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Validating refinements to laboratory housing: asking the animals

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Abstract

The housing and husbandry of laboratory animals should be considered as an integral component of any research protocol. There is evidence that the current minimum legal requirements for laboratory housing and husbandry can have adverse effects - not only on animal welfare, but also on the development and biological functioning of animals in ways that might compromise scientific validity. Changes to housing should therefore be considered as 'refinements' to the experimental protocol, as suggested in the 3Rs by Russell and Burch. Such refinements need to be validated, taking into account that laboratory animals have different senses and motivations to humans. This article discusses behavioural methods used to validate refinements to laboratory housing, focussing on asking animals questions about their preferences and strength of motivation for refinements. Refinements such as additional space, social contact with conspecifics, nesting material, nest boxes, solid floors, burrowing substrate, running wheels, and the opportunity for exploration are discussed. Future refinements might need to account for strain differences in underlying behavioural traits, and allow behaviours such as novelty seeking or territory patrolling. In addition, studies adopting a multidisciplinary approach should be encouraged.

Keywords: refinement, motivation, preference, housing, cage, welfare, scientific validity

Introduction

Russell and Burch (1) suggested that experimental protocols should be refined to minimise the suffering of laboratory animals. It is relatively easy to recognise that some aspects of experimentation need to be refined to minimise suffering, for example, several common methods of euthanasia are not instantaneous and it seems likely that they induce a certain amount of potentially avoidable suffering. However, suffering can be caused by other less obvious aspects of the experimental protocol. The animal's housing is an integral part of an experiment, but laboratory housing is designed primarily for human considerations, such as economics, practicality, longevity and repeatability of the environment. Current minimum legal standards for housing and husbandry of laboratory animals mean that laboratory housing is generally small and minimalistic. It is now widely acknowledged that such housing can cause suffering to animals but, in addition, it has been argued that such housing can adversely affect the normal development and biological functioning of the animals (2-7). This means that animals reared in housing of minimal legal standards are possibly fundamentally flawed in terms of representing a normal population. This would compromise the scientific quality and perhaps the validity of the research in which they are being used, and negate the primary reason for the animals being housed. Therefore, there are two compelling reasons for refining laboratory housing, i.e. animal welfare and scientific quality. But how can we assess whether the changes we make improve animal welfare and biological functioning – how can we validate housing refinements?

What is animal welfare in relation to housing?

Animal welfare is about an animal's subjective state. It is about how an animal perceives its internal and external environments, and how these fulfil the animal's cognitive needs and desires. At present, it is impossible to directly monitor or record the subjective states of another organism (including other humans). This is further complicated by laboratory animals having different senses to humans (e.g. rats can hear ultrasound whereas humans can not) and different motivations (e.g. mice are highly motivated to dig tunnels whereas humans are not). We need to avoid the traps of anthropomorphism and anthropocentricity in assessing welfare and the validity of housing refinements. This can be achieved by observing an animal's overall 'output' in response to its housing and to putative refinements. Animal usually behave to maintain or improve their welfare. By recording the animal's behaviour, we can indirectly observe how animals assess those factors they themselves consider to be relevant and how these match their cognitive needs and desires. From this, we can judge whether animals perceive housing refinements to be satisfactory. In effect, we are asking the animal to show us what the housing problems are, and then whether our attempts to refine the housing have been effective.

Methods of validating housing refinements – asking the animal

Normality of behaviour

One method which can be used firstly to indicate whether refinements are needed, and then secondly to test the validity of refinements, is to assess the normality of behaviour. This can be done using ethograms and time-budgets. Ethograms are a detailed list of the activities that an animal performs. Time-budgets indicate the duration or proportion of time that animals perform these activities. If an ethogram contains an abnormal activity (e.g. stereotypies), or behaviours that are self-harming (e.g. autophagy) or harmful to others (e.g. barbering), or if an activity is abnormally over- or under-represented in the time-budget (e.g. excessive aggression, bar-chewing, inactivity) this indicates that housing refinements are required. By changing the housing to include a putative refinement, and then re-examining the ethogram and time-budget, we can validate whether the purported refinement has made desired changes in behaviour to indicate that a welfare benefit has been achieved (Figure 1). Another approach to assessing the normality of behaviour is to release laboratory animals into a more natural environment and observe their behaviour. If the animals' ethogram in the natural environment contains behaviours that are not observed in the laboratory housing, it is possible the laboratory housing is preventing or frustrating those behaviours.

Figure 1. *Suspended seed cake to encourage natural foraging behaviour in mice.*

Choice tests – let the animals decide

Another method which can be used firstly to assess whether housing refinements are necessary, and then secondly for validating putative refinements, is to ask animals questions about their needs and desires. Animals usually make decisions and behave in ways that maintain or improve their welfare. So, if animals choose one environment more than another, it is likely that this environment, or what it contains, will benefit the animals' welfare more than an environment which is visited less. Similarly, animals will be more highly motivated for environments that improve their welfare than those which do not. By testing the strength of motivation that animals have for environments, we can determine those which are likely to improve welfare and those which are not. These two methods of asking animals questions about their needs and desires are called preference tests and consumer demand studies.

Preference tests

In a typical preference test, we ask animals to choose between two or more environments which differ in only one feature. Figure 2 shows a typical preference test apparatus. In this particular example, the preferences of laboratory mice for different coloured cages were being tested. We can record how long the animals spends in the black, white, red, green or transparent cage, how many times they choose each, and how the animals behave in each cage. If the animals show a preference for one of the colours, it indicates this colour is offering something beneficial to the animals that the others are not, and therefore indicating which colour is most likely to maximise the welfare of the animals. It is also possible to perform preference tests for very different potential refinements. For example, we might wish to improve the opportunity for extended locomotion in laboratory cages. This could be achieved by a running wheel or a long series of plastic tunnels. A preference test could show us which would be most preferred by the animals and therefore most likely to improve their welfare. The major strength of preference tests are that they ask the animals to make decisions based on their own motivations, desires and assessments of the environment. They circumvent our anthropomorphism and anthropocentricity.

Figure 2. *Apparatus for investigating the preference of mice for different colour cages.*

Consumer demand studies

One limitation of preference tests is that they tell us only about relative preferences and not absolute need. They might tell us that refinement A is preferred relative to refinement B, but the test does not distinguish where A, B, or both are on a continuum from 'absolutely essential' to 'trivial or unimportant' for the animals. This problem can be overcome by placing a cost on the animals gaining access to the refinements. Usually, the cost is a task such as pressing repeatedly on a switch. By increasing the number of switch presses required, it is possible to empirically determine the strength of motivation the animals have for a need (e.g. food, water) or a refinement (e.g. additional space), and therefore validate the importance of a refinement as perceived by the animals. The underlying principle is that animals will be most highly motivated to interact with resources they absolutely need, highly motivated for refinements that they perceive as most improving their welfare, and less motivated for refinements they perceive as less important (8-9). Furthermore, it is more likely that animals will experience negative subjective states (e.g. frustration, anxiety) if they are not provided with the refinements for which they show high motivation.

What housing refinements have been validated?

How have these three methods of asking animals questions been used to validate refinements to animal housing and husbandry? Many of the examples below are from species traditionally considered as farm animals; however, these species are sometimes used in laboratories and, irrespective of this, the principle of asking the animals to validate housing refinements remains the same.

Normality of behaviour

A highly comprehensive example of examining the normality of the behaviour of laboratory animals in laboratory cages was a study by Dr Manuel Berdoy (http://www.ratlife.org/), who investigated in detail the behaviour of laboratory rats after he released them into a large natural enclosure. It was rapidly apparent that the rats expressed many behaviours that were not shown in laboratory cages. Within hours, the rats had started building tunnel systems in which to live, adopted idiosyncratic techniques for drinking, developed highly complex social behaviour, and they even started running in a different way, similar to a dog 'looping' over long grass. It is worth remembering that inbred laboratory rats such as these had been bred to their relatives for at least 20 generations. So, rats that had been in laboratory cages for at least 20 generations were still motivated to perform many behaviours that are prevented in most laboratory cages. This study and others clearly show that laboratory cages designed to minimal legal requirements do not allow animals the freedom to express all their normal behaviours.

Choice tests

Choice tests have been used to assess the validity of a range of housing refinements (please see Table 1 for relevant references).

Additional space: The limited amount of space that we provide research animals can have consequences for both the quality of science and animal welfare. Smaller space allowance influences physiological responses in various species and locomotory performance in rats. This potentially compromises any measure or test influenced by these changes, and therefore reduces the external validity and quality of the science. Smaller space allowance can induce behavioural and physiological changes indicative of reduced welfare such as the physical prevention of behaviours, the occurrence of abnormal behaviours, distorted ethograms, increased aggression, increased free cortisol concentrations and suppressed immune responses (6). However, providing larger cages would be expensive and is likely to create other problems, such as the practicality of caging systems. Therefore, we need to validate additional space as a refinement.

Laboratory mice, domestic hens, and laboratory and meat rabbits were all motivated to work for space additional to that provided under minimal legal requirements of laboratory or farm housing. For laboratory mice, this was true irrespective of whether they were housed as singletons or in groups. However, motivation studies indicate that it is not sufficient to simply provide animals with additional empty space. Mice were highly motivated to enter additional space ranging between 196 cm^2 to 1600 cm^2 but they did not differentiate between the different sizes offered (Figure 3). One interpretation of not distinguishing between the different sizes of additional space is that it is the quality of the additional space that was important to the mice, rather than the quantity *per se*. Therefore, the following text describes refinements that might be placed in the cage or additional space, and whether these appear to have been validated.

Cage environment: Sherwin and Glen (10) reported that mice showed a preference for cage colour. Overall, white was the most preferred cage colour and red the least, although this preference might have been related to pre-weaning experience (Figure 4). Mice reared in red cages

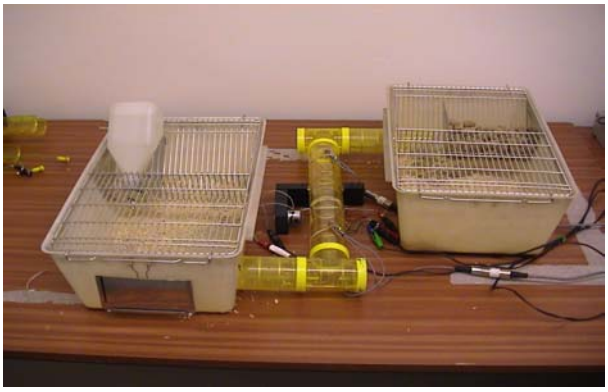

Figure 3. *Apparatus for investigating the strength of motivation of mice for additional space.*

were significantly more anxious than those reared in the white cages, as assessed by an elevated plus-maze test. There is some evidence that mice preferred opaque cages to transparent. Cage colour and opacity might be considered as a housing refinement but more evidence, such as studies on the strength of motivation for these, is required before they should be considered as validated.

Figure 4. *Red cages are less preferred by mice than white, black or green cages.*

The preferences for entire cage system types have been tested. Baumans et al. (11) recorded the preferences for cages in three different types of IVC racks which differed in intra-cage ventilation rate, cage size, location of air supply, and presence of nesting material. It was found that the mice avoided high intra-cage ventilation rates but providing nesting material could counteract this avoidance. In addition, the mice preferred larger cages and an air supply in the cover.

When moving about, some species show a strong preference to remain in contact with a wall and prefer to avoid open spaces (i.e. thigmotaxis). These preferences can be catered for in cages by providing dividers in the vertical and/or horizontal planes. Providing dividers increases the complexity of the cage and might also increase the size of the cage as perceived by the animal. Boyd and Love (12) reported that vertical dividers in cages reduced fearful or anxious behaviour in a novel environment, indicating this was likely to have been a refinement, but this requires further investigation for validation.

Extended locomotion: A major concern of laboratory cages designed to minimal legal requirements is that they are small and offer little opportunity for extended locomotion. Preference tests have been conducted to assess which are the best housing refinements to alleviate this problem. Sherwin (13) reported that running wheels were used prodigiously by pet rodents and also by laboratory animals including the rat, mouse, hamster, hen, fox, bob-cat, domestic cat, macaque, rabbit, ferret, dormouse, flying squirrel, ground squirrel, bandicoot and the Tasmanian devil. Laboratory rodents preferred access to running wheels rather than several metres of plastic tunnels, they used running wheels in semi-naturalistic environments, they preferred running wheels that have corners (e.g. triangles or squares) or ones which contained small hurdles, and preferred to have control over motorised wheels (i.e. to be able to switch them on and off).

It has been shown that laboratory animals, rodents in particular, were highly motivated to unlock a wheel, to turn on a motorized wheel, and to gain access to areas containing a wheel. Essential behaviours such as drinking were often adjusted or foregone to allow wheel running. These studies show that many laboratory animals are highly motivated to perform wheel running, and that running wheels could be considered as a validated refinement.

Flooring: Many laboratory cages have wire mesh or grid floors that prevent us from providing the animals with floor substrate. This can result in health problems such as pressure sores and urological problems, and can also thwart several behaviours such as digging and tunnelling. Preference tests showed that rats chose to dwell on solid floors rather than grid floors, and this preference was regardless of previous housing experience. The preference for solid floors was particularly marked when the animals were resting (88%) but less so during activity (55.4%). Both hamsters and gerbils preferred bedding to wire or bare plastic floor, and mice preferred a solid resting site and generally spent more time on a solid surface rather than a grid floor. Although it might not be possible to change the entire floor structure of a cage, it is sometimes possible to cover a section of the cage floor, or provide a receptacle of substrate.

Manser et al. (14) showed that rats were motivated to lift a door weighing 83% of their bodyweight to rest on a solid floor rather than a grid floor, despite their having been kept on grid floors for over 6 months. This indicates the rats were motivated to gain access to the solid floor and supports the findings of the preference studies that a solid floor could be considered as a validated refinement.

Floor substrate: Preference tests with mice and rats found that a floor substrate of relatively small particles (1.2 x 1.6 mm^2 or less) was generally avoided, whereas substrate consisting of large fibrous particles was preferred. Size and manipulability are among the main determinants of the preferences of substrate particles. Van der Weerd et al. (15) evaluated the preferences of two strains of rat for three types of substrate material (sawdust, softwood shavings and paper particles) compared to wire mesh. The rats showed a significant preference for cages with wood shavings and paper bedding, both consisting of large particles. The cages with sawdust and wire mesh floor were relatively avoided. The rats slept in the cages with large-particle substrate, but used the other cages for active behaviours such as eating and defecating. Many rats preferred different cages during the day and night, indicating that different behavioural activities may require different cage floor substrates. Similarly, Sherwin (16) showed that mice preferred to defecate in areas containing sawdust. These studies indicate that floor substrate could be considered as a validated refinement to a mesh or bare floor.

Burrowing substrate: Many rodents build burrows when given the opportunity. Burrowing substrate would therefore be a likely housing refinement under some circumstances (rodents in burrows are difficult to retrieve making it rather impractical for most laboratories!). Sherwin et al. (17) examined the strength of motivation for burrowing substrate in laboratory mice (Figure 5). Despite an increasing cost of gaining access, the mice continued to work to visit the burrowing substrate. In addition, it was shown that it was the performance of burrowing behaviour that was important to the mice, not simply the functional consequences. King and Welsman (18) showed that when bar pressing resulted in access to sand, deermice increased their rate of bar pressing. In a separate study where sand could be dug from a hopper, the animals performed sand digging at a remarkable rate, some individuals digging over 1,000 times their bodyweight in 24 hours!

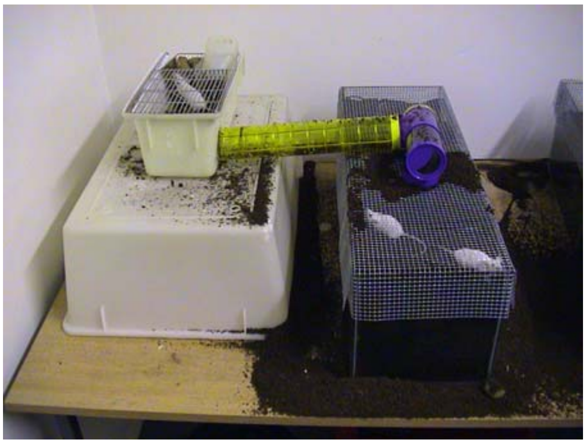

Figure 5. *Apparatus for investigating the strength of motivation of mice for burrowing substrate.*

Dust-bathing substrate: Hens and other species including degus, gerbils, kangaroo rats, jerboas, squirrels and chinchillas, regularly perform dust-bathing. A wide range of studies have shown that hens (Table 1) are highly motivated to gain access to a suitable substrate for this behaviour and that this could therefore be considered as a validated refinement. Further research would show whether this was true for other species that dust-bathe.

Nesting material: Many animals build nests for thermoregulation, security, or protection of themselves or their offspring. Nesting material can influence behavioural and physiological responses, indicating that it improves welfare, and a wide range of species are highly motivated to gain access to nesting material.

Mice will almost always build a nest if manipulable material is available. Choice tests showed a clear preference for cages with tissues or towels compared to paper strips or no nesting material, and for cages with cotton string or wood-wool compared to wood shavings or no nesting material. Paper-derived materials were preferred over wood-derived materials, although the nature (paper or wood) of the nesting material is less important than its structure, as this determines how successfully the material can be incorporated into the nest (Figure 6).

Figure 6. *Chewing block/nesting box/climbing box for mice with paper nesting.*

Mice and hamsters are highly motivated for nesting material and will bar press repeatedly for paper strips to build nests even when the required number of presses is high. Oley and Slotnick (19) reported that rats bar pressed for paper strips usually during the dark phase, but after parturition performed this at a greater frequency and also during the light phase. Guerra and Ades (20) investigated the motivation of hamsters for nesting material by increasing the length of the runway that the animals had to traverse to collect paper strips. Increased travel costs were related to a decreased number of trips to the paper strips and longer intervals between trips, but larger amounts of nest material were transported per trip and the hamsters increased the time spent at the source of the material and in nest-building. Adjustment of behaviours in this way indicates the animals were highly motivated to collect the nesting material. There is overwhelming evidence that rodents, mice in particular, are highly motivated for nesting material and this could therefore be considered as a validated refinement.

Nest boxes/shelters: In nature, some mice species live in burrows with nest chambers where they breed and hide from predators. In the laboratory, a shelter or refuge is a practical and easily provided refinement. In one choice test, male and female mice of two strains were provided with six nest boxes made of different materials. The mice preferred cages with a nest box made of grid metal compared to clear or white Perspex nest boxes, or no nest box. They also preferred cages with a nest box of perforated metal compared to nest boxes made of grey PVC or entirely metal, or no nest box. The preferences for perforated or grid nest boxes presumably related to mice monitoring the environment largely using olfaction – this would be more easily achieved in a non-solid nest box. When offered a nest box with one open side or a nest box with two open sides, most mice preferred the nest box with one open side and were observed to lie in it with their heads directed towards the opening. Sherwin (21) reported that when offered plastic tubes for shelters, mice often attempt to block these with available substrate, or perform nest-building activities in them, indicating that non-manipulable, pre-formed shelters might not always totally satisfy the nesting requirements of mice.

Collier et al. (22) investigated the strength of motivation of rats to bar press to open the door to a nest box. The rats continued to press even when required to do so 40 times per entry. Duncan and Kite (23) showed that hens were also highly motivated to gain access to a nest box, particularly immediately prior to oviposition. The hens would push a weighted door, or walk through water or an air blast to reach a nest box. Duncan and Kite suggested the strength of this motivation was equivalent to that of the strength of motivation to feed after 20 hours deprivation, indicating a nest box could be considered as a validated housing refinement for this species.

Social contact: Some research requires animals to be housed in isolation. However, Van Loo et al. (24) showed that male mice prefer to sleep in close proximity to familiar cage mates, and the need to engage in active social behaviour increases with age. Similarly, group-housed male hamsters spend more time in social proximity than out of proximity, especially if they had prior group-housing experience and they sleep in the proximity of at least one cage-mate. Although social contact was preferred by these hamsters, the costs were enlarged adrenal glands and wounding due to fighting, indicating that on some occasions, refinements must by used with caution as they might be appropriate for some individuals within a species but not others.

There have been several studies on the strength of motivation for social contact in mice, rabbits, hens, pigs and cows, all of which show that animals can be highly motivated to gain or retain social contact. The extent or type of social contact can influence the strength of motivation to work for this refinement. Holm et al. (25) showed that calves were more highly motivated to gain full contact with conspecifics than for head contact only. Mathews and Ladewig (26) showed that for pigs, the motivation for limited access to another pig was similar to that for opening a door to show an empty pen, i.e. the pigs found limited contact to be a poor refinement. Sherwin (27) showed that housing mice as individuals influenced the strength of motivation for a running wheel but not additional space. This shows that studies which have examined motivation for refinements in animals housed individually might not be representative of group housed animals.

The strength of motivation to retain social contact with offspring has also been studied. Van Hemel (28) trained mother mice to press one bar for the opportunity to retrieve pups to the nest and another bar for sensory contact with non-retrievable pups behind a screen door. All the mice pressed more on the bar that yielded retrievable pups, and when this was reversed, five of the six mice learned to press the opposite bar, indicating that the major reinforcing value of pup presentations was the opportunity to retrieve the pup. In a similar study, Wilsoncroft (29) allowed bar pressing by mother rats to be reinforced by pups being delivered into a trough. The mother rats pressed repeatedly on the bar to retrieve their own six pups, and then continued to press in anticipation of retrieving hundreds of babies! Given the high motivation for social contact with offspring, these studies raise concerns about the welfare of mother animals in systems that wean offspring at an early age, and re-iterate that the extent of social contact is important if this is being considered as a refinement.

Lighting: Most conventional fluorescent lighting that we provide laboratory animals has two characteristics that could be refined. First, fluorescent lights flicker on and off at a rate that is imperceptible to humans, but which is below the critical flicker-fusion rate of some laboratory animals (i.e. these animals might perceive the lights as flashing on and off, rather than constant). Second, conventional fluorescent and incandescent lights emit little or no ultraviolet (UV). Adult humans with normal eyes can not see UV so this does not cause problems for us, but it could have consequences for laboratory animals that are visually sensitive to UV. To assess whether there might be adverse consequences of these lights, animals have been placed into preference apparatus offering the choice between low frequency fluorescents (flickering), high frequency fluorescents which flicker at a much greater frequency and would likely be perceived as constant, or, incandescent (non-flickering) lights. Under these conditions, animals generally prefer the non-flickering light source, although the strength of preference is generally not great indicating that at least in terms of flickering, conventional fluorescent lights are unlikely to be a major welfare concern. However, it has been shown that the behaviour of animals in laboratories can be influenced by low or high frequency fluorescent sources, indicating that appropriate lighting type would be a refinement in studies where this might be an influence.

The preferences of animals that are visually sensitive to UV have also been tested. Mice showed an unexpected slight aversion to a UV-enriched environment (Sherwin, unpublished), and although starlings had an initial preference for a UV enriched environment, this soon waned. Turkeys also showed a preference for UV enriched environments, although this preference was not strong. This indicates that an absence of UV might also not be a great welfare concern; however, it might be indicative of our lack of understanding of how these animals use UV in their behavioural biology. Preference tests have also been used to test other refinements of the light environment, such as light intensity. Mongolian gerbils have been shown to prefer partially darkened cages.

Light sources and timing of the day : night schedule in laboratories are usually designed for the human care-takers rather than for the research animals. If lighting is not appropriate for animals, this can result in behavioural and physiological changes indicative of stress. Rats were tested with a 2-bar procedure that allowed on and off control of lights of several intensities. The rats pressed the off switch almost twice as frequently as the on switch; light-related rearing experience influenced the intensity chosen and the duration of light and dark. Baldwin (30) showed that when animals were given control of their lighting with the equivalent of an on/off switch, pigs kept lights on for 72% of the time and sheep for 82%. However, when the pigs had to work for the light by keeping their snout within a photo-beam, they only kept the lights on for 0.5% of the time, indicating that light was a weak refinement for this species. Savory and Duncan (31) showed that individual hens kept in a background of darkness were prepared to work for 4 hours of light per day.

It appears that under some circumstances, animals are prepared to work for light and this might be considered as a validated refinement, although this is influenced by experience and the conditions of testing. Future research should examine the role that UV has in the behavioural biology of laboratory species.

Novelty and searching: Several studies have indicated that exploration of novel areas is a highly motivated behaviour, however, the small, barren nature of most laboratory animal cages means that any exploration or searching is likely to have limited reward for the animal. Manser et al. (14) showed that rats were motivated to lift a weighted door to gain access to a novel cage, and Sherwin (32) showed that mice would work to enter empty cages even though these were small and never contained anything of appreciable biological significance. Cooper and Appleby (33) assessed the strength of motivation of hens to perform pre-laying searching behaviour by having a doorway of variable width between the home pen and an open area. Narrower doorways reduced the number of visits to the open area, but they did not eliminate it.

Some animals are considered to be neophilic (attracted to novelty) whereas others are neophobic (fearful of novelty). This means that novelty can have beneficial or adverse effects on the welfare of animals. Nicol et al. (34) showed that when two strains of mice were provided with a range of novel enrichments, each available for one week, one strain (ICR-CD-1) made more use of the enrichments compared to the other (C57Bl/6J). This was attributed to the latter strain having a higher trait anxiety tendency. It was concluded that while attempts should be made to devise universal enrichments (refinements) that improve welfare across a wide range of strains, if this is not possible then refinements might need to be implemented in a strain-specific manner. Low anxiety, exploratory strains might benefit from the repeated provision of novel objects, but strains exhibiting high trait anxiety might require a more stable cage environment.

Table1. Housing refinements and references to research which have attempted to validate these.

Refinement	Animal	References
Additional space	Mice	32,35
	Rabbits	36-38
	Hens	39-41
Cage environment	Mice	10-12,42
Extended locomotion	Various	13,44
Flooring	Rodents	45-49
Floor substrate	Rodents	15-16,49
Burrowing substrate	Rodents	17,18,21
Dust-bathing substrate	Hens	50-56
Nesting material	Mice	57-61
	Hamsters	20,62
	Rats	19
Nest boxes/shelters	Mice	21,63
	Rats	22
	Hens	23,64,65
Social contact	Mice	24,28,29,66,67
	Hamsters	68
	Rabbits	69
	Hens	23,70
	Pigs	26
	Cows	25
Lighting	Birds	31,71-78
	Gerbils	79
	Pigs and Sheep	30
	Rats	80
Novelty or searching	Mice	32,34
	Rats	14
	Hens	33

The future

The principle behind the choice studies described above is that the animals are showing us what is best for their welfare. If we can validate housing refinements by these methods and provide them to the animals, their welfare should be improved. If this is the case, then animals should be less motivated to leave cages which provide these refinements. Unfortunately, it does not appear to be that simple a relationship. Sherwin (81) housed laboratory mice in large cages which contained food, water, cage-mates, nesting material, a running wheel, plastic shelter, cardboard tube, hanging food stick and flavoured chew-sticks. The mice were still motivated to repeatedly press a switch to enter a small, empty additional cage that offered no appreciable resources or potential enrichments. This means that studies which measure the motivation of animals to validate housing refinements need to take into account this background motivation. Furthermore, future research needs to elucidate the reasons for the mice wanting to leave the refined cage, and whether these needs can be provided for by further refinements.

This article has discussed only behavioural methods of validating refinements to improve laboratory animal welfare. There are, of course, a wide range of physiological indicators of welfare, and there is a growing trend in studies of animal welfare to adopt a multidisciplinary approach including both behavioural and physiological techniques. This is to be applauded, and future studies on validating housing refinements should adopt a similar approach.

Conclusions

Laboratory animals have different senses and motivations to humans. This can make it difficult for us to understand whether refinements to research protocols, including housing refinements, benefit the animal by improving its welfare. By asking questions of the animals we can circumvent these problems. The animals can tell us what refinements they want and how much they want these, and therefore validate those which are the most likely to benefit animal welfare. Laboratory animals are demonstrably highly motivated to obtain additional space, social contact with conspecifics, nesting material, nest boxes, solid floors, burrowing substrate, running wheels, and the opportunity for novelty or exploration. These housing refinements should be provided for laboratory animals wherever appropriate and possible. Further research on validating housing refinements would be more robust if they included physiological indicators to also show improved welfare, alongside the behavioural responses discussed here.

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