Report of the 2004 RSPCA/UFAW rodent welfare group meeting

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Summary

The RSPCA/UFAW Rodent Welfare Group holds a one-day meeting every autumn so that its members can discuss current welfare research and exchange views on rodent welfare issues. A key aim of the Group is to encourage people to think about the whole lifetime experience of laboratory rodents, ensuring that every potential impact on their wellbeing has been reviewed and refined.

The 2004 meeting covered a range of issues including the feasibility of using ultrasonic vocalisations to assess rat welfare; the impact on rats of different cage-cleaning frequencies; temperature fluctuations during mouse transport; a welfare benchmarking scheme for establishments using mice; and laboratory animal allergy. A special session explored the potential to group house male mice by refining husbandry, with a practical example of social housing within toxicology studies and guidance on the roles and expectations of the Home Office Inspectorate when new protocols for housing and care are evaluated and implemented.

Rat ultrasound and welfare assessment: preliminary results

JOANNE NICHOLSON, King's College London

There is now a considerable body of literature demonstrating that male rats produce ultrasonic calls within the 20 to 30 kHz range when they are placed in a variety of aversive situations. These include both natural stimuli, such as an aggressive encounter with another rat, and artificial stimuli such as a puff of air to the face, handling or acoustic startle stimuli. Rats have also been shown to produce calls within the 30 to 50 kHz range in response to positive situations, such as mating (see the 2001 Rodent Welfare Meeting report[1]) and in negative situations such as aggression.

There is still much debate over the biological function of these calls, but it is thought that they might play a role in communication between rats by signalling negative states such as pain or anxiety. If this is true, then ultrasound could be a valuable tool for the assessment of welfare – if we can discover how to interpret the calls. So far, some studies have suggested that rats in painful and/or stressful situations emit ultrasonic calls, but the relationship is far from clear and other studies have found no such calling behaviour.

The aim of the present study, which was funded by the RSPCA, was to investigate the relationship between pain and stress and ultrasonic vocalisation in adult male laboratory rats, by recording any ultrasonic emissions produced during and/or after exposure to procedures of varying severity. This involved visiting a number of research institutions and monitoring procedures that were being carried out on existing project licences under the Animals (Scientific Procedures) Act 1986. No procedures were conducted for the purpose of this project.

We recorded the rats' vocalisations using a U30 (Ultrasound Advice) ultrasound detector connected to a Sony (TCM-359V) cassette tape recorder. The sound recordings were analysed by computer using the program Avisoft™ (R Specht) to determine physical characteristics including the minimum and maximum frequency and length of each call (Figure 1). The recordings are still being analysed, so the preliminary results below are from one part of the study which investigated the efficacy of analgesic drugs following the injection of Complete Freund's Adjuvant (CFA) into the left hind paw. Calls were monitored at various times after the injection from (i) rats who received the analgesic, (ii) those given a dose of vehicle and (iii) a control group that received a saline injection instead of CFA.

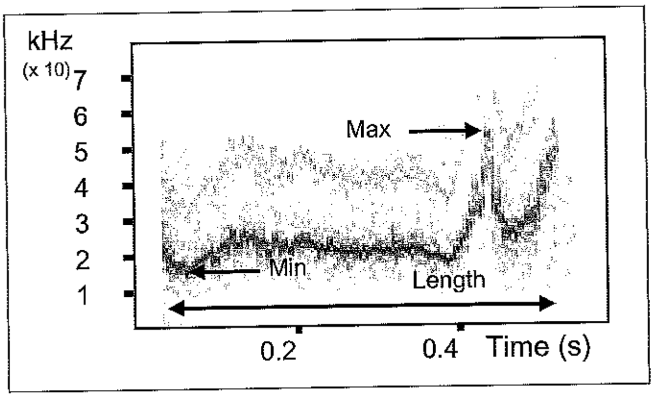

Figure 1. Trace of a typical ultrasonic vocalisation by a male rat.

Our most unexpected finding was that, in contrast to much of the literature, there was no evidence to suggest that only 20 to 30 kHz calls were produced in this aversive situation. The frequencies that we measured were mainly above 30 kHz and some extended to well over 100 kHz. There appeared to be little difference between experimental and saline control groups with respect to changes in the frequency characteristics and duration of calls over the baseline measurements. However, there was a greater decrease in the latency and duration of calls in the experimental group compared with the saline group. The analgesic appeared to reduce the amount of calling and increase the latency to call.

Individual differences in calling were noted. We were able to categorise rats as 'callers' or 'non-callers' on the basis of their vocalisations in response to the injections of CFA and the saline controls that were used in that particular protocol. The reason for this is not known – the Rodent Group discussed possible factors including individual social status (could subordinates vocalise less than dominant animals?) and potential differences in individual pain thresholds.

The Group also discussed the purpose of vocalisations in response to pain or fear in the rat, which is a social animal but cannot necessarily depend on help from others in the way that other species such as dogs or some primates can. Again, it is still far from clear why rats should vocalise in this way at all. Taking all of this into account, it was recognised that a great deal of further research will be necessary before ultrasonic vocalisations can be used to help assess pain and/or distress in the rat. However, it was also agreed that the preliminary results of this research are very interesting and encouraging and will certainly help us to understand the nature of the rat. We will be submitting the full study for publication in the near future.

Long-term effects of cage-cleaning frequency and bedding type on laboratory rat welfare: a cross-laboratory study

CHARLOTTE BURN and GEORGIA MASON,
University of Oxford

Cage-cleaning is necessary to maintain a healthy, hygienic environment for laboratory animals, but rodents make extensive use of odours for communication and so cleaning can be extremely disruptive for them. Studies have shown that cage-cleaning is an acute stressor for rodents and has an impact on both physiology and behaviour, such as increased aggression in mice.[2,3,4,5] Also in mice, more frequent cleaning increased pup mortality and reduced weight gain.[6] It is now widely accepted that a balance needs to be struck in practice between the need to keep animals healthy and to minimise any impact on their psychological wellbeing. However, the literature on the effect of cleaning on rats, rather than mice, can be contradictory and even counterintuitive. For example, more frequent cleaning led to higher numbers of "usable offspring" but more cannibalism in rats.[7] Also, stronger acute stress reactions, as measured by cardiovascular parameters and activity levels, have been recorded in rats following fortnightly rather than weekly cleaning.[8] It is therefore difficult to make a judgement on appropriate cleaning frequencies at present.

Rat cage-cleaning occurs between 3 times a week and once every 2 weeks in most establishments. With this in mind, we set up a study with funding from the APC to evaluate the impact of cleaning frequency on different groups of rats in different laboratories, aiming to see whether we could recommend a cleaning frequency that would optimise both health and welfare. This is also relevant for animal house management, in that if less frequent cleaning is best, it would save money, waste and labour. On the other hand, if more frequent cleaning were better for animal health and welfare, we would know that the effort and resources were justified!

Method

We used male Wistar and Sprague-Dawley rats, housed in groups of 4 and replicated in 4 different conventional animal units within different establishments for 5 months. The rats were housed on two different types of substrate, aspen woodchips and Alpha-dri™ paper (Shepherd, Tennessee), and cleaned out (i) twice-weekly, (ii) weekly and (iii) for Alpha-Dri™ only, fortnightly. The literature suggested that ammonia levels would have become unacceptably high in cages with aspen substrate with fortnightly cleaning, so this was not carried out. Rat welfare was assessed by monitoring behaviour, chromodacryorrhoea (see below), health and handleability.

Behaviours used for assessing welfare included general activity and resting, sneezing (due to high ammonia levels or illness) and 'skirmishing'. Skirmishing behaviour following cleaning out has been widely observed in rats, but it is not clear whether it is play or aggression.[9] As the target site for biting during post-cleaning skirmishes is the back of the neck, while the rump is the usual site for aggressive bites[9], we noted the target sites during skirmishing bouts, along with the locations of any wounds. Behaviour was recorded for 45 minutes following cleaning and during the equivalent time period on the day before cleaning, *i.e.* the dirtiest day.

Chromodacryorrhoea or 'red tears' is a porphyrin-rich secretion from the harderian gland of the rat (see 2003 Rodent Welfare Meeting report[10]). It appears around the nose and/or eyes minutes after the rat is subjected to a stressor such as pain, restraint or changes in background noise.[11] We used a 6-point system, similar to the 5-point one that we had devised previously, to collect numerical scores for the degree of chromodacryorrhoea.[10,11]

Health measurements used in the present study were body weight; general health (coat condition, presence of wounds, eye damage from ammonia or dust, or illness *e.g.* diarrhoea or lethargy); in-cage ammonia levels on the day before cleaning; and *post mortem* respiratory (nasal, tracheal and lung) pathology and stomach ulceration.

Handleability was assessed by testing one rat per cage after 5 months. Each rat was restrained by a familiar technician, who scored tension, struggling, squeaking and attempts to bite.

Results

Some selected results are presented below as a summary of the project's findings; our full paper has been submitted to *Laboratory Animals*.

Behaviour: Skirmishing was all play-like and non-aggressive and was significantly higher in the weekly cleaning treatment than twice-weekly or fortnightly (P = 0.022). The rats sneezed more on wood chip substrate than on Alpha-Dri™ ($P \leq 0.001$).

Chromodacryorrhoea: Scores were higher for Sprague-Dawley rats than for Wistars ($P < 0.001$), and for heavier rats than for lighter ones ($P < 0.001$).

Health: The rats were significantly heavier on the aspen woodchips than on Alpha-Dri™ ($P = 0.006$). They ate more of the aspen substrate, but there may have been other factors involved, such as the thermal properties of the aspen chips, since overall behavioural activity levels did not differ between rats kept on different substrates. However, there was significantly more caudal lung pathology on aspen chips than on Alpha-Dri™ ($P = 0.001$), with more animals showing moderate to marked interstitial pneumonia. Ammonia levels increased with time after cleaning but were generally low, only very rarely rising above 25 ppm, which is the UK legal limit for the human working environment.[12] In one animal unit, they did not rise above 2 ppm even in the fortnightly-cleaned cages.

Handleability: The fortnightly-cleaned rats were easiest to restrain. They struggled, squeaked and bit slightly, but significantly less than the more frequently cleaned rats ($P = 0.032$), although tension scores did not follow any obvious pattern.

Animal unit variability: The animal unit had a significant effect on 7 of the 9 variables tested in the present study. Significant animal unit interactions existed for 6 variables, such that the direction and/or the existence of the effects depended on the unit in which the rats had been housed and the measurements made.

Conclusions

Cage-cleaning frequency does not have a clear effect on welfare in male rats. It is not obvious from the behavioural results which cage-cleaning frequency is best for reducing social stress between rats. With respect to interactions with humans, fortnightly-cleaned rats were easiest to handle, but they could have been either more relaxed or more inhibited.

Aspen substrate was associated with higher weight gain, yet also with higher sneezing rates and lung pathology. Possible agents responsible for this respiratory damage include volatiles from wood or chemicals (aspen has fewer volatiles than other woods but more than paper[13]), micro-organisms or their metabolites, or cellulose dust. Despite this, it is not clear which bedding is better for *welfare* because, as far as we are aware, there have been no preference tests carried out with Alpha-Dri or any similar product. On the basis of the present study, however, we suggest that woodchips, aspen or otherwise, should be avoided for long-term housing, and particularly respiratory and nutritional studies. We suggest that paper bedding should be used where possible, rather than wire flooring, to avoid foot injuries and handleability problems in older rats[14] and to allow digging and foraging behaviours.[15]

Although in-cage parameters were standardised, there were significant animal unit effects, which raises questions about the feasibility of standardisation. Cross-laboratory studies may provide more robust results, but careful experimental design will be necessary to minimise the inevitable increase in the number of animals used, which is contrary to the principle of the Three Rs.

Despite the logistical benefits, we would not recommend fortnightly cleaning for male rats housed in most conventional, open cage systems because:

- ammonia levels were unusually low, but could be harmful after 2 weeks in other units;
- subjectively, fortnightly-cleaned cages seemed more allergenic, although empirical evidence for this is lacking;
- workers involved in this study subjectively found the smell of cages in this treatment noticeably unpleasant.

Mice on the move!

JANE MEAN, GlaxoSmithKline

As the number of transgenic lines used in research grows, so does the need to transport them both in the UK and worldwide. Transport is stressful and we were concerned that the carefully controlled environment in which our mice live could be compromised when they were transported in shipping crates. As well as our desire for the mice to be maintained in optimal environmental conditions during transportation, UK and international laws also require that certain standards are maintained during transport. Until recently, we had no way of measuring any temperature fluctuations that the animals may have experienced during their journey.

The advent of miniaturised temperature dataloggers has now allowed us to monitor any 'in crate' temperature changes that mice may experience in transit. We have begun using the Digitron Thermatag™ datalogger, which is an economical, re-usable piece of equipment that is set up using its accompanying software. Each logger weighs 20g and measures 42 by 27 by 13 mm, so is easy to attach to the inside of the transport box, marking its location on the outside of the container. We start recording at the point when the mice are placed into the box* and then sample data continually at 20 minute intervals until the mice arrive at their destination. Once the Digitron has been returned, its data are downloaded using the same software and exported to Excel so that we can produce graphs of the temperature within the boxes throughout the journey.

Some graphs clearly show significant temperature fluctuations during shipment. For example, the temperature ranged between 19.8 and 12.5°C during a flight from the UK to the East Coast of the USA and fell to below 8°C following unloading into very low local temperatures. We hope that the substrate and nesting material, together with the fact that they are transported in groups, helped the mice to maintain their body temperature without discomfort. Although the duration of low temperature did not contravene the US Animal Welfare Act, we obviously do not want our mice exposed to such extremes of temperature and seek to prevent this in the future.

We are actively using the data to review and refine our shipping procedures, giving us evidence with which to question collaborators and shippers over reasons for delays and temperature fluctuations. Under the UK Welfare of Animals Transport Order 1997 and IATA regulations, it is the responsibility of the carrier to ensure that the aircraft hold is maintained at a satisfactory temperature and that animals are protected from the elements. We now have the data that we need to work with carriers and ensure that our mice have as smooth a journey as they possibly can.

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*All mice are transported with substrate, nesting material and gel to prevent dehydration and all containers comply with International Air Transportation Association (IATA) guidelines on animal transport.

Development of a welfare benchmarking scheme for laboratory mice

MATT LEACH and DAVID MAIN, University of Bristol

A comprehensive welfare 'benchmarking' scheme should enable establishments to compare their welfare standards with other, equivalent facilities, which in turn will help to improve standards of laboratory mouse welfare and to maintain those high standards. We aimed to develop and implement such a benchmarking scheme, which involved the development of a valid, repeatable and practical method of assessing welfare.

The objectives of the present project, which was funded by the APC, were: to develop an assessment protocol as defined by experts in the field; to use it to assess the welfare of laboratory mice in the UK; develop a rolling database of results; finalise the benchmarking scheme; and then set out an ongoing implementation strategy for the finished scheme. The mouse was chosen as the most commonly used laboratory species, but we restricted the initial project to conventional (as opposed to genetically modified, or GM) mice not undergoing procedures.

For effective welfare assessment, it is necessary to measure both *resource input*, or the resources provided by an institution (such as cage size, environmental conditions, enrichment, human staffing levels) and *animal-based outcomes*, as in the effect that the resources have on the mice (such as behaviour, provoked responses and health). There is a spread of opinion on which resources are necessary and how animal-based outcomes should be interpreted and measured, so a range of experts was consulted on this using the Delphi consultation process. This is a recognised, systematic approach for gathering information and achieving a balanced consensus position, which has been used in a number of different fields including farm animal welfare assessment.[16] Responses were obtained from animal technicians, Named Persons, Home Office Inspectors, behaviourists and scientists including welfare scientists. A range of organisations was also consulted including the IAT, LASA, LAVA, RSPCA, UFAW and designated establishments.

The consultation produced 119 measures of mouse welfare, 68 of which were resource input measures and 51 animal-based outcomes. Categories included cage specifications, husbandry procedures, environmental conditions, animal room activity, staff details, provoked and unprovoked responses and unit wide health. The final assessment protocol consists of (i) a questionnaire for the establishment to fill in (largely consisting of resource input measures); and (ii) a one-day assessment which consists almost entirely of animal-based measures, with some resource measures that cannot be covered in the questionnaire.

This assessment protocol has been used in 46 animal units in the UK to date. These house numbers of mice ranging from a few hundred to thousands and represent a number of different levels of biosecurity status. The results have been entered into a secure and anonymous, on-line rolling database from which the participating establishments can view their own results and a summary of the national results. However, they are *not* able to view the individual results of any other establishment.

A range of resources has also been produced to help participating facilities measure and benchmark their own welfare performance. These include computer software, guidance notes and training in the use of the assessment protocol and database. The aim is to motivate and encourage all staff to become involved in monitoring and improving welfare, which will benefit animals, science and staff morale. Now that the mouse scheme is operational, the same core principles could be used to develop welfare benchmarking for GM mice, mice undergoing procedures and other species.

We believe that we have developed a valid, feasible and reliable scheme for welfare assessment, but we recognise that establishments and the individuals within them have to be willing to use it. The scheme is flexible and under continuous review and further consultation, comments and participation would be very welcome. Please contact Dr. Matt Leach at m.c.leach@bristol.ac.uk if you would like further information or if you would be willing to complete a one-page questionnaire on the implementation of the scheme.

Control of laboratory animal allergy and rodent welfare: the HSE inspection project

IAN STRUDLEY, Health and Safety Executive (HSE)

Working with laboratory animals is one of the top eight known causes of occupational asthma (OA), and there are an estimated 1,500 to 3,000 new cases of OA each year across all UK industry sectors. Reducing the incidence of OA has been designated a field priority topic within two key HSE initiatives, Revitalising Health and Safety and Securing Health Together. The overall aim is to significantly improve health and safety by 2010, including a 30% reduction in work-related ill health such as OA.

There is, of course, already legislation that should ensure workplace conditions adequately control the risk of OA. The Health and Safety at Work Act 1974 sets out both employers' duties to protect their employees and the public, and employees' duties to protect themselves and others, as is "reasonably practicable". The Control of Substances Hazardous to Health Regulations 2002 includes more detailed requirements with respect to risk assessment, prevention and control of exposure to hazardous substances, health surveillance, training and arrangements for emergencies.

The HSE's Employment Medical Advisory Service (EMAS) is conducting an inspection project to evaluate how effectively the current legislation is being complied with and whether there are areas that need to be addressed. The project began in April 2002 and aims to visit all relevant licensed premises in the UK. It is looking closely at where exposure to animals and their waste products occurs, including the types and duration of human-animal interactions such as handling and general husbandry.

The project has found that, at present, establishments are using and developing a range of engineering controls and workplace techniques to prevent and control exposure to animal allergens. These include:

- ventilation, which can be facilitated in a number of ways *e.g.* cage design including the use of individually ventilated cages (IVCs);
- modifying systems of work, particularly using local exhaust ventilation (LEV) systems when cleaning cages and rooms; modifying the conduct of animal procedures to reduce exposure; restricting access to animal units;
- using personal protective equipment, including respiratory protective equipment and suitable gloves;
- ensuring that staff rest and changing room facilities are kept clean and separate from 'dirty' work activities, so that accidental exposure, *e.g.* from contaminated clothing, is prevented.

These are all very important practical initiatives to safeguard human health, but the HSE is also well aware that creating barriers between technicians and animals can give rise to both human and animal welfare issues. This was examined in more detail at the 2002 Rodent Welfare Meeting, which focused on the health and welfare implications of IVC use, including its impact on the human-animal bond and how this affects technicians who want to work with animals but may rarely get to handle them.[17]

The EMAS initiative has already shown that there are a number of ways of reducing the risk of OA, including engineering solutions that still permit good quality housing and care, environmental enrichment and levels of interaction that benefit both humans and animals. Do not assume that all animals have to be in IVCs or that all staff have to be in "space suits" – communicate with the HSE so that we can negotiate acceptable solutions that will not impact on animal welfare or staff morale.

It is envisaged that the inspection project will be completed in 2006. Annual progress reports have been produced to date and a final report will be made available describing the findings and making recommendations for action.

Coping with aggression in group-housed male mice

PASCALLE VAN LOO, University of Utrecht

It is essential to implement good practice in laboratory animal husbandry for a number of reasons. Animals spend most of their time in their holding cages, so housing conditions inevitably have a major impact on physiological and psychological wellbeing. Appropriate housing can also help animals to cope with stressful procedures, which in turn will improve the reliability of experimental results. One particularly important aspect of good husbandry practice is the need to house social species in groups.

It is widely recognised that rodents are social animals and need to be group-housed wherever possible. In a natural environment, mice live in family groups, with one dominant male, residing in clearly defined territories.[18] Aggression between males in a stable group of wild mice can benefit some individuals, while other subordinates are generally tolerated within the territory or can migrate to set up new colonies.[19,20] In the laboratory, however, the 'territory' is usually reduced to a standard cage and the environment does not allow subordinates to take refuge from dominant animals. Odour cues that identify individuals and mediate aggressive behaviour are similar between families in inbred strains, which disrupts social behaviour,[21] while some strains have been accidentally or deliberately selected to be more aggressive.[22]

Female mice can be successfully group housed in a laboratory setting, but males can be extremely aggressive towards one another which leads to serious health and welfare problems. This is generally dealt with by:

- using females – but this necessitates euthanasia of unwanted males, leading to ethical issues;
- using docile strains – but these may not be the most appropriate to answer the scientific question, resulting in experimental bias and wastage of animal life;
- housing males individually – but there is evidence that male mice prefer social housing and are both stressed and depressed by social isolation.

All of these solutions have undesirable ethical or welfare consequences, so we set up a project to evaluate whether changing husbandry could reduce aggression in male mice to an acceptable level. The study looked at the three environmental factors that we believe are most likely to influence aggression: olfactory cues, group size and enrichment. We chose a

Table 1
Suggested good practice for housing and caring for male mice

Recommendation	Reason
Avoid individual housing wherever possible	Single housing leads to behavioural and physiological changes that result in poor welfare and reduce the validity of results
When cage cleaning, transfer material from the nesting area to the clean cage but do not transfer substrate	Mice avoid urinating on nesting material, so it mainly contains aggression-inhibiting pheromones from the plantar and other body glands. Substrate or other materials contaminated with urine and/or faeces will contain urinary pheromones, which provide social odour cues and can increase aggression
House males in groups of three	Groups of three males have been found to show the least aggression because the dominance hierarchy is more stable than in larger groups. Pair housing can increase aggression and is more stressful to the subordinate because he will not receive social comfort from a mid-ranking mouse
Provide nesting material as enrichment, rather than a rigid structure such as a shelter	Nesting material permits species-specific behaviour, decreases aggression and may help the mice to cope with stressful situations in the long term. Unlike a single, rigid structure, it can be manipulated and used to structure the cage. This provides the mice with a distracting activity and reduces competition
Minimise disturbance as much as possible – this includes husbandry procedures, such as cage cleaning, as well as experimental procedures	Disturbances cause stress, which leads to aggression. However, more research is needed to determine how the frequency, duration, types and severity of disturbances influence the development of excessive aggression. There will obviously be a compromise between effective animal care and monitoring and minimising disturbance to the group

moderately aggressive strain, BALB/c. The results below are only a very brief outline of a much larger body of work[23] which has also been summarised in *Laboratory Animals*.[5]

Olfactory cues after cage cleaning

Mice were housed in trios and allocated to three different cage change protocols: (i) all new substrate and nesting material in the new cage; (ii) substrate with urine and faeces transferred to the new cage; or (iii) nesting material transferred to the new cage. We found that the number of agonistic encounters was significantly lower when the nesting material was transferred than when the cage was completely cleaned or sawdust was transferred.

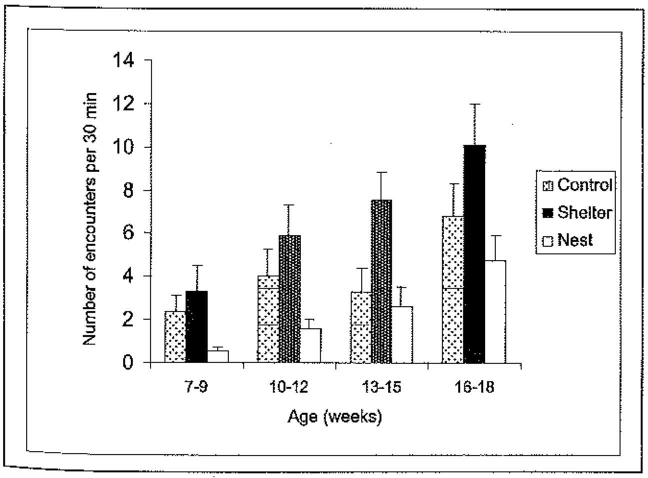

Figure 2. Mean number of agonistic encounters observed in male mice with different enrichment items.

Group size

We housed mice in groups of 3, 5 or 8 per cage, with variable cage sizes to ensure that each mouse had the same area of floor space. In groups of 8 mice, both dominant and subordinate males initiated significantly more aggressive encounters. In general, the frequency of aggressive encounters per mouse was lowest in groups of 3.

Enrichment

Mice were housed in trios and provided with substrate and additionally (i) no enrichment, *i.e.* just substrate, (ii) nesting material or (iii) a shelter. Agonistic encounters were significantly most frequent in the cages with a shelter and significantly lowest with nesting material.

Conclusion

On the basis of our findings using BALB/c mice, we can make the recommendations for male mouse husbandry set out in Table 1. This system has been used in the long term and aggression has remained very low in both the BALB/c strain and the highly aggressive CD-1 strain. Levels of the 'stress' hormone corticosterone are also significantly lower in mice housed according to