomechanics, veterinary education and, of course, the clinicians themselves, and it benefits from having Europe's largest small animal hospital right on its doorstep. For further information, see http://www.rvc.ac.uk/Research/Groups/CAW/

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