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Rattling the cage: Behaviour and resource use of mice in laboratory and pet cages

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ABSTRACT

Conventional housing for laboratory mice limits the expression of species-specific behaviours and restricts the control over their environment, thus failing to guarantee the animals' welfare. To better understand the behaviour and resource use of laboratory mice, we housed mice (n = 64) of two common laboratory strains (C57BL/6 and Swiss), both sexes and two group sizes (3 and 5) in large extensively enriched pet cages and conventional laboratory cages, respectively, and assessed their behaviour, resource use, and space use under these different housing conditions. Mice in pet cages showed more running, mostly on the running disc, and other locomotor behaviour, but also spent much time hidden in deep bedding, while mice in conventional laboratory cages climbed more on the cage grid, reared more and exhibited more stereotypic behaviour. Our findings emphasize the significance of a proper substrate for shelter, as well as other resources that facilitate species-specific behaviour.

1. Introduction

The common house mouse (*Mus musculus)* is the most used laboratory animal worldwide (Carbone, 2021; European Commission, 2022), yet surprisingly little is known about the behavioural needs of mice in captive environments (Bailoo et al., 2018a, 2018b; Latham and Mason, 2004). Conventional housing conditions of laboratory mice are vastly different from their natural habitats, which range from the desert, over tropical rainforests and temporal areas to subarctic regions. Depending on resource availability, mice may form and defend large territories (Crawley, 2007; König, 2012; Latham and Mason, 2004), where they explore, climb, burrow and forage to find food, avoid predation, competition and open spaces (Latham and Mason, 2004). Furthermore, mice are synanthropes living in human-made environments, where they are faced with frequent changes, competition and constant dangers (König, 2012; Latham and Mason, 2004), and can modify their surroundings, creating microenvironments to increase their well-being and fitness (König, 2012).

Despite their ability to adapt to different environments, the motivation to use a range of resources and perform specific behaviours remains in laboratory settings (Mieske et al., 2022). Laboratory mice prefer complex cages, which provide opportunities for climbing, sheltering, running, grooming, resting and manipulation (Hobbiesiefken et al., 2021; Olsson and Dahlborn, 2002). A more complex design may also give mice the choice to segregate different areas (Makowska et al., 2019) and better cope with social life (Streiff et al., 2024; Tallent et al., 2024). Mice also show preferences for nesting material (Gaskill et al., 2012) and deep bedding (Freymann et al., 2017, 2015) and will actively burrow (Adams and Boice, 1981; Ratuski and Weary, 2022; Sherwin et al., 2004), build nests (Cintra et al., 2024; Hess et al., 2008) and run on running wheels (Manzanares et al., 2019). These behaviours are highly motivated which may be indicative of an intrinsic drive to engage in them (Greenwood et al., 2011; Muguruza et al., 2019; Olsson and Dahlborn, 2002; Ratuski and Weary, 2022).

However, most of these resources are still commonly absent in laboratory settings. Conventional housing conditions for laboratory mice are primarily regulated by the quantity of space in relation to body-mass rather than the resources needed to facilitate species-specific behaviour (European Commission.., 2010; Garber, 2011; National Research Council of the National Academies, 2011; "Swiss Animal Welfare Ordinance (SR 455.1)," 2008). Under current standards, mice are housed in small and relatively barren cages for economic, ergonomic and sanitary reasons, as well as a desire for standardisation (Bailoo et al., 2018a; Ratuski and Weary, 2022). The resulting lack of critical resources and

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the inability to meet the animals' behavioural needs can cause stress, and thus can compromise animal welfare (Bailoo et al., 2018b; Dawkins, 2023; Fraser, 2009). Mice kept in conventional laboratory cages are prone to develop abnormal behaviours (e.g. stereotypies, barbering, waking inactivity), that reflect poor welfare (Maclellan et al., 2022; Mason, 1991; Würbel, 2006). Furthermore, conventional housing has been associated with depression-like states (Fureix et al., 2022), increased anxiety (Van Praag et al., 2000; Würbel, 2001) and enhanced morbidity and mortality in rodent disease models (Cait et al., 2022; Walker et al., 2012).

Environmental enrichment can not only attenuate these adverse effects, but can also promote positive affective states, through the provision of adequate stimuli and resources that meet the animals' behavioural and physiological needs (Würbel and Novak, 2022). However, while some countries have included nesting material as a minimal requirement (European Commission.., 2010; "Swiss Animal Welfare Ordinance (SR 455.1)," 2008), other resources have not been implemented widely, as the food pellets are considered to provide sufficient opportunities for gnawing and the cage lid for climbing (European Commission, 2010; Swiss Animal Welfare Ordinance (SR 455.1), 2008). In Switzerland, however, housing requirements for laboratory mice differ substantially from the housing recommendations for pet mice, not only in minimum space, but also in resources provided (Table S1, (Schweizer Tierschutz STS, 2019; Swiss Animal Welfare Ordinance (SR 455.1), 2008). Although previous studies found that space allowance did not affect measures of welfare (Bailoo et al., 2018b), larger cages may be needed to accommodate the resources that are necessary to guarantee animal welfare (Bailoo et al., 2018a).

The distinct legal standards in Switzerland for housing pet and laboratory mice fuelled a debate as to whether minimal housing requirements for pet mice should also be applied to laboratory mice or whether they might be considered a luxury.

As a first step to address this question and better understand the behavioural needs and resource use of laboratory mice, we housed mice under both, conventional housing conditions based on the Swiss minimal housing requirements for laboratory mice (Swiss Animal Welfare Ordinance (SR 455.1), 2008), and housing conditions considered to be ideal for pet mice, according to Switzerland's largest animal protection organisation (Schweizer Tierschutz STS, 2019).

Thus, the aims of this study were (I) to compare the behavioural repertoire of mice housed under these two conditions and (II) to assess the use of space and resources under extensive housing conditions. Based on current evidence, we expected to observe fewer behaviours indicative of impaired welfare, such as stereotypies, and active use of the various enrichment items, in enriched pet cages compared to conventional lab cages. However, we deliberately chose an exploratory approach and, therefore, refrained from formally testing specific hypotheses.

To increase the generalisability of our results we used two common laboratory mouse strains, one inbred and one outbred, and both sexes, and housed them in two different group sizes for the duration of the study.

2. Material and methods

2.1. Subjects and housing

Sixty-four laboratory mice of both sexes and two strains (one inbred: C57BL/6NRj (C57BL/6) and one outbred: RjOrl:SWISS (Swiss)) were ordered from Janvier Lab in France and arrived at our animal facility at three weeks of age (N = 64 mice, 8 mice per sex, strain and housing condition). The mice were randomly allocated to groups of three and five (stratified by sex and strain; n = 2 cages per strain, sex and group size) by using the RAND command in Microsoft Excel. All groups were housed in open-top, laboratory type III cages for one week to monitor health and growth after transport. At four weeks of age, the two groups per sex, strain and group size were randomly assigned to either laboratory (lab) cages or pet (pet) cages.

The lab cages were 40 cm long, 23 cm wide and 15 cm high and contained Pura flake bulk aspen bedding from Labodia, nesting material (10 g Datesand Sizzlenest shredded paper and a PlexxEU cotton nestlet), a wooden gnawing stick from Kliba Nafag, a red Tecniplast mouse house and a red tunnel from Bio-Serv for handling (Fig. 1). Mice were provided with Kliba Nafag type 3430 rodent food and tap water *ad libitum*.

The pet cages were 115 cm long, 85 cm high and 55 cm wide. The bottom 50 cm were made of transparent PVC plates with a 20 cm wide band of 5 mm wide holes for air ventilation and filled with Pura flake bulk aspen bedding from Labodia up to 40 cm high (Fig. 2), in which mice could create stable burrows. The top was (a) a 35 cm high grid (Lupo 120 cage grid from Qualipet) with four small doors on one of the long sides and two large doors on top. The top two doors were replaced by (b) custom made food hoppers containing food pellets and water bottles. One of the small side doors was connected to a tube leading to (c) an attached cage. Mice had continuous access to this attached cage, but could be contained there for health checks, weighing and during cleaning of the pet cages, by closing the access tunnel. The pet cages were designed according to the recommendations for the housing of pet mice by Swiss Animal Protection (Schweizer Tierschutz STS, 2019) and enriched with various resources, thought to facilitate species-typical behaviour in mice. Resources were distributed across several locations within cages, and the location of each resource was randomised across cages (if not restricted by space or function). Resources included (d) two custom built PVC shelves fixed on the grid, (e) a large and (f) a small rectangular house, custom made from red acrylic glass, (g) a red triangular house from Tecniplast, (h) a red, round house from Datesand, (i) a red tunnel from Bio-serv, (j) a hammock from Nobby Sputnik, (k) a wood-ladder from Karlie, (l) a sandbath (rodent sand in a chrome-steel dish from Qualipet), (m) a vertical running wheel (Ø 20 cm) form Savic, (n) a plastic snack ball, (o) a disc shaped running wheel (Ø 15 cm), (p) a rope, (q) a wood-and-rope hanging ladder, (r) a hanging foraging ball (filled with 10 g of Datesand Sizzlenest shredded paper, 10 g of hay and a paper tissue) from Trixie, (s) a rodent saltlick from ZooKakadu, (t) a PlexxEU cotton nestlet, (u) a wooden gnawing stick from Kliba Nafag and (v) an artificial tunnel to a laboratory cage buried in deep bedding (For more details see Table S2 in supplements).

Ambient temperature within the housing room was 22 ± 2 °C and humidity 45 ± 11 %. Mice were housed under a reversed 12:12 h light: dark cycle, with the dark phase starting at 8:00 h. Dim red lights were on continuously and during the light phase white indoor light at max 150 lux was on.

2.2. Animal husbandry

Mice were housed in either lab or pet cages from 4 weeks of age until 13 weeks of age. The mice were marked individually by fur shaving before moving to experimental cages. To facilitate health checks by

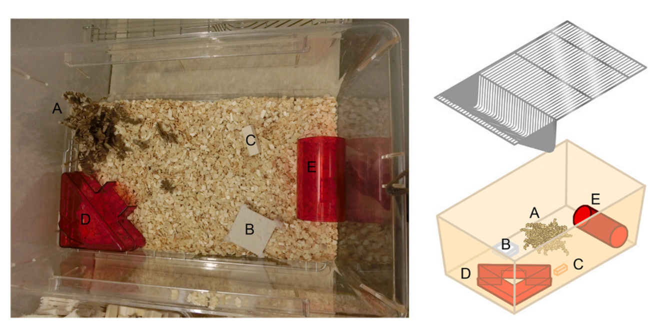


Fig. 1. Laboratory cage as used in the experiment. Lab cages contained woodchip bedding, enriched with (A) shredded paper, (B) cotton nestlet, (C) a wooden gnawing stick, (D) a mouse house and (E) a red tunnel.

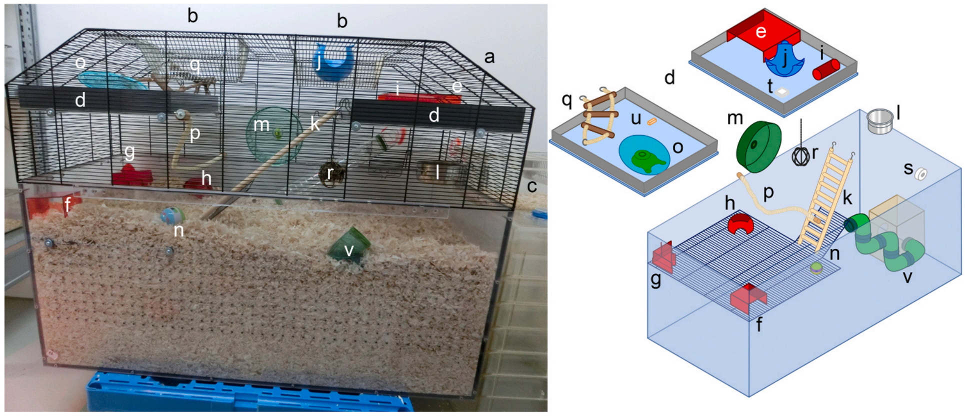


Fig. 2. Pet cage as used in the experiment with various resources (see Section 2).

visual inspection, mice in pet cages were trained to enter the attached cage once per day using chocolate flavoured pellets (dustless Precision Pellets, Bio-Serv) as reward. Mice in lab cages received the same amount of chocolate pellets. Cages were either changed (lab cages) or cleaned (pet cages) every week on Friday. For this, mice were removed from the cages, (re)marked, weighed and inspected for health issues. In pet cages, the top 10 cm of bedding was removed and replaced by clean bedding to remove staining and scent marks. Grids, shelves and items were washed. After cleaning, all items were placed back into the cages in the same position as before. During the first four weeks the mice were acclimatised to the presence of experimenters. Furthermore, video recording material was set up and tested on various occasions to habituate animals to the cameras and recording procedure. All animals were tunnel handled throughout the study and were only fixed by the tail, supported by a cage grid, to apply fur marks for individual identification. For shaving, an electric razor designed for dog grooming (Wahl, Super Trim) was used. In cages with five animals, four mice were shaved, and one was left unshaved, while in cages with three animals, all mice were shaved. The shaving positions were as follows: Animal 1 on the left side, Animal 2 on the right side, Animal 3 at the neck, Animal 4 at the tail base, and Animal 5 was not shaved. The unshaved mouse (Animal 5) underwent a "sham" shaving procedure, where the razor was held in reverse to imitate shaving motions, ensuring that the unshaved mouse experienced the same handling as the shaved mice to account for any potential stress from the shaving process.

2.3. Data collection

All data were collected during a 4-week period from 8 to 12 weeks of age. Behaviour and resource use were assessed by live observations, carried out by two observers (MG and JN) on the fourth and fifth day after cage change. Observations started one hour after lights were off and lasted for four hours, when mice were most active (Latham and Mason, 2004; Mackintosh, 1981; Rowe, 1981). Cages were allocated to the two observers randomly, balanced for strain, sex and housing condition. We changed the cage order each week to ensure that any potential observer bias was minimized. In addition, each pet cage was video recorded on the sixth day after cage change for 24-hours for later assessment of space use by the mice. Due to space constraints, each half of the cages were video recorded on alternate weeks.

2.3.1. Behaviour and resource use

JN and MG conducted all live observations. Each coder practiced individually using the same Standard Operating Procedure (SOP) during the acclimatisation period. During live observations, each mouse was observed for 5 min using instantaneous focal sampling with a 10 second interval. The order of coding cages and animals was randomised for each coding session. Mice were coded as in or on a resource, if at least three legs were in or on the resource. The mouse was coded as inside the bedding if it was not seen anywhere else in the cage. Grooming, stereotypy, and gnawing were recorded only when they persisted for three seconds or longer. Consequently, a total 240 scans per mouse were recorded across four weeks, resulting in a total of 15'360 scans. The ethogram for coding was based on live observations during an initial pilot study, incorporating definitions and descriptions from various sources (Bailoo et al., 2018b; Garner, 2023; Novak et al., 2016; Ratuski et al., 2021). As some behaviours were infrequent, they were aggregated into categories for effective recording (Table 1). During the acclimatisation period, both MG and JN watched the same cages simultaneously and coded the behaviours and resource use to assess observer agreement. This was done to ensure consistency and reliability in the data collection process. Therefore, 3 cages were randomly selected, using the RAND command in Microsoft Excel. These cages contained 11 animals which were watched in randomised order. Cohen's Kappa was then calculated in R (see 2.5 Statistical analysis). Inter-observer reliability for the frequencies of both behaviours and resource use were good (Cohen's kappa for behaviour was 0.78 and for resource use 0.86). Intra-observer reliability could not be assessed for live observations.

2.3.2. Space use in pet cages

Space use in pet cages was assessed from videos by a single coder (MSF) using Solomon coder (version: beta 19.08.02). Using scan sampling, the number of mice visible in the pet cage was recorded every 10 min across the 48 h of recording. To this end, the pet cages were divided into five areas; the attached cage, area below the shelves, area above the shelves including the grid, the middle area (middle floor area and area between the shelves, including the grid), and the deep bedding (when mice were hidden in the bedding; Fig. 4). The videos were split into dark and light phase and coded in randomised order. No information was given to the coder about the sex, strain or group size in the video; however, the coder could sometimes identify strain and group size. To assess inter-observer agreement a second coder (MG) was also trained to code from videos. Both coders practiced individually using a Standard Operating Procedure (SOP) before inter-observer reliability was assessed. Therefore, MSF and MG watched and coded the same video recordings (12 h of the light phase and 12 hours of the dark phase for each area in one cage) and Cohen's Kappa was calculated using R (see 2.5 Statistical analysis). To calculate intra-observer reliability, MSF rewatched 10 % of all recordings. These Videos were randomly selected from all recordings, stratified by cage area and phase of the day using the RAND command in Microsoft Excel beforehand. Inter-observer reliability (Cohen's kappa = 0.87) and intra-observer reliability (Cohen's kappa = 0.95) for the proportion of mice visible per area were very high.

Table 1
The ethogram used for behavioural coding during live observations. This Ethogram was illustrated by Michelle Gygax © 2024.

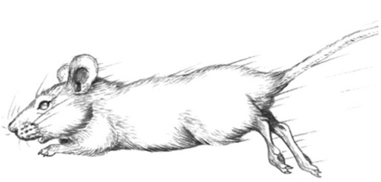
Behaviour	Definition
Grooming 	This behaviour is characterized by repetitive movements for 3 s or more. **Scratching** (depicted)**:** The mouse scratches itself using any paw or mouth. **Face wiping:** Combing the fur and vibrissae on its head with the front paw, progressing from the nose towards the back of the head and ears. **Licking:** The mouse contorts its body to reach various areas with its snout, including the sides, back, genital area and the tail.
Social Behaviour 	Affiliative behaviours shown during interactions with conspecifics. **Sniffing** (depicted)**:** Mice approach and nose at each other. **Huddling:** Mice sit close and sleep or lay together their bodies touching, either next or on top of each other. **Allogrooming:** Mice groom each other. At least one animal is in snout contact with a recipient and repetitively moves its head over the area, licking at the fur, lasting for 3 s or more.
Climbing 	The mouse is moving or hanging on a given structure while holding on to it with the tail, paws, or mouth. All four paws are off the ground or not in contact with any other item.
Running 	The mouse is moving forward with all four legs moving. Can occur in hopping motions, straight or zigzag lines.

Table 1 (*continued*)

Behaviour	Definition
Digging 	The mouse uses its forelimbs and snout to move substrate material, by scrabbling at the substrate with alternating motions of its forepaws, often pushing the material backward or to the sides. Its snout is actively involved, sometimes used to probe and loosen the substrate. As the digging progresses, the mouse may create a pile or a hole. The action is vigorous and can be accompanied by rapid body movements and occasional repositioning to target different digging areas.
Aggression 	Aggressive behaviours shown in social contexts. **Tail rattling:** Mice might rapidly shake the tail which might produce a sound, if the tail is rattled against an object, the cage wall or floor. **Thrusting:** Mice rapidly thrust towards another individual to chase or attack it. **Chasing:** one individual closely follows another. **Mounting:** One individual climbs onto the other mouse with the forelimbs on its hindquarters and makes pelvic thrusts. **Parring** (depicted)**:** An attack, the mouse turns its body sideways to an aggressor or rear its front while repeatedly kicking its forepaws towards the other animal. **Biting:** the teeth of the mouse grab fur or skin of another, mostly directed toward the tail or rump of the recipient. **Attack/fight:** Biting, accompanied by kicks and jumps. Mice hold on to each other rolling around in a fight until one submits or flees. Animals try to pin each other to the ground. A submissive mouse might then crouch by pressing its body closer to the ground or expose its belly by raising its head and forelegs into the air.

(*continued on next page*)

Table 1 (*continued*)

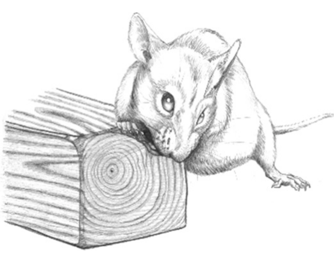
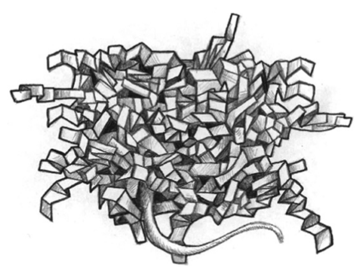
Behaviour	Definition
Locomotion 	Involves all locomotive behaviour, that is not otherwise defined.
Feeding/Drinking 	The mouse's head is directed towards the food hopper or water bottle nozzle and snout is brought in contact with food and water bottle nozzle. Food items can be held in front of the mouth in the animals' front paws and be gnawed on.
Gnawing 	The mouse gnaws on an object. The mouth is placed on the object and the mouse bites into it for 3 s or more. Small objects might be held in the front paws and handled while gnawing.
Stereotypy 	Behaviours that are repeated continuously for 3 s or more (bar mouthing) or at least three times in a row (circling, twirling, backflipping, route tracing). **Circling** (depicted): running around the cage floor or grid in circles. **Bar mouthing:** gnawing on bars with bar held between incisors and molars, while standing on hind paws or hanging from the cage lid by all or front paws only. May be performed on the spot or by moving along the bar. **Twirling:** Spinning around the longitudinal body axis while hanging from the cage lid. **Back-flipping:** Backward flip from the ground, the food rack or one cage wall into the opposite direction. **Route-tracing:** Moving along the same route repeatedly on the cage floor or grid.

Table 1 (*continued*)

Behaviour	Definition
Rearing 	Mouse stands on its hind legs, raising the front of the body from the ground, supporting the front by placing its front paws on a social partner, item or cage wall, or unsupported, when the mouse keeps its front paws in the air.
Inactive 	Movement is absent, for 3 s or more. **Sleeping** (depicted)**:** The animal rests with eyes closed. **Still but awake:** The animal is motionless but keeps its eyes open.
Hidden 	The animal is not visible due to being inside the nest or bedding. Unknown what the animal is doing.

2.4. *Ethical statement*

This study was conducted in accordance with the Swiss Animal Protection Ordinance (Tierschutzverordnung, TSchV 455.1, (Swiss Animal Welfare Ordinance (SR 455.1), 2008) and was approved by the Cantonal Veterinary Office in Bern, Switzerland (permit number: BE1/2022). The reporting of the study follows the ARRIVE 2.0 guidelines for reporting animal research (Percie du Sert et al., 2020).

2.5. *Statistical analysis*

Since this was a purely exploratory study, we did not use statistical hypothesis testing. However, we created statistical models for estimating odds ratios, describing differences in behaviour and resource use between mice in pet and lab cages, respectively. Thus, we also provide outputs from these models for frequencies of behaviour and resource use in the supplementary material (for detailed description see Table 2 and S3 a-c in supplements). We used generalised linear mixed effect models with a binomial distribution for each behaviour and resource used. Data were transformed to a 0–1 dataset for each behaviour, where 1 indicated the behaviour of interest was shown and 0 any other behaviour was shown (same for resource use). The model included behaviour or resource use as outcome variable, sex, strain, groups size and cage type as fixed factors and cage number, mouse ID and day of coding as random

Table 2
The table presents a comparison of behavioural and resource utilization patterns observed in pet and lab cages. Each row lists a specific behaviour or resource, followed by the setting where it is more frequently observed, an odds ratio (OR), a 95 % confidence interval (CI) for the OR, and the associated p-value indicating statistical significance.

Behaviour/Resource	More in:	OR	CI	p-value
Aggression	PET	1.19	0.32–4.48	p = 0.795
Climbing	LAB	0.11	0.06–0.21	p<0.001
Digging	PET	6.77	1.61–28.40	p = 0.009
Feeding/drinking	LAB	0.91	0.5–1.68	p = 0.767
Locomotion	PET	2.04	1.57–2.65	p<0.001
Gnawing	PET	7.50	2.47–22.76	p<0.001
Grooming	PET	1.07	0.76–1.51	p = 0.698
Inactivity	PET	4.98	0.26–95.05	p = 0.286
Rearing	LAB	0.18	0.12–0.25	p<0.001
Running	Running was not seen in LAB cages.			
Social behaviours	PET	1.47	0.58–3.71	p = 0.411
Stereotypy	LAB	0.00	0.00–0.01	p<0.001
Hidden	PET	275.93	35.43–2149.19	p<0.001
Bedding (under)	PET	171.17	19.74–1484.27	p<0.001
Floor (lab)/On Bedding (pet)	LAB	0.11	0.07–0.17	p<0.001
Grid (cage lid)	LAB	0.07	0.04–0.12	p<0.001
Nestlet	Nestlets were not used in LAB cages.			
Red tunnel (in)	LAB	0.05	0.00–0.56	p = 0.016
Shredded Paper	Shredded Paper were not used in LAB cages.			
Mouse House (in)	LAB	0.05	0.01–0.23	p<0.001
Gnawing Stick:	Never used.			

Note: Only resources that were available in both lab and pet cage are included here

factors, with mouse nested in cage (see equation 1). The models were run in R version 4.1.2 (2021–11–01). Sample size for the video analysis was calculated using the *LaplacesDemon* package and models and visualisations were made using the *MASS, tidyverse, ggplot2* and *DHARMa* packages in R. The Cohen's kappas were calculated using the *irr* package and the CohenKappa function in R, providing a measure of how consistently the coders identified the same behaviours or number of mice visible per area. Randomizations were conducted using the RAND() function in Microsoft Excel to ensure unbiased allocation of treatments.

Behaviour resp. Resource used ~ CageType+Strain+Sex+Group+(1|CageID/mouseID)+(1|Date)

Equation 1: Logistic regression model (glm) as used to look at differences in behaviours shown/resources used. CageType (lab or pet), strain (Swiss or C57BL/6), sex (male or female) and group (3 or 5 animals) were treated as fixed factors and CageID (number of cage), mouseID (animal number) and Date (coding day) were treated as random factors.

3. Results

3.1. Behaviour and resource use

Based on live observations during their most active period, mice in pet cages displayed more running and other locomotion (OR = 2.04, CI_{95} = 1.57–2.65), more gnawing (OR = 7.5, CI_{95} = 2.47–22.76) (more in Swiss) and more digging (OR = 6.77, CI_{95} = 1.61–28.40), than mice in lab cages (Fig. 3 A). In contrast, mice in lab cages showed more climbing (OR = 0.11, CI_{95} = 0.06–0.21) (more in C57BL/6 mice and in females), rearing (OR = 0.18, CI_{95} = 0.12–0.25) (more in Swiss), and stereotypic behaviour (OR = 0, CI_{95} = 0.00–0.01) (more in Swiss and groups of 3), while we did not detect differences between lab and pet cages in other behaviours (see Table 2. For more details see Table S3 a-c in supplements).

In terms of resource use (Fig. 3 B), live observations revealed that mice in pet cages spent much of their time on disc wheels, on the elevated shelves (more so in Swiss males), or inside the deep bedding (OR = 0, CI_{95} = 0.00–0.01), and this was consistent across the strains, sexes and group sizes.

In contrast, mice in lab cages spent most of their time on the cage grid (OR = 0.07, CI_{95} = 0.04–0.12) (more in C57BL/6 and in females) and spent only little time in the red tunnel (OR = 0.05, CI_{95} = 0.00–0.56) and the mouse house (OR = 0.05, CI_{95} = 0.01–0.23), but more than mice in pet cages. We found no differences in the use of shredded paper, cotton nestlet, and gnawing stick (Table 2). However, these items may have been moved inside the burrows in the deep bedding and where unseen during coding in pet cages (For more details see Table S3 a-c in supplements). All other objects were not present in the lab cages.

3.2. Space use in pet cages

Based on the 48 h video recordings, mice in pet cages spent most of their inactive phase in the deep bedding. In the active phase, they spent most time on the shelves and on the grid above the shelves, and in the deep bedding. They spent less time in the attached cage, under the shelves and very little in the middle area (Fig. 4). This pattern was consistent across sexes and group sizes, but Swiss mice emerged later from the bedding in the dark phase and entered the bedding earlier in the light phase than C57BL/6 mice.

4. Discussion

With this study, we aimed to assess how behaviour, resource use, and space use differ between mice housed under "optimal" housing conditions as proposed by Swiss Animal Protection association for pet mice (Schweizer Tierschutz STS, 2019) and mice housed with minimal enrichment in conventional laboratory cages. Live observations during the most active phase revealed comparable levels of activity and inactivity, respectively, in both housing systems. However, the types of activities differed markedly between the two environments. Mice in pet cages displayed much more locomotor activities, including running both on the running wheels and among resources. In contrast, active behaviour of mice in laboratory cages was dominated by climbing on the cage grid, rearing and a range of stereotypic behaviours.

The feral relatives of laboratory mice are known to move extensively for both exploratory purposes and defending the borders of their territories, for which they sometimes travel at great speeds (Latham and Mason, 2004; Randall, 1999). They are also very agile climbers and have been seen climbing even vertical brick walls in the wild (Randall, 1999), which helps them access food sources or nesting places. Our observations suggest that mice maintain high locomotor activity when given the opportunity. In line with other authors, we also observed high running wheel activity (Goh and Ladiges, 2015; Hobbiesiefken et al., 2021; Manzanares et al., 2019; Meijer and Robbers, 2014; Sherwin, 1996), a behaviour which laboratory mice are highly motivated to perform and is rewarding to them (Novak et al., 2012). Running wheels may also serve as effective means to facilitate physical activity within confined cages, which is known to positively impact health (De Waard and Duncker, 2009; Engesser-Cesar et al., 2005) and cognition (Cotman and Berchtold, 2002; De Waard and Duncker, 2009; Diederich et al., 2017; Engesser-Cesar et al., 2005; Nichol et al., 2007; Van Praag et al., 2005).

In contrast, mice housed in conventional laboratory cages without various resources or sufficient space appear to redirect locomotor activity mostly towards the cage grid, spending much of their time climbing and rearing. Both climbing and rearing are spontaneous exploratory behaviours in laboratory mice (Büttner, 1991; Nicol et al., 2008) and have been reported to decrease in enriched cages (Bailoo et al., 2018a) as animals utilise different resources. However, despite the greater absolute area of bars available in the larger pet cages, mice housed in these conditions engaged significantly less in climbing, suggesting that grid climbing observed in laboratory cages does not merely reflect an intrinsic motivation for climbing. Earlier research found that climbing in laboratory cages may represent attempts to escape the cage (Würbel, 2006; Würbel et al., 1996). Excessive climbing on the cage lid

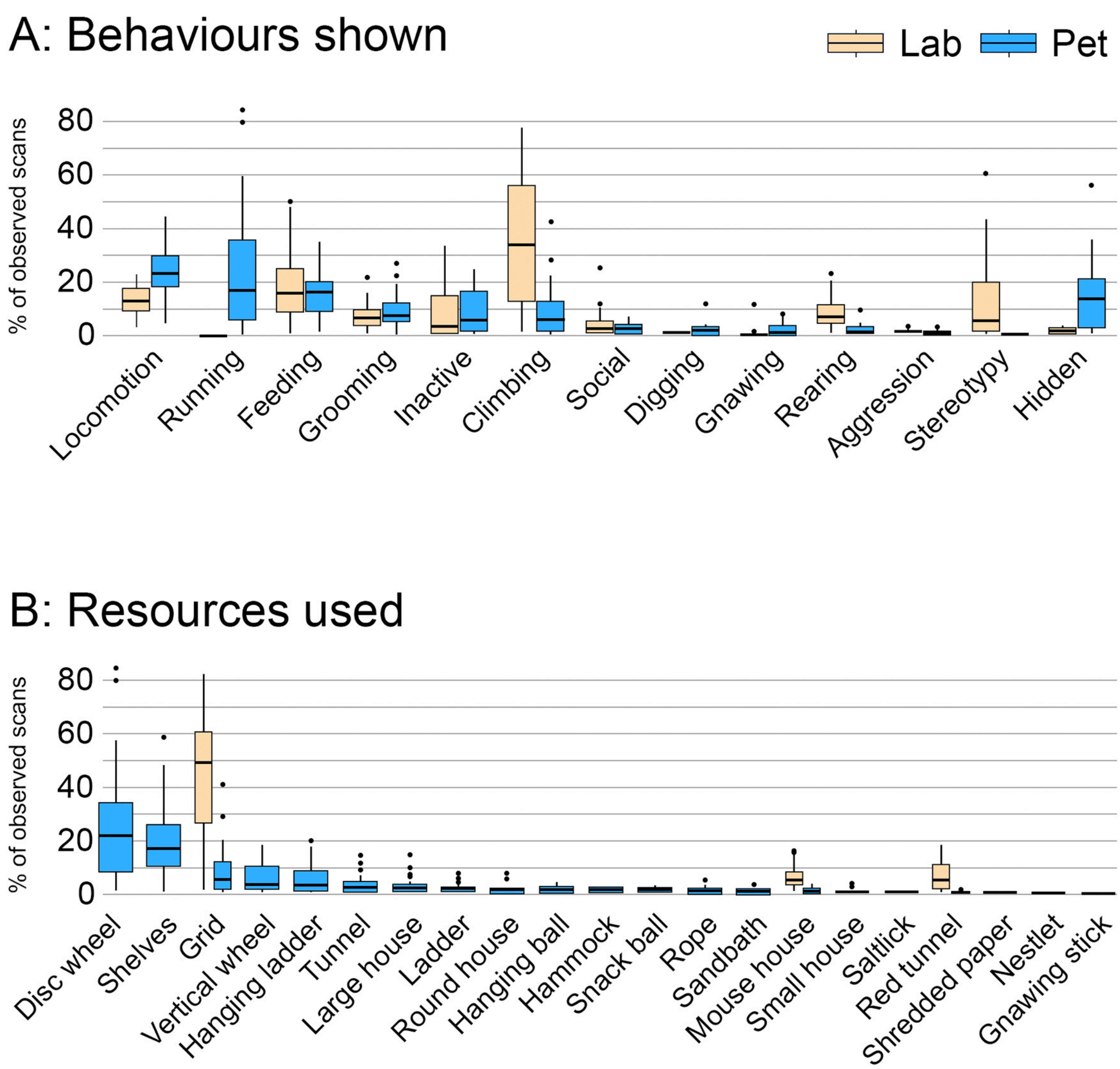


Fig. 3. Relative frequency of home cage behaviours and resource use. Boxplots show the percentages (of observed scans) of behaviours (A) and resource use (B) observed in both pet cages (blue) and lab cages (beige). The box represents the interquartile range (IQR), with the bottom and top edges indicating the first (Q1) and third (Q3) quartiles, and the band inside the box depicting the median. Whiskers extend to the furthest data points within 1.5 times the IQR from the quartiles, and points beyond this are plotted in black as outliers.

of laboratory cages might thus be a response to an inadequate environment rather than an expression of their species-typical behaviour. This is further supported by the fact that in barren laboratory cages, climbing on the cage grid often develops into stereotypies (e.g. bar mouthing, cage lid twirling; (Nevison et al., 1999; Würbel et al., 1998, 1996)). Stereotypies typically develop from behavioural attempts to cope with the lack of critical resources or inadequate environmental stimuli (Mason, 1991; Wolfer et al., 2004). In laboratory mice, stereotypies can be reduced by adequate environmental enrichment (Bailoo et al., 2018a; Gross et al., 2012; Würbel et al., 1998), which may explain why it was mostly absent in pet cages in our study.

Enrichment not only decreases abnormal behaviour, but it also increases complex social interactions and expression of behaviours related to positive welfare (Würbel and Novak, 2022). In contrast to wheel running and climbing, which are linked to specific resources, many behaviours in pet cages may have occurred when mice where in the deep bedding (e.g. social behaviours, grooming, general activity and inactivity), therefore it is possible that the time budget assessment for pet mice underestimated the frequency of some of these behaviours.

Mice in pet cages predominantly utilized deep bedding as a hiding refuge, especially during the light phase. While we know that laboratory mice actively burrow (Ratuski et al., 2021; Sherwin et al., 2004), little is known on how much time mice spend in these underground burrows. Our data show that despite provision of a range of commercial shelters, mice spend most of the light phase in the deep bedding, either in self-made burrows or in buried cages and tunnels. Laboratory mice have a strong preference for nesting material (Van De Weerd et al., 1998), however, we did not observe direct use of nesting material neither in lab nor in pet cages. One possible explanation for this is the timing of our observations. Behaviour and resource use were coded four to five days after cage change, allowing the animals to habituate to their environment. However, we saw that in lab cages, mice constructed a nest, and we observed no nesting material in the pet cages, as it was likely taken to the underground areas, further indicating that deep bedding may not only fulfil the motivation to burrow but is also used as a shelter. Underground shelters not only offer darkness and burrowing opportunities, but may also decrease heat loss from the nesting site (Gaskill et al., 2013). It is well known that ambient temperatures at research facilities are well below the mouse thermoneutral zone (Gaskill et al., 2013, 2012) and mice must resort to behavioural adjustments such as huddling and nest building to maintain thermal homeostasis (Latham and Mason, 2004). However, even nesting material is not sufficient to completely reduce heat loss (Gaskill et al., 2012), and mice have to burn more energy to generate heat, which can affect physiology (Gaskill et al., 2013).

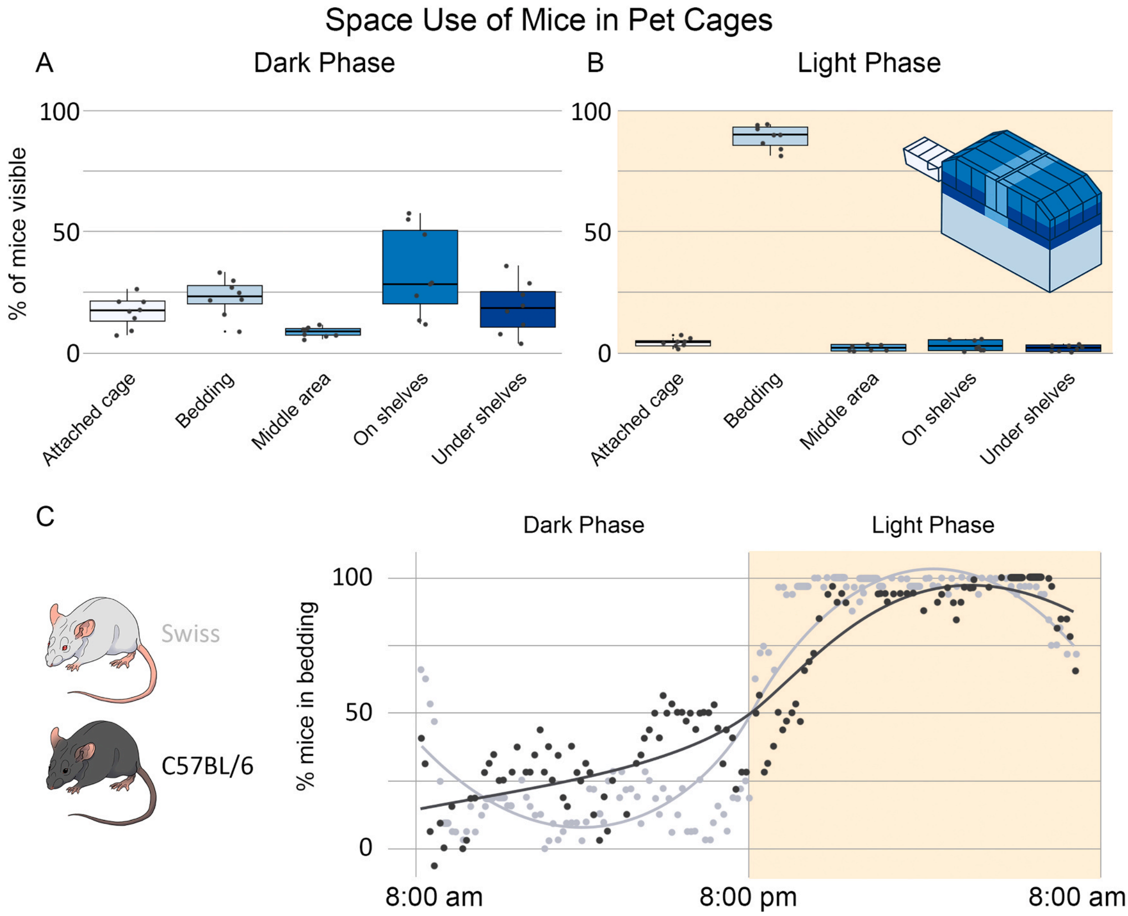


Fig. 4. Space use in the pet cage. A) and B) The box plot shows the percentage of mice visible in each area of the pet cage per day. The boxes represent the interquartile range (IQR), with the bottom and top edges indicating the first (Q1) and third (Q3) quartiles, and the band inside the box depicting the median. Whiskers extend to the furthest data points within 1.5 times the IQR from the quartiles, and points beyond this are plotted in black as outliers. A) During the dark phase and B) during the light phase. C) The line plot shows the percentage of Swiss (light) and C57BL/6 (dark) mice inside the bedding across 24 h starting at 8:00 am.

Deep bedding may therefore enable mice to actively modulate their environment to maintain their desired microclimate, thus fostering an environment that supports their overall health and welfare. While digging and retreating to a safe place are species-specific behaviours (König, 2012), we could not measure the animals' activity, behaviour or space use in the deep bedding. However, the fact that all mice spent most of the light phase underground, implies the significance of the resource.

Apart from the running wheels and deep bedding, mice in pet cages also spent much time on the shelves and the grid above them, as well as the hanging ladder. These findings align with Hobbiesiefken et al. (2021), who noted that mice have a preference for elevated structures. We found that Swiss males spent more time on the elevated shelves compared to other groups, which might have been associated with the inherent tendency of male mice to establish hierarchically organized socio-spatial networks and segregate into distinct network communities. (Williamson et al., 2016). However, the area above the two elevated shelves in the pet cages was associated with attractive resources, including food, water, and other enrichments, such as running disks, as the shelves were used as a surface to place resources on. Therefore, preference for areas within the pet cages may not be completely independent of preferences for specific resources, such as food and water.

Sexes and strains of mice usually differ in behaviour and resource use (Tran et al., 2021; Võikar et al., 2001), making recommendations for improving the housing environment difficult. To enhance generalisability, we used two group sizes, both sexes and two common strains in behavioural neuroscience research (Marchette et al., 2018), that differ in many aspects of home cage behaviour (Crawley, 2007; Nicol et al., 2008; Weber et al., 2023), and housed them in groups of three or five animals. Even though most of our findings were consistent across sex, strain and group size, our data suggest some trends depending on these variables, consistent with published literature. We see more aggression and social investigations in males and Swiss mice (Lidster et al., 2019; Weber et al., 2023), more climbing on the cage grid in females (Borbélyová et al., 2019; Pietropaolo et al., 2007) and in C57BL/6 mice (Crawley, 2007). No strain and sex differences were seen in running wheel activity, although we observed that Swiss mice used the larger vertical wheel more than C57BL/6 mice, possibly due to their larger body size, although mice prefer some types of wheels over others (Banjanin and Mrosovsky, 2000; Walker and Mason, 2018). Mice were housed in groups of three or five animals, as it has been reported that housing three animals per cage was associated with increased prevalence of aggression (Lidster et al., 2019) and competition for resources (McQuaid et al., 2012; Mesa-Gresa et al., 2013); however, we observed no effect of group size on social behaviour and aggression. Mice in groups of three exhibited more stereotypic behaviour compared to those in groups of five. This finding may be attributed to an underestimation of stereotypic behaviour in the larger groups, likely due to higher stocking density leading to more frequent interruptions of stereotypic behaviour bouts. Since stereotypy was coded only when it persisted for at least three seconds, any interruption within this duration prematurely ended the behaviour bout before it was recorded. Therefore, caution is advised in interpreting the relationship between group size and stereotypic behaviour based on these observations.

5. Conclusion

Our study shows that laboratory mice housed in enriched cages exhibit increased locomotion and selective use of available resources, such as the deep bedding and running disc, irrespective of strain and sex. These findings suggest that standard housing conditions may inhibit the natural activity and movement patterns of mice, possibly failing to provide adequate shelters and space for activity. Given that housing conditions significantly impact animal welfare there is a pressing need for further studies. These should explore the implications of inadequate bedding depth and limited opportunities for physical activity on the well-being and health of laboratory mice. Such research is critical in view of refining the housing standards and enhancing the overall welfare of these animals.

CRediT authorship contribution statement

Milena Sanches Fortes: Investigation. **Michelle Gygax:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hanno Würbel:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Bernhard Voelkl:** Writing – review & editing, Investigation, Formal analysis. **Janja Novak:** Writing – review & editing, Project administration, Methodology, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2024.106381.

References

Adams, N., Boice, R., 1981. Mouse (Mus) burrows: effects of age, strain, and domestication. Anim. Learn. Behav. 9, 140–144. https://doi.org/10.3758/BF03212036.

Bailoo, J.D., Murphy, E., Boada-Saña, M., Varholick, J.A., Hintze, S., Baussière, C., Hahn, K.C., Göpfert, C., Palme, R., Voelkl, B., Würbel, H., 2018a. Effects of cage enrichment on behavior, welfare and outcome variability in female mice. Front. Behav. Neurosci. 12, 1–20. https://doi.org/10.3389/fnbeh.2018.00232.

Bailoo, J.D., Murphy, E., Varholick, J.A., Novak, J., Palme, R., Würbel, H., 2018b. Evaluation of the effects of space allowance on measures of animal welfare in laboratory mice. Sci. Rep. 8, 1–11. https://doi.org/10.1038/s41598-017-18493-6.

Banjanin, S., Mrosovsky, N., 2000. Preferences of mice, Mus musculus, for different types of running wheel. Lab. Anim. 34, 313–318. https://doi.org/10.1258/002367700780384681.

Borbélyová, V., Janišová, K., Myslivеček, J., Riljak, V., 2019. Sex-related differences in locomotion and climbing of C57Bl/6NTac mice in a novel environment. Physiol. Res. 68, S353–S359. https://doi.org/10.33549/physiolres.934348.

Büttner, D., 1991. Climbing on the cage lid, a regular component of locomotor activity in the mouse. J. Exp. Anim. Sci. 34, 165–169.

Cait, J., Cait, A., Scott, R.W., Winder, C.B., Mason, G.J., 2022. Conventional laboratory housing increases morbidity and mortality in research rodents: results of a meta-analysis. BMC Biol. 20, 1–22. https://doi.org/10.1186/S12915-021-01184-0.

Carbone, L., 2021. Estimating mouse and rat use in American laboratories by extrapolation from Animal Welfare Act-regulated species. Sci. Rep. 11, 1–6. https://doi.org/10.1038/s41598-020-79961-0.

Cintra, L., Alexandre-Ribeiro, S.R., Teixeira, J.T.X., Megid, M.M., Coucolis, T.V., Zanatto, D.A., Leal, V.N., Massironi, S.M.G., Mori, C.M.C., 2024. Nest-building in breeding mice: Impact of macro- and micro-environment. Lab. Anim. 0, 1–12. https://doi.org/10.1177/00236772231171191.

Cotman, C.W., Berchtold, N.C., 2002. Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci. 25, 295–301. https://doi.org/10.1016/S0166-2236(02)02143-4.

Crawley, J.N., 2007. Social Behaviors, in: What's Wrong with My Mouse?: Behavioral Phenotyping of Transgenic and Knockout Mice. John Wiley & Sons., pp. 206–210.

Dawkins, M.S., 2023. Natural behaviour is not enough: farm animal welfare needs modern answers to tinbergen's four questions. Animals 13, 988. https://doi.org/10.3390/ANI13060988.

De Waard, M.C., Duncker, D.J., 2009. Prior exercise improves survival, infarct healing, and left ventricular function after myocardial infarction. J. Appl. Physiol. 107, 928–936. https://doi.org/10.1152/japplphysiol.91281.2008.

Diederich, K., Bastl, A., Wersching, H., Teuber, A., Strecker, J.K., Schmidt, A., Minnerup, J., Schäbitz, W.R., 2017. Effects of different exercise strategies and intensities on memory performance and neurogenesis. Front. Behav. Neurosci. 11, 1–9. https://doi.org/10.3389/fnbeh.2017.00047.

Engesser-Cesar, C., Anderson, A.J., Basso, D.M., Edgerton, V.R., Cotman, C.W., 2005. Voluntary wheel running improves recovery from a moderate spinal cord injury. J. Neurotrauma 22, 157–171. https://doi.org/10.1089/neu.2005.22.157.

European Commission, 2010. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Off. J. Eur. Union 28, 82–128.

European Commission., 2022. ALURES—Animal Use Reporting—EU System. EU NTS Database on the Use of Animals for Scientific Purposes Under Directive 2010/63/EU [WWW Document]. Eur. Comm. URL https://webgate.ec.europa.eu/envdataportal/content/alures/section2_number-of-uses.html (accessed 4.19.24).

Fraser, D., 2009. Animal behaviour, animal welfare and the scientific study of affect. Appl. Anim. Behav. Sci. 118, 108–117. https://doi.org/10.1016/j.applanim.2009.02.020.

Freymann, J., Tsai, P.P., Stelzer, H., Hackbarth, H., 2015. The amount of cage bedding preferred by female BALB/c and C57BL/6 mice. Lab Anim. 44 (NY), 17–22. https://doi.org/10.1038/laban.659.

Freymann, J., Tsai, P.P., Stelzer, H., Hackbarth, H., 2017. The impact of bedding volumes on laboratory mice. Appl. Anim. Behav. Sci. 186, 72–79. https://doi.org/10.1016/j.applanim.2016.11.004.

Fureix, C., Trevarthen, A.C., Finnegan, E.M., Bučková, K., Paul, E.S., Mendl, M.T., 2022. Do greater levels of in-cage waking inactivity in laboratory mice reflect a spontaneous depression-like symptom? A pharmacological investigation. Pharmacol. Biochem. Behav. 212, 1–10. https://doi.org/10.1016/j.pbb.2021.173311.

Garber, J.C., 2011. On the care and use of US lab animals. Nature 476, 152. https://doi.org/10.1038/476152a.

Garner, J., 2023. www.mousebehaviour.org [WWW Document]. URL https://mousebehavior.org/ethogram/.

Gaskill, B.N., Gordon, C.J., Pajor, E.A., Lucas, J.R., Davis, J.K., Garner, J.P., 2012. Heat or insulation: Behavioral titration of mouse preference for warmth or access to a nest. PLoS One 7, 1–11. https://doi.org/10.1371/journal.pone.0032799.

Gaskill, B.N., Gordon, C.J., Pajor, E.A., Lucas, J.R., Davis, J.K., Garner, J.P., 2013. Impact of nesting material on mouse body temperature and physiology, 110–111 Physiol. Behav. 87–95. https://doi.org/10.1016/j.physbeh.2012.12.018.

Goh, J., Ladiges, W., 2015. Voluntary Wheel Running in Mice. Curr. Protoc. Mouse Biol. 5, 283–290. https://doi.org/10.1002/9780470942390.mo140295.

Greenwood, B.N., Foley, T.E., Le, T.V., Strong, P.V., Loughridge, A.B., Day, H.E.W., Fleshner, M., 2011. Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway. Behav. Brain Res. 217, 354–362. https://doi.org/10.1016/j.bbr.2010.11.005.

Gross, A.N., Richter, S.H., Engel, A.K.J., Würbel, H., 2012. Cage-induced stereotypies, perseveration and the effects of environmental enrichment in laboratory mice. Behav. Brain Res. 234, 61–68. https://doi.org/10.1016/j.bbr.2012.06.007.

Hess, S.E., Rohr, S., Dufour, B.D., Gaskill, B.N., Pajor, E.A., Garner, J.P., 2008. Home improvement: C57BL/6J mice given more naturalistic nesting materials build better nests. J. Am. Assoc. Lab. Anim. Sci. 47, 25–31.

Hobbiesiefken, U., Mieske, P., Lewejohann, L., Diederich, K., 2021. Evaluation of different types of enrichment - their usage and effect on home cage behavior in female mice. PLoS One 16, 1–22. https://doi.org/10.1371/journal.pone.0261876.

König, B., 2012. The Behaviour of the House Mouse, in: Hedrich, H. (Ed.), The Laboratory Mouse. Academic Press, pp. 367–381. https://doi.org/10.1016/B978-0-12-382008-2.00016-7.

Latham, N., Mason, G., 2004. From house mouse to mouse house: The behavioural biology of free-living Mus musculus and its implications in the laboratory. Appl. Anim. Behav. Sci. 86, 261–289. https://doi.org/10.1016/j.applanim.2004.02.006.

Lidster, K., Owen, K., Browne, W.J., Prescott, M.J., 2019. Cage aggression in group-housed laboratory male mice: an international data crowdsourcing project. Sci. Rep. 9, 1–12. https://doi.org/10.1038/s41598-019-51674-z.

Mackintosh, J.H., 1981. Behaviour of the House Mouse, in: Berry, R.J. (Ed.), Biology of the House Mouse. Zoological Society of London, pp. 337–365.

Maclellan, A., Nazal, B., Young, L., Mason, G., 2022. Waking inactivity as a welfare indicator in laboratory mice: Investigating postures, facial expressions and depression-like states. R. Soc. Open Sci. 9, 1–21. https://doi.org/10.1098/rsos.221083.
Makowska, I.J., Franks, B., El-Hinn, C., Jorgensen, T., Weary, D.M., 2019. Standard laboratory housing for mice restricts their ability to segregate space into clean and dirty areas. Sci. Rep. 9, 1–10. https://doi.org/10.1038/s41598-019-42512-3.
Manzanares, G., Brito-Da-Silva, G., Gandra, P.G., 2019. Voluntary wheel running: Patterns and physiological effects in mice. Braz. J. Med. Biol. Res. 52, 1–9. https://doi.org/10.1590/1414-431X20187830.
Marchette, R.C.N., Bicca, M.A., Santos, E.C. da S., de Lima, T.C.M., 2018. Distinctive stress sensitivity and anxiety-like behavior in female mice: strain differences matter. Neurobiol. Stress 9, 55–63. https://doi.org/10.1016/j.ynstr.2018.08.002.
Mason, G.J., 1991. Stereotypies: a critical review. Anim. Behav. 41, 1015–1037.
McQuaid, R.J., Audet, M.C., Anisman, H., 2012. Environmental enrichment in male CD-1 mice promotes aggressive behaviors and elevated corticosterone and brain norepinephrine activity in response to a mild stressor. Stress 15, 354–360. https://doi.org/10.3109/10253890.2011.623249.
Meijer, J.H., Robbers, Y., 2014. Wheel running in the wild. Proc. R. Soc. B Biol. Sci. 281 https://doi.org/10.1098/rspb.2014.0210.
Mesa-Gresa, P., Pérez-Martinez, A., Redolat, R., 2013. Environmental enrichment improves novel object recognition and enhances agonistic behavior in male mice. Aggress. Behav. 39, 269–279. https://doi.org/10.1002/ab.21481.
Mieske, P., Butzke, D., Rudeck, J., Lewejohann, L., Diederich, K., 2022. Bored at home ? - A systematic review on the effect of environmental enrichment on the welfare of laboratory rats and mice. Front. Vet. Sci. 9, 1–31. https://doi.org/10.21203/rs.3.rs-1411623/v1.
Muguruza, C., Redon, B., Fois, G.R., Hurel, I., Scocard, A., Nguyen, C., Stevens, C., Soria-Gomez, E., Varilh, M., Cannich, A., Daniault, J., Busquets-Garcia, A., Pelliccia, T., Caillé, S., Georges, F., Marsicano, G., Chaouloff, F., 2019. The motivation for exercise over palatable food is dictated by cannabinoid type-1 receptors. JCI Insight 4, 1–17. https://doi.org/10.1172/jci.insight.126190.
National Research Council of the National Academies, 2011. Guide for the care and use of laboratory animals., 8th ed. The National Academies Press, Washington, DC.
Nevison, C.M., Hurst, J.L., Barnard, C.J., 1999. Strain-specific effects of cage enrichment in male laboratory mice (Mus musculus). Anim. Welf. 8, 361–379.
Nichol, K.E., Parachikova, A.I., Cotman, C.W., 2007. Three weeks of running wheel exposure improves cognitive performance in the aged Tg2576 mouse. Behav. Brain Res. 184, 124–132. https://doi.org/10.1016/j.bbr.2007.06.027.
Nicol, C.J., Brocklebank, S., Mendl, M., Sherwin, C.M., 2008. A targeted approach to developing environmental enrichment for two strains of laboratory mice. Appl. Anim. Behav. Sci. 110, 341–353. https://doi.org/10.1016/j.applanim.2007.05.006.
Novak, C.M., Burghardt, P.R., Levine, J.A., 2012. The use of a running wheel to measure activity in rodents: Relationship to energy balance, general activity, and reward. Neurosci. Biobehav. Rev. 36, 1001–1014. https://doi.org/10.1016/j.neubiorev.2011.12.012.
Novak, J., Bailoo, J.D., Melotti, L., Würbel, H., 2016. Effect of cage-induced stereotypies on measures of affective state and recurrent perseveration in CD-1 and C57BL/6 mice. PLoS One 11, 1–22. https://doi.org/10.1371/journal.pone.0153203.
Olsson, I.A.S., Dahlborn, K., 2002. Improving housing conditions for laboratory mice: a review of "environmental enrichment. Lab. Anim. 36, 243–270. https://doi.org/10.1258/002367702320162379.
Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W. J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S.T., Howells, D.W., Karp, N.A., Lazic, S.E., Lidster, K., MacCallum, C.J., Macleod, M., Pearl, E.J., Petersen, O.H., Rawle, F., Reynolds, P., Rooney, K., Sena, E.S., Silberberg, S.D., Steckler, T., Würbel, H., 2020. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research*. J. Cereb. Blood Flow. Metab. 40, 1769–1777. https://doi.org/10.1177/0271678X20943823.
Pietropaolo, S., Mintz, M., Feldon, J., Yee, B.K., 2007. The behavioral sequela following the prevention of home-cage grid-climbing activity in C57BL/6 mice. Behav. Neurosci. 121, 345–355. https://doi.org/10.1037/0735-7044.121.2.345.
Randall, C., 1999. Vertebrate pest management - a guide for commercial applicators. Ext. Bull. 3–2050.
Ratuski, A.S., Weary, D.M., 2022. Environmental enrichment for rats and mice housed in laboratories: a metareview. Animals 12, 414. https://doi.org/10.3390/ANI12040414.
Ratuski, A.S., Makowska, I.J., Dvorack, K.R., Weary, D.M., 2021. Using approach latency and anticipatory behaviour to assess whether voluntary playpen access is rewarding to laboratory mice. Sci. Rep. 11, 1–13. https://doi.org/10.1038/s41598-021-98356-3.
Rowe, F.P., 1981. Wild house mouse biology and control, in: Biology of the House Mouse. Academic Press, pp. 575–589.
Schweizer Tierschutz STS, 2019. STS Merkblatt - Farbaus [WWW Document]. URL http://www.tierschutz.com/publikationen/heimtiere/infothek/kleintiere/maus.pdf.
Sherwin, C.M., 1996. Laboratory mice persist in gaining access to resources: a method of assessing the importance of environmental features. Appl. Anim. Behav. Sci. 48, 203–213. https://doi.org/10.1016/0168-1591(96)01027-1.
Sherwin, C.M., Haug, E., Terkelsen, N., Vadgama, M., 2004. Studies on the motivation for burrowing by laboratory mice. Appl. Anim. Behav. Sci. 88, 343–358. https://doi.org/10.1016/j.applanim.2004.03.009.
Streiff, C., Herrera, A., Voelkl, B., Palme, R., Würbel, H., Novak, J., 2024. The impact of cage dividers on mouse aggression, dominance and hormone levels. PLoS One 19, e0297358. https://doi.org/10.1371/JOURNAL.PONE.0297358.
Swiss Animal Welfare Ordinance (SR 455.1) [WWW Document], 2008. AS 2008 2985. URL https://www.fedlex.admin.ch/eli/cc/2008/416/de (accessed 4.19.24).
Tallent, B.R., Law, L.M., Lifshitz, J., 2024. Partially divided caging reduces overall aggression and anxiety which may indicate improved welfare in group housed male C57BL/6J mice. BMC Vet. Res. 20, 1–11. https://doi.org/10.1186/S12917-024-03918-Y.
Tran, T., Mach, J., Gemikonakli, G., Wu, H., Allore, H., Howlett, S.E., Little, C.B., Hilmer, S.N., 2021. Male-female differences in the effects of age on performance measures recorded for 23 hours in mice. J. Gerontol. - Ser. A Biol. Sci. Med. Sci. 76, 2141–2146. https://doi.org/10.1093/gerona/glab182.
Van De Weerd, H.A., Van Loo, P.L.P., Van Zutphen, L.F.M., Koolhaas, J.M., Baumans, V., 1998. Strength of preference for nesting material as environmental enrichment for laboratory mice. Appl. Anim. Behav. Sci. 31, 133–143. https://doi.org/10.1016/S0168-1591(97)00043-9.
Van Praag, H., Kempermann, G., Gage, F.H., 2000. Neural consequences of enviromental enrichment. Nat. Rev. Neurosci. 1, 191–198. https://doi.org/10.1038/35044558.
Van Praag, H., Shubert, T., Zhao, C., Gage, F.H., 2005. Exercise enhances learning and hippocampal neurogenesis in aged mice. J. Neurosci. 25, 8680–8685. https://doi.org/10.1523/JNEUROSCI.1731-05.2005.
Võikar, V., Kõks, S., Vasar, E., Rauvala, H., 2001. Strain and gender differences in the behavior of mouse lines commonly used in transgenic studies. Physiol. Behav. 72, 271–281. https://doi.org/10.1016/S0031-9384(00)00405-4.
Walker, M., Mason, G., 2018. A comparison of two types of running wheel in terms of mouse preference, health, and welfare. Physiol. Behav. 191, 82–90. https://doi.org/10.1016/j.physbeh.2018.04.006.
Walker, M.D., Duggan, G., Roulston, N., Van Slack, A., Mason, G., 2012. Negative affective states and their effects on morbidity, mortality and longevity. Anim. Welf. 21, 497–509. https://doi.org/10.7120/09627286.21.4.497.
Weber, E.M., Zidar, J., Ewaldsson, B., Askevik, K., Udén, E., Svensk, E., Törnqvist, E., 2023. Aggression in group-housed male mice: a systematic review. Animals 13. https://doi.org/10.3390/ani13010143.
Williamson, C.M., Franks, B., Curley, J.P., 2016. Mouse social network dynamics and community structure are associated with plasticity-related brain gene expression. Front. Behav. Neurosci. 10, 1–16. https://doi.org/10.3389/fnbeh.2016.00152.
Wolfer, D.P., Litvin, O., Morf, S., Nitsch, R.M., Lipp, H.-P., Würbel, H., 2004. Laboratory animal welfare: cage enrichment and mouse behaviour. Nature 432, 821–822. https://doi.org/10.1038/432821a.
Würbel, H., 2001. Ideal homes? Housing effects on rodent brain and behaviour. Trends Neurosci. 24, 207–211. https://doi.org/10.1016/S0166-2236(00)01718-5.
Würbel, H., 2006. The motivational basis of caged rodents' stereotypies. : Stereo Anim. Behav.: Fundam. Appl. Welf.: Second Ed. 86–120. https://doi.org/10.1079/9780851990040.0086.
Würbel, H., Novak, J., 2022. Environmental enrichment: animal welfare and scientific validity, in: Golledge, H., Richardson, C. (Eds.), The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals. John Wiley & Sons, Ltd, p. 137. https://doi.org/10.1002/9781119555278.CH10.
Würbel, H., Stauffacher, M., Von Holst, D., 1996. Stereotypies in laboratory mice—quantitative and qualitative description of the ontogeny of 'wire-gnawing'and 'jumping'in Zur: ICR and Zur: ICR nu. Ethology 102, 371–385.
Würbel, H., Chapman, R., Rutland, C., 1998. Effect of feed and environmental enrichment on development of stereotypic wire-gnawing in laboratory mice. Appl. Anim. Behav. Sci. 60, 69–81. https://doi.org/10.1016/S0168-1591(98)00150-6.